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Research Paper

Nutritional Analysis, Phytochemical and Antifungal Study of *Equisetum arvense L.* From Village Kharkay Pak Afghan Border District Dir Lower, Pakistan

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Abstract

The objective of the present study was to study the nutritional analysis, and antifungal activities and find out the presence of phytochemicals in the aqueous, ethanol, and methanol extracts of *Equisetum arvense* L 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 showed positivity compared to the aqueous and methanolic extracts for the 13 phytochemicals. The important secondary metabolite total phenol and flavonoid content were tested in quantitative analysis. The ethanolic extract of total flavonoids and total phenol content were the highest. Also comparatively studied for nutritional analysis. Ash in Sample from junikalay 26.44%, 22.83%, in sample from Shahi Benshay and 6.01% in sample from Bara Bala. Moisture was found at 18.69% in a sample from Bara Banda and the lowest amount was found in Shahi at 10.27%. Protein highest amount was found in a sample from Chmarks at 4.37% and the lowest amount was found in a sample from Kas

Kuruna at 0.85 %. Fats' highest amount was 74.27% in a sample from Bara Banda and the lowest amount was found in a sample from Shahi Benshay 47.17%. The antifungal activity of all samples collected from different areas showed inhibition against each fungal strand. The most active among the plants was a sample from Joni Klay with a 17.00mm zone of inhibition.

Introduction

Ferns and their relatives belong to a significant division of the Plant Kingdom known as Pteridophyta, with a history spanning millions of years. There are over 250 genera and approximately 12,000 species of ferns documented globally (Chang et al., 2011). Notably, pteridophytes appear resistant to microbial pathogens, which may contribute to their evolutionary success and longevity of over 350 million years (Sharma and Vyas, 1985). Additionally, pteridophytes have been utilized in folk medicine for more than 2,000 years and are referenced in ancient texts (Ullah et al., 2023a). The Ayurvedic system of medicine recognizes the medicinal properties of pteridophytes, while the Unani system also incorporates these plants in its practices (Rani et al., 2018). Pteridophytes exhibit resistance to microbial infections, which may be a key factor in their evolutionary success, allowing them to thrive for over 350 million years (Shinozaki et al., 2008). Recently, numerous traditional medicinal ferns have been studied, revealing a range of bioactivities, including antioxidant, antitumor, anti-HIV, antimicrobial, anti-inflammatory, and antiviral properties (Shakoor et al., 2013). It showed bioactivity properties such as antimicrobial, anti-inflammatory, anti-tussive, antitumor, etc (Khan et al., 2018). However, very little research has been carried out on the evaluation of the bioactive properties of Pteridophytes (Ullah, 2019a). Hence an attempt has been made to evaluate the phytochemical and nutritional properties of native Pteridophytes of Khyber Pakhtoon Khwa (Subhan et al., 2024). Phytochemicals are naturally occurring chemical, biologically active compounds found in plants, which are responsible for health benefits for humans further recognized as micronutrients and macronutrients (Ullah et al., 2021). Phytochemicals play a crucial role in protecting plants from damage and disease, while also enhancing their color, flavor, and aroma. These plant chemicals defend plant cells against environmental threats, including stress, drought, pollution, pathogenic attacks, and UV exposure (Ullah et al., 2019b). Recent studies indicate that phytochemicals also contribute to human health when consumed as part of the diet. Over 4,000 phytochemicals have been cataloged and classified based on their protective functions, physical properties, and chemical characteristics (Zaman et al., 2022; Ullah et al., 2023b). Notably, approximately 150 of these phytochemicals

