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Environmental Influence on Phytochemicals, Phenolic Compounds and Antioxidant Activity in Flower, Leaf and Root Extracts of *Fumaria indica* L.

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ABSTRACT

Background and Objective: The *Fumaria indica* of the family Fumariaceae had huge medicinal value in their parts and was used in folk medicine. The plant is used in fever, diarrhea, goiter, blood purifier, cooling agent, diabetes, and bladder disease infection. The objective of the study was to identify the phytochemical contents in various parts of *Fumaria indica* and to measure its ethnomedicinal value. **Materials and Methods:** The screening of phytochemicals, antioxidant activity, total phenolic compounds, proximate analysis, and ethnobotanical activities of *Fumaria indica* was conducted. The experimental materials were collected from four diverse locations in the Haripur District. Plant parts used in the studies were leaves, flowers, and roots. **Results:** High amounts of phytochemicals including Alkaloids, Saponins, and Tannins, were detected in all plant parts. Protein, phenols, diterpenes, and phytosterols were absent in flower samples. Khanpur area contained higher contents of phenolic compounds (280.4 mg GAE/g) and flavonoids (0.708 mg/100 g), while a higher quantity of crude fiber (16.1 mg) was recorded in leaves collected from Haripur City. Values regarding carotenoid content in leaves showed that the maximum value (1834 mg/100 g) was recorded in samples of the Srikot area. Leaf samples collected from Tarbela showed a maximum pH value (9.1). Superoxide dismutase (SOD) (6135 µg/g) was higher in roots, while Catalase (CAT) (5000 µg/g) was higher in leaves and Peroxidase (POX) (10638 µg/g) was higher in flower. **Conclusion:** The research was based on health-related elements investigation. The concentration of phytochemicals varies from place to place and in different parts of the plant body. The ethnobotanical study showed that the *Fumaria indica* are highly medicinal values and are widely used by the local community as a whole plant and in powder form for treatments of various diseases.

KEYWORDS

Haripur, antioxidant, phytochemical screening, phenolic compound

INTRODUCTION

Fumaria is a genus of the family Papaveraceae and consists of about 60 species. The variety of Fumaria species presents a high risk of puzzlement due to their analogous appearance. The classification of the *Fumaria* species related to this genus belongs to some definite morphological characters in which the

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absence or presence of the sepals, their length, shape of the fruit, length of the fruit pedicel, and length of the fruit pedicel bracteole[1]. Several species of the genus have been examined for the healing of hepatobiliary pathologies as traditional medicine. Different species of Fumaria have been used for antifungal, antibacterial, anti-inflammatory, and antimicrobial activities. These actions are particularly appropriate with protopine is mainly normally initiated along with isoquinoline alkaloids, moreover the isoquinoline alkaloids in their composition[2]. It is reported that Fumaria species have been important resource flavonoids and polyphenols, which indicated their antioxidant capability, diuretic, and antiprotozoal action[3].

Fumaria capreolata L., has been known for its essential bioactivities among the other species. The antioxidant activity of this genus is found due to the presence of important alkaloids Anticholinesterasic and analgesic activities are different biological activities that have been reported in this genus[4]. It has been reported that *Fumaria officinalis* has a large number of biological activities like antibacterial and antioxidant activities[5]. It contains various compounds like isoquinoline alkaloids and polyphenols etc. *Fumaria rostellata*, *Fumaria vaillantii*, and *Fumaria parviflora* are the antioxidant ones although many studies express the antidiarrheal, antispasmodic, bronchodilator behavior, diuretic and antiprotozoal activity[6].

Fumaria officinalis has the capability of antibacterial and antioxidant actions that have been proven in different research articles. isoquinoline alkaloids are mostly used for these activities, but there are also instigators that state the association of the polyphenols in some of these biological actions[7].

An indigenous plant in Western Romania, Fumaria jankae has a significant role in protecting against oxidative stress, which has been identified as one of the primary causes of many ailments in the modern world[1,2]. The current study provides scientific evidence that *Fumaria* species are a significant and beneficial source of polyphenols, which may contribute to the creation of natural diuretics and antioxidants. New antioxidant and diuretic compounds that may be useful in a range of oxidative stress-related diseases may be introduced into the food and pharmaceutical industries as a result of more research. Therefore, the current research is carried out to identify the phytochemical components in various parts of *Fumaria indica* and its total phenolic compounds used to determine the health-promoting elements in *F. indica* and also check its antioxidant activity.

