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# Phytochemical Evaluation and Antioxidant Activity of Different Samples of Pteris Vittata in Doon Valley, Uttarakhand Region

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## ABSTRACT

*Pteridophytes including ferns are vascular cryptogams,show various economic values towards food and fodder indicators, biofertilizers, insect repellents, medicine and folk medicines. Various pteridophytes have antimicrobial and antioxidant activity, to test the phytochemical and antioxidant activity of these economically plants, the present study was conducted with one of the pteridophyte named as Pteris vittata commonly known as Chinese brake in Doon valley.The yield of various crude extract of different samples ranged from 0.48-0.96%. Phytochemical studies showed that protein, alkaloids, amino acid, carbohydrates, flavonoids, tannins, phenolics, and triterpenoids were present whereas glycosides were absent in all samples.The total phenolic content of six samples of Pteris vittata showed large variation ranges from 0.21875±0.467 mg GAE/g to 1.687±1.299.The content of flavonoid varied from 0.811±0.900 to 0.21865±.467mg QE/g.The study showed that all the samples of Pteris vittata have as compare to the standard ascorbic acid having $IC_{50}$ value 300 µg/ml. The $IC_{50}$µg/ml ranged from 260-395 µg/ml in the samples.Thus, these findings suggest that Pteris vittata cultivation should be promoted as a medicinal plant for the development of health care products for aging and chronic disease as they are rich in phenolic and flavonoid which are good source of antioxidants. Thus, these findings suggest that Pteris vittata cultivation should be promoted as a medicinal plant for the development of health care products for aging and chronic disease as they are rich in phenolic and flavonoid which are good source of antioxidants.*

***Key words:*** *Pteris vittata, pteridophyte, Antioxidants, Free radicals.*

## INTRODUCTION

Pteridophytes are vascular cryptogams, important for phylogenetic and evolutionary point of view, and form an untouched group of plants in biodiversity as far as their economic value is concerned[1]. Pteridophytes including ferns show various economic values towards food and fodder indicators, biofertilizers, insect repellents, medicine and folk medicines[2,3,4]. Later on modern biological and pharmaceutical studies were carried out on pteridophytes by different workers[3]. Various pteridophytes have antimicrobial and antioxidant activity, to test the phytochemical and antioxidant activity of these economically plants, the present study was conducted with one of the pteridophyte named as *Pteris vittata* commonly known as Chinese brake.

*Pteris vittata* isnative and widespread, found from the east to the south tropicalandSouthern Africa. *Pteris vittata* grows readily in the wild, is sometimes cultivated, it is grown in gardens for its attractive appearance, or used in pollution control. *Pteris vittata* has unique characteristics of heavy metal accumulation such as arsenic[5]. The aim of the present study are an endeavor to find the phytochemical

constitution and antioxidant activity of different extracts of organic solvents of *Pteris vittata* in Doon valley, Uttarakhand region.

## MATERIAL AND METHOD

**Sample collection and processing:**

The present investigation was carried out during January-June 2014 at Biotechnology laboratory in Department of Life Sciences, Sri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun, Uttarakhand. The fresh whole plant was collected from different places of Doon valley. The plant sample was dried in shade at $25^0$C to $35^0$C for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant material was stored in paper bags.50gm of the dried plant material powder was successively extracted with 250mL of solvents i.e. petroleum ether, benzene, chloroform, ethyl acetate, acetone, and ethanol using Soxhlet apparatus for 24 hours. Finally, the residue left was extracted with distilled water by infusion method i.e. by soaking the powdered plant extract in 250ml of distilled water for 24 hours. All the extracts were filtered and evaporated on the water bath till they were finally reduced to dryness to get dry extracts. The extracts were then transferred to previously weigh airtight containers and stored in the refrigerator until they were screened for their phytoconstituents and antibacterial activity.

**Phytochemical Analysis:**

The various solvent extracts of *Pteris vittata* were subjected to preliminary qualitative phytochemical investigation including test for alkaloids, proteins, carbohydrates, flavonoids, cardiac glycosides, saponins, steroids and triterpenoids, tannins and phenols and oils.

