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Estimation of genetic diversity among *Berberis* spp. from Karakoram Mountain Ranges using morpho-pathological and floral characters

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Key words: Berberis, Genetic diversity, Karakoram, morphology, pathology, dendrogram, cluster analysis, phylogeny, floral characters

Abstract

The present research describes genetic diversity analyses of medicinally important Berberis species (spp.) from great mountainous altitude i.e. Karakoram Mountain Ranges. High range (0-100%) of Genetic Distance estimates using 31 morphological, pathological and floral characters indicated possibilities of improving the material using genetic selection in Berberis spp. Eleven Berberis accessions were classified in 2 main clusters A and B comprising 5 and 6 accessions, respectively. Accessions from relatively lower altitude (< 6800 feet) were predominantly clustered in main group A. While main group B primarily comprised accessions from comparatively higher altitude (> 6800 feet) except accessions # 2 (5637 feet) and accession # 3 (5410 feet). Present research indicated that accessions collected from lower altitudes should be classified as *B. pseudumbellata* subsp. *pseudumbellata;* instead of previously reported species *B. Lyceum, B. brandisiana, B. orthobotrys, B. kunwarensis etc.* Predominant habitats (Himalaya and Karakoram Ranges) and spatial distribution of *Berberis* spp. has also been discussed. Study contributes into effective identification of Berberis spp. found in the area which is uncertain to a greater extent like rest of the world. *Berberis* spp. are highly medicinal and serve wildlife as 'wild-herbal-clinic' in natural habitat. *Berberis pseudumbellata* subsp. *gilgitica* is endemic to Gilgit-Baltistan and has become critically endangered.

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Introduction

Family Berberidaceae contains about 15 established genera and 650 species worldwide (Bottini *et al.*, 2002). Members of the family and genus Berberis are distributed mostly across Northern Hemisphere (Chandra and Purohit, 1980). *Berberis* is the richest genus among other 15 genera comprising upon approximately 500 species (Negi, 2013; Jin, 2011; Sing *et al.*, 2009; Naef *et al.*, 2002; Agrios, 1988; Hooker, 1982). Berberis is represented by 29 species from Pakistan and distributed in the mountainous parts of the country (eFlora, 2014). In the study area (CKNP), there are only two subspecies of *Berberis pseudumbellata* Parker i.e. *B. pseudumbellata* subsp. *pseudumbellata* and *Berberis pseudumbellata* Parker subsp. *gilgitica* Jafri. *Berberis pseudumbellata* Parker subsp. *gilgitica* Jafri is endemic to Gilgit-Baltistan (Khan *et al.* 2014; Alam and Ali, 2010; Jafri, 1975). Area is surrounded by mighty Himalaya, Karakoram and Hindukush Ranges. The area has recently been established as a new province of Pakistan.

Berberis species (spp.) are perennially deciduous unlike several evergreens (Khan *et al.* 2014). They are woody shrubs erect up to 3.3 m height (khan *et al.* 2014; Agrios, 1988; Herrera, 1984; Hooker, 1982) with a thick woody spiny shoot and covered with a thin brittle bark (Kulkarni *et al.*, 2012; Mehrhoff *et al.*, 2003; Agrios, 1988; Herrera, 1984; Hooker, 1982). Berberis spp. exhibit several morphological similarities across species and subspecies, which make their identification difficult (Bottinin *et al.* 2007; Mazumder *et al.* N.M.).

Berberis spp. are integral parts of traditional and modern medicine systems (khan *et al.* 2014; Sing *et al.*, 2009; Chopra *et al.*, 1981; Chandra and Purohit, 1980). It has shown significant usefulness towards treatment of various diseases i.e. jaundice, enlargement of spleen, AIDS, osteoporosis, heart, ocular trachoma, hypertension, infectious diseases, cholera, diarrhea, dysentery, eye troubles, leprosy, bone fractures and cancer (Khan *et al.*, 2013; Sing *et al.*, 2009; Asif *et al.*, 2007; Fatehi *et al.*, 2005; Caraballo *et al.*, 2004; Kuo *et al.*, 2004; Villinski *et al.*, 2003; Janbaza and Gilanib, 2000; Hwang *et al.*, 2002; Ivanoska and Philipov, 1997; Koo and Seang, 1996; Chopra *et al.*, 1981; Chandra and Purohit, 1980).

