INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2024, Vol. 3, NO. 2, 217-225
doi: 10.56612/ijaaeb.v1i1.71
https://eminentscientists.com/

Floristic and conservation studies of pteridophytes of Maidan Region, Tirah, Khyber Agency, Pakistan

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Abstract
This study was designed to investigate the floristic diversity of the pteridophytes of Maidan Region, Tirah, Khyber Agency, Pakistan. The research activities were carried out in various phases. Before starting the field-related research activities, a detailed field program was designed. Information regarding the respective taxa was obtained through several field surveys. During field surveys, about 200 specimens were collected with proper information. The collected plant specimens were then authentically identified with internationally well recognized floras. Twenty-nine (29) species were identified under 7 genera and 6 families. Of these, one species, i.e., *Asplenium hainanense* was new to Pakistan. For families, Aspleniaceae was the dominant family with 17 species (58.62%). For the remaining families, less than 6 species were recorded for each. *Asplenium* was the largest genus with 17 species (58.62%). Altitude-wise, 7 species (24.13%) were recorded between 600-1500 m and minimum number of species were seen in the range between 4000-4500 m. Some promising threats, i.e., land modification for agricultural purposes, stone excavation, fire in the forest land, overgrazing, and deforestation were identified as causing adverse effects on the species explored. These factors should be reduced in order to protect the taxa from local extinction. Serious conservation measures are necessary to avoid eradication of the species from the study area.

ARTICLE TYPE
Research Paper (RP)

SECTION
Plant Biology (PB)

HANDLING EDITOR
İbadullayeva, S.J. (PB)

ARTICLE HISTORY
Received: 29 May, 2023
Accepted: 01 Jan, 2024
Published: 04 Jul, 2024

KEYWORDS
Biodiversity;
Ferns;
Habitat loss;
Seedless vascular plants;
Systematics

Introduction

Pteridophytes are recognized as flowerless and seedless spore-bearing vascular plants. They are divided into homosporus and heterosporus groups based on types of spores (Kumar, 2000; Wang and Bai, 2019; Modena de Medeiros et al., 2023). In general, spore-bearing vascular plants have some similarity with pteridophytes in spore characteristics (Lashin, 2012; Sessa and Der, 2016). The spores are not only responsible for dispersal, but also play a vital role in distribution of pteridophytes (Vijayakanth et al., 2016). They had produced a leading piece of vegetation in the significant past about hundred million years ago (Vasuneva and Bir, 1993a; Modena de Medeiros et al., 2023). They are cosmopolitan and grow in diverse climatic zones (Vasunevam and Bir, 1993b; Ibiye et al., 2023; Korsa et al., 2024). Pteridophytes are spore producing plants, formed by two lineages, i.e., Lycophyte fronds with no leaf gap in the stem stele (Lycophylls) and Monilophytes or fern fronds with a leaf gap in the stem stele (Pryer et

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TO CITE THIS ARTICLE: Aziz, T., Gul, A., Alam, J., Qaiser, M. (2024). Floristic and conservation studies of pteridophytes of Maidan Region, Tirah, Khyber Agency, Pakistan. *International Journal of Applied and Experimental Biology* 3(2): 217-225.

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al., 2001, 2004; Smith et al., 2006).

The pteridophytic ancestors of the present-day vegetation of ferns and fern allies are the plants which have evolved the seed habitat, and passed on this characteristic to the present-day vegetation, and hence, the pteridophytes could be genuinely considered as the forefathers of the present-day vegetation (Haufler, 2002).

Previously, about 300 genera, 9600 ferns and about 1400 lycophytes were reported worldwide (Smith et al., 2006, 2008). Currently, more than 12000 species have been evaluated globally. However, all over the world, a variety of pteridophytes are still not clearly known (Bandyopadhyay and Dey, 2022) and the expected number of earth ferns is around 9000-15000 (Haufler, 1996; Mabberlay, 1997; Smith et al., 2008). Hassler and Swale (2001) and Moran (2008) mentioned about 13,600 kinds of ferns, which have been named worldwide. In Pakistan, 250 species of ferns were documented by Gul et al. (2017).

