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Uses, preparation and phytochemical analysis of anti-diabetic medicinal plants in the rural Murree (Pakistan)

Hina Fatimah[1], Sana Naveed[1], Samar Naseer[1], Tauseef Anwar[2] and Huma Qureshi[3*]
[1]Department of Biology, Allama Iqbal Open University (Main Campus), Sector H-8, Islamabad, Pakistan
[2]Department of Botany, The Islamia University of Bahawalpur (Baghdad-ul-Jadeed Campus), Bahawalpur, Pakistan
[3]Government Associate College (W), Choonawala, Hasilpur-63000, Bahawalpur, Pakistan

Abstract: Medicinal plants in most of societies have been a source of quality health care. The indigenous populations are getting many ethnomedicinal products form local biodiversity. The current study aimed to investigate anti-diabetic plants in 8 villages (Patriata, Surasi, Charhan, Lower toppa, Ghikagali, Kashmir point, Pindi point and Shawala) of rural Murree. The methodology included preparation of questionnaire, identification of plants and phytochemical analysis. The informants were asked about vernacular name, part used and folk recipe. The quantitative study included fidelity level, use value and relative frequency of citation of plant species. Data indicated that 30% of patients use local herbs to treat diabetes. The most significant plant species were *Berberis lycium* with use value of 0.50 followed by *Melia azedarach* with use value of 0.43 and *Himalaiella heteromalla* with use value of 0.33. *Himalaiella heteromalla* was selected for phytochemical analysis. Total flavonoid content was 7.25±0.08mg (ethanol extract) and 6.03±0.13mg (chloroform extract) quercetin equivalent/gm of extract; total phenolic content was 53.92±0.47mg (ethanol extract) and 72.75±0.48mg (chloroform extract) quercetin equivalent/gm of extract; total β–Carotene content was 29.7µg/g in ethanol extract, and 27.26µg/g in chloroform extract while total lycopene content was 46.33µg/g (ethanol extract) and 41.54µg/g (chloroform extract) in *H. heteromalla*. The results suggest that medicinal plants of Murree region may be potential natural resources for antidiabetic compounds.

Keywords: Ethnopharmacology, herbal remedies, phytochemical analysis, traditional knowledge.

INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. The deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves (Mrabti *et al.,* 2018). About 80% of diabetes mellitus patients live in under-developed and developing countries. About 3.2 million deaths per year are caused due to complications of diabetes. The top most ten countries (in term of numbers) of DM patients are: India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh. According to the current statistics of the International Diabetes Federation (IDF), it has been projected that approximately 1 in 11 adults (415 million) has diabetes and is expected to reach 1 in 10 adults (642 million) by 2040. Statistically, the prevalence of DM in Pakistan is high; ranging from 7.6% (5.2 million) to 11% in 2011, it is estimated to reach 15% (14 million) by 2030. This places Pakistan at number 7 in the list of countries with a prevalence of DM and if the present situation continues, is expected to move to 4thPlace. The overall ratio of DM is about 22.04% in urban and 17.15% in rural areas. The pattern of DM prevalence is as follows: Punjab; male, 16.6%, female, 19.3%, Khyber PakhtunKhwa; 11.1% both genders, Baluchistan; 10.8% both genders, Sindh; male 16.2% female 11.7% (Hussain *et al.,* 2016).

The plants that possess healing properties or exert advantageous effects are generally nominated as “medicinal plants”. The medicinal plants are used by the people and also recommended by the doctors for the treatment of diabetes because they have no side effects. Most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and can often have anti-diabetic effects (Kooti *et al.,* 2016). In traditional medicine, the number of plants used in the treatment of diseases associated with physiological disorder such as diabetes is limited (Erasto *et al.,* 2005). According to the World Health Organization (WHO), traditional medicine is used by up to 90% of the population in developing countries for primary health care. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. There are about 800 plants which have been reported to show antidiabetic potential (Rizvi and Mishra, 2013). The plant species are highly valued and capable of treating diabetes. Although various plants have been traditionally used to reduce the blood glucose level and improve diabetic complications. The medicinal plants are easily available, have low cost and are also effective (Salehi *et al*., 2019).

It is important to explore the potential of traditional phytotherapies for diabetes control worldwide generally

**Corresponding author:* e-mail: drhuma@gu.edu.pk

and especially in developing countries like Pakistan. The limited efficacy and annoying side effects of anti-diabetic allopathic drugs draw the attention of patients, physicians and researchers towards alternative therapy especially medicinal plants-based drugs. The search for anti-diabetic drugs focused on medicinal plants because of their efficacy in human clinical trials and the less side-effect of drugs and easy availability. In Pakistan, many traditional healers are expert in using and practicing herbal recipes for the treatment of diabetes mellitus (Yaseen *et al.,* 2015). The aim of present study is to introduce the indigenous plants to control diabetes in the study area (rural Murree) which is not much explored with reference to ethnobotanical study on diabetes mellitus.