Several Berberis spp. exhibit invasive nature (USDA, 2014). Similarly, 18 species have been reported as alternate host for rust pathogen and have contributed into world-wide epidemics and famine (USDA NRCS, 2011; Lubell *et al.*, 2008a; Lubell *et al.*, 2008b; Mehrhoff *et al.*, 2003; Negi, 2013; Jin, 2011; Naef *et al.*, 2002; Agrios, 1988; Barbu-Diaconescu, 1961; Watson and Luig, 1958).

In the past, taxonomic classification of Berberis spp. reported from present study area i.e. Central Karakoram National Park, (CKNP) of Gilgit Baltistan province was based mostly on qualitative morphological characters. Since 1939, at least 10 researchers have reported 8 Berberis spp. i.e. *B. lyceum, B. brandisiana, B. kunwarensis, B. parkeriana, B. pseudumbellata pseudumbellata, B. pseudumbellata gilgitica, B. orthobotrys orthobotrys and B. orthobotrys capitata* (Khan *et al.* 2014). Sometimes these reports are inconsistent to each other and in many cases they are self-contradictory.

The present study was aimed at potentially re-assessment of such an uncertain situation, which is to a greater extent inherent to genus Berberis, through more elaborate, quantitative and statistically guided manner. Moreover, study was undertaken to estimate amount of available genetic diversity of 11 accessions collected from the natural populations for the first times using floral, morphological and pathological characters. Furthermore, study helps in more precise elaboration of phylogenetic relationships among Berberis spp. found in the area which is still uncertain (Bottini *et al.*, 1999, 2000, 2007).

Material and method

Geography

Central Karakorum National Park (CKNP) is part of

Karakorum Ranges located in the North of Pakistan. It extends over 35°N to 36.5°N Latitude and from 74°E to 77°E Longitude. (Hussain *et al.*, 2012, 2010). Elevation ranges from 1200m-6000m above sea level. It is the largest national park in Pakistan covering an area of 10,000 km². To protect its unique wildlife and natural ecosystem park was established in 1993. Study area covers almost 2000 sq.km in the park (Khan *et al.* 2013) including Bagrot valley (432 sq.km), Rakaposhi (613 sq.km), Rahimabad-Juglot (450 sq.km) and Naltar (273 sq.km).

Climate

Climate of the study area is predominantly cold arid and temperate in the lower elevations. Prevalent season is winter, occupying the valleys eight to nine months a year (WWF Pakistan, 2009). Area lacks significant rainfall, averaging only 120 to 240 millimeters (4.7 to 9.4 inches) annually (Karrar and Iqbal, 2011). Most of rainfall occurs during winter and early spring.

Sampling

Following stratified random sampling technique, morpho-pathological details were recorded from representative mature shrubs of Berberis spp. from amongst the natural populations (figure 1). Non floristic measurements were taken in post flowering period (after May 20 from lower altitudes i.e. below 2000 m and July 20 from higher altitudes i.e. above 2000 m). Sampling was carried out in representative valleys of CKNP viz: Bagrot, Rahimabad-Juglot, Rakaposhi and Naltar.

Data collection

Data collection from 11 Berberis accessions (table 1) was carried out in the field and in laboratory. Details on leaves, spines and plant height were recorded in the field. For this purpose 100 leaves and 100 spines were measured from each representative accession. Moreover, 10 different plants from the same population were measured for height scale. Berries and seed details were taken in the laboratory using scale and electric weight balance. For comparison of fresh and dried berries, berries were dried using thermal oven at 40°C (104 °Fahrenheit) for seven days (Bottini *et al.*, 2000, 2002). 100 berries and 100 seeds of each accessionwere measured for length and width, however, for weight measurement 100 berries and 1000 seeds were weighed. Infection prevalence was recorded using berry infection rate out of 200 berries which were collected randomly from each accession. Global Positioning System (GPS-Garmin) was used to record geographical coordinates and elevations (mean height above sea level). Climatic data was retrieved from local weather stations.