MATERIALS AND METHODS

Study area: The study was conducted from 2022-23. District Haripur is situated in Hazara Division at an elevation of 610 m. It lies between 33°44' to 34°22' Latitude and 72°35' to 73°15' Longitude. The geographical significance of the district lies in the fact that its boundaries touch the Mardan, Abbottabad, Mansehra, Buner, Swabi, Attock, and Rawalpindi districts. The federal capital Islamabad is also adjacent to the district in the South. Geographically, Haripur District is divisible into four regions i.e., Maidan-e-Hazara, Tanaval Region, Khanpur Punjkahta and Chhachh (Maidan-e-Khari), which is mainly the study area, located in the West of the city. All are submerged under the reservoir of Tarbela Dam. The soil of the area is dissected loess plain or dissected piedmont plains in silty loam, and silty clay loam texture, respectively[8].

Sample collection: *Fumaria indica* samples were collected from four different locations which two were plane areas (Tarbela, City (Haripur) and two were hilly areas (Khanpur and Sirikot Hills) of District Haripur during spring 2023. After a complete survey of the selected locations, mature plants were selected and harvested. The harvested samples were collected and preserved in the lab for analysis. Dry plant samples were crushed into powder form. To make a solution mix 15 mL of distilled water in 1 g of the powder plant sample mix with a mechanical shaker and clean through filter paper. The clean and transparent solution was put in a separate bottle in the refrigerator for future use.

Screening of phytochemicals in herbal plants: The screening of phytochemicals was carried out by using analytical screening techniques. Prepare leaves, roots and flowers, extracts in methanol at a ratio of 1.12. A total of 6 g powdered sample was taken and soaked in 50 mL of overhead affirmed complex and to be set up for 5-6 days. The solution was regularly shaken 4-5 times a day, and purified through filter paper, termed Wattman's filter paper, and the filtrate of respectively trial was used remains of corresponding tests be carried out for the detection of alkaloids, saponins, tannins, flavonoids, proteins, diterpenes, phytosterols and detection of phenol.

Samples were liquefied in dilute hydrochloric acid, and the filtrate was treated with Wagner reagent (iodine in potassium iodide). The appearance of brown or radish precipitation indicated the presence of alkaloids[9]. Samples were diluted with 20 mL of distilled water and mixed in a cylinder for 16 min. The appearance of a 2 cm foam layer indicated the presence of saponins[10]. The plant extraction of 1% gelatin solution containing sodium chloride was dissolved. The appearance of white precipitate specifies the occurrence of tannins[11]. For flavonoid measurements, the solution was treated with a few drops of sodium hydroxide solution. The formations of yellow color, become colorless after the accumulation of dilute acid, specifying the occurrence of flavonoids. Extract was treated with some drops of concentered nitric acid. Yellowish colors appear which specifies the existence of proteins. The extract was treated with copper acetate solution and liquefied in water. A bright green color has appeared which showed the existence of diterpenes. Plant juice was accumulated with chloroform and purified then treated with some drops of concentrated sulphuric acid, and after some time formation of a golden color signifies the occurrence of triterpenes[12]. Plant juice was treated with 4-5 drops of ferric chloride solution. Appearances of bluish-black color specify the occurrence of phenols[13].

Quantitative analysis: Folin-Ciocalteu reagent was used for the detection of total phenolic contents. Formulate the solution of 100 mL in pure water mixed with Folin-Ciocalteu reagent. In every sample, FC-reagent (100 µL), was added and mixed thoroughly. The 700 mM Na_2CO_3 (800 µL) was added into each sample and incubated at room temperature for 2 hrs. Sample (100 µL) was calculated at 765 nm. The amount of TPC was calculated using a calibration curve for gallic acid. The results were expressed as gallic acid equivalent. Total antioxidant activity was assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radical. Reading was examined beside a blank at 517 nm using an ELISA reader (Biotech, USA). Through 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free radical inhibition in percent (%) was measured by following formula[14]:

