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Antifungal, nutritional and phytochemical investigation of *Actiniopteris radiata* of district Dir Lower, Pakistan

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Abstract
The objective of the present study was to study the nutritional analysis, antifungal activities and find out the presence of phytochemicals in the aqueous, ethanol and methanol extracts of *Actiniopteris radiata* collected from different areas of Khyber Pakhtoon Khwa by both quantitative and qualitative screening methods. In qualitative analysis, the phytochemical compounds such as alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, volatile oils, resins, glycosides and terpenoids were screened. In quantitative analysis, the phytochemical compounds such as total phenolic and total flavonoids were quantified. The ethanolic fern extract performed well to show positivity rather than aqueous and methanolic extracts for the 13 phytochemicals. In quantitative analysis the important secondary metabolite total phenol and total flavonoids content were tested. The ethanolic extract of total flavonoids and total phenol content were highest. Also comparatively studied for nutritional analysis. Ash in Sample from Tahtbahi 26.44%, 22.83%, in sample from Luqman Banda and 6.01% in sample from Dermal Bala. Moisture was found 18.69% in sample from Luqman Banda and lowest amount was found Jandul 10.27%. Protein highest amount found in sample from Hall 4.37% and lowest amount was found in sample from Dermal Bala 0.85%. Fats highest amount 74.27% in sample from Dermal Bala and lowest amount found in sample from Shahi Benshay 47.17%. The antifungal activity of all sample were collected from different areas showed inhibition against each fungal strands. The most active among the plants was sample from Tahtbahi with 17.00mm zone of inhibition.

Keywords: Phytochemistry, antifungal, nutritional analysis, *Actiniopteris radiata*, Dir lower, Pakistan

1. Introduction
Ferns and their allies are in a major division of the Plant Kingdom called Pteridophyta and they have been around for millions of years. There are over 250 different genera and 12,000 species of ferns reported all over the world (Chang *et al*., 2011) [7]. It has been observed that pteridophytes are not infected by microbial pathogens which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years (Sharma and Vyas 1985) [37]. As per folk medicine, the pteridophytes have been known for more than 2000 years and also been mentioned in ancient literature (Kirtikar and Basu, 1935) [23]. The medicinal use of the pteridophytes was suggested by Ayurvedic systems of medicine. In the Unani system of medicine these are also used (Uddin *et al*., 1998) [43]. Pteridophytes are resistant to microbial infection which may be one of the crucial factors for their evolutionary success and the fact that they lasted for more than 350 million years (Shinozaki *et al*., 2008) [39]. In the recent past, many traditional medicinal ferns were analyzed and reported to have various bioactivities, such as antioxidant, antitumor and anti-HIV, antimicrobial, anti-inflammatory and antiviral (Shakoor *et al*., 2013) [36]. It showed bioactivity properties such as antimicrobial, anti-inflammatory, anti tussive, antitumor, etc. But a very little research has been carried out on the evaluation of bioactive properties of pteridophytes. Hence an attempt has been made to evaluate the phytochemical and nutritional properties of native pteridophytes of Khyber Pakhtoon Khwa. Phytochemicals are naturally occurring chemical, biologically active compounds found in plants, which be responsible for health benefits for humans further these recognized to micronutrients and macronutrients (Hasler & Blumberg, 1999) [15]. They protect plants from damage and disease and contribute to the plant's color, flavor and aroma.