Parameters

Thirty one morpho-pathological and floral characters studied are given in table 2. These parameters are basically different characters of seeds, berries, leaves, spines, pod borer attack and plant height. Most of the parameters are given in their averages (mean) to reflect the actual picture of series of calculations of 1000 seeds (weight), 100 leaves, 100 spines and 200 berries etc. from each accession.

Statistical analysis

Data was recorded in the purposely designed field and laboratory note book and compiled in MS Excel. Cluster analysis/ construction of dendrogram were performed/ constructed using Statistical Package for Social Sciences (SPSS version 16.0). UPGMA (Unweighted Pair Group Arithmetic Mean) was used for calculation of GD (genetic distance) matrix (Nei and Li, 1979). Geographical Information System (ArcMap ver. 10.1) tools were used to analyze spatial distribution pattern of disease prevalence.

Result and discussion

Parameters studied

Mean, standard error and ranges for 31 morpho-pathological and floral characters are given in table 2. Important details are described in the following subheadings.

Leaf Characters

Mean number of leaves per branch was 58.69 and

ranged from 40.100-84.700). Mean length of lamina was 2.98 cm (SE ± 0.13) and ranged from 2.480-3.850 cm; mean width of lamina recorded was 1.05 (SE ± 0.12) and ranged between 0.630-2.000 cm; mean leaf size was 3.23 (SE ± 0.40) and ranged from 1.658-5.826 cm.

Thorns/spines

Mean number of thorns per branch documented were 49.74 (SE ± 5.03) and ranged 16.300-68.300; mean length of spines (thorns) was 1.30 cm (SE ± 0.06) and ranged 0.980-1.670 cm.

Table 1. Geo-ecological information of sample populations and study area (CKNP).

Acc. #	Sample site	Long.	Lat.	Altitude (ft)
RG01	Rahimabad	36.107	74.304	5722
RG02	Goro	36.181	74.290	5637
RG03	Nomal	36.091	74.282	5410
NM04	NaltarMW	36.156	74.199	8989
NE05	NaltarEnd	36.173	74.166	9708
BU06	BagrotUp	36.040	74.588	8660
BD07	BagrotDwn	36.034	74.567	8491
RG08	Juglot	36.179	74.311	6711
RA09	Hupaye	36.236	74.442	6739
RA10	Ghulmet	36.240	74.484	6564
RA11	Thol	36.237	74.435	6351

Table 2. Basic statistics for 31 morpho-pathological and floral characters in Berberis species.

