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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1189-1197. **OPEN ACCESS**

Ethnobotanical, Phytochemical, Antioxidant Study of *Persicaria barbata* (L.) Hara (PBH) from District Bhimber (AJK), Pakistan

Mehwish Maqbool[1]*, Muhammad Ajaib[1], Muhammad Ishtiaq[1], Khizar Hayat Bhatti[2], Waheeda Mushtaq[1], Muhammad Waqas Mazhar[1], Tanveer Hussain[1] and Mubsher Mazhar[1]

[1]Department of Botany, Mirpur University of Science & Technology (MUST), Mirpur-10250 (AJK), **Pakistan**
[2]Department of Botany, University of Gujrat, Gujrat, **Pakistan**
[3]Department of Botany, University of Sargodha, Sargodha, **Pakistan**
[4]Department of Education, Directorate of Education, Govt. of Azad Jammu and Kashmir, **Pakistan**

***Correspondence:** mehwish.botany@must.edu.pk **Received 07-02-2021, Revised: 16-03-2021, Accepted: 29-03-2021 e-Published: 05-04-2021**

Plants are very important for existence of life on this planet because plants provide all fundamental necessities. Plants have pivotal role in the survival of man and all other organisms on this planet as plants give food, fodder, medicines, shelter and other variety of benefits to continue the ecosystem web-chain. The current research study was designed to document ethnobotanical, phytochemical and antioxidant potential of the medicinal plant "*Persicaria barbata* (L.) Hara" of family Polygonaceae. The ethnobotanical expedition confirmed that it is used in traditional ethnomedicines (TEMs). On basis of TEMs information, its phytochemical profiling was determined using maceration protocol in four solvents viz: petroleum ether (PE), chloroform (Chl), methanol (MeOH) and aqueous (Aq.) which confirmed presence of various chemicals like alkaloids, saponins, terpenoids, flavonoids and flavonons. The presence of different chemical constituents instigated to see its efficacy as herbal drug having significant antioxidant potential. The DPPH analysis proved that leaf extract in PE has highest scavenging effect of 92.50 and stem fraction showed 75.40. The leaf crude extract in PE showed highest total antioxidant activity (TAC) by phosphomolybdenum method with value of 2.55±0.20 mm while stem fraction depicted 2.45±0.20 mm in water. While total phenol content (TPC) was highest for leaf in PE with 2.56±0.6 mM while stem portion showed 1.90±2.0 mM value. All these findings confirm the presence of various phenolic compounds which act as antioxidants. Hence, the plant have great potential to be used as herbal medicine confirming authenticity of TEMs and it provides clues for further ethnopharmacological and pharmaceutical analysis for discovery of drugs to combat increasing trend of multidrug (MDR) resistance of many microbes.

Keywords: *Persicaria barbata*; Antioxidant activity; Ethnobotany; Total phenolic content; Bhimber; Phytochemical analysis; Total antioxidant content; Azad Jammu and Kashmir

INTRODUCTION

Since emergence of man on the planet, plants have been used as source of life sustenance because all life needs are generated directly or indirectly by plants. Plants provide us food, medicines, fodder, fuel, fibers, shelter and the most important "oxygen" for life activities. Plants are being playing significant role in the food chain and food web systems of ecosystems. Since the earliest recorded history, ancient civilizations have

been totally depended on forests and wild or natural life for food and other life necessities. It is very true that plants have always been playing a major and key role in providing food, fodder, shelter, clothes, fuel and other needs like medicines to cure life diseases (Bishop, et al. 1978; Mehwish et al. 2019a; Ishtiaq et al. 2020).

It is well documented and proved that plants have been used as easiest and cheapest mode of medicines to cure different infirmities which different societies of world are being facing (Sandberg et al. 2001; Ishtiaq et al. 2020). Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids in different ethnic cultures of world and particularly Pakistan's major (80%) population hitherto relies upon on herbal drugs due to strong belief of communities on their traditional cultural rituals and thought that plant-based drugs are cheap and without or less toxicity or side effects. Thus, in conjunction with cultural bonds world's different societies are being depending on use of traditional phytomedicines (TPMs) and it has attained a commanding role in health system all over the world. This is also confirmed and supplemented with theme that use of plants as TPMs not only for the treatment of diseases but also as potential nutritive material for maintaining good health and conditions as looking smart and young. It is declared that many countries of world, about two-third of the world's population still primarily depends on herbal medicines for primary health care and life boosting tonics. The reasons for this is because of their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects (Oladeji, 2016; Ishtiaq et al. 2020) Plants are used as a source of medicine due to presence of certain chemicals or active ingredients in their body which are known as secondary metabolites and also called as "mechanism of offense and defense". The variable qualitative and quantitative concentration of phytochemicals is keynote cause of their efficient mode of usefulness in the treatment of certain disorders or diseases of human and animal bodies (Ishtiaq et al. 2017; 2020).