MATERIALS AND METHODS

Study area

The study was carried out in 8 rural villages (Patriata, Surasi, Charhan, Lower toppa, Ghikagali, Kashmir point, Pindi point, Shawala) of Murree. Murree is located in Galyat region of the Pir Punjab Range with the Rawalpindi district of Punjab Pakistan. It is mountainous area, forming part of outer Himalayan and it is located at a comfortable altitude of 2,291 meters (7,517 feet) in the Himalayan foothill at 33◦54'30" north latitude and 73°26'30" east longitude (Khan *et al.,* 2014). Murree features a subtropical highland climate (cwb) under the Koppen climate classification (Climate-Data/Murree). This area has cold, snowy winters, relatively cool summers with drastically escalated rain. The annual precipitation is 1,789 mm (70.0 in). The average annual temperature in Murree is 12.7°C (Khan *et al.,* 2019). Area of Murree is rich in species diversity, with over 700 plant species have been recorded (Khan *et al.,* 2014).

Method of data collection

According to the objective of the present study, the methodology included several steps ranging from preparation of questionnaire to conduction of survey, identification of plants and phytochemical analysis of the novel species selected form the mentioned list. The structured questionnaire was filled from hundred 100 diabetic patients and semi structured interviews were taken from 10 Hakims and homeopathic doctors in the study area. The questionnaire was designed according to this study. The questionnaire contained personal information of diabetic patients, disease profile, their style of life and type of medication they used. To collect data on medicinal plants the informants were asked about vernacular name, part used, habitat of plant, mode of preparation and folk recipe. To evaluate the success chances of the study pre-testing was done. For pre-testing 10 patients were cross-examined out of which 3 used local medicinal plants and 3 used non-native medicinal plants. This percentage was enough for further study.

Study design

The survey was done from the month of April to August, 2019. Hundred diabetic patients were interviewed out of those thirty patients used local medicinal plants and eighteen of patients used plants those were not native. So basically, the questionnaire addressed three groups of people: those who used local medicinal plants against diabetes, those who used medicinal plants which were not local and those who did not use medicinal plants. The mentioned plant species were photographed and collected with informants. The collected medicinal plants were preserved and herbarium sheet was prepared for identification. The medicinal plants were identified by taxonomist of Quaid-e-Azam University Islamabad.

Ethnomedicinal data compilation

The ethnomedicinal data collected during survey was analyzed by using quantitative ethnomedical indices in order to evaluate the importance of the mentioned plant species in the inspected area. The quantitative study was carried out by fidelity level (FL), use value (UV) and relative frequency of citation (RFC) (Umair *et al.,* 2017, Bhat *et al.,* 2019).

Phytochemical screening

Himalaiella heteromalla plant was selected for phytochemical analysis

Extraction, evaporation and dilution: The seeds were dried and grinded to make powder. The powdered material was extracted successively in two solvents Ethanol and Chloroform. 10g powdered material was soaked in 100ml of each solvent for seven days. The extracts were filtered using Whatmann filter paper No. 1. The extracts were evaporated by using water bath for 48 hours. The evaporated solid material (10mg) of each solvent was diluted in methanol and Dimethyl Sulfoxide (10ml), and these dilutions were further used for qualitative and quantitative tests (Aruju *et al*., 2017). Total flavonoid and total phenolic content of plant sample were determined by following standard protocols (Vishwakarma *et al.,* 2014).

Total Flavonoids content: Total Flavonoids content was determined by dissolving 10mg of plant extract in 10ml of dimethyl sulfoxide (DMSO). The samples were introduced into eppendorf tubes. 100µl of plant extract (chloroform and ethanol) was added to 50µl of 10% aluminum chloride solution. After that, 50µl of 1M potassium acetate solution was added. The mixtures were incubated at room temperature for about 30 min and optical density was recorded using spectrophotometer at 415nm. Stock solution of Quercetin was prepared by using DMSO. Quercetin concentrations were prepared in Eppendorf tubes. 500µl DMSO was added to six Eppendorf tubes then labeled each as Ei, Eii, Eiii, Eiv, Ev and Evi. After that, 500µl Quercetin stock was added to Ei. After mixing this mixture 500µl from this was added

into Eii, and then 500µl from Eii was added into Eiii and so on. Six more eppendorf tubes were used for the Quercetin preparation, which run as standard. These eppendorf tubes were labeled as A, B, C, D, E and F. For this purpose, 50µl of 10% aluminum chloride was mixed into 50µl of 1M potassium acetate in each Eppendorf tube. Then 100µl of Ei mixture was added to A, 100µl of Eii mixture was added to B, 100µl form Eiii mixture was added to C and so on. In order to obtain total volume of 1ml, 800µl distilled water was added in each Eppendorf tube. This was run as standard and results for TFC were articulated as quercetin equivalent mg/g of plant sample.