$$I\,(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 10$$

Flavonoids were determined by the method described by Kim *et al*.[4]. Distilled water (4 mL) was added to 1 mL of plant extract. Then, 5% sodium nitrite solution was added, followed by 10% aluminum chloride solution. Test tubes were placed at a specific temperature for 5 min, and after that 2 mL of 1 M sodium hydroxide was added to the combination, and then the volume of the mixture was made up of 8 mL by the accumulation of distilled water. The mixture was thoroughly vortex and the absorbance of the pink color was observed at 510 nm. All the measurements were taken and the mean values were calculated. Total carotenoid contents were estimated according to the method of Lichtenthaler and Buschmann[15]. Plant extract (5 mL) was extracted with 2 mL of pure acetone and then the mixture was homogenized for 1 min and incubated at 4°C in darkness until the cap turned white. The homogenate was centrifuged at 16,000×g for 15 min and 200 µL of supernatant from each tube the absorbance was read at 470 nm in a micro-plate reader (Power Wave HT, Bio.Tec). The concentration of total carotenoids was calculated as follows[15]:

$$TC\ (\mu g/mL) = \frac{1000 \times A470}{214}$$

And expressed as mg/100 g fresh weight.

Sample preparation of enzymes: The plant extracts were prepared by taking 2 g of plant sample and then adding 2 mL of phosphate buffer by adjusting the (pH 7-7.8), after adjusting the pH centrifuge for 3 min. Superoxide dismutase enzyme was assayed spectrophotometrically. The incubation medium contained a final volume of 3.1 mL, 55 mM potassium phosphate buffer (pH 7.8), 44 µM methionine, 6.3 mM riboflavin, 83 µM NBT, and 22 µm potassium cyanide. The quantity of blend added toward this standard was reserved to make definite accurateness. At 25°C the tubes were kept in an aluminum foil, prepared with 16 W fluorescent lamps. Exposure to light for 15 min, the reduced NBT was observed spectrophotometrically at 600 nm. The value was measured as units/mg protein. Catalase activity was estimated by the technique of Kau *et al*.[16]. To start reaction 1 mL blend had 50 mM H_2O_2, 260 µL of enzyme extract, and potassium phosphate buffer (pH 7.0) were used. The reaction proceeded at 240 nm for 4 min and H_2O_2 utilization was measured using extinction coefficient, 39.4 mM/cm. Peroxidase activity was done by using the guaicol oxidation method. The mixture contains 12 mM potassium phosphate buffer (pH 7.0), 8 mM guaicol, and 200 µL enzyme extract. With the addition of 0.5 mL of 1% H_2O_2 reaction was started. Enhancement in absorbance was measured in 30 S at 430/470 nm. The unit of peroxidase enzyme (POX) activity was described with the change in absorbance per min and specific activity as enzyme units per mg soluble protein (extinction coefficient 6.39 mM/cm).

RESULTS

Screening of phytochemicals in *Fumaria indica*: The phytochemical test of the plant samples (leaves, root, and flower) of *Fumaria indica* was collected from 4 locations which two were hilly areas (Srikot and Khanpur) and two were plane areas (City and Tarbela) from District Haripur. Screening of phytochemicals i.e., Alkaloids, Tannins, Saponins, Flavonoids, Proteins, Diterpenes, Phytosterol, and Phenols were performed. Alkaloids and saponin were highly detected in three different localities while tannins were in two localities. Flavonoids were found in low quantities in all localities (Table 1).

Screening of phytochemicals in different parts of *Fumaria indica*: Alkaloid concentration in leaves, roots, and stem of 3 samples was high while moderate in one sample (Tarbela). Tannin concentration in all parts of the plant was high in two localities while moderate in Sirikot and City. Saponin concentration was high in the leaves of all samples except the root and flower of the Khanpur sample. The concentration of flavonoids was low in all parts of all samples. Protein was absent in all flower samples while in low quantity in leaves and roots. Diterpenes were found in high concentration in the root of the city sample while absent in all flower samples. Phenols were moderate in leaves of two samples and absent in flowers. Phytosterol was moderate in the leaves and roots of Sirikot and Khanpur while absent in all flower samples. Over leaves and root contains a different concentration of phytochemicals as compared to flowers (Table 1).

Total antioxidant activity: The maximum antioxidant content of the flower extract of *Fumaria indica* was recorded from Srikot (49.278%) followed by Haripur (49.892%). Among leaf samples, the maximum value was (18.402%) for Tarbela while the minimum value was (13.64%) recorded for the Khanpur sample. The highest value for root samples was (18.095 %) recorded for Khanpur while the minimum value was (10.568%) from Sirikot. Phytochemicals constituents from sites observed that from the hilly more phytochemicals are found in Khanpur compared to Sirikot on the other hand from plane areas more phytochemicals are found in Haripur City as compared to Tarbela in Fig. 1.