Plants have been an incredible reservoir of remedy for multitudinous of times for coping different necessities of life for human being. It has been recognized by researchers as an ancient form of traditional ethnomedicines (TEMs) to cure various disease of the common population (Behera et al. 2018).The plant *Persicaria barbata* (L.) Hara (PBH) commonly known as "Jori buti or Bistakali" belongs to family Polygonaceae and it is prevalently occurring different areas of the study area (District Bhimber of AJK). Plants of the genus are also commonly known as knotweeds, or smartweeds. The plants of this species occur in wild habitat of small hilly and shady places. Many plants of Polygonaceae have potential source of herbal medicines and the plant under study is also used to cure different ailments by indigenous communities. In past it has been studied by many researchers. Family Polygonaceae contain 43 genera 1100 species (Lamb et al.2003).

In this research, main focus was to review ethnobotany of *Persicaria barbata*, do its phytochemical profile and determine its role as antioxidant agent or drug. The study area is one the Districts of Azad Jammu and Kashmir which is hub of plant diversity and many plant species are endemic to this region of AJK. Some sporadic studies have been on ethnobotanical studies of different areas of AJK and District Bhimber which is mere in form of checklist with some traditional ethnomedicinal recipes. The comprehensive study in biological order of ethnobotanical (E) information, phytochemistry (P) and antioxidant (A) analysis commonly known as EPA process is yet to be explored and this first attempt to conduct research in EPA order of single species confirming its efficacy as medicine using EPA model.The District Bhimber is the southern part of Azad Jammu and Kashmir and eastern side has Indian-administered Kashmir. The study area (District Bhimber) is located between latitude 32-48° to 33-34° and longitude 73-55° to 74-45°. It has an area of 1516 Km_2 with an altitude of 1118 ft from sea level (Ishtiaq et al. 2017; 2020). According to latest information, population of District Bhimber is 45000 while the annual growth rate is 2.6% (Adeel et al. 2011; Mehwish et al. 2019b; Ishtiaq et al. 2020). The purpose this research was (i) to document the ethnobotanical uses (ii) to explore phytochemical profile of stem and leaf parts using four solvents; (iii) to determine the antioxidant potential of leaf and stem extracts of the indigenous plant *Persicaria barbata* (L.) Hara (PBH) of District Bhimber (AJK) by using different approaches.

MATERIALS AND METHODS

Ethnobotanical study:

The ethnobotanical study was conducted using questionnaire methodology involving open-ended interview (OEI) and close-ended interview (CEI) protocols as per guidelines of Mehwish et al.

2019b and Ishtiaq et al. 2020 with some alterations as per requirement. In this study informant consensus factor (ICF) and fidelity level (FL) was calculated to confirm the authenticity of herbal therapeutics told be the local communities of the area. The FL and ICF were calculated using following equations:

Fidelity level (FL)

The fidelity level (FL) is the level of witnesses guaranteeing the utilization of certain plant for a similar object or purpose. The FL describes how commonly or certainly is being used for cure of a specific disease or infirmity in the stud area. The FL was determined by using follow equation as per protocol cited by Mehwish et al. (2019a) and Ishtiaq et al. (2020).

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

where, Np indicates the number of informants that claim "a use of plant species" used for "a particular purpose/ disease" and N is mentioned "the number of informants" that use the plant as a medicine to treat any given disease.

Informants consensus factor (ICF)

ICF identifies the agreement of the informants on the reported herbal medicines for the cure of group of ailments. It was calculated by the following procedure of Mehwish et al. (2019b) and Ishtiaq et al. (2020) in form of following equation:

$$ICF = n_{ur} - n_t / n_{ur} - 1$$

where n_{ur} is "the number of used citation" in each category and n_t denotes "number of specie used" to cure particular group of infirmities. This method is used for further examinations in drug investigation and other exploration ventures (Mehwish et al. 2019b).