The floristic studies of pteridophytes in Pakistan are still lagging. Most of the work has been done on angiosperms, and awfully only sporadic work is available on lower plants, especially cryptogams. Consequently, the pteridophyte flora of Pakistan is still somewhat unexplored (Gul et al., 2017). This study is an attempt to assess the pteridophytic diversity in Mansehra district. Prior to this attempt, very few workers have performed the diversity analysis in various potential regions of Pakistan, especially in Mansehra district (Gul et al., 2016). Stewart (1972) was the first person who compiled a detailed checklist of ferns and fern allies of Pakistan and Kashmir; he reported 133 species, of which 39 were also listed from Mansehra district. Later on, a Japanese team became active in 1990-1991 and collected ample material of lower plants from Pakistan; they especially focused the Northwestern Himalayan region. Their findings were published by Nakaike and Malik (1992, 1993). They provided a comprehensive list of 87 pteridophytes under 18 families and 30 genera. They also showed the occurrence of 62 taxa from district Mansehra. In the same years, Fraser-Jenkins (1991) studied the ferns and allies of the West Himalaya and reported 189 species in Pakistan. Thus, the current investigation aimed to collect and identify all pteridophyte species from Mansehra district and describe briefly their potential uses for multiple purposes.

Materials and Methods

Study Area

Tirah is known as Tirah Valley; it is in the Khyber, Kuram and Orakzai Agencies (33.73N 71.01E) of the Federally Administered Tribal Area of Pakistan (**Figure 1**). Its smaller part across the border to the north is in the province of Nangarhar, Afghanistan. Tirah covers an area of approximately 4,144 sq. km and is located in the eastern, southern and northern parts of Peshawar, Kohat and Khyber (Kheli et al., 1975). The area is located between the Khyber Pass and the Khanki Valley (**Figure 1**). It is inhabited by the Afridi, Orakzai and Shinwari tribes of Pashtun. The area is rural, and the Bagh Malikdin Khel in Maidan Tirah is a traditional meeting place for Afridi Jirgas or gatherings. Afridi is the major tribe of Maidan (Tirah Khyber Agency). This tribe is further divided into 8 categories which are Malik Din Kheil, Kamber Kheil, Kamar Kheil, Zakha Kheil, Kuki Kheil, Aka Kheil, Sipah, and Adam Kheil. This is also the area where the government has been working to reduce poppy cultivation. Minorities are known as Hamsayas (protected people), such as the Sikh community, which is primarily involved in trade and other occupations (Anonymous, 2011). After a field survey, various territories of Maidan, Tirah, Khyber Agency were chosen for the study.