Total phenolic compounds (mg GAE/g): Data regarding the hilly areas showed that location Sirikot exhibited a high ratio of phenolic compounds (184.09 mg GAE/g) in flowers followed by roots (175.9 mg GAE/g). Values recorded for Khanpur demonstrated higher phenolic content (280.4 mg GAE/g) in flowers, followed by (169.5 mg GAE/g) leaf. Results generated for plane area Haripur city showed that maximum values for phenolic contents were found in flowers (255.5 mg GAE/g), followed by leaf (225 mg GAE/g). Tarbela contained higher phenolic content (265.9 mg GAE/g) in the flower (Fig. 2).

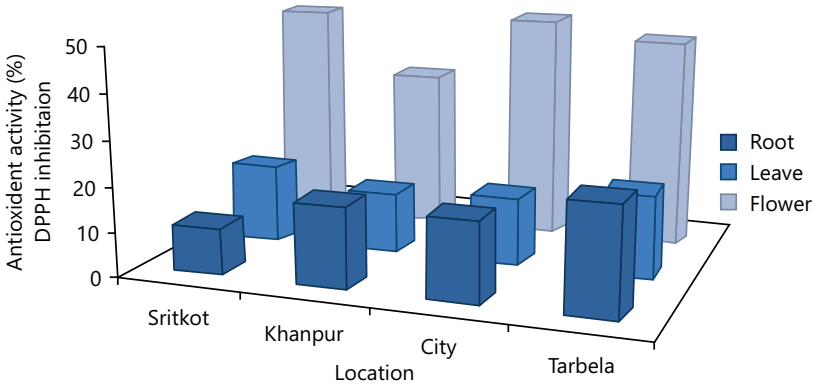

Fig. 1: Total antioxidant content of *Fumaria indica* at different locations

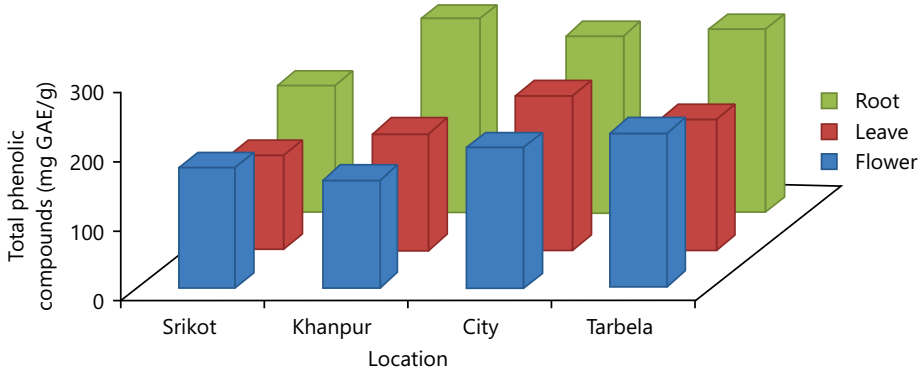

Fig. 2: Total phenolic compound of *Fumaria indica* at different locations

Table 1: Screening of phytochemicals compounds in various parts of *Fumaria indica* from different four sites

		Screening of phytochemical							
Sites	Parts	Alk	Tannins	Saponin	Flavonoid	Proteins	Diterpenes	Phenol	Phytosterol
Srikot	Leaves	+++	++	+++	+	+	++	+	++
	Root	+++	++	+++	+	+	+	+	++
	Flower	+++	++	+++	+	_	_	_	_
Khanpur	Leaves	+++	+++	+++	+	+	++	++	++
	Root	+++	+++	++	+	+	++	++	++
	Flower	+++	+++	++	+	_	_	_	_
City	Leaves	+++	++	+++	+	++	_	++	_
	Root	+++	++	+++	+	++	+++	_	+
	Flower	+++	++	+++	+	_	_	_	_
Tarbela	Leaves	++	+++	+++	+	_	_	+	_
	Root	++	+++	+++	+	+	++	_	++
	Flower	++	+++	+++	+	_	_	_	_

+++: Highly detected, ++: Moderate, +: Specifies existence and -: Specifies deficiency