In common, the plant chemicals that defend plant cells from environmental threats such as stress, drought, pollution, pathogenic attack and UV exposure are called as phytochemicals (Gibson *et al*, 1998) [13]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher *et al*., 1999) [28] and in detail studied About 150 phytochemicals have been studied. In wide-ranging dietary phytochemicals are found in fruits, vegetables, whole grains, nuts, seeds, legumes, fungi, spices and herbs (Mathai, 2000) [27]. Broccoli, tomatoes, grapes, onions, garlic, cabbage, carrots, whole wheat bread, cherries, strawberries, beans, legumes and raspberries, soy foods are common sources (Moorachian, 2000) [29]. Phytochemicals accumulate in different parts of the plants, such as in the stems, leaves, roots, flowers, fruits. Various phytochemicals, mostly the pigment molecules, are often concentrated in the outer layers of the several plant tissues. Levels different from plant to plant depending upon the processing, variety, growing conditions and cooking (King & Young, 1999) [22]. Phytochemicals are also existing in additional forms, but proof is lacking that they make available the same health benefits as nutritional phytochemicals (Rajesh *et al*., 2016) [34]. *Actiniopteris radiata* (Swartz) Linn belongs to the family Actiniopteridaceae subfamily Pteridoideae. It is found throughout India, Pakistan, Afghanistan, Sri Lanka, Persia, Arabia, Yemen, South Eastern Egypt, Tropical Africa, Australia and Madagascar (Naik1 and Jadge, 2010). The plants are 8-25cm high rooting in the crevices of rocks or in between the joints of brick walls in moist and shady places. The rhizome is oblique to horizontal 1.5 to 2.0 cm in length, densely covered with wiry roots, the young leaves show circinate venation but the lamina become flat early stage of development. Plant known to possess antioxidant (Manjunath *et al*., 2009). Antibacterial, antifertility activity (Mathad *et al*., 2015).

2. Material and Methods

2.1 Collection of plant materials and Botanical Identification

In the present study, *Actiniopteris radiata* was collected in October, 2016 from district Malakand, Tahtbahi, Luqman Banda, Hall, Jandul, Dermal Bala and Shahi Benshay of Khyber Pakhtunkhwa Province. Plant samples were collected and with the help of Flora of Pakistan and already data present in the herbarium of Hazara University, Mansehra Plant were taxonomically identified and placed in the Herbarium of Abdul Wali Khan University Mardan.

2.2 Solvent system used

For the preparation of crude extract of the *Actiniopteris radiata* methanol, ethanol and distilled water was used.

2.3 Crashing and filtration of the plant

The dried plant was powdered with the help of electric grinder. The powder were kept in air tight plastic bottles for further phytochemical analysis. 10 gm of plant powdered was retained in distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol and aqueous) was added to the powdered separately. With the help of aluminum foil the flask were covered and retained in shaker for 72 hrs for the shaking purposes. After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through filtration process plant extracts were removed (Pirzada *et al*., 2010) [33].

2.4 Phytochemical analysis

2.4.1 Qualitative study

The plant extract i.e. methanol, ethanol and aqueous were tasted for the absence or presence of phytochemical constituents' like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, volatile oils, resins glycosides and terpenoids (Soni *et al*., 2011) [41].

2.4.2 Tests for Alkaloids

For detection of alkaloids, a few drops of Wagner's reagent (Potassium iodine) are add to 2 ml of all three methanol, ethanol and aqouse extracts. The formation of reddish brown precipitate showed the presence of alkaloids (Khandewal *et al*., 2015) [21].

2.4.3 Tests for Tannins

For the detection of tannins Ferric chloride test was done. Ferric chloride (FeCl3) solution was mixed with all three extracts separately. Formation of blue green coloration indicated the presence of tannins. (Kokate *et al*., 2008) [24].

2.4.4 Tests for Phlobatannins

In test tubes 0.5 ml of all the three extracts was taken separately, added 3ml distilled water and shaken for a few minutes then 1% aqueous hydro chloride (HCl) was added and boiled on water both. The presence of phlobatannins is indicated by the formation of red color (Wadood *et al*., 2013) [44].

2.4.5 Tests for Flavonoids

For flavonoids detection, all the three extracts were treated with sodium hydroxide (NaOH) solution. Red precipitation formation of indicate the presence of flavonoids (Kokate *et al*., 2008) [24].

2.4.6 Tests for Carbohydrates

For detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's regent. The solution were heated for 2 minutes on a water bath. By the formation of reddish brown precipitate the presence of carbohydrate was confirmed (Bussau, *et al*., 2002) [6].

2.4.7 Tests for Phenols

For phenol detection, 2 ml of ferric chloride (FeCl3) solution was added to 2 ml of all the three extracts in a test tube separately. Formations of deep bluish green solution showed the presence of phenol (Dahiru *et al*., 2006) [9, 10].