have been extensively studied. Wide-ranging dietary phytochemicals are found in fruits, vegetables, whole grains, nuts, seeds, legumes, fungi, spices, and herbs (Bartolome et al., 2013). Broccoli, tomatoes, grapes, onions, garlic, cabbage, carrots, whole wheat bread, cherries, strawberries, beans, legumes and raspberries, soy foods are common sources (Moorachian, 2000). Phytochemicals accumulate in different parts of the plants, such as in the stems, leaves, roots, flowers, and fruits. Various phytochemicals, mostly pigment molecules, are often concentrated in the outer layers of several plant tissues. Levels differ from plant to plant depending upon the processing, variety, growing conditions, and cooking (Ullah,2019c, 2021). Phytochemicals also exist in additional forms, but proof is lacking that they make available the same health benefits as nutritional phytochemicals (Rajesh *et al*., 2016). *Equisetum arvense* L is a fern (pteridophyte) found with a rosette of fronds from a rhizome, leaves are rachis green, scaly beneath, blade pinnatifid, 5-15 cm long, with 6 to 13 pairs of lobes. The lobes are 5-12 mm wide. They are found in rocky, shady, and ravines in moist soil (Ullah et al., 2021a, 2023c). *Equisetum arvense* L is generally distinguished by its allied species through its once pinnatifid leaves from *Equisetum arvense* L exiguum which has pinnatifid leaves. It is a genus (*Equisetum arvense* L) of about 700 species of ferns found extensively worldwide, mostly native to northern Mexico and disjunction to the Himalayas mountains in Asia (Iwashina and Matsumoto, 2011). In India, the general habitat at IHR (Indian Himalayan Region) includes Kashmir, Himachal, Uttarakhand, and many more. The plant is found to possess many medicinal activities. Roots of the plant are used in snake bite (Kumar *et al*., 2013). The present research aims to investigate and evaluate nutritional, phytochemical, and antifungal properties of Equisetum arvense L. from Dir Lower and Understand potential health benefits and traditional medicinal applications.

Materials and Methods

Collection of plant materials and Botanical

In the present study, *Equisetum arvense* L was collected in October 2016 from District Dir Kharkay of Khyber Pakhtunkhwa Province. Plant samples were collected with the help of Flora of Pakistan and are already data present in the herbarium of Ghazi Umar Khan Degree College Samarbagh. The plant was taxonomically identified and placed in the herbarium of Ghazi Umar Khan Degree College Samarbagh Distract Dir Lower.

Solvent system used

For the preparation of crude extract of the methanol, ethanol, and distilled water were used.

Crashing and filtration of the plant

The dried plant was powdered with the help of an electric grinder. The powder was kept in air-tight plastic bottles for further phytochemical analysis. 10 gm of plant powder was retained in a

distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol, and aqueous) was added to the powder separately. With the help of aluminum foil, the flask was covered and retained in a shaker for 72 hrs for shaking purposes. After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through the filtration process plant extracts were removed (Pirzada *et al.*, 2010, Ullah et al., 2024a).

Phytochemical analysis

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 (Ullah *et al.*, 2020, 2023d).

Tests for Alkaloids

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

Tests for Tannins

For the detection of tannins Ferric chloride test was done. Ferric chloride ($FeCl_3$) solution was mixed with all three extracts separately. The formation of blue-green coloration indicated the presence of tannins. (Kokate *et al*., 2008, Ullah et al., 2024d).

Tests for Phlobatannins

In test tubes 0.5 ml of all three extracts was taken separately, added 3ml of distilled water, and shaken for a few minutes then 1% aqueous hydrochloride (HCl) was added and boiled in water. The presence of Phlobatannins is indicated by the formation of a red color (Ullah, et al., 2023e, Wadood *et al.*, 2013).

Tests for Flavonoids

For flavonoid detection, all three extracts were treated with a sodium hydroxide (NaOH) solution. Red precipitation formation indicates the presence of flavonoids (Kokate *et al.*, 2008, Khan et al., 2018).

Tests for Carbohydrates

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

Tests for Phenols

For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml of all three extracts in a test tube separately. Formations of deep bluish-green solution showed the presence of phenol (Dahiru *et al.,* 2006).

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 a*l., 2016).

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 (H_2SO4) and iron tri chloride ($FeCl_3$). The formation of a brown ring indicated the presence of glycosides (Soni *et al.*, 2011).

Tests for Proteins

Xanthoproteic test: For the detection of protein, 1 ml of all three extracts was treated with 1 ml of concentrated nitric acid ($HNO_{3)}$ solution. The presence of proteins is indicated by the formation of a yellow color (Rajesh *et al.,* 2016).

Test for volatile oils

For volatile oils detection, 2ml of plant extracts were shaken with 0.1ml of dilute sodium hydroxide (NaOH) added small amounts of dilute hydrochloride (HCl). A white precipitate formation indicated the presence of volatile oils (Dahiru *et al*., 2006).