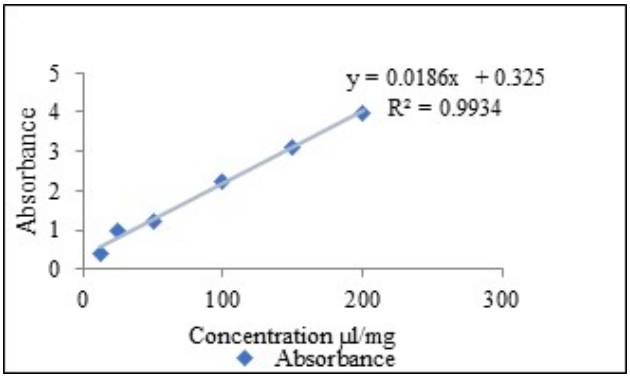

Fig. 1: Calibration Curve of Quercetin for total flavonoid determination

An equation was obtained from the standard QE graph. This equation was used for the determination of quercetin concentration in sample. The equation is given below:

y = 0.0186x + 0.325
y = absorbance of sample
x = concentration of quercetin
m = slope = 0.0186
c = intersection = 0.325
x = y - 0.325 / 0.0186

Then, the concentration of total flavonoid content in sample was determined as milligram of quercetin equivalent by using the following equation.

A = (c x v) / m
A = Total flavonoid content (mg/gm quercetin equivalent)
c = Concentration of quercetin in mg/ml
v = Volume of extract
m = Mass of extract (gm)

Total Phenolic Content: Total phenolic content was determined by dissolving 10mg of plant extract in 10ml of dimethyl sulfoxide (DMSO). The samples were introduced into eppendorf tubes. 100µl of plant extract (chloroform and ethanol) was added to 450µl of Folin-Ciocalteu reagent. After that, 450µl of 6% sodium carbonate solution was added. The mixtures were incubated at room temperature for 60 min and optical density was recorded using spectrophotometer at 760nm. Stock of Gallic acid was prepared by using DMSO. Gallic acid concentrations were prepared in Eppendorf tubes. 500µl DMSO was added to six eppendorf tubes then labeled each as E1, E2, E3, E4, E5 and E6. After that, 500µl Gallic acid stock was added to E1. After mixing this mixture 500µl from this was added into E2 and then 500µl from E2 was added into E3 and so on. Six more eppendorf tubes were used for the Gallic acid preparation, which run as standard. These eppendorf tubes were labeled as a, b, c, d, e, and f. For this purpose, 450µl of Folin-Ciocalteu reagent was mixed into 450µl of 6% sodium carbonate solution in each eppendorf tube. Then 100µl of E1 mixture was added to a, 100µl of E2 mixture was added to b, 100µl form E2 mixture was added to c and so on. This was run as standard. Consequently, total phenolic content was expressed as Gallic acid equivalents mg/of plant extract.

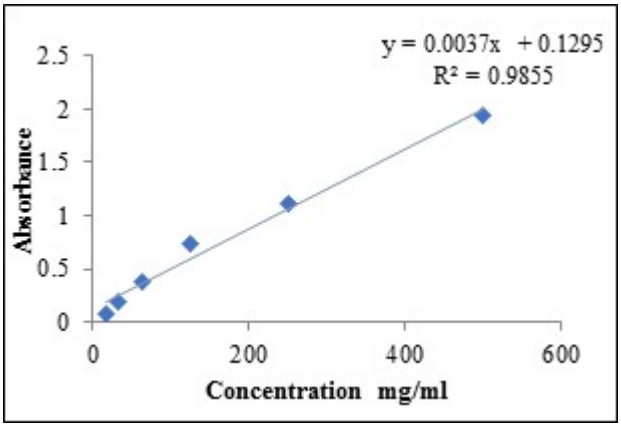

Fig. 2: Calibration Curve of Gallic acid for total phenolic content determination

The concentration of gallic acid in samples was determined by using an equation that was obtained from standard gallic acid graph. The equation is given bellow:

y = 0.0037x + 0.1295
y = absorbance of sample
x = concentration of Gallic acid
m = slope = 0.0037
c = intersection = 0.1295
So, to find concentration
x = y - 0.1295/ 0.0037

Then, the concentration of total phenolic content in sample was determined as milligram of gallic acid equivalent by using the following equation.

A = (c x v) / m
A = Total phenolic content (mg/gm gallic acid equivalent)
c = Concentration of gallic acid in mg/ml
v = Volume of extract
m = Mass of extract (gm)

Test for β-carotene: B-carotene was estimated by mixing 2mg of extract in 10ml of acetone and n-hexene (4:6) and shaking the mixture vigorously for 1 min. After that the mixture was filtered and absorbance was recorded at different wavelengths (405, 453, 505, 645 and 663nm). Content of β-carotene was calculated using following equation (Sravanthi and Rao, 2015).

$$\beta\text{-Carotene (mg/g)} = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