2.4.8 Tests for Saponins

For the detection of saponin, in test tube 5 ml of all three extracts were shaken vigorously. The formation of froth indicated the presence of saponins (Rajesh *et al*., 2016) [34].

2.4.9 Tests for (Cardiac) Glycosides

For cardiac glycosides detection, 2 ml of all three extracts solution were shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid (H2SO4) and iron tri chloride (FeCl3). The formation of a brown ring indicated the presence of glycosides (Soni *et al*., 2011) [41].

2.4.10 Tests for proteins
Xanthoproteic test: For the detection of protein, 1 ml from of all three extracts were treated with 1ml of concentrated nitric acid (HNO3) solution. The presence of proteins indicated by the formation of yellow color (Rajesh *et al*., 2016) [34].

2.4.11 Test for volatile oils
For volatile oils detection, 2ml of plant extracts were shaken with 0.1ml of dilute sodium hydroxide (NaOH) than added small amounts of dilute hydro chloride (HCl). A white precipitate formation indicated the presence of volatile oils (Dahiru *et al*., 2006) [9, 10].

2.4.12 Tests for resins
Turbidity test: For the detection of resins, 5 ml distilled water were added to the plant extracts. The presence of resins conformed by turbidity (Chaouch *et al*., 2011).

2.4.13 Tests for terpenoids
Salkowski test: One ml of Actiniopteris radiata plant extracts (methanol, ethanol and aqouse) was added with 2 ml of chloroform and carefully added concentrated sulphuric acid (H2SO4) along the sides of tube to form a layer. The formation of reddish brown coloration indicated the presence of terpenoids (Dahiru *et al*., 2006) [9, 10].

2.4.14 Tests for Glycosides
For the detection of glycosides, 5% of Ferric chloride solution and 1 ml glacial acetic acid were added to 5 ml of all three extracts and then further addition of few drops of concentered sulphuric acid (H2SO4). The presence of glycosides was conformed through the formation of greenish blue color (Rajesh *et al*., 2016) [34].

2.4.15 Quantitative analysis of total flavonoids and phenols Contents
2.4.16 Determination of total flavonoids contents Ethanol, methanol and aqueous extracts were used for the detection of total flavonoids contents. Total flavonoids quantification was done by taking 0.5 g of plant extracts. Than the sample were mixed with 4.3 ml methanol and then more addition of 0.1 ml of aluminum tri chloride from 10% prepared solutions of aluminum tri chloride laterally. Potassium acetate (0.1 ml) was added the volume was reached to 5 ml. The mixtures were shaken by vortex to make uniform solution and then these mixture were placed at room temperature for 30 minutes for the purpose of incubation. After the completion of incubation process, the absorption was checked at 415 nm in spectrum. The Quercetin was used as a standard (Daffodil *et al*, 2013) [8].

2.4.17 Determination of Total Phenolic Contents
Total phenolic quantification was done by the addition of 0.5g plant extract to 1 ml of 80% ethanol. Then the mixture were centrifuged for 15 minutes at 12,000 rpm. After that the supernatant were kept in test tube and these process were repeated 6 times. After collecting the supernatant were placed in water bath for drying. The distilled water was added to the supernatant until its volume reached to 3 ml. 2 ml (Na2CO3) of 20% were added in this solution. To this 0.5 ml Folin ciocalteau regent was added and after 5 minutes more addition of 2 ml (Na2Co3) from 20% Na2Co3 solutions. The solution were mixed homogenously and then the test tube were brought in to the water bath in boiling water. At 650 nm their absorbance were checked. The Catechol was used as a standard (Hagerman *et al*., 2004).

3.6 Nutritional Analysis
3.6.1 Proximate Analysis
The proximate analysis (fats, proteins, moisture, carbohydrates and ash) plant was determined by using AOAC methods (AOAC, 1990; AOCS, 2000).