Plant collection and Identification:

Plant samples were collected from different areas of District Bhimber using local guides and assistants. Plant samples were identified by taxonomist (Dr. Muhammad Ajaib, Associate Professor) of Department, assigned accession number (MUH-) and specimens were deposited to Herbarium of Department of Botany MUST, Mirpur Azad Jammu and Kashmir, Pakistan.

Sample Preparation for Extraction of Phytochemical

For phytochemical analysis, the identified plant samples were shadow dried and powdered. The leaf and stem parts were ground in powder form separately. For phytochemical analysis, four solvents viz: petroleum ether (PE), chloroform (Chl), methanol (MeOH) and water (aqueous) were used in order of polarity (Table 4) to extract maximum chemical constituents (Mehwish et al. 2016). For extraction maceration or dipping process was employed. The plant powder of weight 250 g was first soaked in 250 ml of PE in beakers as tightly sealed and dipped material was filtered by using Whatmann filter paper No. 42 after seven days at room temperature (RT). Then filtrate was concentrated by using rotary evaporator and residue/remnant was re-soaked in Chl. solvent, then in MeOH and Aq. solvents, subsequently repeating the same process as for first one (Mehwish et al. 2019b).

Phytochemical analysis

For phytochemical analysis, qualitative tests were used to conform the presence of different phytochemicals like saponins, flavonoids, terpenoids, alkaloids and flavonons etc. In extraction process, four solvents petroleum ether (PE), Chloroform (chl) ,Methanol (MEOH), water (aq) were used in order of polarity. Phytochemical profile of stem and leaf has been narrated following methods of Mehwish et al. 2017 and Ajaib et al. 2020).

Antioxidant Analysis (AOA)

For antioxidant analysis following methods was used: (i) 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, (ii) total antioxidant capacity (TAC) by use of Phosphomolybdenum method and (iii) total Phenolic Contents (TPC).

Procedure of DPPH Protocol

Preparation of stock solution (SS)

In the analysis, stock solutions were prepared by dissolving ca. 0.02g of solidified extract of the plant for each solvent was dissolved in 10mL of MeOH in cylinder and then its final volume was raised upto 20mL by adding MeOH (Maqbool et al.,2017; 2019).

Preparation of dilutions series (DS)

For preparation of dilutions solutions each of extract was prepared as described earlier and its serial dilutions were prepared *viz*: 125 µl, 250 µl, 500 µl, and 1000 µl volumes. The final volume of DPPH assay was made upto 500 µl and then its final reading was measured following protocol of Mehwish et al. (2017; 2019a).

Preparation of 0.1 mM DPPH solution

Amount ca. 0.04 g of DPPH was dissolved in 100 mL of MeOH in graduating cylinder. Its final volume was raised up to 1L by adding MeOH. For analysis, following dilution solutions *viz*: 60 µl, 125 µl, 250 µl and 500 µl were made as shown in Table 1. The solution of DPPH with 0.1 mM conc. was processed and kept at 4°C for future use. But it is important to state that fresh solution of DPPH gives better results as compared to stocked /stored solutions (Maqbool et al. 2017).

Table 1: Preparation of dilution serial solutions of DPPH assay for measuring antioxidant activity

Concentration Required µg/ml	Stock solution used (µl)	Methanol used (µl)	Final volume of dilution (µl)
1000	1000	0	1000
500	500	500	1000
250	250	750	1000
125	125	875	1000
50	50	950	1000

Total antioxidant activity analysis by Phosphomolybdenum complex procedure

Phosphomolybdenum (PPM) solution preparation

Total antioxidant activity (TOA) of each plant extract was calculated by using Phosphomolybdenum (PPM) complex protocol by admixing different conc. of Sodium phosphate (SP), Ammonium molybdate (AM) solutions and adding of Sulphuric acid (SA) as per protocol of Ajaib et al. (2016); and Maqbool et al. (2017) and described in Table 2.

Table 2: Phosphomolybdenum complex's composition and ratio of solution prepared for analysis

Chemicals Used	Conc. Used	Final amount used (500mL)
Sodium phosphate	28 mM	5.32g
Ammonium molybdate	4 Mm	2.47g
H_2SO_4	0.6 M	16.7 mL

For preparation of Phosphomolybdenum (PPM) Complex reagent, nearly 5.32g of Sodium Phosphate (SP) and 2.47 g of Ammonium Molybdate (AM) were weighed precisely and dissolved in 100 mL of double distilled (d.d.) water. Later on, ca. 16.7 mL of Sulphuric acid (H_2SO_4) was incorporated in the mixture by pippete mode. The total volume of this reagent was prepared equal to 500 mL by pouring more d.d. H_2O (Ajaib et al. 2016; Mehwish et al. 2019b).