Tests for resins

Turbidity test: For the detection of resins, 5 ml distilled water was added to the plant extracts. The presence of resins conformed by turbidity (Chaouch *et al*., 2011, Ullah et al., 2023c).

Tests for terpenoids

Salkowski test: One ml of plant extracts (methanol, ethanol, and aqueous) was added with 2 ml of chloroform and carefully concentrated sulphuric acid (H_2SO_4) along the sides of the tube to form a layer. The formation of reddish-brown coloration indicated the presence of terpenoids (Dahiru *et al.,* 2006).

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 (H_2SO_4). The presence of glycosides was confirmed through the formation of a greenish blue color (Rajesh *et al.*, 2016).

Quantitative analysis of total flavonoids and phenols Contents

Determination of total flavonoid contents

Ethanol, methanol and aqueous extracts were used for the detection of total flavonoids contents. Total flavonoid quantification was done by taking 0.5 g of plant extracts. Then 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 mixtures 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. Quercetin was used as a standard (Daffodil *et al*, 2013, Ullah et al., 2023f).

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 was centrifuged for 15 minutes at 12,000 rpm. After that the supernatant were kept in test tube and these processes 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 (Na_2CO_3) 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 (Na_2Co_3) from 20% Na_2Co_3 solutions. The solution was mixed homogenously and then the test tube were brought in to the water bath in boiling water. At 650 nm their absorbance was checked. The Catechol was used as a standard (Ullah et al., 2019, 2023g).

Nutritional Analysis

Proximate Analysis

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

Determination of Moisture contents

Petri plates were autoclaved for 35 minutes at 105^0C. 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 105^0C. From oven, the plates were removed, in a desiccator cooled, and then weighed. 5 times repeated this process. The moisture content was calculated by using the following formula (Ullah et al., 2018d).

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

Ash Determination

The crucible was washed, cleaned and placed at 550 ^{0}C for 30 minutes in furnace for drying. In desiccators the crucibles were 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 550^0C 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, Ullah et al., 2023).

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

Curd Fat Determination

6 gm of plant powder was taken in paper thimble, and attaching to a goldfish of Soxhlet 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, Ullah, 2019d).

$$\text{Crude Fat (\%)} = \frac{\text{Weight of beaker with fat} - \text{Weight of blank beaker}}{\text{Weight of original sample}} \times 100$$

Protein Determination

The determination of protein in plant sample is categorized into three stages:

Digestion of the powder

In a digestion flask One gm of the dried powder of plant was taken. Approximately 2 gm 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, then 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 (Ullah et al., 2018e).

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 was completed and indicated by conversion. The color of boric acid becomes yellow due to the formation of ammonium borate.

Titration of the powder

In the presence of trapped ammonia, the addition of 0.1N hydrochloric acid to boric acid. When the color of boric acid changes again to pink the presence of ammonia will be confirmed. For the calculation of protein amount, the following formula was used (Ullah et al., 2018f).

$$\text{Protein (\%)} = \frac{6.25 \times 1.4 \times 0.1\text{N HCl} \times \text{Vol OF } H_2SO_4 \text{ (used)}}{\text{Weight of sample}}$$

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

Anti-fungal activity

Media preparation for fungal growth Dissolve 39 g of Potato dextrose agar (PDA) in 1 liter of distilled water, sterilized by autoclaving at 15psi (121^{o}C) for 15 minutes. Cool to room temperature and pour into sterilized Petri plates to solidify. Kept at room temperature to solidify for 30 minutes (Ullah et al., 2018a and 2018b.

Agar well diffusion method

The agar well diffusion method was followed as described by Sammie *et al*., (2010). 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 corn borer, hole) were made in each of the culture plates. 75µl of crude extract of selected plants was 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 the standard deviation was calculated (Ullah et al., 2014).

Statistical analysis

All the tests were performed as individual triplicate experiments. All the data are expressed as mean ± standard deviation.

Results

Phytochemical analysis

In the present research work both qualitative and quantitative investigation of methanolic, ethanolic and aqueous extracts of was carried out.

Qualitative Detection of Bioactive compound in the whole plant

Qualitative analysis of 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 were found in methanolic and ethanolic extracts, while alkaloids, phlobatannins and glycosides were found absent in the aqueous extracts. Flavonoids, carbohydrates, saponins, phenols, terpenoids and proteins were found present in aqueous extracts (Table 4.1, 4.2 and 4.3).