3.6.2 Determination of Moisture contents
Petri plates were autoclaved for 35 minutes at 105 ^{0}C. In a desiccator the petri plates were cooled and then weighed. 3 gram dry plant powder was taken and for 6 hours in an oven placed at 105 ^{0}C. From oven the plates were removed, in a desiccators cooled and then weighed. 5 times repeated this process. The moisture content was calculated by using the following formula.

$$\text{Moisture contents (\%)} = \frac{\text{Fresh sample Weight} - \text{dry sample Weight}}{\text{Weight of sample}} \times 100$$

3.6.3 Ash Determination
The crucible were washed, cleaned and placed at 550 ^{0}C for 30 minutes in furnace for drying. In desiccators the crucibles was placed for cooling purpose. 2 gm of dried plant powder was taken in a crucible. Over a low fire the sample were burnt. In a Muffle furnace then at about 550 ^{0}C the crucible was placed and remain left for 3 hours until white ash was formed. From crucibles the ash was taken out and placed in desiccators for cooling. At last the crucible was weighted. Ash percentage was calculated by the following formula (AOCS, 2000).

$$\text{Ash (\%)} = (\%)\frac{\text{weight of sample after ashing}}{\text{Weight of sample}} \times 100$$

3.6.4 Curd Fat Determination
6 gm of plant powder was taken in paper thimble, and attaching to a goldfish of sox let extractor. 250 mL of petroleum either or n-hexane solvent was transferred in to the top of the thimble. The thimble was run for 6 hours on heating mantle. The thimble was removed when the Crude fat of plant were formed. The paper thimble was cooled in a desiccator and then weighed. By using the following formula the percent crude fat was calculated (AOCS, 2000).

3.6.5 Protein Determination
The determination of protein in plant sample is categorized into three stages
1. Digestion of the powder
2. Distillation of the powder
3. Titration of the powder

3.6.6 Digestion of the powder
In a digestion flask One gm of the dried powder of plant was taken. Approximately 2gm of the digestion mixture (potassium sulphate, ferrous sulphate, copper sulphate: 18; 1:.5:0.25 (w/w/w) and then added to the flask 20 ml of concentrated sulphuric acid. the solution was boiled, than cooled and digest and about 30 ml distil water was added in 5 ml portions with mixing, and transferred in to a 100 mL volumetric flask and made the volume up to the mark.

3.6.7 Distillation of the powder
The pranas Wagner distillation muster was set, added 2 drops of methyl red indicator than about 40 mL of 4% boric acid was added to appear pink colored. 5 milliliter of the digest was transferred to distillation assembly. In the assembly 10 mL of 40% sodium hydroxide solution were added to digest. After 10 minutes the process of distillation were completed and indicated by conversion. The color of boric acid become yellow due to formation of ammonium borate.

3.6.8 Titration of the powder
In the presence of trapped ammonia the addition of 0.1N hydrochloric acid to boric acid. When color of boric acid changed again to pink the presence of ammonia will be confirmed. For the calculation of protein amount the following formula were used.

Where 6.25 = Protein factor for vegetable 1.4 = Weight of Nitrogen in gram

3.7 Anti-fungal activity
3.7.1 Media preparation for fungal growth Dissolve 39 g of Potato dextrose agar (PDA) in 1litre of distilled water, sterilized by autoclaved at 15psi (121 ºC) for 15 minutes. Cool to room temperature and pour into sterilized Petri plates to solidify. Kept at room temperature to solidify for 30 minutes.

3.7.2 Agar well diffusion method
Agar well diffusion method was followed as described by Samie *et al*., (2010) [35]. Using the micropipette, 100µl of different fungal cultures in sterile distilled water (SDW) was placed over the surface of an agar plate and spread using a sterile inoculation loop. Using a sterile Cork borer, hole) were made in each of the culture plates. 75µl of crude extract of selected plants were added. The culture plates were then incubated at 37 °C, and the results were observed after 24 hours depending on the fungal growth. The clear zone around each well was measured in mm, indicating the activity of the plant extract against the fungus. Each test was triplicated and standard deviation was calculated.

4. Statistical analysis
All the tests were performed as individual triplicate experiment. All the data are expressed as mean ± standard deviation.