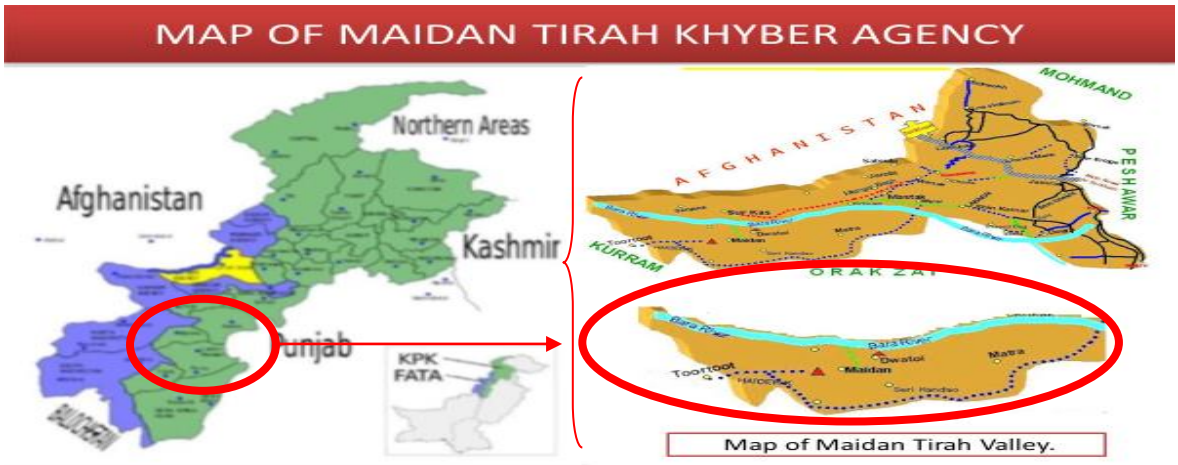

Figure 1. Map of the study area

Field work

Before starting sports ground visits, a detailed field plan was made and sits were managed. Various plant collections were also selected. Arranged visits were efficient in different times in various sites of the study area, and hence field data collected. All data, like collection numbers, markings, relevant species habitats, habits and other features were recorded. Global position system (GPS) was used to trace the exact location and elevation of the respective species during the field work. Details of the field visits in different zones of the study area are presented in **Table 1**.

Table 1. Details of field visits in the study area conducted during 2018-2019

Localities Visited	Date
Mirdarrah	3rd August 2018
Takhtakai	4th August 2018
Landawar	5th August 2018
Khadang	10th September 2018
Kawarah	14th September 2018
Botan Sharif	14th September 2018
Darphir, Warsak	14th September 2018
Masthy	20th September 2018
Kalawouch	21st September 2018
Malanga Sholobar	7th June 2019

The collected specimens were properly processed as per internationally recommended procedures (Farmen and Bedson, 2000). The collected material was properly pressed using a wooden field presser, old newspapers, and corrugated sheets. Bigger samples were folded into M, N or V shaped forms. After 24 hours, the material was shifted to another pressing process to reduce moisture content. All samples were placed in open for drying. The completely dried material was then poisoned with a mixture of different chemicals following Jain and Rao (1976).

The solution was prepared by dissolving 2 g mercuric chloride, 10 g copper sulphate, and 10 g phenolic crystals in one litre of ethanol. Mercuric chloride and copper sulphate were ground to powder and added to ethanol. Later, phenol crystals were added to the solution and were properly shaken for a few minutes. Each sample was dipped in the poison solution for 8-10 minutes. The poisoned material was then shifted on a dried newspaper and air-dried for a few minutes. The dried poisoned samples were mounted on to appropriate herbarium sheets.

Morphological characteristics, i.e., micro- and macro-characters were critically examined. A hand lens (30X) stereoscope (model: SZM-LED2. Italy) and a microscope (Model: OPTIKA B-383Pli, Italy) were used for examining the above-mentioned characters. Quantitative characters were also measured using a scale. Plant length, size of frond, type of hairs, type of scales on costa and rachis, pinnule and pinna, size, number, and diameter, number of sori and arrangement of spores, and sporangium size, shape and other relevant attributes were examined in detail. The stereoscopic and microscopic images were captured by a digital camera (Model: C.B 10).

Each specimen was identified using diagnostic/distinct features of a respective specimen. Various authentic floras of various regions were used for this purpose, especially Cryptogamic Flora of Pakistan (Nakaike and Malik, 1993) and different illustrations available on internet sources.

Qualitative and quantitative characters were utilized to examine the distribution pattern of the taxa. For qualitative data, habit, life form and nature of habitat were examined. Life form was determined according to Raunkiaer (1934) criteria.

Likewise, quantitative information was also used. For this purpose, first, a location map of the study area was obtained to distinguish different localities in the study area, and each location consisted of plains, forests and peaks. Various identified/classified localities of the study area were Mirdarrah, Takhtakai, Khadang, Malanga, Sholobar, DarpherWarsak, Kalawouch, Masthy, landawar, Kawara, Botan, Shamshy and Aurhanga. Altitudinal range of species, habitat type, i.e., rocks, soil and aquatic nature were considered. Number of species found in different habitats and at various altitudes were counted. Number of species in genera and genera in families were counted and their percentages (%) calculated.

Various threat concerns to the species were directly observed during the field studies. Direct observations were made keeping in mind the IUCN guidelines for detecting threats to the species.

Habitat loss was analysed by observing habitat/land modification for cultivation purpose. Stone excavation for construction, deforestation, over-utilization of other species in the habitat, overgrazing, etc., were observed. Fire incidences were also observed in a few sites of the study area, which might have caused elimination of several pteridophyte species.

