



Genetic diversity encourages conservation of threatened ethno medicinally important plant species in Koz Abakhel District Swat, KP, Pakistan

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Abstract

Swat valley being naturally blessed with tremendous biodiversity; altitudinal and topographic variations is exposed to increasing human pressure, social injustice and low literacy rate that are destabilizing the biodiversity status especially species survival, habitat and ecosystem. These medicinal plant species in the study area were commonly used as an ethno medicine against 19 various disorders out of which most frequently occurred are; Anti-tumor, High blood pressure, Pile, Rheumatism etc. Dominated medicinal plants with most use values were *Berberis lycium* having (UVi= 0.438), *Ziziphus oxyphylla* (0.393), *Myrtus communis* (0.337), *Punica protopunica* (0.281). While minimum used values of collected medicinal plants were *Olea ferruginea* (0.258), *Alnus nitida* (0.224) and *Quercus incana* (0.146). Based on the RFC values, the most cited medicinal plant species by the traditional drivers were *Berberis lycium* (RFCs= 0.786), *Punica protopunica* (0.731), *Myrtus communis* (0.617), *Ziziphus oxyphylla* (0.551), *Olea ferruginea* (0.514), *Alnus nitida* (0.337) and *Quercus incana* (0.224). The medicinal plant species with highest fidelity level was of *Berberis lycium* cited 100% for Jaundice, Diarrhea, Pile, Dysentery, Anti-tumor, Urinary infections, Digestive Problems, Dry Cough, Rheumatism, Dysentery, Diarrhea, Ulcer respectively. Due to representation by only single medicinal plant taxa ($Mt = 1$), the various diseases *viz.* Anti-tumor, High blood pressure, Pile, Rheumatism had maximum FIC value. Conservation studies of 7 threatened medicinal plants were carried out for assessment of their conservation status, threats and recommendations growing in Swat valley. Phytogeographically, the valley is present in the Sino Japanese floristic region and is a hot spot of endemism. Among total of 7 taxa 3 (42.85%) vulnerable, 4 (57.14%) endangered species. The protein profiling was carried out on 12% gel electrophoresis; 10, 8, 9, 10, 10, 10, 10 and seven reproducible bands were detected for *B. lyceum*, *O. ferruginea*, *M. communis*, *Z. oxyphylla*, *P. protopunica*, *A. nitida* and *Q. incana* respectively; with molecular weight ranges from 10 to 180 KDa. The locus contribution toward genetic disagreement (LCTGD) of *B. lyceum*, *M. communis*, *P. protopunica* and *A. nitida* was (40% each) whereas the genetic diversity among the genotype of *O. ferruginea*, *Z. oxyphylla*, was 30% and the genetic disagreement in the genotypes of *Q. incana* was 57.142%. This genetic diversity within genotypes of the studied specie is the basis for selection as well as for plant improvement and conservation. The current work delivers indication that these medicinal plants have an important role in the healthcare system of swat urban public. The people this valley still endures to depend on medicinal plants for the treatment of healthcare problems. The existing paper denotes significant ethno botanical information on medical plants which provides baseline data for future pharmacological studies and genetic diversity is helpful in selection elite genotypes for future conservation.

Keywords: threatened medicinal plants, ethno medicinal uses, conservation, genetic diversity, Koz Abakhel Swat

1. Introduction

Plant-based medicines enjoy a reliable position today, mainly in the developing countries, where basic health facility is insufficient. Native medications are more effective, safe and cheap and are gaining fame among both rural and urban areas. Information from native traditional medicine or ethnic groups has played a crucial role in the finding of novel products from plants as chemotherapeutic agents (Katewa *et al.*, 2004; Muhammad *et al.*, 2019) [14, 19]. The world health organization (WHO) has highlighted the importance of the traditional indigenous medicines, since a large majority of rural people in the developing countries still rely on these medicines as the first protection in health care (Goleniowski *et al.*, 2006) [7]. Pakistan is rich with a distinctive biodiversity, consisting of nine major ecological

zones. Due to its pleasant climate, Pakistan is rich in medicinal floras which are distributed over a large area. The origins of the therapeutic use of herbal medicine can be traced back to China about 5000 years ago. The extracts of several plants have been used as a folk medicine throughout the world and many drugs prescribed by physicians are either directly isolated from plants or commercially modified of natural products (Wang *et al.*, 2007; Muhammad *et al.*, 2019) [38, 19]. About 80% indigenous people of all over the world as well as Pakistan rely on traditional medicines for health practices (Hocking, 1958) [9], but now it is restricted to the rural areas (Ibrar *et al.*, 2007) [11]. Because the rural areas inhabitants greatly depend on natural resources due to lack of modern medical facilities (Sandhya *et al.*, 2006) [27] while in contrast, the urban

community member deviates towards modern health facilities. But presently traditional knowledge is facing severe threat due to changing life styles and modernization in rural societies (Shinwari *et al.*, 2003) [29]. Plants are vital sources of traditional medicines and used for the treatment of various ailments without having side effects and these plants resources are easily affordable and accessible. Approximately 4, 22,000 flowering plants reported from the world, more than 50,000 have been used worldwide for medicinal purposes (Walter & Hamilton, 1993) [37] and from Pakistan 6000 plant species have been documented among which only 600 plants have been reported for ethno medicinal studies (Shinwari *et al.*, 2003) [29]. These medicines are safe and environment friendly (Prasad *et al.*, 2013) [23]. Ethno medicinal investigations have established emphasis on the relationship between the plants uses and ethnic communities (Verpoorte *et al.*, 2005) [34]. It is therefore imperative to increase ethno medicinal studies in order to preserve this valuable knowledge before its extinction (Khan *et al.*, 2011; Muhammad *et al.*, 2018) [18, 19].