5. Results and Discussion
5.1 Phytochemical analysis
In the present research work both qualitative and quantitative investigation of methanolic, ethanolic and aqueous extracts of Actiniopteris radiata was carried out.

5.1.1 Qualitative Detection of Bioactive compound in the whole plant
Qualitative analysis of Actiniopteris radiata was carried out for the detection of alkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides, proteins, volatile oils and resins. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins and glycosides were found absent in the aqueous extracts. Flavonoids, carbohydrates, saponins, phenols, terpenoids and protein were found present in aqueous extracts (Table 1, 2 and 3).

Table 1: Phytochemical analysis of different samples of *Actiniopteris radiata* in methanolic extracts

S. NO	Phytochemical test	sample from Mlakand	sample from Tahtbahi	sample from Luqman Banda	sample from Hall	sample from Jandul	sample from Dermal Bala	sample from Shahi Benshay
1	Alkaloid	+	+	+	+	+	+	+
2	Flavonoids	+	+	+	+	+	+	+
3	Carbohydrate	+	+	+	+	+	+	+
4	Phlobatannins	+	+	+	+	+	+	+
5	Glycosides	-	-	-	-	-	-	-
6	Saponins	+	+	+	+	+	+	+
7	Phenol	+	+	+	+	+	+	+
8	Terpenoids	+	+	+	+	+	+	+
9	Tannins	+	+	+	+	+	+	+
10	Cardiac glycosides	_	_	_	_	_	_	_
11	Proteins	+	+	+	+	+	+	+
12	Volatile oils	_	_	_	_	_	_	_
13	Resins	_	_	_	_	_	_	_

Key: - : absent +: present

Table 2: Phytochemical analysis of different samples of *Actiniopteris radiata* in ethanolic extracts

S. No	Phytochemical test	sample from Mlakand	sample from Tahtbahi	sample from Luqman Banda	sample from Hall	sample from Jandul	sample from Dermal Bala	sample from Shahi Benshay
1	Alkaloid	+	+	+	+	+	+	+
2	Flavonoids	+	+	+	+	+	+	+
3	Carbohydrate	+	+	+	+	+	+	+
4	Phlobatannins	+	+	+	+	+	+	+
5	Glycosides	+	+	+	+	+	+	+
6	Saponins	+	+	+	+	+	+	+
7	Phenol	+	+	+	+	+	+	+
8	Terpenoids	+	+	+	+	+	+	+
9	Tannins	+	+	+	+	+	+	+

10	Cardiac glycosides	+	+	+	+	+	+	+
11	Proteins	+	+	+	+	+	+	+
12	Volatile oils	_	_	_	_	_	_	_
13	Resins	_	_	_	_	_	_	_

Key: - : absent +: present

Table 3: Phytochemical analysis of different samples of *Actiniopteris radiata* in aqouse extracts

S. No	Phytochemical test	sample from Malakand	sample from Tahtbahi	sample from Banda	sample from Hall	sample from Jandul	sample from Dermal Bala	sample from Shahi Benshay
1	Alkaloid	_	_	_	_	_	_	_
2	Flavonoids	+	+	+	+	+	+	+
3	Carbohydrate	+	+	+	+	+	+	+
4	Phlobatannins	_	_	_	_	_	_	_
5	Glycosides	_	_	_	_	_	_	_
6	Saponins	+	+	+	+	+	+	+
7	Phenol	+	+	+	+	+	+	+
8	Terpenoids	+	+	+	+	+	+	+
9	Tannins	+	+	+	+	+	+	+
10	Cardiac glycosides	_	_	_	_	_	_	_
11	Proteins	+	+	+	+	+	+	+
12	Volatile oils	_	_	_	_	_	_	_
13	Resins	+	+	+	+	+	+	+

Key: - : absent +: present

5.2.2 Quantitative investigation of Flavonoids and total Phenolic constituents

Actiniopteris radiata was comparatively studied for their total flavonoids contents and total phenolic compounds in the solvents methanol. (The Quercetin were used as a standard for flavonoids having y = 0.0031× + 0.0159 while the value of R2 = 0.9997. The Catechol was used as a standard for phenol having the values of R2 = 0.999 y= 0.0012 × + 0.0659).