**Determination of total phenolic content:**

The total phenolic content was determined using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi (1965) with some modifications[6]. 0.1 ml of sample and 50µl of 2N Folin-Ciocalteu reagents was added to a 5 ml volumetric flask. The solution was mixed and allowed to stand for 3-5 minutes at room temperature. Next, 0.3ml of 20% sodium carbonate solution (w/v) was added, and the solution was mixed and kept aside for 15 minutes. Finally, 5ml of distilled water was added. The blue colour was measured against reagent blank at 725nm using a UV-spectrophotometer. The total phenolic content of the samples were determined by comparison with the optical density values of different concentrations of the standard phenolic compound gallic acid. Each sample was analysed in triplicate, and a calibration curve of gallic acid was constructed by plotting absorbance versus concentration. The total phenolic content was expressed as gram of gallic acid equivalents (GAE) per 20 gm extract.

**Determination of total flavonoid content:**

The total flavonoid content was determined with aluminum chloride ($AlCl_3$) method using quercetin as a standard. The extract (0.25 ml each) was mixed with 1.25 ml double distilled water which was followed by addition of 75µl of 5% $NaNO_2$. This mixture was incubated for 5 minutes at room temperature and then 0.15ml of 10% $AlCl_3$ was added. The reaction mixture was treated with 0.5ml of 1mM NaOH after incubation of 6 minutes at room temperature. Finally, the reaction mixture was diluted 5ml of double distilled water followed by an incubation of 20 minutes at room temperature. The absorbance was measured at 510nm. The flavonoid content was calculated from a quercetin standard curve. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of samples.

**Determination of reducing power activity:**

The reducing power of the samples were determined by the Oyaizu (1986) method with some modifications[7]. Reducing power activity is based on ferric cyanide ($Fe^{3+}$) in stiochiometric excess relative to the amount of antioxidants. Samples (50µl) with different concentration were mixed with 0.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferric cyanide (w/v)and incubated at 50°C for 20 minutes. After incubation, 2ml of 10% trichloroacetic acid (w/v) were added to the mixture, followed by the centrifuge at 3000 rpm for 10 minutes. The upper layer (2.5ml) was mixed with 2 ml of deionized water and 0.5 ml of 0.1% ferric cyanide (w/v), and the absorbance of the resultant solution was measured at 700nm. Ascorbic acid was used as reference.

**DPPH radical scavenging assay:**

The scavenging activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The plant extract was redissolved in 70% ethanol. The 5ml assay mixture contained 3.98 ml methanol, 20µl extract (50µl, 100µl, 150µl, 200µl)and 1ml DPPH (0.15mM in methand). After incubation at room temperature for 30 minutes, the decrease in absorbance was measured at 517nm using a spectrophotometer. Ascorbic acid was used as reference. The $IC_{50}$ value indicates the concentration of tested sample required to free radical concentration by 50%. The experiment was performed in triplicate.

$$\text{DPPH scavenging activity}(\%) = A_0\text{-}A_1 / A_0 \times 100$$

## RESULT AND DISCUSSION

Free radicals, generally activated oxygen species contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS[8,9]. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system, change in gene expression and induce abnormal proteins. Antioxidant agents that prevented the consumption of molecular oxygen in biological systems have multiple purposes, including defending against oxidative damage and participating in the major signaling pathways of the cells. Currently, the natural antioxidant α-tocopherol and some synthetic antioxidants such as butylated hydroxyl toluene, butylated hydroxyl anisole and propyl gallate are commonly used to act against free radicals in food and biological systems. However, the use of synthetic antioxidants in food products is under strict regulation owing to their potential health hazards[10]. These synthetic antioxidants also show low solubility and moderate antioxidant activity[11].