Table 1: Phytochemical analysis of different samples of *Equisetum arvense* L methanolic extracts

S. NO	Phytochemical test	Mayyar	Kotkay	Luqman Banda	Hall	Jandul	Dermal Bala	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 *Equisetum arvense* L in ethanolic extracts

S. No	Phytochemical test	Mayyar	Kotkay	Luqman Banda	Hall	Jandul	Dermal Bala	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 *Equisetum arvense* L aqueous extracts

S. No	**Phytochemical test**	**Mayyar**	**Kotkay**	**Luqman Banda**	**Hall**	**Jandul**	**Dermal Bala**	**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

Quantitative investigation of Flavonoids and total Phenolic constituents

Equisetum arvense L was comparatively studied for their total flavonoid's contents and total phenolic compounds in the solvent's 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).

Total Flavonoids Contents:

Highest number of flavonoids was found in the methanolic extract in sample from Jandool (0.86±0.924 mg/g) followed by sample from Dermal Bala (0.810±0.90 mg/g) and lowest number of flavonoids was found in sample from Shahi Benshay (0.121±0.81mg/g) (figure 1).

Total Phenolic Contents

The highest amount of phenol was determined in a methanolic sample from Mayyar **(**1.68.5±1.29 mg/g), followed by a sample from Luqman Banda (0.96±0.92 mg/g) and the lowest amount of phenol was found in a sample from Hall (0.21±0.463 mg/g) (Figure.1).

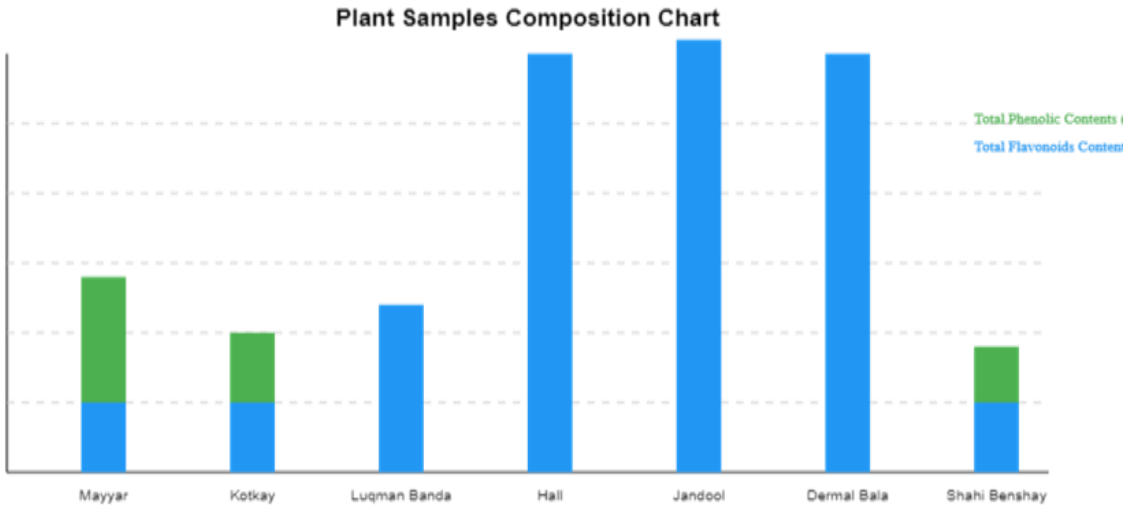

Figure 1: Qualitative Phytochemistry *Equisetum arvense* L of in Methanolic Extracts

Nutritional analysis

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

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

The percentage of ash, moisture, protein and fats was determined in *as* sample from Kotkay 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 Jandool 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% (figure 2).