S.No.	Character/Variable	$\bar{x}$ ± SE	Max	Min
Leaf Characters				
1.	Avg. No. of leaves per branch	58.69 ± 4.60	84.700	40.100
2.	Avg. Lamina length (cm)	2.98 ± 0.130	3.850	2.480
3.	Avg. Lamina width (cm)	1.05 ± 0.120	2.000	0.630
4.	Avg. Leaf Size (cm)	3.23 ± 0.400	5.826	1.658
5.	Avg. No. of thorns per branch	49.74 ± 5.03	68.300	16.300
6.	Avg. Size (length) of spine (cm)	1.30 ± 0.060	1.670	0.980
Berry Characters				
7.	Avg. No. of berries per branch	5.94 ± 1.860	16.400	0.000
8.	Avg. No. of Seeds per berry	2.48 ± 0.210	3.500	1.500
9.	Avg. Wt. (g)per Berry	0.06 ± 0.010	0.130	0.035
10.	Avg. wt. (g) Dry Pulp per berry	0.04 ± 0.010	0.106	0.028
11.	Avg. Wt. (g) total Seeds per berry	0.02 ± 0.000	0.028	0.007
Seed Characters				
12.	Avg. Wt. (g) one seed	0.01 ± 0.000	0.012	0.004
13.	Avg. Wt. (g) per 1000 seeds	6.59 ± 0.660	11.831	4.013
14.	Seed Floating Percentage (%)	27.35 ± 5.42	57.692	5.714
15.	Seed Submerged Percentage (%)	72.65 ± 5.42	94.286	42.308
16.	No Seed (%)	11.82 ± 2.23	24.000	2.000
17.	1 Seeded (%)	17.27 ± 4.43	48.000	5.000
18.	2 Seeded (%)	18.82 ± 3.53	36.000	2.000
19.	3 Seeded (%)	18.00 ± 3.26	35.000	0.000
20.	4 Seeded (%)	9.18 ± 2.810	33.000	1.000
21.	5 Seeded (%)	1.45 ± 0.670	6.000	0.000
22.	6 Seeded (%)	0.09 ± 0.090	1.000	0.000
Pathological Characters				
23.	Seed Infection (%)	23.36 ± 7.48	73.000	0.000
24.	Berry infection (%)	23.36 ± 7.48	73.000	0.000
Floral Characters				
25.	Number of Outer Whorl Sepals	3.74 ± 0.191	5.000	2.900
26.	Number of Inner Whorl Sepals	4.03 ± 0.199	5.000	2.900
27.	Number of Petals	5.26 ± 0.152	6.000	4.600
28.	Number of Stamens	5.37 ± 0.175	6.000	4.600
29.	Number of Carpals	1.00 ± 0.000	1.000	1.000
30.	Number of Flowers at each Axil	11.69 ± 0.845	16.80	7.900
Plant Height				
31.	Plant Height (m)	2.71 ± 0.180	3.370	1.750

Berries

Mean number of berries per branch was 5.94 (SE ± 1.86) and ranged between 0.000-16.400; mean number of seeds per berry was 2.48 (SE ± 0.21) ranging from 1.500-3.500; mean weight per fresh berry was 0.06 g (SE ± 0.01) and range from 0.035-0.130; mean weight dried pulp per berry was recorded 0.04 (SE ± 0.01) and range in between 0.028-0.106.

Table 3. Genetic diversity estimates calculated using Morpho-pathological and floral characters (using SPSS ver. 16.0).

Case	1	2	3	4	5	6	7	8	9	10
2	.677									
3	.459	.706								
4	.854	.872	1.000							
5	.560	.740	.861	.053						
6	.625	.830	.873	.029	.062					
7	.776	.898	.798	.205	.468	.089				
8	.419	.734	.794	.500	.474	.497	.702			
9	.281	.739	.619	.738	.578	.517	.922	.268		
10	.531	.766	.905	.436	.200	.506	.835	.010	.297	
11	.472	.431	.544	.238	.224	.301	.452	.000	.297	.045

This is a dissimilarity matrix.

Seed

Mean weight (g) total seeds per berry, mean weight (g) one seed and mean weight (g) per 1000 seeds were 0.02 (SE ± 0.00), 0.01(SE ± 0.00) and 6.59 (SE ± 0.66) ranging from 0.007-0.028, 0.004-0.012 and 4.013-11.831 g respectively. Mean seed floating (%) and submerged (%) were 27.35 (SE ± 5.42) and 72.65 (SE ± 5.42) respectively while ranging from 5.714-57.692 and 42.308-94.286 percent respectively. Mean seedlessness (%), one seeded (%), two seeded (%), three seeded (%), four seeded (%), five seeded (%) and six seeded (%) recoded were 11.82 (SE ± 2.23), 17.27 (SE ± 4.43), 18.82 (SE ± 3.53), 18.00 (SE ± 3.26), 9.18 (SE ± 2.81), 1.45 (SE ± 0.67) and 0.09 (SE ± 0.09) correspondingly.