Data for wood consumption, i.e., fuel wood and timber wood were taken from educated persons,

transporters, wood merchants, and common people from different localities through prearranged interviews and questionnaires. The procedures described by Nawab (2018) were followed for fuel wood consumption analysis.

The level of palatability of different species was documented by the grazing livestock in the field. Preferences of cattle were visually examined from the study area. Local herders were also interviewed. Two categories of plants were identified based on livestock use, i.e., palatable, and non-palatable. The palatable plant species are further classified into highly palatable, mostly palatable, less palatable, and rarely palatable according to Hussain and Durrani (2009). Grazing intensity of the study area was examined using different parameters like browsed foliage, animal dung and hoof marks and was categorized as ungrazed, moderately grazed, highly grazed, and intensively grazed. At the end of the research work, all specimens collected and identified were deposited in the Herbarium of the Hazara University Pakistan (HUP) as a permanent record reference.

Results and Discussion

Exploration of the pteridophyte flora of Pakistan is still at initial stage. For proper records of this important plant (Ferns) group, extensive inventory is extremely indispensable. Though, several preliminary surveys have been accomplished in a few selected regions of the country (Stewart, 1972; Nakaike and Malik, 1992-1993; Gul, 2016; Gul et al., 2017), major parts of Pakistan are still under exploration, especially Baluchistan, the Frontier region, Chitral and Gilgit (Baltistan province). The current study is an attempt to reduce this gap and explore an entirely virgin location of Pakistan for this important plant group (Ferns). Only a single district has earlier been systematically discovered (Gul, 2017). Maidan, Tirah Khyber Agency is mostly mountainous area and lies in the Sino-Japanese phytogeographical zone of Pakistan (Ali and Qaiser, 1986) having a rich vegetation. This area is totally unexplored for ferns flora.

About 200 species have been documented from Pakistan and most species (ca. 90 %) have been recorded from the Sino-Japanese phytogeographical region. Pteridaceae and Dryopteridaceae are dominant families having over 40 species each (Stewat, 1972; Nikaike and Malik, 1993, 194; Gul et al., 2016). In the consequence of several field surveys, twenty-nine (29) species have been recognized for this plant group (**Table 2**). This figure makes about 14.5% of the whole known species of pteridophytes of Pakistan. Moreover, these types of species are disseminated in six families and seven genera. Our results further suggest that Aspleniaceae (17 spp.; 58.62%) seems to be the dominant family. The remaining families, however, have less than five species each. It is important to note here that Aspleniaceae is a moderately large family among the known families of the pteridophyte group in Pakistan (Gul et al., 2016). As members of this family are lithophytic, so they prefer rocky microclimatic conditions (Wu et al., 2013). Our documented species of the family (**Table 2**) were collected form rocky habitats. Hence, these species dominate in the study area. It has also been observed that pteridophytes mostly occur in fragile and specific habitats (Jalas and Suominen, 1972; Anderson, 2022) due to less competitive abilities with higher plants. Evolutionarily, this group has spent its dominant period in the Devonian to Permian period (DiMichele et al., 2001.), but now these are remnants of that flora. Therefore, these taxa usually preferred and grew on rocks, along stream banks and shady places (Dixit, 2000). The documented species from the study region are also found in a particular fragile habitat, i.e., rock crevices, rock bases or the rock cliffs (lithophytic). Maximum species (i.e. 20 species; 68.96%) have been reported as lithophytic. Nine species were in thick soil growing on forest ground, spring sides or stream sides (**Figure 3**).

As ferns usually hunt a wide range of altitude, so maximum diversity of this plant group exists between tundra to tropical region, being more distributed in central area (Tryon, 1972; Treyon and Treyon, 1982; Morane, 2008; Possamai Della, 2023). Although so far Pakistan is concerned, maximum fern species have also been documented between 1500 to 2500 m (Gul et al., 2017). Our findings also agree with the altitude-wise distribution pattern of Pakistan and maximum species were reported to occur between 1000-3000 m, i.e., 17 species (58.62 %). This range seems suitable for the growth and establishment of most of the species.