Total flavonoid compounds (mg/100 g): The results indicated that there was a wide range of variation in the flavonoid compounds concentration of different plant samples which are collected from (Sirikot, Khanpur, Haripur City, and Tarbela). From Sirikot the flavonoid content was higher (0.682 mg/100 g) in the leaf extract as compared to the flower (0.481 mg/100 g). Flavonoids from Khanpur samples were higher (0.656 mg/100 g) in the leaf extract. From the plane area of Haripur City, the flavonoid content was higher (0.708 mg/100 g) in the leaf extract. Data for samples collected from Tarbela exhibited maximum flavonoid contents in (0.676 mg/100 g) leaf extract followed by flower extract (Fig. 3).

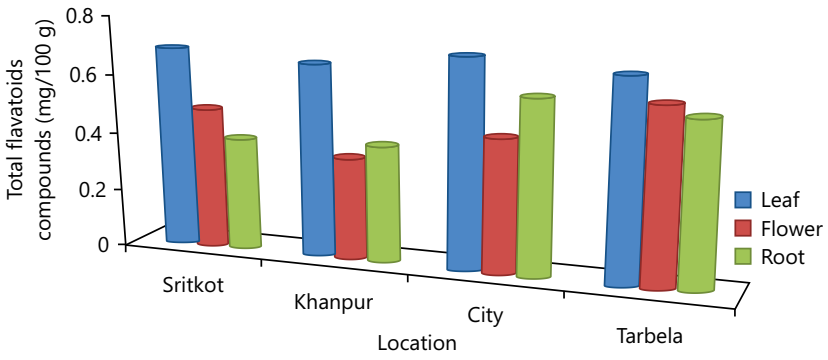

Fig. 3: Total flavonoids compound of *Fumaria indica* at different locations

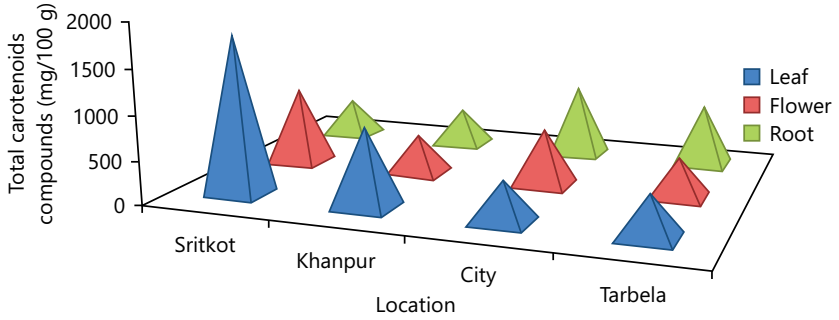

Fig. 4: Total carotenoid compounds of *Fumaria indica* at different locations

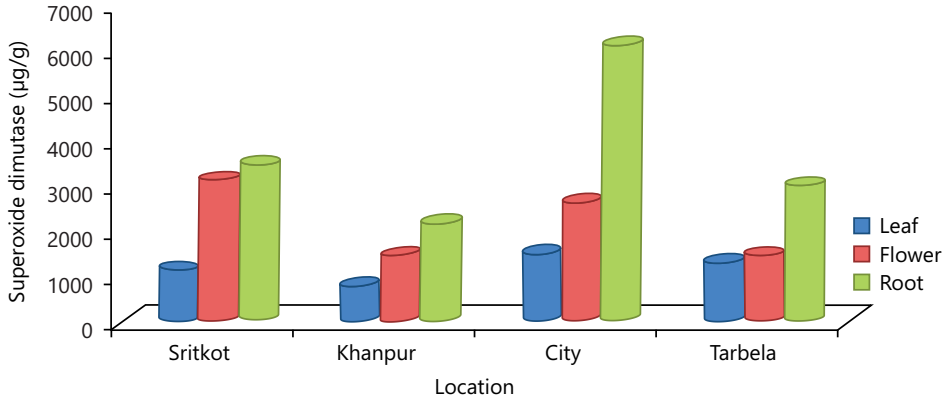

Fig. 5: Superoxide dismutase contents of *Fumaria indica* at different locations

Total carotenoids compounds (mg/100 g): The carotenoid compounds were maximum in leaf extract (1834 mg/100 g) followed by flower extract (919 mg/100 g) of Sirikot samples. The minimum carotenoid content (459 mg/100 g) was found in the flower extract (Fig. 4).