Infection

Mean seed infection (%) was observed 23.36 (SE ± 7.48) and ranging from 0.000-73.000.

Plant Height

Mean plant height was observed was 2.71 m (SE ± 0.18) and range was from 1.75-3.37.

Fig. 1. A representative shrub of Berberis (left), inflorescence inset and it's unripen berries (right) from Karakoram ranges (CKNP). Photographs by Tika Khan author).

Statistical Analysis and GD Estimation

Hierarchical cluster analysis of SPSS was used to construct Genetic Distance matrix (Table 3) and dendrogram (Fig. 2). Clustering method "between groups linkage cluster method" with the major of squared Euclidean distance while values were transformed to standardized range of 0-1 (Levesque,

2007).

Genetic Distances among all possible combinations ranged from 0-100 %, indicating opportunity of improving the material using genetic selection.

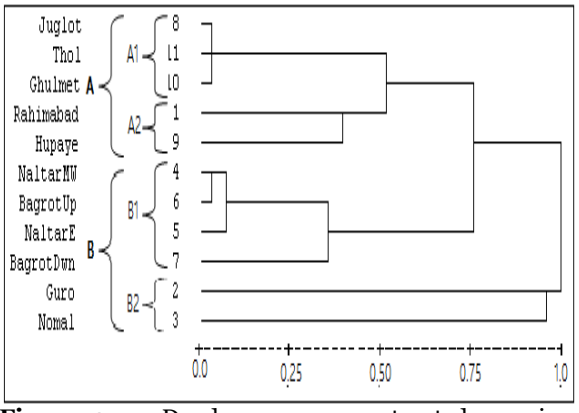

Fig. 2. Dendrogram constructed using morphological, pathological and floral, characters in Berberis spp. Clustering method "between groups linkage cluster method" of SPSS ver. 16.0 was employed.

Dendrogram based on morpho-pathological and floral characters is presented in Fig. 2. Eleven accessions were classified in 2 main clusters viz; A and B. Main cluster A was further subdivided into 2 subgroups A1 (comprising upon 3 accessions) and A2 (comprising upon 2 accessions). While main group B was subdivided into subgroups B1 and B2 comprising on 4 and 2 accessions respectively.

Subgroup A1 (comprising accession # 8 from Juglot and # 11 from Thol and # 10 from Ghulmet). Subgroup A2 comprised upon accessions (# 1 from Rahimabad, # 9 from Hupaye). Subgroup B1 comprised accessions (# 4 from Naltar MW, # 6 from Bagrot up, # 5 from Naltar E and # 7 from Bagrot Dwn). Subgroup B2 comprised accession # 2 from Goro and # 3 from Nomal.

It is evident from table 1 that Main group A comprised 5 accessions all of them collected from relatively lower altitude collection sites (< 6800 feet). While main group B predominantly comprised accessions from relatively higher altitude (> 6800 feet) except accessions # 2 (collected from 5637 feet) and accession # 3 (collected from 5410 feet). These 2 accessions constituted subgroup B2.

Based on dendrogram (presented in Fig. 2, using present data and observation of floral characters (using present research and previously reported characteristics described in flora of Pakistan (eFlora 2014, Jafri 1975). It was previously concluded (Khan *et al.*, 2014) that 5 accessions included in subgroups A1 and A2 more likely falls in *B. pseudumbellata* subspecies *pseudumbellata;* instead of previously reported species *B. Lyceum* (Qureshi *et al.*, 2006). The observation is further strengthened by the fact that *B. Lyceum* has been reported previously native to Himalayan ranges and not Karakoram ranges. Two accessions collected from Goro and Nomal (both low altitude collection sites) which are included in subgroup B2 are also more closely related to *B. pseudumbellata* subspecies *pseudumbellata*.

Numerical traits and principal component analysis used during present study have previously used to classify and measure the pattern of genetic diversity as in various crops of agronomic importance including black gram (Ghafoor *et al.*, 2001), chickpea (Naghavi and Jahansouz, 2005) and lentil (Sultana *et al.*, 2006).