A = Absorbance recorded at specific wavelength

Table 1: Demographic data and medication status of diabetic patients

Demographic data of patients			
Percentage of interviewer's gender ○ 65% - female diabetic patients ○ 35% - male diabetic patients	Age group distribution of diabetic patients ○ 3% - young (13-29 years) ○ 46% - adults (30-49 years) ○ 51% - old (≥50years)	Marital status of diabetic patients ○ 98% - married ○ 2% - unmarried	
Type 1 and Type 2 diabetic patients ○ 16% - type I ○ 84% - type II	Number of complications faced by diabetic patients ○ 34% - one complication ○ 19% - 2 complications ○ 5% - 3 complications ○ 41% - no other complication	Inheritance from other family members ○ 8% - father ○ 15% - mother ○ 5% - both ○ 72% - none	
Disease in respondent's siblings ○ 33% - diabetic siblings ○ 67% - normal siblings	Glucose level monitoring ○ 12% - daily ○ 58% - once a week ○ 19% - twice a week ○ 11% - rare monitoring	Weight of diabetic patients ○ 16% diabetic patients - weight gain ○ 64% diabetic patients - weight lose ○ 29% diabetic patients - unaffected	
Smoking status of patients			
Percentage of smokers having diabetes ○ 34% - smokers ○ 66% - nonsmokers	Smoking period of diabetic patients ○ 100% - smoking since more than 5 years	Smoking per day ○ 42% smoke 1-2 cigarettes per day ○ 25% smoke 3-4 cigarettes per day ○ 33% smoke 5 or more cigarettes per day	
Exercise status of diabetic patients			
Percentage of patients doing exercise ○ 91% patients - do exercise ○ 9% patients - do not exercise	Types of exercise ○ 92% - walking ○ 4% - active job ○ 3% - both	Days of exercise per week ○ 87% - everyday ○ 4% - 1 day/week ○ 5% - 2 days/week ○ 3 % - 3 or 4 days/week	Total time of exercise ○ 15% exercise 10-15 minutes ○ 26% exercise 11-15 minutes ○ 26% exercise 16-20 minutes ○ 32% exercise 21-30 minutes
Medication status of patients			
Types of medication used by diabetic patients ○ 79% - Allopathic ○ 16% - Insulin ○ 5% - none	Additional medicine taken by diabetic patients ○ 7% - Homeopathic ○ 30% - Local herbs ○ 18% - home remedies	Different plant part used by respondents ○ 60% - leaves ○ 20% - roots ○ 13% - fruits ○ 7% - seeds	
Preparation methods of listed species ○ 40% - infusion ○ 27% - decoction ○ 27% - powder ○ 7% - raw form	Time period of using medicinal plants ○ 66% - < 1 year ○ 27% - < 5 years ○ 7% - > 5 years	Experience about medicinal plants ○ 10% - no improvement ○ 60% - improvement to some extent ○ 30% - improved and satisfied	Respondents' views about medicinal plants ○ 57% - agreed ○ 17% - strongly agreed ○ 27% - disagreed

STATISTICAL ANALYSIS

The primary data was arranged in Excel sheets (2019) and values for following indices were calculated and compared by applying below-mentioned formulae for different plants.

Fidelity Level: FL is the percentage of respondents who claimed the uses of certain plant species to treat a particular disease in a study area. The FL was calculated as:

$FL\ (\%) = N_P/N \times 100$

N_P is the number of respondents that claimed a use of a plant species to treat a specific disease and N is the number of respondents that used plants as a medicine to treat any given disease.

Use Value: Use value indicates the relative importance on use of plant species. It was calculated as:

$UV_i = \sum U_i / N$

Where, U_i represents the number of use report(s) of particular plant species, N represents the total number of respondents interviewed for a specific plant species.

Relative Frequency of Citation: It signifies the local importance of each species in a study area. The RFC index was calculated as:

$RFC = FC/N\ (0<RFC<1)$

Where, FC is the number of respondents who cited the use of specific species and N is the total number of respondents.

RESULTS

In this study, hundred diabetic patients were questioned. The data analysis as presented in table 1 showed that the percentage of female diabetic patients (65%) was more than the male diabetic patients (35%).