Plants are under tremendous pressure in Pakistan due to a variety of threats. Narrow distribution area, small population size, habitat conversion/modification, deforestation for timber and fuelwood collection, over-grazing and over-collection of medicinal plants have been reported as main threats. To date, only the conservation status of 142 flowering plant species (ca. 2.36 %; **Table 3**) could be evaluated and most of the species (97.64%) are yet to be evaluated (Alam, 2009; Abbas, 2010; Ali and Qaiser, 2012; Majid, 2015; Muhammad, 2017). However, even a single species of pteridophytes could not be evaluated in terms of conservation status for Pakistan as per IUCN criteria. Therefore, this aspect should be considered at top priority. In the current study, some direct field observations were made to highlight various threats. Habitat degradation is the main threat and this phenomenon is due to over-grazing by

domestic animals, stone excavation, land modification for agriculture purpose, and deforestation to meet timber and fuel wood requirements. However, in conservation related studies, data is taken at least for 2-3 years to assign any particular threatened category for a taxon (IUCN, 2001). Detailed information could not be collected due to limited time, so further information is needed in this connection.

Table 2. Checklist of the taxa studied

FAM	Species	Altitude (m)	Distribution in the area
ASP	*Asplenium varians* Wallich ex Hooker & Greville	700	Khadang, Masthy, Maidan, Tirah
	Asplenium nesii Christ	1500	Kalawouch, Maidan Tirah
	Ceterach officinarum Willd.	700-1800	Khadang, Kalawouch, Tirah Takhtakai, Landawar, Mirdarrah, Masthy, Maidan Tirah
	Asplenium tenuicaule Hayata	1200-1500	Kawara, Kalawouch, Maidan, Tirah
	Asplenium trichomanes L.	600-2000	Takhtakai, Landawar, Kalawouch, Mirdarrah
	Asplenium quadrivalens (D.E. Meyer) Landolt.	1800-2200	Kawara, Maidan Tirah
	Asplenium ruta-muraria L.	1200-3000	Kalawouch, Takhtakai, Mirdarrah, Kawarah, Darpher Warsak, Landawar
	Asplenium dalhousiae Hooker	1200-2500	Kalawouch, Kawarah, Khadang, Mirdarrah.
	Asplenium humistratum Ching ex H.S. Kung	1000-2300	Mirdarrah, Kawarah, Kalawouch.
	Asplenium microtum Maxon.	800-1500	Khadang, Takhtakai
	Asplenium viride Hudson	2500-3000	Kalawouch
	Asplenium yananense Franch.	1800-2600	Landawar, Botan, Khadang, Masthy
	Asplenium punjabense Bir	800	Masthy, Darpher, Warsak
	Asplenium deqenense Ching	1000-1800	Takhtakai, Mirdarrah.
	Asplenium kansuense Ching	1200-1800	Masthy, Kalawouch
	Asplenium hainanense Ching	500-800	Masthy Kalawouch
	Asplenium montanum Willd.	400-1000	Malanga Sholobar
DRY	*Dryopteris fructosa* Christ	1200	Takhtakai
	Dryopteris porosa Ching	1500	Takhtakai, Kalawouch
	Dryopteris intermedia A. Gray	1200	Darpher Warsak
	Dryopteris clintoniana (D.C. Eaton) Dowell	2000	Kawarah
	Dryopteris arguta (Kaulf.) Watt.	2000	Kalawouch
DEN	*Pteridium aquilinum* (L.) Kuhn	2500-3500	Aurhanga
PTE	*Adiantum capillus-veneris* L.	400-800	Kawarah, Takhtakai, Kalawouch, Darpher Warsak
	Adiantum venustum D. Don.	400-3500	Mirdarrah, Takhtakai, Kalawouch, Khadang
	Cheilanthes feei T. Moore	800	Khadang
SLE	*Selaginella sanguinolenta* (L.) Spring	400-800	Mirdarrah, Aurhanga
WOO	*Cystopteris fragilis* (L.) Bernhardi	700-1200	Kawarah, Sholobar
	Cystopteris dickieana R.Sim.	900	Sholobar