Enzymes studies (µg/g): These enzymes contained superoxide dismutase, catalase, and peroxidase. Higher content of superoxide dismutase was contributed by the roots sample of Haripur City (6135 µg/g), followed by root extract (3484 µg/g) of Sirikot while the minimum value was contributed by leaf extract (805 µg/g) of Khanpur sample (Fig. 5).

Hilly area Sirikot catalase contents were higher (5000 µg/g) in the leaf extract, moderate (4926 µg/g) in roots, and lower (4878 µg/g) in flowers. Khanpur demonstrated higher catalase contents (4831 µg/g) in leaf extract, plane area of Haripur City was higher (2639 µg/g) in root extract, while in Tarbela maximum

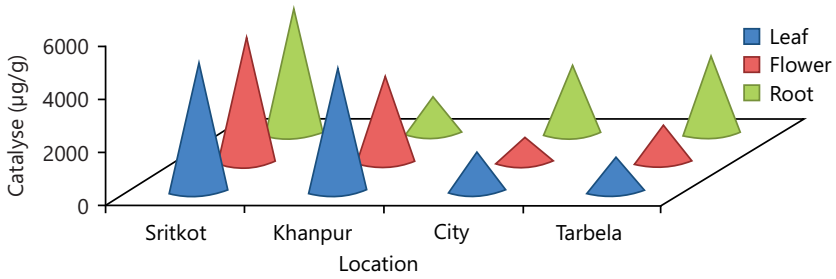

Fig. 6: Catalase content of *Fumaria indica* at different locations

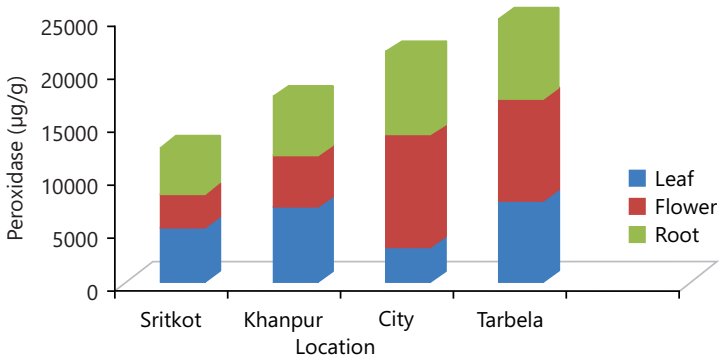

Fig. 7: Peroxidase contents of *Fumaria indica* at different locations

contents (3022 µg/g) were recorded in roots. The variation in the catalase enzyme of *Fumaria indica* might be due to the chemical changes in plant and plant parts (Fig. 6).

Results regarding plane areas show that the maximum value for peroxidase content in Haripur City was recorded in flowers (10638 µg/g) followed by Tarbela (9524 µg/g). The peroxidase contents were lower (3111 µg/g) in the flower extract of *Fumaria indica* collected from Sirikot (Fig. 7).

DISCUSSION

Plant samples were collected from four locations in Haripur (Srikot, Khanpur, City, and Tarbela). Data regarding hilly areas showed that the composition of phytochemical contents was high in Khanpur compared to Sirikot. Different types of phytochemicals were observed in different parts of *Fumaria Indica* including Alkaloids, Saponins, and Tannins. These phytochemicals were highly detected in all plant samples of different localities similarly flavonoids also showed positive results. These results were supported by the earlier studies of Humayun *et al.*[17] who reported reasonable phytochemicals in various plant parts of *Fumaria indica*. In the phytochemical screening test, they observed different health-promoting elements like carbohydrates, alkaloids, flavonoids, tannins, saponins, anthraquinones, and starch which are beneficial for human health and have the potential to cure different diseases. The TPC (280.4 mg GAE/g) was higher in flowers of Khanpur while lowest (138.6 mg GAE/g) in leaves samples of Sirikot. Data for hilly areas showed that location Khanpur contained higher phenolic compounds as compared to Sirikot, while from plane areas higher phenolic compounds were found in samples collected from Tarbela as compared to City (Haripur) area plant samples. The changes in TPC in these areas were due to different environmental conditions. This is in line with the results of Sharma *et al.*[3], who previously investigated the total phenolic contents and antioxidant activity of *Fumaria indica* in different plant extracts. The antioxidant content in urban flowers (49.892% DPPH inhibition) was higher. The higher antioxidant might be due to the existence of several constituents like phenolics, flavonoids, coumarin, quinones, etc., which is closest to Jaberian *et al.*[18] investigated that antioxidant activities of plant extracts were usually explained with the presence of phenolic acids and flavonoids in them found strong antioxidant activities of methanol and methanol-water extracts of *F. vaillantii*, determined by DPPH