Table 2: Medicinal plants use by the informants of study area to cure diabetes

#	Botanical Name	Family	Vernacular Name	Life Form	Plant part	Use Value	Traditional recipes
1.	*Aloe vera* (L.) Burm.f.	Liliaceae	Kanwar gandal	Herb	Leaves	0.13	1-2 teaspoon of paste obtain from fresh leaves of *Aloe vera* is taken daily.
2.	*Artemisia scoparia* Waldst. & Kitam.	Asteraceae	Chahoo	Herb	Leaves	0.03	The leaves of plant are placed in water overnight. This water is taken daily before breakfast.
3.	*Berberis lycium* Royle	Berberidaceae	Sumbal	Shrub	Roots	0.5	The roots of plant are dried and grinded to prepare powder. 1 teaspoon is taken with water at morning. The roots of plant are also taken by soaking them in water overnight.
4.	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Bath-phay	Herb	Roots	0.2	The roots of plant are soaked in water overnight followed by taking the water in the morning. The roots are grinded to from powder and taken with water.
5.	*Justicia adhatoda* L.	Acanthaceae	Baik-karh	Shrub	Leaves	0.06	The leaves are washed, boiled in water for 30 minutes. Half cup of this water is taken daily.
6.	*Melia azedarach* L.	Miliaceae	Dharek	Tree	Leaves	0.43	Leaves are placed in water for 48 hours and taken daily before breakfast.
7.	*Mentha spicata* L.	Lamiaceae	Kala podina	Herb	Leaves	0.06	Fresh leaves are boiled for 10-15 minutes. One cup of this tea is taken after meal daily.
8.	*Pinus roxburghii* Sarg.	Pinaceae	Chir	Tree	cone	0.06	The cone of *Pinus roxburgii* is soaked in water overnight. This water is taken in the morning before breakfast.
9.	*Pyrus pashia* Buch.-Ham. ex D.Don	Rosaceae	Tang	Tree	Fruit	0.03	Fresh fruits are taken daily when in season.
10.	*Rumex hastatus* D. Don	Polygonaceae	Katimber	Herb	Leaves	0.03	Fresh plant material is boiled in water and taken 2-3 days a week.
11.	*Himalaiella heteromalla* (D.Don) Raab-Straube	Asteraceae	Kali zeri	Herb	Seeds	0.33	Seeds are grinded to prepare powder. 1 teaspoon is taken with water daily.
12.	*Solanum villosum* Mill.	Solanaceae	Kachmach	Herb	Leaves	0.03	The leaves of plant are boiled in water. Then boiled leaves are grinded to prepare paste. This paste is taken with bread.
13.	*Swertia cordata* (Wall. ex G. Don) C.B. Clarke	Gentianaceae	Choraita	Herb	Leaves	0.03	Leaves of *Swertia cordata* are boiled in water and taken daily.
14.	*Ziziphus acuminata* Royle	Rhamnaceae	Amnuii	Shrub	Roots	0.06	Roots are dried and grinded to make powder. Or roots are boiled in water and this water is taken daily after lunch.

The patients were divided into four different categories of age to assess dominant age group of diabetic patients i.e., child 12 years, young 13-29 years, adult 30-49 years and old 50^{+} years. Analysis of results on age showed that the most dominant age of diabetic patients was ≥50. The proportion of patients aged 30-49 was 46% and 13-29 aged patients was 3%. Diabetic patients were inquired about type of their diabetes.

Table 3: Absorbance of quercetin (Standard)

Concentration mg/µl	Absorbance
200	3.993
150	3.124
100	2.24
50	1.21
25	0.96
12.5	0.395

Patients with type 1 were 16% according to data collected during inventory. Type 2 diabetic patients were more than type 1 that were 84%. The patients were asked about their corresponding complication. Seven complications were mentioned in questionnaire. Those were cardiovascular, eye damage, kidney damage, foot damage, skin condition, hearing loss, Alzheimer's disease. 34% of diabetic patients suffered from only one complication and 19%

patients suffered from two complications. However, 5 suffered from three complications.

This shows that majority of patients suffered from one complication. Data analysis showed that the percentage of patients having no diabetic family member was more (72%). But the family member with diabetes were father (8%), mother (15%) and both (5%). Data indicated that 33% siblings of patients were suffering from diabetes and other (67%) were healthy having no diabetes. This expressed that 67% siblings of patients were non-diabetic. The patients were inquired about how often they monitored their sugar level. Out of 100 patients, 12% check their glucose level daily, 58% once a week, 19% twice a week and only 11% rarely.

This shows that the most of patients monitor their glucose level at least once a week. The analysis of data shows that the 15% of patients gain weight to diabetes most of them were type 1 diabetic patients. 64% of type 2 diabetic patients were overweight. However, 29% of patients did not experience any change in weight. Hundred patients were interviewed of which 35% were male patients and 65% were female patients. The collected data indicated that 34% of diabetic patients were smokers and 66% of diabetic patients were non-smokers (out of 35% of male patients). This was inquired from diabetic patients to know their type of medication. Allopathic medicines were most commonly used. According to analysis of survey 79% of patients used allopathic medicine. 16% of diabetic patients injected insulin. Only 5% of patients did not use any medicine. 30% of patients used local herbs to treat diabetes. 18% of diabetic patients used home remedies. Homeopathic medicines were being used by only 7% of patients. Local herbs or weeds were most commonly used. 10% of patients did not feel improvement after using medicinal plants. The percentage of diabetic patients who improved somewhat was 60% and 30% of patients improved significantly.

Table 6: Absorbance of gallic acid (standard)

Concentration mg/ml	Absorbance
500	1.949
250	1.11
125	0.729
62.5	0.389
31.25	0.199
15.625	0.086

The present study reported fourteen plants species with antidiabetic properties. Botanical name, family, vernacular name, plant material used, use value and traditional recipes are presented in table 2. In the present study, the people of the study area treat diabetes through indigenous medicinal plants. They believed that traditional medicines are better, permanent cure of their disease. So, the indigenous knowledge is better to provide the building blocks for the treatment and development of rural communities. The study on phytochemical tests shows that plants crude extract contains phenolic, terpenoids, steroid and saponin.