Plants contain numerous primary and secondary metabolites. Several secondary metabolites such as polyphenols and flavonoids appreciated as chemo preventive agents against oxidative damage and participating in the major signaling pathways of the cells. Polyphenols are natural occurring substances and the most abundant antioxidants found in both higher and lower plants[12,13,14,15]. The antioxidant activity of polyphenols is considered to be due to their properties as free radical terminators. This activity depends mainly on different structural features such as O-H bound dissociation energy, resonance delocalization of the phenol radical and steric hindrance derived from hydrogen substitution in the aromatic ring[16]. The effect of polyphenols antioxidants on DPPH free radical scavenging is thought to be primary due to their hydrogen donating ability owing to the presence of hydroxyl groups[17]. The non-phenolic part of the molecule appears also to influence the activity of the conjugates with thiols have been showed to exhibit a higher antiradical capacity than their counterparts in the DPPH suggesting the favorable role played by the non-phenolic part of the molecules in the antioxidant activity[18,19,20].The present study focuses on the estimation of total phenolic and flavonoid content with the emphasis of determination of antioxidant activities of the samples of *Pteris vittata*. The findings of the present study are discussed under the following headings:

**Yield and phytochemical estimation of crude extracts:**

The appearance of the ethanol extracts of six different *Pteris vittata* was varied from green to dark green. The yield of various crude extract of different samples ranged from 0.48-0.96% (Table-1).

Phytochemical are the chemical which are derived from the plant sources, generally affect health, but are not yet established nutrients. Alkaloids, terpenoids, coumarins, tannins, quinines, flavonoids, glycosides, steroids, saponins etc. are some classes of phytochemicals. They play role in plants but they are biologically active as well as used to cure various ailments. These compounds show anticancer, antioxidants and anti-inflammatory activities. The plant contains biological active compounds including tannins, phenols, anthocyanin, flavonoids which has been linked with potential antioxidant activities (Table-2).

**Total Phenolic content**

Phenolics are one major class of phytonutrients that have been widely studied, thus they are well known antioxidants compounds that work in multiple ways to prevent disease[13].Phenolics are well established to

show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin-Ciocalteu method, with the gallic acid as a standard. The content of phenolics was evaluated from the regression equation of the calibration curve ($y = 0.62x + 0.78$, $R^2 = 0.884$), expressed in GAE as milligram per gram of extract (mg GAE/g extract) (Table-3) (Figure-1). The total phenolic content of six samples of *Pteris vittata* showed large variation ranges from 0.21875±0.467 mg GAE/g to 1.687±1.299 (Figure-2). The highest amount of phenol was found in Mussorie road sample of *Pteris vittata* and the lowest phenol was found in the Tapkeshwar sample of *Pteris vittata.*

**Total flavonoid content**

Flavonoids are well known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties. Therefore, the total flavonoid content was evaluated from the calibration curve ($y = 0.072x-0.023$, $R^2 = 0.845$) expressed in QE in milligrams per gram of extracts (mg QE/g extract) (Table-3) (Figure-3). The content of flavonoid varied from 0.811±0.900 to 0.21865±.467mg QE/g(Figure-4). The highest amount of flavonoid was found in Dharampur sample of *Pteris vittata* (0.21865±0.46mg QE/g) and the lowest flavonoid content was found in Miyawala (0.811±0.900 mg QE/g).

**Reducing power activity of different samples of *Pteris vittata:***

All the extract of *Pteris vittata* was determined from distinct color changes (yellow, green and blue) at 3000 rpm, depending on the reducing power of the sample concentration (Table-4) (Figure5). The high absorbance of the reaction mixture indicates high reducing power. Total flavonoid concentration of different sample of *Pteris vittata* were expressed as regression equation of the calibration curve.

**DPPH radical scavenging assay:**

DPPH scavenging activity of all the samples of *Pteris vittata* was compared with standard (ascorbic acid) by evaluating antioxidant efficiencies, known as $IC_{50}$ (Table-5) (Figure-6). $IC_{50}$ is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed. The lower the $IC_{50}$ number,the greater the overall effectiveness of the antioxidant in the samples of *Pteris vittata*.The study showed that all the samples of *Pteris vittata* have as compare to the standard ascorbic acid having $IC_{50}$ value 300 µg/ml. The $IC_{50}$ µg/ml ranged from 260-395 µg/ml. The highest value of Mussorie Road sample of *Pteris vittata* and lowest value of Tapkeshwar sample of *Pteris vittata.* The highest $IC_{50}$ was obtained in Mussorie Road sample of *Pteris vittata.* Similar types of study were conducted by different workers on various fern species which showed vigorous antioxidant activities more than vitamin C or BHT (synthetic antioxidant)[21,22,23].