Family (FAM): ASP= Aspleniaceae; DRY = Dryopteridaceae, DEN = Dennstaedtiaceae; PTE = Pteridaceae; SLE = Selaginellaceae; WOO = Woodsiaceae

Table 3. Family-wise detail of the studied taxa

Family	Genera	Species	Percentage of the species
Aspleniaceae	1	17	58.62
Dryopteridaceae	1	5	17.24
Dennstaedtiaceae	1	1	3.44
Pteridaceae	2	3	10.34
Selaginellaceae	1	1	3.44
Woodsiaceae	1	2	6.89

Distribution pattern of taxa

Habitat-wise distribution was found in three different growth forms, i.e., terrestrial, lithophytic and epiphytic. Altitudinal range and dissemination design of species were distinctive as per propensity and living place; 7 species were found in elevation of 400-1000 m, 3 species within 1001-1500 m, and 5

species in 1501–2000 m, and 6 species within each of altitude range 2001-2500 m and 2501-3000 m (**Figure 2**; **Table 4**). Only two species were found within the altitudinal range of 3001-3900 m.

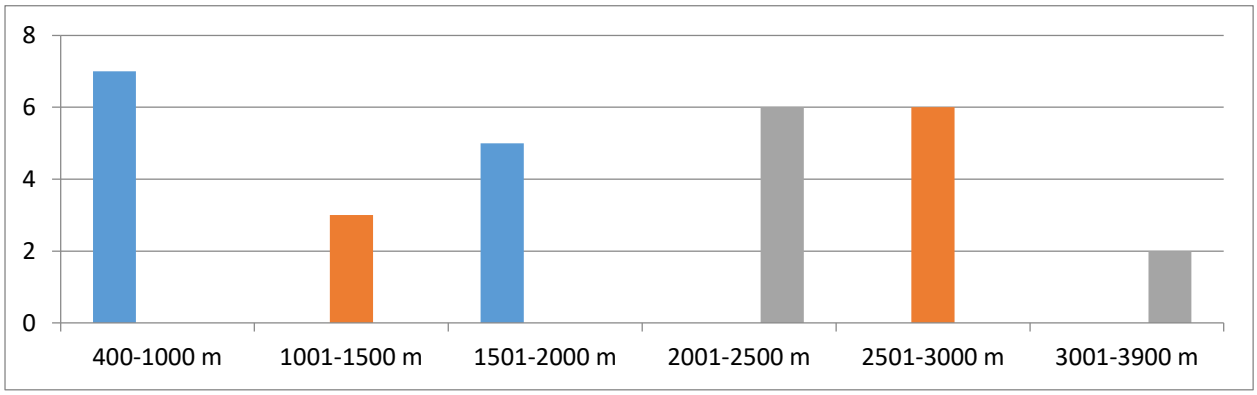

Figure 2. Altitude-wise species distribution

Substrate-wise species distribution

A total of 29 species were found in the study area in four different substrate forms (**Table 4**), 14 species were found in rock crevices (lithophytes) being dominant (48%), 6 species were on the forest soil (20.6%), 5 (17%) species on spring side, and the remaining 4 (13.7%) species were on the base of the trees (epiphytes) (**Figure 3** and **Figure 4**).