The most significant plant species was *Berberis lycium* with use value of 0.50 followed by *Melia azedarach* with use value of 0.43 and *Himalaiella heteromalla* with use value of 0.33. Phytochemical screening of *Himalaiella heteromalla* (seed extract) was performed. For the determination of flavonoid, quercetin was used as standard. The absorbance of quercetin was measured at 415 nm on spectrophotometer used for the formation of standard quercetin graph (table 3, fig. 1). Total flavonoid content in *Himalaiella heteromalla* was 7.25±0.08mg (ethanol extract) and 6.03±0.13mg (chloroform extract) quercetin equivalent/gm of extract shown in table 4 and 5.

Phenolic content was determined by gallic acid (standard). The absorbance of gallic acid were measured at 760 nm is shown in table 6. These were used for the formation of standard gallic acid graph (fig. 2). Total

Table 4: Flavonoid content of *Himalaiella heteromalla* seed of ethanol extract (n=3 was taken and shown below)

Sample solution (µg/ml)	Wt. of dry extract per ml m(gm)	Absorbance	QE cons. C (µg/ml)	QE cons. C (mg/ml)	TFC as QE, A = C x V/m (µg/ml)	Mean & SEM	STDEV
1000	0.001	1.699	7.39	0.00739	7.39	7.25	0.13
1000	0.001	1.649	7.12	0.00712	7.12		
1000	0.001	1.673	7.25	0.00725	7.25	0.08	

Table 5: Flavonoid content of *Himalaiella heteromalla* seed of chloroform extract (n=3 was taken and shown below)

Sample solution (µg/ml)	Wt. of dry extract per ml m(gm)	Absorbance	QE cons. C (µg/ml)	QE cons. C (mg/ml)	TFC as QE, A = C x V/m (µg/ml)	Mean & SEM	STDEV
1000	0.001	1.475	6.18	0.00618	6.18	6.03	0.22
1000	0.001	1.399	5.77	0.00577	5.77		
1000	0.001	1.464	6.12	0.0059	6.12	0.13	

phenolic content in *Himalaiella heteromalla* was 53.92± 0.47mg (ethanol extract) and 72.75±0.48mg (chloroform extract) gallic acid equivalent/gm. of extract shown in table 7 and table 8.

β-Carotene & lycopene content was determined by recorded absorbance. The recorded absorbance revealed that the total β - Carotene content of *Himalaiella heteromalla* was 29.7µg/g in ethanol extract and 27.26µg/g in chloroform extract (table 9). However, total lycopene content was 46.33µg/g (ethanol extract), and 41.54µg/g (chloroform extract).

DISCUSSION

The present study finds out that mostly aged people used medicinal plants and other remedies to treat diabetes. The most cited plants species were *Berberis lycium, Melia azedarach, Himalaiella heteromalla, Bergenia ciliata* and *Aloe vera*. *Berberis lycium* was reported by Mustafa *et al.* (2011) with same traditional use. Another study conducted in three regions of Pakistan showed 120 plant species belonging to 50 families used by diabetic patients. The study shows the most used part of the plants was leaves and mode of preparation was decoction (Yaseen *et al*., 2015). Ahmed *et al*. (2009) investigated the traditional herbal remedies for diabetic treatment in District Attock Pakistan. This study showed 37 plant species belonging to 33 genera and 33 angiosperm plant families used for diabetes. This study showed the traditional recipes include extracts, leaves, powders, flour, seeds, vegetables, fruits and herbal mixtures. In another study, the traditional medicinal plants are reported to be used to treat the diabetes by local people of western Anti-Atlas, Morocco. This study has shown that the folk medicines are important for the healthcare system especially for diabetes. 48 plant species are used by the local communities (Barkaoui *et al*., 2017).

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Njoku and Obi, 2009). In most cases these substances appear to be non-essential to the plant producing them. For example, penicillin produced by a few species of fungi has great value to man as antibiotic, but appears to serve no useful purpose in the microorganisms producing it (Sofowora, 1984). Many of these natural products have vital roles as mediators of ecological interactions; that is, they have functions in ensuring a continued survival of particular organisms in often hostile environments where there is competition with other organisms (Doss, 2009).