**Table 1: Appearance and Yield of crude extract from ethanol solvent of different samples**

| S.No. | Samples from different places | Appearance of the extract | Quantity of plant material (gm) | Weight of extract (gm) | Percentage yield |
|---|---|---|---|---|---|
| 1 | Mussorie | Green | 25 | 0.12 | 0.48 |
| 2 | Rajpur Road | Dark Green | 25 | 0.18 | 0.72 |
| 3 | Dharampur | Medium Green | 25 | 0.24 | 0.96 |
| 4 | Tapkeshwar | Medium Green | 25 | 0.19 | 0.76 |
| 5 | Miyawala | Dark Green | 25 | 0.21 | 0.84 |
| 6 | Gullarghati | Dark Green | 25 | 0.24 | 0.96 |

**Table 2. Phytochemical analysis of different samples of *Pteris vittata***

| S.No. | Constituents | Mussorie sample | Rajpur Road sample | Dharampur sample | Tapkeshwar sample | Miyawala sample | Gullarghati sample |
|---|---|---|---|---|---|---|---|
| 1 | Alkaloids | + | + | + | + | + | + |
| 2 | Proteins | + | + | + | + | + | + |
| 3 | Amino acid | + | + | + | + | + | + |
| 4 | Carbohydrates | + | + | + | + | + | + |
| 5 | Flavonoids | + | + | + | + | + | + |
| 6 | Glycosides | - | - | - | - | - | - |
| 7 | Tannins&phenolics | + | + | + | + | + | + |
| 8 | Titerpenoids | + | + | + | + | + | + |

*(+) and (-) signs indicate presence and absence of the compound, respectively.

**Table 3. Total phenol and flavonoids concentration of different samples of *Pteris vittata***

| S.No. | Plant samples | Total phenolics (mg GAE/g) | Total flavonoids (mgQE/g) |
|---|---|---|---|
| 1 | Mussorie road sample | 1.6875±1.299 | 0.0765±0.276 |
| 2 | Rajpur road sample | 0.5912±0.768 | 0.0673±0.259 |
| 3 | Dharampur sample | 0.96585±0.982 | 0.21865±0.467 |
| 4 | Tapkeshwar sample | 0.21875±0.467 | 0.6839±0.826 |
| 5 | Gularghati sample | 0.3766±0.613 | 0.873±0.934 |
| 6 | Miya wala sample | 0.50025±0.707 | 0.811±0.900 |

**Table 4. Reducing power activity analysis of different samples of *Pteris vittata***

| S.N. | Samples | Concentration (µg/ml) | Absorbance (700nm) |
|---|---|---|---|
| 1. | Mussorie road sample | 250 | 0.0232 |
| | | 500 | 0.0523 |
| | | 750 | 0.0763 |
| | | 1000 | 0.2654 |
| 2. | Rajpur road sample | 250 | 0.3157 |
| | | 500 | 0.3398 |
| | | 750 | 0.4321 |
| | | 1000 | 0.4211 |
| 3. | Dharampur sample | 250 | 0.0174 |
| | | 500 | 0.0259 |
| | | 750 | 0.0299 |
| | | 1000 | 0.1458 |
| 4. | Tapkeshwar sample | 250 | 0.1270 |
| | | 500 | 0.1511 |
| | | 750 | 0.2059 |
| | | 1000 | 0.2543 |
| 5. | Gullarghati sample | 250 | 0.2567 |
| | | 500 | 0.3896 |
| | | 750 | 0.4329 |
| | | 1000 | 0.1247 |
| 6. | Miyawalla sample | 250 | 0.0210 |
| | | 500 | 0.0432 |
| | | 750 | 0.1294 |
| | | 1000 | 0.1582 |