More than 270,000 vascular plant species are supporting varied types of ecosystem (Walter and Gillet, 1998) [36]. Because of severe man made activities, the natural flora is under stress and in many regions it is declining. Pakistan has miscellaneous climatic, topographic, altitudinal and phytogeographical dissimilarity supporting more than 6000 species with 428 endemics (Ali and Qaiser, 2010) [3]. There is a little work carried out on the conservation studies and the available data is deficient. More than 580 plants are reported by Nasir (1991) [21] as threatened. In additional investigation, it has been described that 709 plants species are threatened and endangered in Pakistan (Chaudhri and Qureshi, 1991) [6]. Whereas according to Walter and Gillet (1998) [36], 14 flowering plants are threatened in Pakistan. It has been stated by IUCN (1994) [25] that 20 species are measured to be target species in Pakistan (Shah and Baig, 1999). According to the IUCN Red list (2008) [13], 19 plants are threatened in Pakistan. While according to Ali and Qaiser (2010) [3], it has been recounted that 21 species are threatened. Genetic diversity evaluation through molecular practices such as biochemical assessment at protein level and DNA based techniques have a number of advantages over the traditional morphology (Ndiaye *et al.*, 2012, Muhammad *et al.*, 2018) [22, 19] but matched to biochemical assessment at protein level, molecular investigation of DNA markers are too expensive (Win *et al.*, 2011, Muhammad *et al.*, 2018) [39, 19]. Among biochemical techniques, SDS-PAGE process is a simple, reliable, cheap and free of environmental fluxes (Muhammad *et al.*, 2018) [19]. SDS-PAGE is now widely used as biochemical procedure to describe the genetic structure of plant species (Hameed *et al.*, 2009). Massive consideration has been focused on the use of SDS-PAGE over the last decades for evaluation of genetic diversity, reliable judgment and identification of plant varieties. Seed storage protein markers have been effectively used to resolve taxonomic relationships and characterize cultivated varieties in a number of medicinal plant species (Muhammad *et al.*, 2018) [19]. Proteins are being the end products of gene expression; SDS-PAGE can be employed to identify varieties, determine polygenetic relationship in different species, biosystematics analysis,

conservation study and evaluate the passport data (Sammour, 1991; Muhammad *et al.*, 2018a) [26, 19]. A concentrated study for the determination of the conservation status, threats and recommendations for various species and fluctuation in its population has never been carried out. Various parameters have adversely donated to make certain species vulnerable, endangered and critically endangered. It is observed during the present study that anthropogenic activities are the main contributor in the unsustainable utilization of the plant natural resources growing in the area. Therefore, study concerning the conservation status, threats and recommendations, genetic diversity for some threatened medicinal plants were planned.

2. Materials and Methods

Study area

The Koz Abakhel (Remote Area) is in District Swat. It is reached from the main Saidu Sharif City road at the junction 'Kabal' and is about at a 3 Km distance in west of Kabal. The community in the Koz Abakhel has multiple urgent needs resulting from economic poverty, geographical isolation and very poor provision of health, education and communication services. The research work cannot address all these issues but hopes to make a significant impact on a number of key signs for the health of the valley community. Akhun Kalay, Kotlai, Melagah, Dagay, Gadi, Sharif Abad, Swegalai, Ziarat, Dadahara, Zawra, Yakhtangay, Qambo, Nasapai, Jawand are major villages of Kuz Abakhel valley. The Valley is full of natural resources and sceneries but many factors like no transportation, health care centers, markets, schools etc. as well as environmental factors like harshness are the factor due to which it didn't gain any importance yet. For data collection several visits were made on the basis of seasonal variation to different areas of Koz Abakhel, Swat.

Necessary equipment

During field work, the equipment used were; field note book, study area map, pencils, field presser, blotting papers, polythene bags, knife, twig cutter and digital Camera.

Medicinal plants collection, questionnaire and data collection

89 local informants were interviewed. The study was conducted from February till December 2017. The study was based on direct communication with the local informants of the area. A survey was established to interview local people concerning the medicinal values of plants. On the basis of this information, the medicinal value of plants of the study area was noted. The methodology was adopted by following the work of Qureshi *et al.* (2009) [1]; Qureshi *et al.* (2008) [2] and Ahmad *et al.* (2009) [1].

The specimens were identified referring several Floras, viz., Flora of Pakistan, Hooker (1872-1897). The updated nomenclature of the identified species followed Siddiqui *et al.* (2007) [30] and Ahmed *et al.* (2009) [1]. Ethnomedicinal data has been collected through Participatory Rural Appraisal (PRA), which is based on communication with indigenous people and direct observation in the ground (Martin, 1995) [17]. The data have been recorded through semi-structured interviews with populaces involved in the plants management (Alexiades, 1996) [2].

Statistical Analysis

Used value (UVi)

Used Value index is a quantitative approach that evaluates the relative importance of each medicinal plant species based on their relative use among informants and it was calculated by using the formula:

$$UV_i = \sum U_i / N$$

U_i = each informant cited number of use reports for a given medicinal plant species where, N_i is the total number of informants interviewed for a given medicinal plant species.

Relative frequency of citations (RFCs)

To evaluate the traditional value and medicinal importance of each plant species in an area, the Relative Frequency of Citations formula was used (Tardio and Pardo-de-Santayana, 2008).

$$RFC_s = FC_s / N$$

FC_s = Number of local informants who reported traditional medicinal use of the species and

N is the total number of informants of the study. (In this study, $N = 89$)

Consensus index (CI %)

Percentage of local informants having traditional knowledge of plant species medicinal use against diseases (In this ethno veterinary study) was calculated by Consensus index (CI %) which indicating citation by % of informants:

$$CI = n / N \times 100$$

Where, n is the number of informants citing medicinal plant species, while N is the total number of respondents of the study.

Fidelity level (FL) value

The fidelity level (FL) is the percentage of indigenous informants claiming the given plant's use report for the same major ailment. It was calculated by the following formula (Alexiades, 1996)^[2].

$$FL = I_p / I_u \times 100$$

I_p = Use of plant species suggested by the number of informants for a particular disease and I_u is the total number of informants who cited same plant for any disease.