5.2.3 Total Flavonoids Contents

Highest amount of flavonoids was found in the methanolic extract in sample from Jandul (0.86±0.924 mg/g) followed by sample from Dermal Bala (0.810±0.90 mg/g) and lowest amount of flavonoids was found in sample from Shahi Benshay (0.121±0.81mg/g) (Table 4).

5.2.4 Total Phenolic Contents

The highest amount of phenol was determined in methanolic sample from Mlakand (1.68.5±1.29 mg/g), followed by sample from Luqman Banda (0.96±0.92 mg/g) and lowest amount of phenol was found in sample from Hall (0.21±0.463 mg/g) (Table 4).

Table 4: Qualitative Phytochemistry of *Actiniopteris radiata* in Methanolic Extracts

S. No	Plants sample	Total phenolic contents mg/g	Total flavonoids contents mg/g
1	sample from Malakand	1.68±1.29	0.076±0.276
2	sample from Tahtbahi	0.59±0.76	0.067±0.26
3	sample from Luqman Banda	0.96±0.92	0.218±0.46
4	sample from Hall	0.21±0.463	0.684±0.82
5	sample from Jandul	0.36±0.613	0.86±0.924
6	sample from Dermal Bala	0.50±0.70	0.810±0.90
7	sample from Shahi Benshay	0.32±0.65	0.121±0.81

6. Nutritional analysis

Proximate composition of plant provides a valuable information about its medicinal and nutritional quality.

6.1.1 Determination of percentage of ash, moisture, protein and fats.

The percentage of ash, moisture, protein and fats was determined in Actiniopteris radiata as sample from Tahtbahi 26.44%, followed by 22.83%, in sample from Luqman Banda and lowest amount is found in 6.01% in sample from Dermal Bala respectively. Moisture was found 18.69% in sample from Luqman Banda and lowest amount was found in sample from Jandul 10.27%. Protein highest amount found in sample from Hall 4.37% and lowest amount was found in sample from Dermal Bala 0.85%. Fats highest amount found in 74.27% in sample from Dermal Bala and lowest amount found in sample from Shahi Benshay 47.17% (Table 5).

Table 5: Proximate compositions of *Actiniopteris radiata* Hook

S. No	Sample collected Area	% age of Ash	% age Moisture	% age Protein	% age Fat
1	sample from Malakand	20	16.26	2.55	47.19
2	sample from Tahtbahi	26.44	11.41	1.66	57.49
3	sample from Luqman Banda	22.83	18.69	1.75	52.73
4	sample from Hall	9.92	15.50	4.37	62.79
5	sample from Jandul	17.67	10.27	3.39	64.68
6	sample from Dermal Bala	6.01	11.17	0.85	74.27
7	sample from Shahi Benshay	19	16.17	2.57	47.17

7. Antifungal activity of crude extracts of *Actiniopteris radiata* against selected fungal strains

The results of antifungal activity showed that (Crude methanolic Extract) of all plants sample were active against all fungal species and showed different range of zone of inhibition. The most active among the plants was sample from Tahtbahi with 17.00mm zone of inhibition. Sample from Mlakand against Acremonium with 16.50mm zone of inhibition, followed by sample from Tahtbahi against *Trichoderma* with zone of inhibition of 16.41mm. Sample from Jandul was most active against *Trichoderma* with 16.06mm of zone of inhibition. Sample from Hall showed 15.33mm zone of inhibition against *Acremonium*, sample from Luqman Banda showed 14.77mm inhibition zone against *Trichoderma* while sample from Dermal Bala showed 12.33mm zone of inhibition against *Verticellium***.** The data are shown in table (6).