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the samples of plant were measured by preparing the solutions of Folin-Ciocalteu (FC) Reagent (Sigma Co.) and Sodium Carbonate (SC) compounds and its stock solution was made according to the following recipe of the reagent as given in following Table 3.

Table 3: Composition of different solutions used for total phenolic content (TPC) assay

Chemicals Used	Conc. Used	Final Quantity used
Folin-Ciocalteu reagent	2 N	0.1 mml
Na_2CO_3	10 %	2.8 ml
Plant Extract	0.5 mg/ml	100 µg/ml

Table 4: Polarity indices of solvents used in maceration process of extraction

Petroleum ether (Lab Grade)	0.1
Chloroform (Lab Grade)	4.1
Methanol (Lab Grade)	5.1
Water (D. Distilled)	10.2

RESULTS AND DISCUSSION

As plants have been used in every sphere of life and particularly the plants are used as source of herbal therapeutics to cure different chronic and acute infirmities. Hence, due to this reason, a plan was devised to collect and determine the ethnobotanical, phytochemical and antioxidant potential of the plant *Persicaria barbata* which is very commonly occurring in wild areas of District Bhimber of AJK.

The ethnobotanical survey proved that the plant is used in cure of different diseases. It is also used in different ethnobotanical uses like fodder, hedging and thatching material for livestock huts and cottages in villages (Table 5).

In this study, medicinal potential of *Persicaria barbata* was explored by ethnomedicinal survey conducted in different areas of District Bhimber AJK (Table 5). The research was focused on ethnobotanical survey, phytochemical analysis and antioxidant activity of plant, to check the medicinal potential for future drug development. Before conducting antioxidant activity,

phytochemical analysis was performed by using qualitative method, four solvents: petroleum ether (PE), chloroform (Chl), methanol (MEOH) and water (Aq.) as shown in Table 4. Presences of different phytochemical indicated that plant have good medicinal potential and can be used to cure different ailments (Table 6).

In phytochemical analysis, saponins, flavonoids, terpenoids Tanins and cardiac-glycosides, flavonoids and flavonons were present. Terpenoids were absent while cardiacglycosides were least present in all solvents and found absent in aqueous solvent. Alkaloids were at higher concentration in all solvents. Phytochemical screening showed that plant is rich source of alkaloids and flavonoids and used as source of medicines in local area (Table 5) and these results are in congruent with previous work of (Morris, 2008; Ajaib et al. 2020).

The chemical profile of the plants predicted that plants do possess various secondary metabolites which vary in quantity and quality plant-wise or parts-wise and these explorations have been cited in the past works (Ndam et al. 2014; Ajaib et al. 2016; Mehwish et al. 2019b). The commonly found compounds were saponins, flavonoids, tanins, terpenoids, alkaloids and others as shown in tables, and same type of previous works support these outcomes (Doss et al. 2009; Chugh et al. 2012; Mehwish et al. 2019a). Terpenoids are active to treat or mitigate the diseases like allergy, diabetics, bacterial and viral infections (Wagner et al. 2003; Rabi and Bishayee, 2009; Chugh et al. 2012).

The flavonoids which are frequently used for cure of cancer, mutagenic disorders, oxidative stress by reactive oxygen species (ROS) and these findings are coincidence with previous works cited by different researchers (Ishtiaq et al., 2007; Dhale and Markandeya, 2011; Mehwish et al. 2020).

Determination of Antioxidant Activity (AOA)

Antioxidants are chemical substances that the inhibit oxidation process by preventing the formation of free radicals that cause damage to healthy cells, thus treating and managing chronic diseases such as cardiovascular diseases, diabetes, obesity, and some forms of cancers. Antioxidant activity of leaf and stem of *Persicaria barbata* was carried out by following protocol of Lee and Shibamoto (2001) with required changes In DPPH analysis, it was found that petroleum ether extract of stem showed highest scavenging effects, with percentage value was 75.40±0.5. While extract showed highest scavenging value of 92.50±0.5 in PE shown in Table 7. These findings proved that plant have good antioxidant potential to reduce the harmful impacts of ROS and chronic infirmities like cancer. These results showed that leaf fractions showed more antioxidant activity than stem. It is also said in other previous findings that leaf has more phytochemicals and is effectively used in preparation of TEMs (Ishtiaq et al. 2007; Ajaib et al. 2013b; Jaroennoni, et al. 2017; Mehwish et al. 2020).