Informant consensus factor (FIC)

Informant consensus factor (FIC) value was used to analyze the consensus between use of plants for various ailment categories and respondents of the study area, it was calculated by the following formula Bhat *et al.*, 2013)^[4].

$$FIC = \text{Nur} - N_t / \text{Nur} - 1$$

Where, Nur = Number of use citations for a particular disease category while N_t is the number of botanical species used for a particular use category by all informants, the values of FIC range from 0 to 1. High value specifies that the informants are in favor on the use of plant species for a

disease category and low value shows that plant species are randomly selected / informants do not exchange their traditional medicinal use knowledge.

IUCN Categories for Threatened species

IUCN criteria for threatened categories Version 3.1 (IUCN, 2001) were applied and Category A of vulnerable, Category A of endangered and Category A of critically endangered species was used for assessment. A taxon is vulnerable when the best available evidence specifies that it meets any of criteria A, and it is therefore considered to be facing a high risk of extinction in the wild. Category-A reduction in population size based on, 1) an observed, estimated, inferred or suspected population size reduction of $\geq 50\%$ over the last 10 years or three generations, whichever is the longer, where the causes of the reduction are: clearly reversible and understood and ceased, based on (and specifying) any of the following; (a) direct observation, (b) an index of abundance appropriate to the taxon, (c) a decline in area of occupancy, extent of occurrence and/or quality of habitat, (d) actual or potential levels of exploitation and (e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites. A taxon is endangered when the best available evidence indicates that it meets any of criteria A, and it is therefore considered to be facing a very high risk of extinction in the wild. Category-A reduction in population size based on, 1) An observed, estimated, inferred or suspected population size reduction of $\geq 70\%$ over the last 10 years or three generations, whichever is the longer, where the causes of the reduction are clearly reversible and understood and ceased, based on (and specifying) any of the criteria a to e shown under vulnerable category. A taxon is 'critically endangered' when the best available evidence indicates that it meets any of the criteria A, and it is therefore considered to be facing an extremely high risk of extinction in the wild. Category-A reduction in population size based on, 1) An observed, estimated, inferred or suspected population size reduction of $\geq 90\%$ over the last 10 years or three generations, whichever is the longer, where the causes of the reduction are clearly reversible and understood and ceased, based on (and specifying) any of the criteria a to e shown under vulnerable category of IUCN (2001).

Protein profiling

Genetic diversity among the 140 genotypes of Selected Species viz., 20 genotypes of *B. lyceum*, 20 genotypes of *O. ferruginea*, 20 genotypes of *M. communis*, 20 genotypes of *Z. oxyphylla*, 20 genotypes of *P. protopunica*, 20 genotypes of *A. nitida* and 20 genotypes of *Q. incana* were investigated using biochemical description. For the approximation of genetic diversity, SDS-PAGE was carried out. Three seeds from each genotype was taken for total seed storage protein profile and crushed into fine powder. A seed powder (0.01 g) protein extraction buffer (PEB) about 400 μ l were added; with composition of 0.5 M Tris-HCL, 0.2% SDS, 5 M urea, 1% B-mercap-toethanol under pH 8. The E-tube was vortexed to homogenize the PEB and seed fine powder (PEB-FP). To detect the movement of PEB-FP in the separation PAG, the coomassie brilliant blue (CBB) was added to the E-tubes as a tracking dye. The E-tubes were centrifuged at room temperature at 13,000 rpm for 10 minutes. Polyacrylamide gel 12% was carried out for electrophoretic process (composition of resolution gel (3.0

M Tris-HCl, pH 9.0, 0.4% SDS) and staining gel (0.4 M Tris-HCl, pH 7.0, 0.4% SDS)). The electrode buffer containing 0.025 M Tris, 129M glycine and 0.125% SDS was poured in the electrophoresis tank. Similarly, 9 μ l PEB-FP was loaded in each well of 12% PAG. At 100 V the electrophoresis ran until the blue line passed to the bottom of gel plates. For the scoring the PAG were stained and destained.

3. Results and Discussion

Demographic features

A total of 89 informers were interviewed, of which 70 were males and 19 females. Traditional ethno medicinal drivers, shepherds, local informants were interviewed to seek wide range of information about the plants used for medicinal purposes.

Phytodiversity

Cultural diversity and rich flora are the main indicators for the existence of rich traditional medicines (Yesilada, 2002; Muhammad *et al.*, 2018). The present study reported 7 plants belonging to 7 families commonly used to cure various diseases by the local human community. Considering taxonomic characteristics, it was noted that 57.14% of the species trees and 42.85% were shrubs.

Used value (UVi)

The use values of medicinal plant species ranged from 0.146 to 0.438 (Fig. 1). Dominated medicinal plants with most use values were *Berberis lycium* having (UVi= 0.438), *Ziziphus oxyphylla* (0.393), *Myrtus communis* (0.337), *Punica protopunica* (0.281). While minimum used values of collected medicinal plants were *Olea ferruginea* (0.258), *Alnus nitida* (0.224) and *Quercus incana* (0.146). Maximum used values of cited medicinal plant species might be due to their extensive distribution and cultural drivers awareness which resulted those botanical species as the first choice for treatment (Ullah *et al.*, 2014)^[33]. Table 1 designates all the plants recorded with their respective scientific, local and family names, habit, parts used and particular diseases to prepare the phytomedicines. Those plants reported by the interviewees were directed for all the local populaces of the research area.

Relative frequency citations (RFCs)

A relative frequency citation is calculated in order to know the most frequently used medicinal plants for various disorders. In the present analysis, it ranged from 0.224 to 0.786 (Fig. 2; Table. 1). Based on the RFC values, the most cited medicinal plant species by the traditional drivers are *Berberis lycium* (RFCs= 0.786), *Punica protopunica* (0.731), *Myrtus communis* (0.617), *Ziziphus oxyphylla* (0.551), *Olea ferruginea* (0.514), *Alnus nitida* (0.337), and *Quercus incana* (0.224). Maximum relative frequency citations clarify the facts that these medicinal plant species are very well known among the most number of traditional drivers (Butt *et al.*, 2013; Muhammad *et al.*, 2108). Those medicinal plant species having maximum RFC should be further evaluated phytochemically and pharmaceutically to identify their active constituents for drug discovery (Vitalini *et al.*, 2013)^[35].