Table 6: Antifungal activity of crude extracts of *Asplenium dalhousiae* against selected fungal strains

Plant	*Alternaria*	*Acremonium*	*Verticellium*	*Pythium*	*Trichoderma*
sample from Malakand	6.89± 0.11	16.50± 1.89	13.44± 0.53	10.67± 1.30	11.65± 1.75
sample from Tahtbahi	10.33± 0.88	14.12± 0.192	17.00± 0.48	16.17± 0.93	16.41± 0.82
sample from Luqman Banda	10.80± 0.58	9.89± 0.63	13.62± 0.87	9.17± 1.01	14.77± 1.13
sample from Hall	9.76± 1.65	15.33± 1.129	7.93± 0.66	10.23± 0.96	13.12± 1.03
sample from Jandul	14.50± 1.25	13.57± 0.87	12.83± 0.95	14.27± 1.22	16.06± 0.97
sample from Dermal Bala	11.00± 1.00	10.23± 0.88	12.37± 1.86	10.00± 3.60	12.00± 2.08
sample from Shahi Benshay	6.32± 1.00	12.13± 0.78	11.13±1.65	13.16±0.57	10.062±1.32

8. Discussion

The present investigations of phytochemical screening of *Actiniopteris radiata* extracts shown the existence of alkaloids, flavonoids, carbohydrates, phlobatannins, glycosides, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts. Phytochemical ingredients which are present in plant samples are known to be biologically active compounds and they are responsible for diverse activities such as antioxidant, antimicrobial, anticancer, antifungal, and antidiabetic (Hossain & Nagooru, 2011) [17]. Wide variety of pharmacological activities showed by different phytochemicals, which may help in protection against chronic diseases. Tannins, flavonoids, saponins, glycosides, and amino acids have anti-inflammatory and hypoglycemic activities. Steroids and terpenoids shows central nervous system (CNS) activities and analgesic properties. Because of their antimicrobial activity saponins are involved in plant defense system (Ayoola *et al*., 2008) [4]. These phytochemicals showed antimicrobial activity through different mechanisms. With proline-rich protein tannins have been found to form irreversible complexes (Shimada, 2006) [38] resulting in the inhibition of cell protein synthesis. (Parekh and Chanda, 2007) [32]. Reported that tannins are known to react with proteins to deliver the typical tanning effect which is essential for the treatment of ulcerated or inflamed tissues. Herbs that have tannins as their key components are astringent in nature and are used for treating intestinal disorders such as dysentery and diarrhea (Dharmananda, 2003) [11]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile *et al*., 2007) [5]. These observations therefore support the use of Asplenium dalhousiae in herbal cure remedies, thus suggesting that Asplenium dalhousiae has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in Asplenium dalhousiae supports the traditional medicinal use of this plant in the treatment of different ailments. Alkaloid was another phytochemicals constituent's observed in the extract of Asplenium dalhousiae. One of the best common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the reduction and elimination of human cancer cell lines (Nobori, *et al*., 1994) [31]. One of the largest groups of phytochemicals as alkaloids in plants which have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002) [20]. (Just *et al*., 1998) [19] shown the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in Asplenium dalhousiae extracts and has supported the usefulness of this plant in managing inflammation. Flavonoids, another phytochemicals shows a varied range of biological activities like anti-inflammatory, antimicrobial, analgesic, anti-angionic, cytostatic, antioxidant and antiallergic properties (Hodek *et al*., 2002) [16]. Several reports are presented on flavonoid groups which showing high potential biological activities such as anti-inflammatory, antioxidant, antiallergic reactions (Thitilertdecha *et al*., 2008) [42]. The bioactive compounds such as tannins and flavonoids components were present in the crude extracts. However, these bioactive compounds were inducing the antioxidant and antimicrobial activities. The amount of active components in the crude extract may be diluted or increased their concentrations by fractionation (Anyasor *et al*., 2010) [3]. For all living creatures the nature has provided abundant plants which possess medicinal virtues. The significant values of some plants have long been available but a large number of them remain unknown. There is a need to search their uses and to perform pharmacological and phytochemical studies to determine their therapeutic properties the basic nutritional importance of plants is assessed by their protein and carbohydrate contents. Oils, fats vitamins, minerals and water which are responsible for the development and growth in man and animals (Akinniyi & Waziri, 2011) [2]. Protein, fats and carbohydrates are the important nutrients of life. (Haque *et al*., 2014; Nisar and Scott, 2009) [14, 30].

8. References

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