Table 7: Phenolic content of *Himalaiella heteromalla* seed of ethanol extract (n=3 was taken and shown below)

Sample solution (µg/ml)	Wt. of dry extract per ml m(gm)	Absorbance	GA cons. C (µg/ml)	GA cons. C (mg/ml)	TFC as GA, A = C x V/m (µg/ml)	Mean & SEM	STDEV
1000	0.001	0.332	54.73	0.05473	54.73	53.92	0.81
1000	0.001	0.329	53.92	0.05392	53.92		
1000	0.001	0.326	53.11	0.05311	53.11	0.47	

Table 8: Phenolic content of *Himalaiella heteromalla* seed of chloroform extract (n=3 was taken and shown below)

Sample solution (µg/ml)	Weight of dry extract per ml m(gm)	Absorbance	GA cons. C (µg/ml)	GA cons. C (mg/ml)	TFC as GA, A = C x V/m (µg/ml)	Mean & SEM	STDEV
1000	0.001	0.402	73.65	0.07365	73.65	72.75	0.83
1000	0.001	0.398	72.57	0.07257	72.57		
1000	0.001	0.396	72.03	0.07203	72.03	0.48	

Table 9: Total β - carotene & lycopene content in *Himalaiella heteromalla*

Absorbance	Ethanol extract	Chloroform extract
Absorbance at 663	0.089	0.079
Absorbance at 645	0.087	0.078
Absorbance at 505	0.083	0.075
Absorbance at 453	0.079	0.073
Absorbance at 405	0.074	0.075
β-carotene (mg/g)	0.0297	0.02726
β-carotene (µg/g)	29.7	27.26
Lycopene (mg/g)	0.0463328	0.0415464
Lycopene (µg/g)	46.3328	41.5464

Such roles include being attractant to pollinators, allelopathic agents or defense against predators and pathogens. For example, ipsdienol, a major constituent of the floral fragrance of several orchid species and azadirachtin, present in *Azadirachta indica*, have roles as attractant to bees and defense mechanism against insects respectively (Njoku and Obi, 2009). One study shows the phytochemical investigation of medicinal plants has been used by people of Nigeria (Omotayo and Borokini, 2012). According to this study, the extract of 21 plants separately prepared by using ethanol, methanol and distilled water. The study indicated that solvent extracts of only 7 plants have significant concentrations of total polyphenol and flavonoid. In another study, the phytochemical screening of reported medicinal plants has performed in Indonesia. In this study 15 plants are mentioned for the treatment of diabetes. The anti-diabetic activity of plants is attributed to secondary metabolites e.g., phenolic compounds, terpenoids, steroids and saponins (Nasution *et al.,* 2018; Qureshi *et al.,* 2016).

Multiple investigations have been carried out on natural products, mainly plants used to treat diabetes mellitus worldwide. In current study, *Himalaiella heteromalla* was selected for phytochemical analysis being used mostly by local people to cure diabetes. Another reason for selection of this plant was that *H. heteromalla* is one of indigenous medicinal flora of Pakistan with few reports published with a clue of is antidiabetic activity (Batool *et al*., 2019). Majeed *et al*. (2021) have reviewed variety of chemical compounds including polyphenols, flavonoids, alkaloids, terpenoids, phenolic content, tannins, saponin and glycoside present in the various parts of the plants e.g., leaves, roots, stem, wood, fruit, seeds, flowers that alone or with the combination of other compounds helps in the management DM. Alkaloids (e.g., vindolicine III, vindolinine, Catharanthine, vindolinene, vinblastine, vincristine), glycosides (e.g., Cytopiloyne, niazirin A, Galactomannan, Aloe-emodin-8-O-glycoside, Syringin, Rutin), polyphenols (e.g., Quercetin, Cinnamic acid and its derivatives, epigallocatechin and epigallocatechin gallate, Gingerols, Luteolin), Saponins (e.g., Furostanol, momordicine I, momordicine II, kuguaglycoside G) and terpenoids (e.g., Oleanolic acid, Thujone) are reported to have antidiabetic action via enhancing glucose uptake in pancreatic or muscle cells, through free radical scavenging action or ameliorated glycolysis and glycogenolysis (Kasali et al., 2021). In current study, high content of flavonoids, β - carotene & lycopene in ethanol extract of *Himalaiella heteromalla* seed while high phenolic content in chloroform extract indicates presence of diverse nature (polar and non-polar) of antidiabetic compounds in this plant.

CONCLUSION

Diabetes is a global problem and a huge number of patients have diabetes. Over 800 plant species showing hypoglycemic activities can be essential sources for discovering and developing new types of antidiabetic molecules. Medicinal plants reported in this study reflects evidence that Murree is rich in diversity of medicinal plants and people relay on these plants for their healthcare system. Moreover, the presence of phytochemical in reported plants clearly shows that these plants have proactive and disease preventing properties. It can be postulated that diabetes can be treated from these antidiabetic plants.

REFERENCES

Ahmed MF, Rao AS, Ahemad SR and Ibrahim M (2012). Phytochemical studies and antioxidant activity of *Melia azedarach* Linn leaves by DPPH scavenging assay. *Int. J. App. Pharm*. **3**(1): 271-276.

Akhtar S, Nasir JA, Abbas T and Sarwar A (2019). Diabetes in Pakistan: A systematic review and meta-analysis. *Pak. J. Med. Sci*. **35**(4): 1173-1178.