Fidelity level (%)

Fidelity level (FL %) is used to identify species that are

most preferred by the inhabitants for the treatment of certain ailments. In the current study, fidelity level ranges from 44.444% to 100% (Fig. 3; Table 1). The medicinal plant species with highest fidelity level was of *Berberis lycium* cited 100% for Jaundice, Diarrhea, Pile, Dysentery, Anti-tumor, Urinary infections, Digestive Problems, Dry Cough, Rheumatism, Dysentery, Diarrhea, Ulcer respectively. Some other medicinal plant species with most fidelity level were *Myrtus communis* (FL%= 84.269%), *Ziziphus oxyphylla* (74%), *Punica protopunica* (74.358%), *Olea ferruginea* (66.666%), *Quercus incana* (65.789) and *Alnus nitida* (44.444%) for various disorders as mentioned in Table 1. These mentioned plant species may be confirmed as important medicinal plants by further assessment and evaluation through phytochemical, biological and pharmaceutical activities. Many researchers obtained maximum fidelity level values against certain disorders (Srithi *et al.*, 2009; Lulekal *et al.*, 2013). Moreover, plants with minimum FL should not be abandoned as declining to remark them to the future generation could raise the threat of gradual depletion of the cultural knowledge (Chaudhary *et al.*, 2006)^[5].

Consensus index (CI%)

Percentage of respondents questioned with traditional medicinal knowledge about plant species used to treat disorders and Consensus index (CI) of the botanical taxa are mentioned in Table 1, which ranged from 28.089% to 78.651% (Fig. 4). CI results revealed that most respondents percentage was for *Berberis lycium* (CI%= 78.651%), followed by *Ziziphus oxyphylla* (73.033%). Most consensus index for *Berberis lycium* was also reported by Khan and Ahmad, (2015)^[15]. However, *Ziziphus oxyphylla* was followed by *Olea ferruginea* (67.415%), *Myrtus communis* (61.797%), *Punica protopunica* (50.561%), *Alnus nitida* (44.943%), and *Quercus incana* (28.089%). CI indicates consensus on the importance of *Berberis lycium*, well known medicinal plants used in cultural folk medicines and treat a number of disorders in the Swat valley. Similar results were found by Khan and Ahmad (2015)^[15] who reported that 'CI' values are highest for *Berberis lycium*.

Informant consensus factor (FIC)

Informant consensus factor (FIC) ranged from 0.918 to 1 (Fig. 5; Table 2) and their use reports (URs) from 19 to 67. Different diseases *viz.* Anti-tumor, High blood pressure, Pile, Rheumatism had maximum FIC value due to representation by only single medicinal plant taxa/ species ($N_t = 1$). Other most frequent and cited disease categories were Jaundice (FIC= 0.984), followed by ulcer (0.976), Earache (0.9714), Anthelmintic and Diarrhoea (0.969 each), Anti inflammation agent (0.965), Dysentery (0.964), Trauma and Injuries (0.962), Urinary infection (0.961), digestive problems (0.94), Dry cough (0.957), Mouth & throat sore (0.95), Anti diabetics (0.944), Swelling and body pain (0.925) and Skin diseases (0.918). Many disease categories have most citations or maximum number of species but their FIC values vary due to the basic indices of FIC formula as the consensus of informants on the use of medicinal plants against different disorders is described by the computed index FIC. Lowest FIC value was found in Skin diseases (0.918). Many researchers reported Anti-tumor, High blood pressure, Pile, Rheumatism as the most

cited disease categories (Tangjitman *et al.*, 2015) ^[3]

Threats, Conservation status and Genetic diversity of *Berberis lycium* Royle.

Berberis lycium comes under the family berberidaceae. This plant is highly medicinal. An important chemical compound (Berberidine) is obtained from the underground root of this specie. The main hazard to this plant is over exploitation for medicinal purposes. Its' over exploitation as a high valued medicinal plant must be stopped to insure its conservation. According to the local residents, the population has decreased up to 70% due to its distinctive life form and unsustainable collection. It comes under the category A (a, c and d) of the threatened species.

Intraspecific locus variation among 20 genotypes of *Berberis lycium* is represented in Table 3 and Notably, B/L-1 and B/L-6, 7, 8, 9 and 10 were monomorphic in *Berberis lycium* and was treated as specie specific. B/L-2, B/L-3, B/L-4, B/L-5 and B/L-6 were polymorphic. These bands showed 80%, 80, 95 and 35% variation respectively. The locus contribution toward genetic disagreement (LCTGD) of *Berberis lycium* was 40 % (Table 3). Due to High intra-specie locus contribution toward genetic disagreement SDS-PAGE could be a consistent procedure for characterization of this specie (Muhammad *et al.*, 2018) ^[20]. The genetic pool of dissimilarity within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018) ^[20]. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is essential for its conservation and use. It will help greatly in describing what to conserve as well as where to conserve, and will enhance our information and understanding of the taxonomy, origin and evolution of *Berberis lycium*.