**Table 5: DPPH Assay $IC_{50}$ values of different *Pteris* samples**

| S.No. | Plant samples | DPPH Assay $IC_{50}$(µg/ml) |
|---|---|---|
| 1. | Musorie Road sample | 390 |
| 2. | Rajpur Road sample | 320 |
| 3. | Dharampur sample | 365 |
| 4. | Tapkeshwar sample | 265 |
| 5. | Gullarghati sample | 280 |
| 6. | Miyawala sample | 278 |
| | Standard (Ascorbic acid) | 312 |

**Fig.1: Standard curve represent concentration of gallic acid (µg/ml) against absorbance**

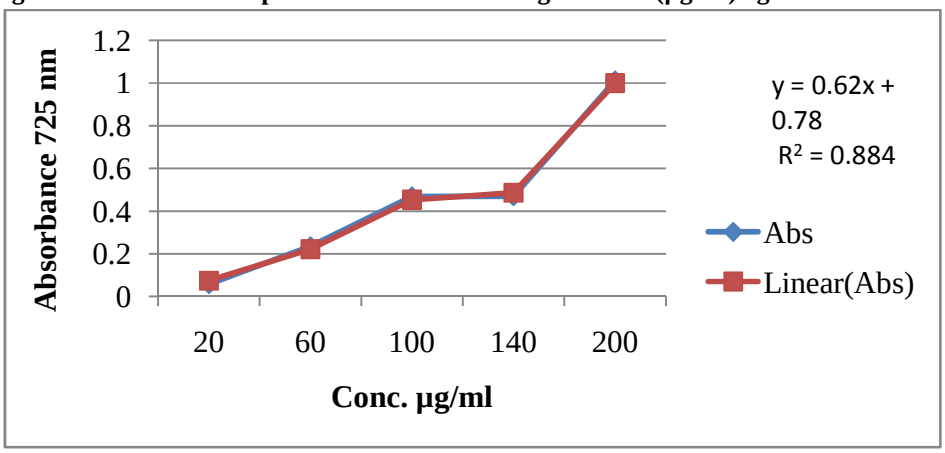