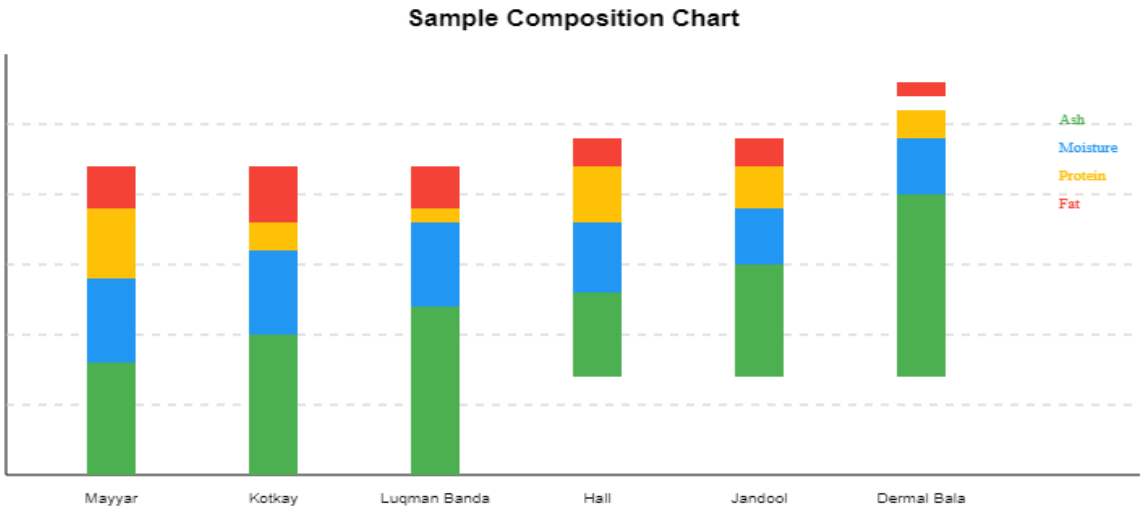

Figure 2: Proximate compositions of Equisetum *arvense* L

7. Antifungal activity of crude extracts of *Equisetum arvense* L 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 Kotkay with *17.00mm zone of inhibition.* Sample from Mayyar against *Acremonium* with 16.50mm zone of inhibition, followed by sample from Kotkay against *Trichoderma* with zone of inhibition of 16.41mm. Sample from Jandool 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 figure 3.

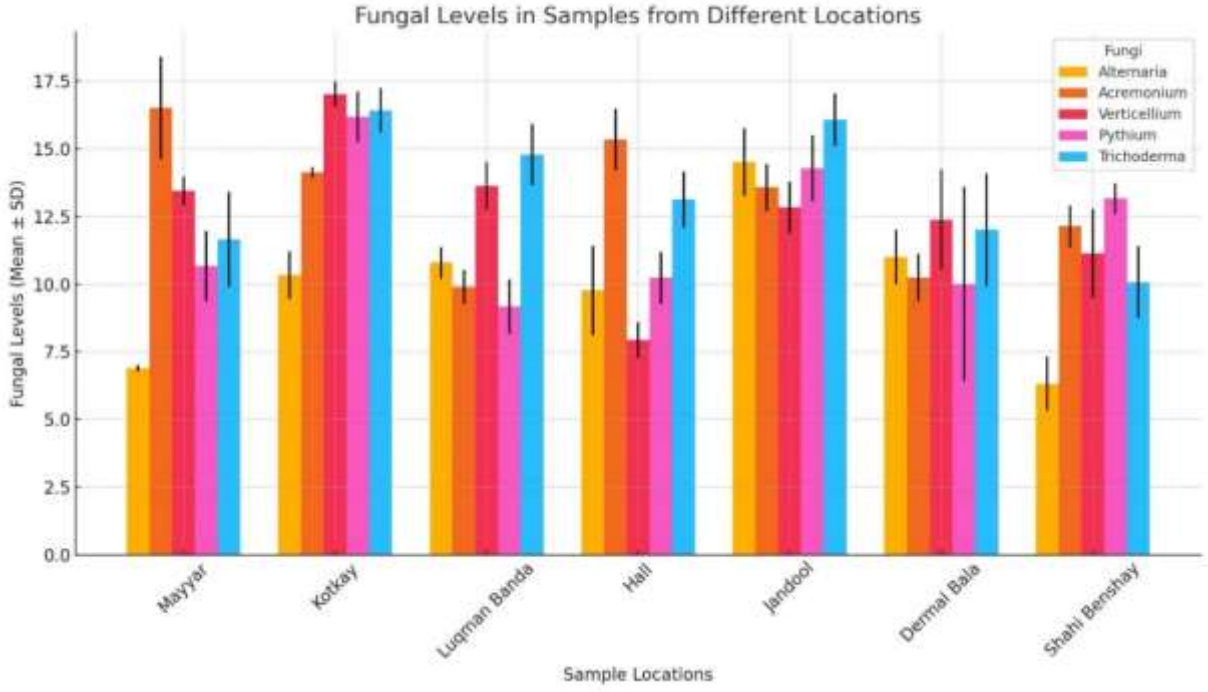

Figure:3: Antifungal activity of crude extracts of *Equisetum arvense* L against selected fungal strains