Threats, Conservation status and Genetic diversity of *Olea ferruginea* Royle

Olea ferruginea belongs to the family Oleaceae. The main threats are use of timber and wood for fuel unsustainable collection and also over exploitation. The population has been reduced by 66% and decline in area of occupancy has occurred. Its collection and over exploitation must be banned. It is falling under the category A (a and c) of the vulnerable species. Intraspecific locus variation among 20 genotypes of *Olea ferruginea* is shown in Table 3 and Especially, B/L-1, B/L-4, 5, 7 and 8 were monomorphic in *Olea ferruginea* and was treated as specie specific loci. B/L-2, B/L-3 and B/L-6 were polymorphic. These bands showed 90%, 50, and 85% polymorphism respectively. The locus contribution toward genetic disagreement (LCTGD) of *Olea ferruginea* was 30 % (Table 3). Due to High intra-specie locus contribution toward genetic dissimilarity SDS-PAGE could be a reliable way for representation of this specie (Muhammad *et al.*, 2018; Nisar *et al.*, 2016) ^[20, 25]. The genetic pool of difference within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018) ^[20]. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is essential for its conservation and use. This diversity will help greatly to explain how and where to conserve, and will enhance our information and insight into taxonomy, origin and evolution of *Olea ferruginea*.

Genetic diversity, Conservation status, threats and recommendations of *Myrtus communis* L.

Myrtus communis belongs to the family Myrtaceae. The main threats are unsustainable collection and over exploitation as a medicinal species. According to the local inhabitant population has decreased up to 74% due to its medicinal value and unsustainable collection. It comes under the category A (a, c and d) of the endangered species Its collection and over exploitation must be banned. Intraspecific locus variation among 20 genotypes of *Myrtus communis* is shown in (Table 3) and particularly, B/L-6 and B/L-7, 8 and 9 were monomorphic in *Murtus communis* and were treated as specie specific loci. B/L-1, B/L-2 B/L-3, B/L- 4 and B/L-5 were polymorphic. These bands showed 40%, 55, and 85%, 45%, 45% variation respectively. The locus contribution toward genetic disagreement (LCTGD) of *Myrtus communis* was 40% (Table 3). Due to intra-specie locus contribution toward genetic dissimilarity SDS-PAGE could be a reliable way for representation of this specie (Muhammad *et al.*, 2018) ^[20]. The genetic pool of difference within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018) ^[20]. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is vital for its conservation and application. This will aid significantly in describing what to conserve as well as where to conserve, and will enhance our information and understanding of the taxonomy, origin and evolution of *Myrtus communis*.

Genetic diversity, Conservation status, threats and recommendations of *Ziziphus oxyphylla* Edgew.

Ziziphus oxyphylla belongs to the family Ramnaceae. The main threats are unsustainable collection and over exploitation as a medicinal species and cutting of plants for making fence around field for protection. According to the local inhabitant population has decreased up to 60% due to its medicinal value and unsustainable collection. It comes under the category A (a, and c) of the vulnerable species. Its collection and over exploitation must be banned. Intraspecific locus variation among 20 genotypes of *Ziziphus oxyphylla* is shown in (Table 3) and particularly, B/L-1 and B/L-6, 7, 8 and 9 were monomorphic in *Ziziphus oxyphylla* and were treated as species specific loci. B/L-2, B/L-4 B/L-5, and B/L-10 were polymorphic. These bands showed 70%, 35, and 80% polymorphism respectively. The locus contribution toward genetic disagreement (LCTGD) of *Ziziphus oxyphylla* was 30% (Table 3). Due to intra-specie locus contribution toward genetic variation SDS-PAGE could be a dependable technique for documentation of this specie (Muhammad *et al.*, 2018) ^[20]. The genetic pool of dissimilarity within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018) ^[20]. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is vital for its conservation and application. This will aid significantly in conservation of this specie.

Genetic diversity, Conservation status, threats and recommendations of *Punica protopunica* (L). Balf. fil

Punica protopunica comes under the family punicaceae.

The plant is highly medicinal species. The main hazard to this plant is over exploitation for medicinal purposes and wood as fuel. Its' over exploitation as a high value medicinal plant must be stopped to insure its conservation. According to the local residents, the population has decreased up to 70% due to its cutting as fuel wood and unsustainable collection. It comes under the category A (a, c and d) of the threatened species.

Intraspecific locus variation among 20 genotypes of *Punica granatum* is represented in Table 3 and notably, B/L-1, B/L-2, 8, 9 and 10 were monomorphic in *Punica protopunica* and were treated as species specific. B/L-3, B/L-4, B/L-5, B/L-6 and B/L-7 were polymorphic. These bands represented 90%, 75, 85, 50 and 90% variation respectively. The locus contribution toward genetic disagreement (LCTGD) of *Punica protopunica* was 40 % table 3. Due to High intra- specie locus contribution toward genetic disagreement SDS- PAGE could be a consistent procedure for characterization of this specie (Muhammad *et al.*, 2018) [20]. The genetic pool of dissimilarity within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018) [20]. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is essential for its conservation and use. It will help greatly in describing what to conserve as well as where to conserve, and will enhance our information and understanding of the taxonomy, origin and evolution of *Punica protopunica*.

Genetic diversity, Conservation status, threats and recommendations of *Alnus nitida* (Spach.) Endel.

Alnus nitida belongs to the family Betulaceae. The main threats are wood cut for fuel, overgrazing, unsustainable collection and over exploitation. According to the local inhabitant population has decreased up to 75% due to its medicinal value and unsustainable collection. It comes under the category A (a, c and d) of the endangered species, its collection and over exploitation must be banned. Intraspecific locus variation among 20 genotypes of *Alnus nitida* is presented in Table 3 and mainly, B/L-1, B/L-4, 5, 8, 9 and 10 were monomorphic in *Alnus nitida* and were treated as specie specific loci. B/L-2, B/L-3, 6 and 7 were polymorphic. These bands showed 18, 18%, 10%, and 10% variation respectively. The locus contribution toward genetic disagreement (LCTGD) of *Alnus nitida* was 40% (Table 3). Due to intra-specie locus contribution toward genetic dissimilarity SDS-PAGE could be a reliable way for representation of this specie (Muhammad *et al.*, 2018; Muhammad *et al.*, 2018a). The genetic pool of difference

within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018). A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is vital for its conservation and application.

Genetic diversity, Conservation status, threats and recommendations of *Quercus incana* Roxb.