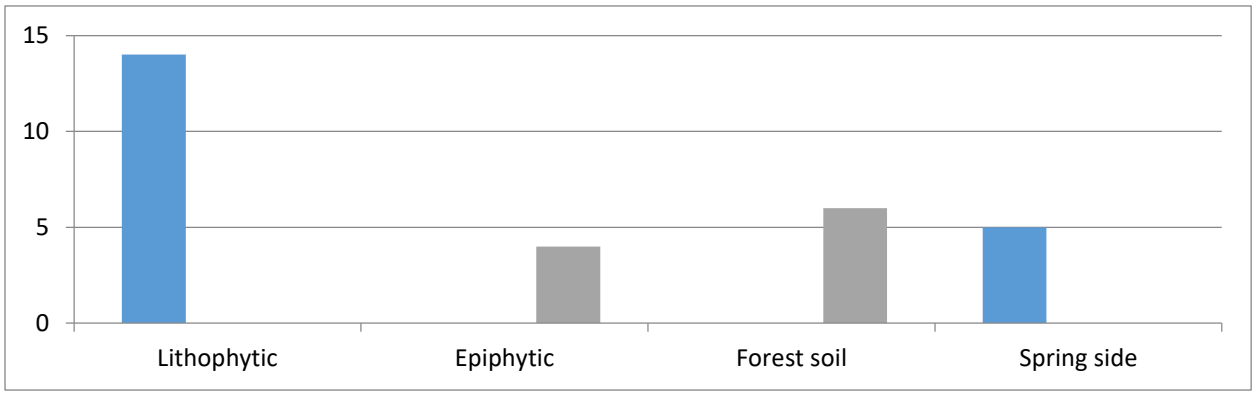

Figure 3. Substrate-wise species distribution

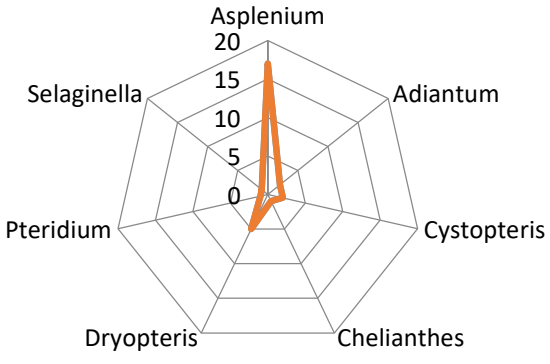

Figure 4. Number of species within genera

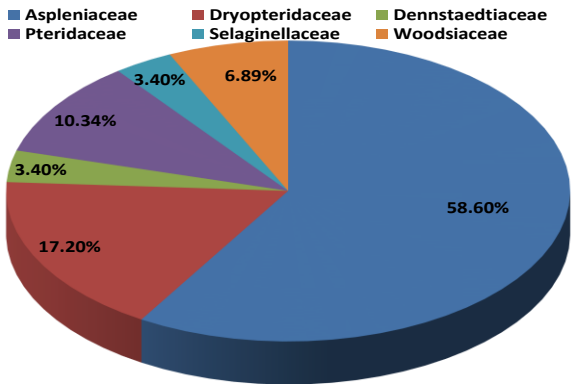

Figure 5. Percentage of each family at the study site

Table 4. Family-wise distribution of the recognized taxa

Families	New reports for Pakistan	New reports for the study area	No. of total species
Aspleniaceae	1	17	17
Dryopteridaceae	-	5	5
Dennstaedtiaceae	-	1	1
Pteridaceae	-	3	3
Selaginellaceae	-	1	1
Woodsiaceae	-	2	2
Current status	1	29	29

Abundance

Abundance was also determined. The majority of the taxa were restricted to less than three locations in the area. Extremely (ER) rare species, i.e., 3 taxa (10%), followed by common (CM) and very common (VC) species each by existing 2 (6.89%). Only 22 species (75%) were widespread (**Table 5**, **Figure 5**)

Table 5. Abundance-wise distribution of the taxa

Abundance of taxa	No. of taxa	Percentage
Widespread	22	75
Very common	2	6.89
Common	2	6.89
Rare	3	10

Author(s), Editor(s) and Publisher's declarations

Acknowledgement

The current study was funded by the IFS project [1-3-F-6135-1] and Higher Education commission ,National Research Programme for Universities Pakistan [No: Ref No. 20-14667/NRPU/R&D/HEC/2021 2021].

Conflict of interest

The authors declare no conflict of interest.

Source of funding

None declared.

Contribution of authors

Conceptualization and designing the study: AZ, AG, JA. Conduction of experiment: AZ, AG, JA. Data collection: AZ, AG. Writing of the frist draft: AZ, AG, JA, MQ. Revision of the manuscript: AZ, AG, JA. Proofreading and approval of the final version: AZ, AG, JA, MQ.

Ethical approval

This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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Declaration of generative AI and AI-assisted technologies in the writing process

It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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