Discussion

The present investigations of phytochemical screening of extracts show the existence of alkaloids, flavonoids, carbohydrates, phlobatannins, glycosides, saponins, phenols, terpenoids, tannins, cardiac glycosides 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 (Ullah et al, 2018). A wide variety of pharmacological activities is

shown 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 show central nervous system (CNS) activities and analgesic properties (Khan et al., 2024). Because of their antimicrobial activity saponins are involved in plant defense systems (Ullah *et al*., 2018). These phytochemicals showed antimicrobial activity through different mechanisms (Ullah and Shakir, 2023). With proline-rich protein, tannins have been found to form irreversible complexes (Shimada, 2006) resulting in the inhibition of cell protein synthesis. (Parekh and Chanda, 2007). 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 and are used for treating intestinal disorders such as dysentery and diarrhea (Dharmananda, 2003). Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile *et al*., 2007, Ullah et al., 2023b). These observations therefore support the use of herbal cure remedies, thus suggesting that has potential as a source of important bioactive molecules for the treatment and prevention of cancer (Ullah et al., 2018a). The presence of tannins supports the traditional medicinal use of this plant in the treatment of different ailments. Alkaloid was another phytochemical constituent observed in the extracts (Ullah et al., 2018c). One of the most 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 (Paikra, *et al*., 2017). Alkaloids represent one of the largest groups of phytochemicals found in plants, renowned for their remarkable effects on human health. Their therapeutic potential has led to the development of powerful pain-relieving medications (Kam and Liew, 2002). Additionally, saponins have demonstrated inhibitory effects on inflamed cells, indicating their role in managing inflammation (Sher et al., 2018). The presence of saponins in extracts supports the traditional use of these plants for inflammatory conditions. Flavonoids, another significant class of phytochemicals, exhibit a diverse range of biological activities, including anti-inflammatory, antimicrobial, analgesic, anti-angionic, cytostatic, antioxidant, and antiallergic properties (Hodek et al., 2002). Numerous studies have highlighted the high potential of flavonoids for biological activities such as anti-inflammatory, antioxidant, and antiallergic responses (Thitilertdecha et al., 2008). Crude extracts of plants often contain bioactive compounds like tannins and flavonoids, which are known to induce antioxidant and antimicrobial activities (Ullah, 2024). The concentration of active components in these extracts can be modified through fractionation, either diluting or increasing their potency (Anyasor et al., 2010). Nature has

provided an abundance of plants with medicinal virtues, offering significant benefits for all living organisms (Shakir et al., 2023). While some plants have long been recognized for their therapeutic properties, many remain underexplored (Ullah et al., 2018g). Therefore, there is a pressing need for further research into their uses, alongside pharmacological and phytochemical studies to elucidate their therapeutic potential.

The nutritional importance of plants is fundamentally assessed through their protein and carbohydrate content, which are essential for the growth and development of both humans and animals (Sajid et al., 2023). Additionally, oils, fats, vitamins, minerals, and water play crucial roles in overall health and development (Akinniyi & Waziri, 2011).

Conclusion

Equisetum arvense L. from various regions of Khyber Pakhtunkhwa revealed the presence of important phytochemicals, including alkaloids, flavonoids, tannins, saponins, and terpenoids, known for their medicinal properties. Quantitative analysis showed that the ethanolic extract had the highest levels of total phenolic and flavonoid content. Nutritionally, significant variations were found in the samples, with the highest protein content (4.37%) in the Chmarks sample and the highest fat content (74.27%) in the Bara Banda sample. Antifungal activity was observed in all samples, with the Joni Klay sample demonstrating the strongest inhibition, with a 17.00 mm zone of inhibition. These results highlight the potential of *Equisetum arvense* as a valuable source of bioactive compounds with medicinal and therapeutic benefits.

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