American Diabetes Association. (2009). Diagnosis and classification of diabetes mellitus. *Diabetes Care*. **32**(Supp 1): 62-67.

Batool A, Miana GA, Muddassir M, Khan MA and Zafar S (2019). *In vitro* cytotoxic, antioxidant, antibacterial and antifungal activity of *Himalaiella heteromalla* indigenous to Pakistan. *Pak. J. Pharm. Sci*., **32**(6): 2771-2777.

Bhat S and Mulgund GS (2019). Ethnomedicinal plants used for the treatment of diabetes mellitus in SiddapurTaluk of Uttara Kannada district, Karnataka, India. *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci.,* **5**(2): 189-200.

Doss A (2009). Preliminary phytochemical screening of some Indian medicinal plants. *Anc. Sci. Life*, **29**(2): 12 -16.

Erasto P, Adebola PO, Grierson DS and Afolayan AJ (2005). An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. *Afr. J. Biotechnol*., **4**(12): 1458-1460.

Kasali FM, Kadima JN, Peter EL, Mtewa AG, Ajayi CO, Tusiimire J, Tolo CU, Ogwang PE, Weisheit A and Agaba AG (2021). Antidiabetic medicinal plants used in Democratic Republic of Congo: A critical review of ethnopharmacology and bioactivity data. *Front. Pharmacol*. **12**:757090.

Katiri A, Barkaoui M, Msanda F and Boubaker H (2017). Ethnobotanical survey of medicinal plants used for the treatment of diabetes in the Tizin' Test Region (Taroudant Province, Morocco). *J. Pharmacogn. Nat. Prod*., **3**: 130.

Khan A and Ahmed M (2019). Vegetation loss in Murree Forest, Pakistan. *FUUAST J. Biol.,* **9**(1): 51-58.

Khan KA, Ansari MJ, Al-Ghamdi A, Sharma D and Ali H (2014). Biodiversity and relative abundance of

different honeybee species (Hymenoptera: Apidae) in Murree-Punjab, Pakistan. *J. Entomol. Zool. Stud,* **2**: 324-327.

Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D and Asadi-Samani M (2016). The role of medicinal plants in the treatment of diabetes: A systematic review. *Electron. Physician*, **8**(1): 1832-1842.

Majeed Y, Shaukat MB, Abbasi KY and Ahmad MA (2021). Indigenous plants of Pakistan for the treatment of diabetes: A review. *Agrobiol. Records,* **4**: 44-63.

Mustafa KG, Ganaia BA, Akbar S, Dar MY, Tantry MA and Masood A (2011). The extracts of *Berberis lycium* and diabetes mellitus in alloxan monohydrate induced diabetic rats. *J. Pharm. Res*., **4**(8): 2570-2573.

Nasution BR, Aththorick TA and Rahayu S (2018). Medicinal plants used in the treatment of diabetes in karoethnic, North Sumatra, Indonesia. *Environ. Earth Sci*., **130**: 012038.

Njoku OV and Obi C (2009). Phytochemical constituents of some selected medicinal plants. *Afr. J. Pure Appl. Chem*., **3**(11): 228-233.

Omotayo FO and Borokini TI (2012). Comparative phytochemical and ethnomedicinal survey of selected medicinal plants in Nigeria. *Sci. Res. Essays*, **7**(9): 989-999.

Qureshi H, Asif S, Ahmed H, Al-Kahtani HA and Hayat K (2016). Chemical composition and medicinal significance of *Fagonia cretica*: A review. *Nat. Prod. Res*., **30**(6): 625-639.

Rizvi SL and Mishra N (2013) Traditional Indian medicines used for the management of diabetes mellitus. *J. Diabetes Res*. Article ID 712092, https://doi.org/10.1155/2013/712092.

Salehi B, Ata A, Kumar NVA, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, Ayatollahi SA, Fokou PVT, Kobarfard F, Zakaria ZA, Iriti M, Taheri Y, Martorell M, Sureda A, Setzer WN, Durazzo A, Lucarini M, Santini A, Capasso R, Ostrander EA, Atta -ur-Rahman, Choudhary MI, Cho WC and Sharifi-Rad J (2019). Antidiabetic potential of medicinal plants and their active components. *Biomolecules.,* **9**(10): 551.

Sofowora A (1984). African Medicinal Plants, University of Ife Press, IIeIfe, Nigeria. p.104.

Umair M, Altaf M and Abbasi AM (2017). An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab-Pakistan. *PLoS One.,* **12**(6): e0177912.

Vishwakarma KL and Dubey V (2011). Nutritional analysis of indigenous wild edible herbs used in Eastern Chattisgarh, India. *Emir. J. Food Agric*. **23**(6): 554e560.

Yaseen G, Ahmad M, Zafar M, Sultana S, Kayani S, Cetto AA and Shaheen S (2015). Traditional management of diabetes in Pakistan: Ethnobotanical investigation from Traditional Health Practitioners. *J. Ethnopharmacol*., **174**: 91-117.