method. Flavonoid compounds analysis results showed that higher flavonoid compounds (0.708 mg/100 g) were recorded in leaf samples collected from Haripur City and the lowest flavonoid content (0.344 mg/100 g) in flower collections of Khanpur. The current results is closest to Orhan *et al.*[19] who indicated that the total phenol flavonoid content in the ethanol extract *Fumaria* species was high in the leaf. Carotenoid compounds were higher (1834 mg/100 g) in Srikot leaves and lower value (440 mg/100 g) in the root of Khanpur. This observation is related to Pal and Habibur Rahaman[20] who observed that different plant species have variations in the carotenoid content due to the genetic potential and spatial differences. Enzyme includes superoxide dismutase, catalase, and peroxide. It was shown that the SOD was the highest content (6135 µg/g) in City root and lower content in the leaf sample (805 µg/g) of Khanpur and locations wise results showed that from hilly areas higher SOD contents were found in samples collected from Srikot as compare to Khanpur area plant samples on the other hand from plane areas higher SOD contents were found in samples collected from City as compare to Tarbela area plant samples. The differences in the superoxide dismutase content might be due to the differences in the genetic makeup of the plants. This observance is similar to Racchi[7] who reported biochemical and molecular features of the antioxidant enzymes superoxide dismutase (SOD) is an enzyme that helps break down potentially harmful oxygen molecules in cells which might prevent damage to the tissues of plants. The catalase was also analyzed and found higher (4926 µg/g) in the root of Srikot and lower in the flower (1000 µg/g) of the city plant and locations results showed that from hilly areas higher CAT contents were found in samples collected from Srikot as compare to Khanpur area plant samples on the other hand from plane areas higher CAT contents were found in samples collected from Tarbela as compare to city area plant. The presence of catalase enzyme indicated the ability of plants to be beneficial for medicinal purposes[21]. The variation in the catalase enzyme of *Fumaria indica* might be due to the chemical changes in the plant and plant part shown in Fig. 2. Catalase activity is the decisive factor in protecting photosynthetic cells from oxidative stress induced by abiotic stress conditions (such as cold, drought, salt, and ozone). This finding is similar to Racchi[7] investigated that the fumaria species have the important enzyme catalase (CAT) plays a role in protecting the cells from oxidative damage by reactive oxygen species. Similarly, the content of peroxidase was also analyzed and found to be higher in urban flowers (10638 µg/g) and lower in Sirikot flowers (3111 µg/g). The results of location analysis showed that in hilly areas, collected from Hampshire the samples have higher POX content. On the other hand, comparing the plant samples from the planar area with the Sirikot area, it was found that the POX content in the samples collected from the city was higher than the plant samples in the Tabera area (Fig. 1), This is line in similar to the observed that enzyme peroxidase (POX) plays a vital role in the removal of ROS in different cell compartments, and under stress conditions, it has the function of decomposing hydrogen peroxide, which uses oxygen one of the byproducts of breathing[7].

CONCLUSION

The research was conducted on phytochemical screening, phenolic compounds, and antioxidant activity of *Fumaria indica* from District Haripur. Alkaloids, tannins, saponins, proteins, and flavonoids were mostly present in all selected plant parts of Khanpur area. Higher contents of phenolic compound in flower, carotenoids in leaf samples, superoxide dismutase, and catalase in root samples while Peroxidase (POX) was higher in flower samples. The ethnobotanical study showed that *Fumaria indica* are highly medicinal values and are widely used by the local community as a whole plant and in powder form for treatments of various diseases.

SIGNIFICANCE STATEMENT

The studies showed that *Fumaria indica* were highly medicinal values these medical values were possessed in all parts (leaves, flowers, and roots). The plant contained the active compounds, as plant possesses enzymes and antioxidant activities in their parts. It was recommended that the parts were further standardized process and future uses as a drug discovery in the pharmaceuticals and pharmacy industry as a source of medicine.

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