Present work (included in this paper and Khan *et al.*, 2014) is in favor of some previously published reports (for example Alam and Ali, 2009) but in contrast to Stewart (1939, 1954), Sheikh (2000), Khan and Khatoon (2007) and Abbas *et al.* (2013). It is recommended that much work (preferably based on DNA technology) is needed to resolve discrepancies regarding taxonomic classification of Berberis species/subspecies (these discrepancies have been discussed previously Khan *et al.*, 2014).

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International University.

Conflict of interest

Authors declare that they have no conflict of interest.

References

Agrios GN. 1988. Plant pathology. San Diego, CA, USA: Academic Press.

Alam J, Ali SI. 2010. Contribution to the red list of the Plants of Pakistan. Pakistan Journal of Botany **42(5),** 2967-2971.

Asif A, Kabub G, Mehmood S, Khunum R, Gulfraz M. 2007. Wound healing activity of root extract of Berberis Lyceum Royle in rats. Phytother. Res. **21,** 589-591.

Barbu-Diaconescu V. 1961. A note on some fungus species parasitic on ornamental shrubs.Lucrarile.Gradiniibotanice din Bucuresti. . 237-241 P.

Bhandari DK, Nath G, Ray AB, Twari PV. 2000. Antimicrobial activity of crude extracts from Berberis asiatica stem bark. Pharmaceutical Biology **38,** 254–257.

Bottini MCJ, Greizerstein EJ, Aulicino MB, Pogg L. 2000. Relationships among Genome Size, Environmental Conditions and Geographical Distribution in Natural Populations of NW Patagonian Species of Berberis L. (Berberidaceae). Annals of Botany **86,** 565-573.

Bottini MCJ, Premoli AC, Poggio L.1999b. Hybrid speciation in Berberis L.An isoenzymatic approach. In: XVI International Botanical Congress, St. Louis, Missouri, USA.

Bottini MCJ, BustosADe, Jouve N, Poggio L. 2002. AFLP characterization of natural populations of Berberis (Berberidaceae) in Patagonia, Argentina. Plant Systematics and Evolution **231(1-4),** 133-142 P.

Bottini MCJ, Bustos ADE, Sanso AM, Jouve N, Poggio L. 2007. Relationships in Patagonian species of Berberis (Berberidaceae) based on the characterization of rDNA internal transcribed spacer sequences. Botanical Journal of the Linnean Society **153(3)**, 321–328. http://dx.doi.org/10.1111/j.1095-8339.2007.00586.x

Buchwald, NF. 1937. The susceptibility of Berberis species to black rust (Puccinia graminis).Gartner Tidende. 5 P.

Caraballo A, Caraballo B, Rodríguez-Acosta A. 2004. Preliminary assessment of medicinal plants used as antimalarials in the southeastern Venezuelan Amazon. Revista da SociedadeBrasileira de Medicina Tropical **37(2),** 186–188.

Chandra GV, Purohit M. 1980. Medicinal plants of India. ICMR, New Delhi, 377 P.

Chandra P, Purohit AN. 1980. Berberine contents and alkaloid profile of Berberis species from different altitudes. Biochemical Systematics and Ecology **8(4),** 379–380.

Chopra M, Chatterji A, Pakrashi SC. 1981. The treatise on Indian medicinal plants CSIR, New Delhi, 33-35 P.

eFlora. 2014. eFlora of Pakistan. Retrieved from http://www.efloras.org/browse.aspx?flora_id=5&start_taxon_id=103816 on May 6, 2014.

Fatehi M, Saleh TM, Fatehi Z, Farrokhfal K, Jafarzadeh M, Davodi S. 2005. A pharmacological study on Berberis vulgaris fruit extract. Journal of Ethnopharmacology **102(1),** 46–52.