Quercus incana belongs to the family fagaceae. The main threats are use of timber and wood for fuel, unsustainable collection and over exploitation as a medicinal species. The population has been reduced by 65% and decline in area of occupancy has occurred. Its collection and over exploitation must be banned. It is falling under the category A (a and c) of the vulnerable species. Intraspecific locus variation among 20 genotypes of *Quercus incana* is shown in Table 3 and specially, B/L-1, B/L-5, and 7 were monomorphic in *Quercus incana* and were treated as specie specific loci. B/L-2, B/L-3, 4 and 6 were polymorphic. These bands showed 85%, 75, and 20, 35% diversity respectively. The locus contribution toward genetic disagreement (LCTGD) of *Quercus incana* was 57.142% (Table 3). Due to High intra-specie locus contribution toward genetic dissimilarity SDS-PAGE could be a reliable way for representation of this specie (Muhammad *et al.*, 2018; Muhammad *et al.*, 2018a) [20, 18]. The genetic pool of difference within genotypes of this specie is the basis for selection as well as for plant improvement and conservation (Muhammad *et al.*, 2018) [20]. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is essential for its conservation and use. It will help greatly in describing what to conserve as well as where to conserve, and will enhance our information and understanding of the taxonomy, origin and evolution of *Quercus incana*.

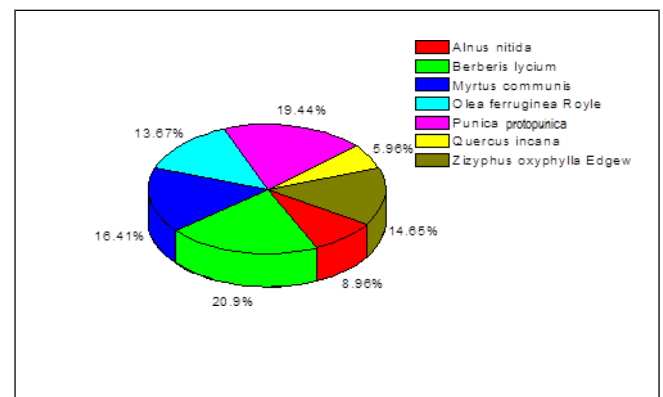


Fig 1: Graphical representation of used value

Table 1: Medicinal plants with vernacular and family name, habit, part used, disease cured, UVi, RFCs, FL, CI

S. No	Botanical Name	Local name	Family	Habit	Part used	Uvi	RFCs	FL%	CI%	Ailments
1	<i>Alnus nitida</i> (Spach.)Endel.	Geray	Betulaceae	Tree	Leaves, bark	0.224	0.337	44.444	44.94	Swelling & body pain, Trauma & injuries, Anti-inflammation agent, Pile, Swelling & body pain
2	<i>Berberis lycium</i> Royle	Kawaray	Berberidaceae	Shrub	Fruits & roots	0.438	0.786	100	78.65	Jaundice, Diarrhea, Pile, Dysentery, Anti-tumor,
3	<i>Myrtus communis</i> L.	Manro	Myrtaceae	Shrub	Fruits, leaves	0.337	0.617	84.269	61.8	Urinary infections, Digestive Problems, Dry Cough, Rheumatism, Dysentery, Diarrhea, Ulcer
4	<i>Olea ferruginea</i> Royle	Khona	Oleaceae	Tree	Fruit, leaves	0.258	0.514	66.666	67.42	Mouth & throat sore.
5	<i>Punica protopunica</i> L.	Anangor ay	Punicaceae	Tree	Fruits peels, Seed, flower	0.281	0.731	74.358	50.61	Urinary infections, Dry Cough, Digestive Problems, Diarrhea, Skin disease, Anthelminthic, Anti-diabetics, Dysentery, Mouth & throat sore
6	<i>Quercus incana</i> Roxb.	Banj	Fagaceae	Tree	Fruits, bark, leaves	0.146	0.224	65.789	28.09	Dry Caugh, Anthelminthic, Dysentery
7	<i>Zizyphus oxyphylla</i> Edgew	Elanai	Rhamnaceae	Shrub	Root, fruits, leaves	0.393	0.551	75	73.03	Mouth & throat sore, Skin diseases, High Blood pressure, Jaundice, Earache,

Table 2: Groups of disorders and informant consensus factor (ICF) for each category.

Disease categories	No. of Taxa (Nt) Used	Use Reports (Nur)	Fic
Anthelminthics	2	34	0.969
Anti-diabetics	2	19	0.944
Anti-inflammation agent	2	30	0.965
Anti-tumor	1	55	1.00
Diarrhoea	3	67	0.969
Digestive Problems	3	51	0.96
Dry Cough	3	48	0.957
Dysentery	3	58	0.964
Earache	2	36	0.9714
High blood pressure	1	37	1.00
Jaundice	2	64	0.984
Mouth & throat sore	3	41	0.95
Pile	1	62	1.00
Rheumatism	1	45	1.00
Skin disease	4	38	0.918
Swelling & body pain	3	28	0.925
Trauma & injuries	2	28	0.962
Ulcer	2	43	0.976
Urinary infections	3	53	0.961