Total Antioxidant Activity (TAA)

In another test, Total antioxidant activity, of leaf and stem was In another analysis, TAC was determined for PBH and it was seen that highest value of TAC was found for PE solvent /extract with 2.55±0.20 nm for stem and for leaf extract showed 2.45±0.20 nm for Aq. extract while standard depicted 3.65±0.25 as shown in (Table 8). These findings prove that the plants have good antioxidant potential and it can be used to reduce the injurious impact of ROS and other relevant diseases such cancer or tumors (Cadena-González et al. 2013; Umair et al. 2017; Mehwish et al. 2020).

Total Phenolic Contents (TPC)

To determine AOA, other parameter was designed in which, TPC was calculated for different plant parts extracted in various solvents. In calculation of total phenolic contents (TPC) of plant two parts was explored. Firstly in PE fraction TPC was found in stem 1.90±2.0 mM, while leaf PE fraction is 2.56±0.6 mM. The phenolic contents are important for use of different medicines being used for treatment of different diseases (Table 9). It was found that phenolic compounds' concentration is linked with antioxidant potential of the plant. These findings have been seen in literature survey where researchers have proved that with higher content of phenolic and flavonoid contents, antimicrobial and antioxidant potential of the plants increases (Anokwuru, et al. 2011; Azizzuddin et al. 2014; Barman et al. 2013; Jaroennoni et al. 2015; Maqbool, et al. 2017; 2020).

The plant is potential source of antioxidant, and used to cure different related diseases like cancer, oxidative stress. To explore the antioxidant potential of plant its two parts stem and leaf were tested for three methods *viz*: DPPH (1,1-diphenyl-2-picrylydrazyl) procedure, total antioxidant activity total phenolic content (Ajaib et al. 2012; 2013b; Sarker, et al. 2019).). It was

noted that leaf fractions of the plant showed better
SP value activity than stem fractions.

Table 5: Ethnobotanical and ethnomedicinal profile of Persicaria barbata (L.) Hara from Different villages of District Bhimber, Azad Jammu and Kashmir

S. No	Ethnobotanical Uses	Ethnomedicinal Uses
1	It is used as spice, food flavoring agent and garnish. It is used as fodder and fuel. It is used for thatching of huts and mud houses of livestock.	The leaf decoction is used for cure of bacterial infections. The root powder is used as antioxidant. The root decoction is used for cure of oestrogenicity and other fertility issues. The seed powder is used to cure colic pain, inflammations, urinary disorders fever, stomachache, ulcers, wounds, bacterial infections, flu, oestrogenicity, infertility and scabies.

Table 6: Physiochemical and qualitative analysis of phytoconstituents of leaf and stem'macerates of *Persicaria barbata* (L.) Hara from Different areas of District Bhimber of AJK

Name of constituents	Phytochemical tests used	Presence of different chemicals in diffident solvents			
		Petroleum ether (P.E.)	Chloroform (Chl)	Methanol (MeOH)	Aqueous (H_2O)
Saponins	Frothing test	+++	+	++	-
Flavonoids	$FeCl_3$ test	++	+++	+++	+
Terpenoids	Salkowski test	-	+	-	-
Cardiac glycosides	Keller-Killiani test	+	++	+++	-
Tannins	$FeCl_3$ test	+	++	+	-
Alkaloids	Mayer's test	+++	+	+++	++

Table 7: Percentage (%) DPPH free radical scavenging activity of stem and leaf of *Persicaria barbata* (L.) Hara from District Bhimber AJK (measured in triplicate (Mean ±SEM)