Ghafoor A, Sharif A, Ahmad Z, Zahid MA, Rabbani MA. 2001. Genetic diversity in Blackgram (Vignamungo) Field Crops Research 69, 183-190. Doi: http://dx.doi.org/101016/S0378-4290(00)00141-6

Herrera CM. 1984. Selective Pressures on Fruit Seediness: Differential Predation of Fly Larvae on the Fruits of Berberis Hispanica. Oikos **42(2),** 166-170.

Hooker JD. 1982. Flora of British India. Reeve and Co, London **640,** 1882 P.

Hussain A, Farooq MA, Ahmad M, Akbar M, Zafar MU. 2010. Phytosociology and Structure of Central Karakoram National Park (CKNP) of Northern Areas of Pakistan. World Applied Sciences Journal **9(12),** 1443-1449.

Hussain E, Khan B, Lencioni V, Mumtaz S, Ali F. 2012. Stream macro-invertebrate assemblages in the Bagrot Valley of Central Karakoram National Park, Pakistan. Records Zoological Survey of Pakistan **21,** 60-64.

Hwang, Jin-Ming, Wang, Chau-Jong, Chou, Feu-Pi, Tseng, Tsui-Hwa, Hsieh, Yih-Shou, Lin, Wea-Lung, Chu, Chia-Yih. 2002. Inhibitory effect of berberine on tert-butyl hydroperoxide-induced oxidative damage in rat liver.Archives of Toxicology **76(11),** 664-670.

Iqbal, A. *et. al.* 2013. Genetic characterization of Berberis species collected from Kunhar River catchment using morphological and molecular markers. International Journal of Biosciences 3(5), 35-42.
http://dx.doi.org/10.12692/ijb/35.35-42

Ivanoska N, Philipov S. 1997. Study on the anti-inflammatory action of Berberis vulgaris root extracts, alkaloid fractions and pure alkaloid. International Journal of Immunopharmacology **18(10),** 553–561.

Jafri SMH. 1975. Berberidaceae. In: Flora of Pakistan No.87. (Eds.): E. Nasir and S.I. Ali. Department of Botany University of Karachi.1-40 P.

Janbaza KH, Gilanib AH. 2000. Studies on preventive and curative effects of Berberine on chemical-induced hepatotoxicity in rodents.Fitoterapia **71(1),** 25–33.

Jin Y. 2011. Role of Berberis species.as alternate hosts in generating new races of Puccinia graminis and P. striiformis. Euphytica **179(1),** 105-108.

Karrar M, Iqbal A. 2011. Gilgit City. Department of Architecture and Planning, NED University of Engineering and Technology, Karachi. 18-20 P.

Khan T, Khan IA, Rehman A, Alam J, Ali S. 2013. Exploration of near-extinct folk wisdom on medicinally important plants from Shinaki Valley Hunza, Pakistan. International Journal of Biosciences **3(10),** 180-186.

Khan T, Khan IA, Ahmed N, Rehman A. 2014. Elaboration of Phylogenetic studies in *Berberis* spp. from Karakorum Ranges. II. Floral and Morpho-pathological data. International Journal of Biosciences **5(1),** 133-142
http://dx.doiorg/10.12692/ijb/5.1.133-142

Koo L, Seang K. 1996. Inhibitory effect of protoberberine alkaloids from the roots of Coptis japonica on chatecolamine biosynthesis in PC12 cells. PlantaMedica **62(1),** 31-34.

Kulkarni GT, Sharma VN, Agrawal S. 2012. Antimicrobial and Anti-inflammatory Activities of Bark of Four Plant Species from Indian Origin.WebmedCentral Pharmaceutical Sciences **3(10)** WMC002010. Retrieved from on October 29, 2013.
https://www.webmedcentral.com/articleview/2010

Kuo CL, Chi CW, Liu TY. 2004. The anti-inflammatory potential of Berberine in vitro and in vivo. Cancer Letters **203(2),** 127–137.

Levesque R. 2007. SPSS Programming and Data

Management: A Guide for SPSS and SAS Users, Fourth Edition (2007), SPSS Inc., Chicago Ill. ISBN 1-56827-390-8.