Fic= Informants consensus factors

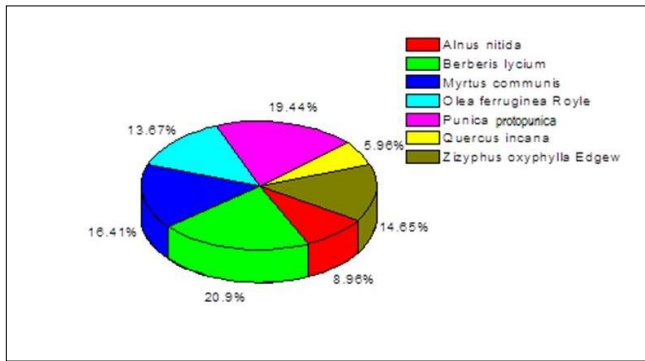


Fig 2: Graphical representation of RFCs

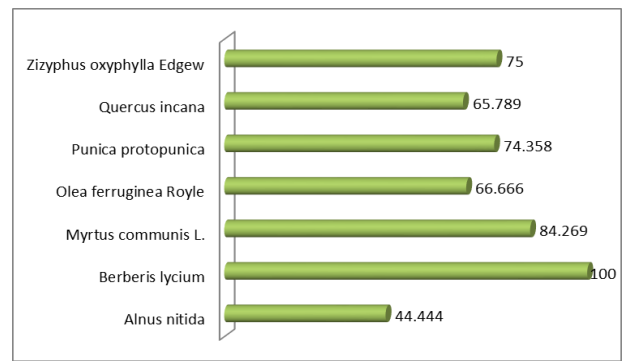


Fig 3: Graphical representation of FL%

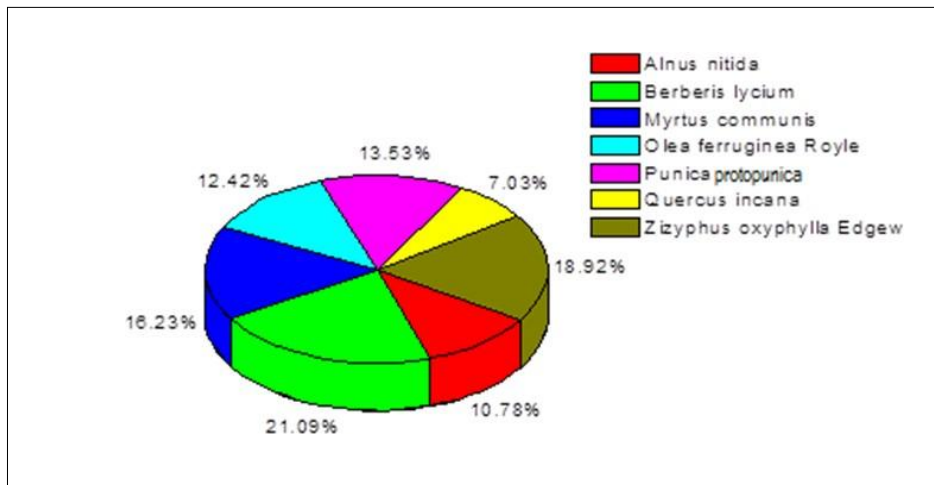


Fig 4: Graphical representation of CI%

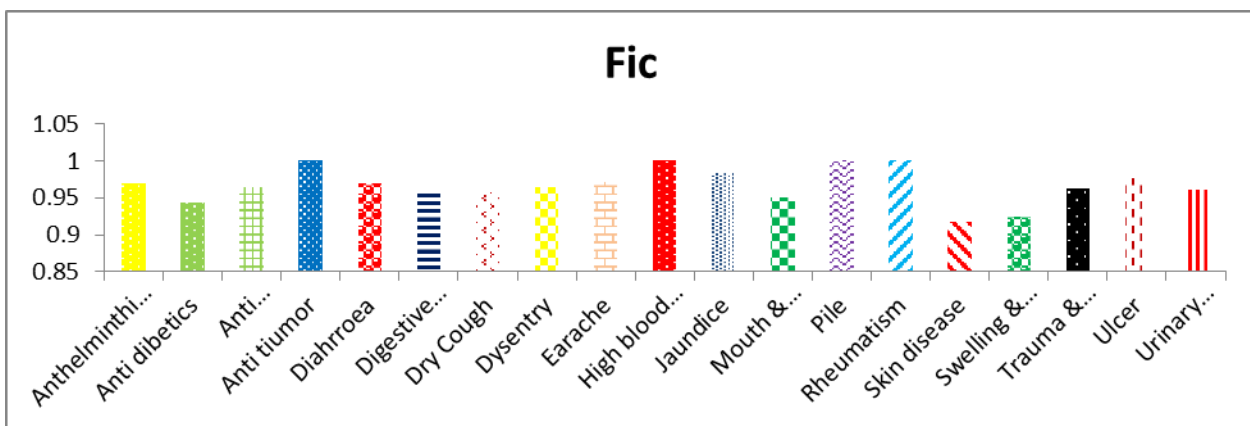


Fig 5: Graphical representation of Informants Consensus factor (FIC)

Table 3: Intra locus variation among various genotypes *B. lycium*, *O. ferruginea*, *M. communis*, *Z. oxyphylla*, *P. protonica*, *A. nitida* *Q. incana*

<i>Berberis lyceum</i>						<i>Olea ferruginea</i>				
Locus	Present	Absent	Variation	Status	GD	Present	Absent	Variation	Status	GD
B/L-1	20(100%)	0	Nil	mono	1.00	20(100%)	0	Nil	mono	1.00
B/L-2	4(20%)	16(80%)	80%	poly	0.2	2(10%)	18(90%)	90%	poly	0.1
B/L-3	4(20%)	16(80%)	80%	poly	0.2	10(50%)	10(50%)	50%	mono	0.1
B/L-4	1(5%)	19(95%)	95%	poly	0.1	20(100%)	0	Nil	mono	1.00
B/L-5	13(65%)	7(35%)	35%	poly	0.1	20(100%)	0	Nil	mono	1.00
B/L-6	20(100%)	0	Nil	mono	1.00	3(15%)	17(85%)	85%	poly	0.15
B/L-7	20(100%)	0	Nil	mono	1.00	20(100%)	0	Nil	mono	1.00
B/L-8	20(100%)	0	Nil	mono	1.00	20(100%)	0	Nil	mono	1.00
B/L-9	20(100%)	0	Nil	mono	1.00	Genetic disagreement GD= 30% (poly loci/total loci *100)				
B/L-10	20(100%)	0	Nil	mono	1.00					
Genetic disagreement GD= 40% (poly loci/total loci *100)										