Plant Parts Used	Extracts' Used	Volume Used (µL)	%age of Scavenging Effect of DPPH
Stem	Petroleum ether	500	75.40±0.5
		250	64.50±1.5
		125	48.10±0.4
		50	45.30±0.2
	Chloroform	500	58.20±0.5
		250	53.50±0.1
		125	50.10±0.5
		50	48.20±0.3
	Methanol	500	77.50±1.0
		250	70.20±0.4
		125	62.40±0.2
		50	43.20±0.5
	Water	500	66.50±0.2
		250	60.10±1.0
		125	57.30±0.5
		50	44.05±0.2
Leaf	Petroleum ether	500	92.50±0.5
		250	87.40±0.3
		125	69.10±0.5
		60	56.05±0.7
	Chloroform	500	72.20±0.5
		250	65.05±0.1
		125	45.50±0.3
		60	58.20±0.8
	Methanol	500	60.40±0.9
		250	61.30±1.2
		125	49.50±0.5
		60	52.02±0.5
	Aqueous	500	79.05±1.2
		250	65.04±1.5
		125	58.15±0.5
		60	90.50±1.2

Table 8: Total antioxidant capacity (TAC) of leaf and stem extracts of *Persicaria barbata* (L.) Hara from District Bhimber AJK (measured in triplicate (Mean ±SEM)

Plant Parts Used	Solvent Used for Extraction	Absorbance at 695 nm (mM)
Stem	Petroleum Ether (P.E.)	2.55±0.20
	Chloroform (Chl)	0.90±0.25
	Methanol (MeOH)	1.90±0.10
	Aqueous (H_2O)	1.55±1.10
Leaves	Petroleum Ether (P.E.)	2.25±0.30
	Chloroform (Chl)	1.95 ±0.30
	Methanol (MeOH)	1.85±0.30
	Aqueous (H_2O)	2.45±0.20
Standard (BHT)	Standard	3.65±0.25

Table 9: Total phenolic content (TPC) of leaf and stem of *Persicaria barbata* L.) Hara from District Bhimber AJK (measured in triplicate (Mean ±SEM)

Plant Parts Used	Solvent Used	Absorbance at 725 nm (mM)
Stem	Petroleum Ether	1.90±2.0
	Chloroform	1.80±0.5
	Methanol	1.25±0.4
	Water	1.10±0.3
Leaves	Petroleum ether	2.56±0.6
	Chloroform	1.52±0.2
	Methanol	1.35±0.5
	Water	1.20±0.5

In DPPH analysis it was found that leaf fractions with highest value was 92.50±0.5 found for PE For 500ml and leaf fractions least value was found for MeOH fraction having 60.40±0.9 for 500 mL. Similarly stem extract showed highest value is 75.40±0.5 In PE for 500 ml and least value of stem was 43.20±0.5 in MEOH for 500 ml. (Table 6). These results showed that leaf fractions showed more antioxidant activity than stem. It is also said in other previous findings that leaf has more phytochemicals and is effectively used in preparation of TEMs (Ishtiaq et al. 2007; Ajaib et al. 2013b; Jaroennoni, et al. 2017; Mehwish et al. 2019b).

In order explore the antioxidant potential of the plant two other procedures viz: total antioxidant capacity (TAC) and total phenol content (TPC) were also used to supplement the DPPH protocol's findings. In TAC, it was found that PE extract depicted better value 2.55±0.20 nm for leaf than to 2.45±0.20 nm of stem, while standard used gave 3.65±0.25 (Table 7). TPC analysis showed that PE fraction produced 2.56±0.6 mM for leaf and 1.90±2.0 mM for stem part (Table 8). It was found that phenolic compounds concentration is linked with antioxidant potential of the plant. These findings have been seen in literature survey where researchers have proved that with higher content of phenolic and flavonoid contents, antimicrobial and antioxidant potential of the plants increases (Anokwuru, et al. 2011; Aziz-ud-din et al. 2014; Barman et al. 2013; Jaroennoni et al. 2015; Maqbool, et al. 2017; 2020).

CONCLUSION

Persicaria barbata L. (Hara) is most important medicinal plant. It is used to cure different ailment in folklore medicinal system. Presence of phytochemicals like alkaloids, flavonoids terpenoids and plant is used as medicinal purpose in different traditional systems of medicines.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Authors are highly obliged to the informants who shared their TEMs knowledge with gracious heart and authors thankful to the lab workers who assisted in these experimental trials. No funding source supported this research project.

AUTHOR CONTRIBUTIONS

All authors have contributed in the research and/or paper preparation. MM conducted field work; MA supervised the work, MI assisted in field and lab work, KHB assisted in phytochemical analysis, WM assisted in Antioxidant work, MWM assisted in EB data analysis, TH assisted in manuscript preparation and MM did proof reading of paper. All authors read and approved the final version and reviewed the manuscript. All authors read and approved the final version.

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