Lubell JD, Brand MH, Lehrer JM. 2008a. AFLP identification of Berberis thunbergii cultivars, inter-specific hybrids, and their parental species. Journal of Horticultural Science & Biotechnology **83,** 55–63.

Lubell JD, Brand MH, Lehrer JM, Holsinger KE. 2008b. Detecting the influence of ornamental Berberis thunbergii var. atropurpurea in invasive populations of Berberis thunbergii (Berberidaceae) using AFLP. American Journal of Botany **95,** 700–705.

Mazumder PM, Das S, Das S, Kumardas M. (N.M). Pharmacognostical And Phytochemical Evaluation Of The Stems Of Berberis Aristata DC. International Bulletin of Drug Research **1(1),** 47-53.

Mehrhoff LJ, SilanderJr JA, Leicht SA, Mosher ES, Tabak NM. 2003. Invasive plant atlas of New England. IPANE. Retrieved from on November 20, 2013.
http://www.eddmaps.org/ipane/

Naef A, Roy BA, Kaiser R, Honegger R. 2002. Insect-mediated reproduction of systemic infections by Puccinia arrhenatheri on Berberis vulgaris. New Phytologist **154(3),** 717–730.

Naghavi MR, Jahansouz MR. 2005. Variation in the agronomic and morphological traits of Iranian chickpea accessions. Journal of Integrative Plant Biology **47,** 375-379.
http://dx.doi.org/10.1111/j1744-7909.2005.00058.x

Negi R. 2013. Four new host records of sooty mold from PauriGarhwal. Annals of Plant Protection Sciences **21(2),** 455-457.

Parvu M, Parvu AE, Craciun C, Barbu-Tudoran L, Vlase L, Tamas M, RoSca-Casian O, Perseca O, Molnar A. 2010. Changes in Botrytis cinerea Conidia Caused by Berberis vulgaris Extract. NotulaeBotanicaeHortiAgrobotanici Cluj-Napoca **38(3),** 15-20.

Rashmi, Rajasekaran A, Pokhriyal R, Singh YP. 2009. Quantitative estimation of Berberine in roots of different provenances of *Berberis aristata* DC by HPLC and study of their antifungal properties. Pharmacognosy Magazine **5(20),** 355-358.

Sing M, Srivastava S, Rawat AKS. 2009. Antimicrobial studies of stem of different Berberis species. Natural Product Sciences **15(2),** 60-65.

Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. 2000a. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5-methoxyhydnocarpin, a multidrug pump inhibitor. In: Proceedings of the National Academy of Sciences of the United States of America **97,** 1433–1437.

Sultana T, Ghafoor A, Ashraf M. 2006. Geographical patterns of diversity of cultivated lentil germplasm collected from Pakistan, as assessed buy seed protein assays. ActaBiologicaCracoviensia, Series Botanica, Poland **48,** 77-84.

USDA. 2014. United States Department of Agriculture. Retrieved from on July 3, 2014.
http://www.invasivespeciesinfo.gov/plants/barberrys html

USDA, NRCS. 2011. The PLANTS Database.National Plant Data Team, Greensboro, North Carolina, USA. Retrieved from on November 20, 2013.
http://plants.usdagov/java/

Villinski RJ, Dumas ER, Chai H-B, Pezzuto JM, Angerhofer CK, Gafner S. 2003. Antibacterial activity and alkaloid content of Berberis

thunbergii, Berberis vulgaris and Hydrastis canadensis. Pharmaceutical Biology **41(8),** 551-557.

Watson IA, Luig NH. 1958. Widespread natural infection of Barberry by *Puccinia graminis* in Tasmania. In: Proceedings of the Linnean Society of New South Wales **83(2),** 181-186.

WWF Pakistan. 2009. Land Cover Mapping of the Central Karakoram National Park, Version 2.0, WWF–Pakistan, Lahore. Retrieved from on September **28,** 2013.
http://www.wwfpak.org/wwf-projects/pdf/190909_report_CKNP_LandCoverpdf