<i>Myrtus communis</i>						<i>Ziziphus oxyphylla</i>				
Locus	Present	Absent	Variation	Status	GD	Present	Absent	Variation	Status	GD
B/L-1	12(10%)	8(40%)	40%	mono	0.1	20(100%)	0.00	Nil	mono	1.00
B/L-2	9(45%)	11(55%)	55%	poly	0.5	6(30%)	14(70%)	70%	poly	0.3
B/L-3	3(15%)	17(85%)	85%	poly	0.2	20(100%)	0.00	Nil	mono	1.00
B/L-4	11(55%)	9(45%)	45%	poly	0.6	13(65%)	7(35%)	35%	poly	0.65
B/L-5	11(55%)	9(45%)	45%	poly	0.6	20(100%)	0.00	Nil	mono	1.00
B/L-6	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
B/L-7	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
B/L-8	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
B/L-9	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
Genetic disagreement GD= 40% (poly loci/total loci *100)						4(20%)	16(80%)	80%	poly	0.2
						Genetic disagreement GD= 30% (poly loci/total loci *100)				
<i>Punica Protopunica</i>						<i>Alnus nitida</i>				
Locus	Present	Absent	Variation	Status	GD	Present	Absent	Variation	Status	GD
B/L-1	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
B/L-2	20(100%)	0.00	Nil	mono	1.00	2(10%)	18(18%)	18%	poly	0.1
B/L-3	2(10%)	18(90%)	90%	poly	0.1	2(10%)	18(18%)	18%	poly	0.1
B/L-4	5(25%)	15(75%)	75%	poly	0.3	20(100%)	0.00	Nil	mono	1.00
B/L-5	3(15%)	17(85%)	85%	poly	0.2	20(100%)	0.00	Nil	mono	1.00
B/L-6	10(50%)	10(50%)	50%	mono	0.1	18(90%)	2(10%)	10%	poly	0.9
B/L-7	2(10%)	18(90%)	90%	poly	0.1	18(90%)	2(10%)	10%	poly	0.9
B/L-8	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
B/L-9	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
B/L-10	20(100%)	0.00	Nil	mono	1.00	20(100%)	0.00	Nil	mono	1.00
Genetic disagreement GD= 40% (poly loci/total loci *100)						Genetic disagreement GD= 40% (poly loci/total loci *100)				
<i>Quercus incana</i>										
Locus	Present	Absent	Variation	Status	GD					
B/L-1	20(100%)	0.00	Nil	mono	1.00					
B/L-2	3(15%)	17(85%)	85%	poly	0.15					
B/L-3	15(75%)	5(25%)	75%	poly	0.75					
B/L-4	16(80%)	4(20%)	20%	poly	0.8					
B/L-5	20(100%)	0.00	Nil	mono	1.00					
B/L-6	13(65%)	7(35%)	35%	poly	0.65					
B/L-7	20(100%)	0.00	Nil	mono	1.00					
Genetic disagreement GD= 57.142% (poly loci/total loci *100)										

4. Conclusion

The medicinal plant species in the study area were used commonly as an ethno medicine against 19 various disorders out of which most frequently occurred disorders are; Anti-tumor, High blood pressure, Pile, Rheumatism etc. Dominated medicinal plants with most use values were *Berberis lycium* having (UVi= 0.438), *Ziziphus oxyphylla* (0.393), *Myrtus communis* (0.337), *Punica protopunica* (0.281). While minimum used values of collected medicinal plants were *Olea ferruginea* (0.258) and *Alnus nitida* (0.224), *Quercus incana* (0.146) Based on the RFC values, the most cited medicinal plant species by the traditional drivers are *Berberis lycium* (RFCs= 0.786), *Punica protopunica* (0.731), *Myrtus communis* (0.617), *Ziziphus oxyphylla* (0.551), *Olea ferruginea* (0.514), *Alnus nitida* (0.337), and *Quercus incana* (0.224). The medicinal plant species with highest fidelity level was of *Berberis lycium* cited 100% for Jaundice, Diarrhea, Pile, Dysentery, Anti-tumor, Urinary infections, Digestive Problems, Dry Cough, Rheumatism, Dysentery, Diarrhea, Ulcer respectively. Due to representation by only single medicinal plant taxa ($N_t = 1$), the various diseases viz. Anti-tumor, High blood pressure, Pile, Rheumatism had maximum FIC value. Conservation studies of 7 threatened medicinal plants were carried out for assessment of their conservation status, threats and recommendations growing in Swat valley. Phytogeographically, the valley is present in the Sino

Japanese floristic region and is a hot spot of endemism. Among total of 7 taxa 3 (42.85%) vulnerable, 4 (57.14%) endangered species. The protein profiling was carried out on 12% gel electrophoresis; 10, 8, 9, 10, 10, 10 and seven reproducible bands were detected of *B. lycium*, *O. ferruginea*, *M. communis*, *Z. oxyphylla*, *P. protopunica*, *A. nitida* and *Q. incana* respectively; with molecular weight ranges from 10 to 180 KDa. The locus contribution toward genetic disagreement (LCTGD) of *B. lycium*, *M. communis*, *P. protopunica* and *A. nitida* was (40% each) whereas the genetic diversity among the genotype of *O. ferruginea*, *Z. oxyphylla*, was 30% and the genetic disagreement in the genotypes *Q. incana* was 51.142%. This genetic diversity within genotypes of the studied specie is the basis for selection as well as for plant improvement and conservation. The current work delivers indication that these medicinal plants have an important role in the healthcare system of swat urban public. They still endure to depend on medicinal plants for the treatment of healthcare problems. The existing paper denotes significant ethno botanical information on medical plants which provides baseline data for future pharmacological studies and genetic diversity is helpful in selection of elite genotypes for future conservation.

Competing interests

The authors declare that they have no competing interests. Authors' contributions NM conducted the field work and

Laboratory work, analyzed the data write the paper. NA, SU and ML oversaw all stages of the work presented in this report and provide comments on the report, NU, MKK and MU, ZX and IH helped in analyzing the graphical data. All authors read and approved the final manuscript.

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