**Fig.2: Total phenol concentration mean of different samples of *Pteris vittata***

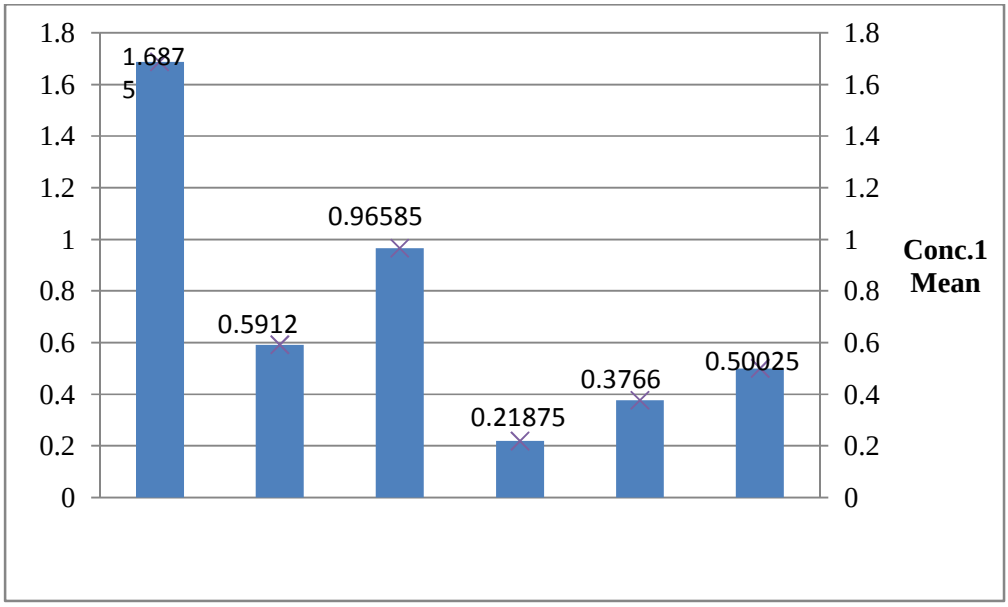



**Fig.3: Standard curve represent concentration of quercetin (µg/ml) against absorbance**

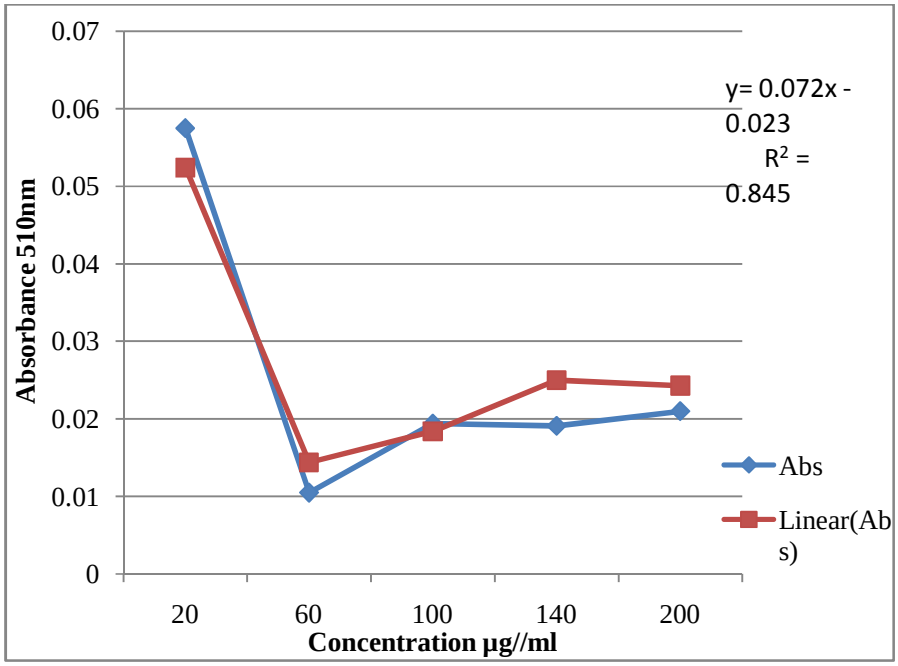


**Fig.4: Total flavonoid concentration mean of different samples of *Pteris vittata***

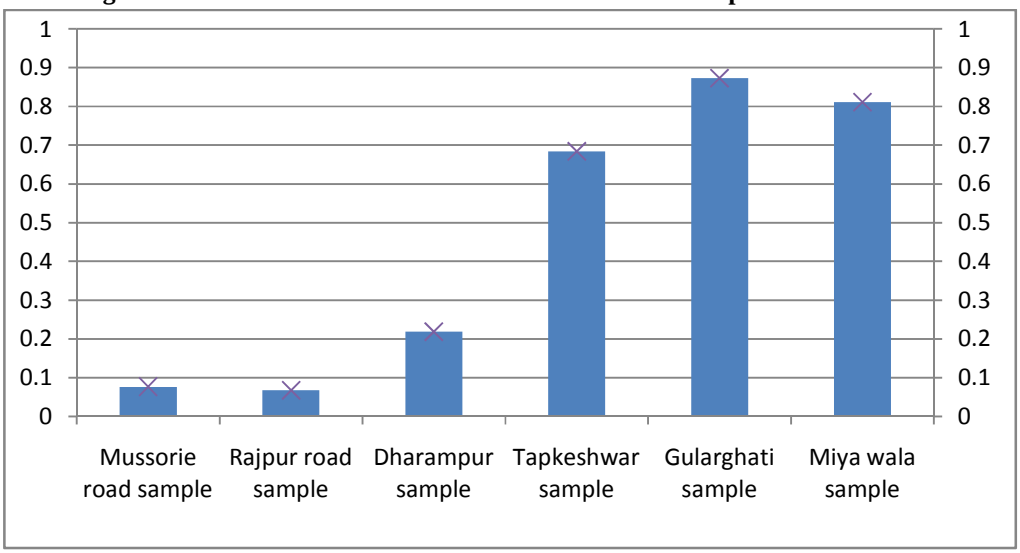



**Fig. 5: Reducing Power Compare of Different *Pteris vittata* Samples**

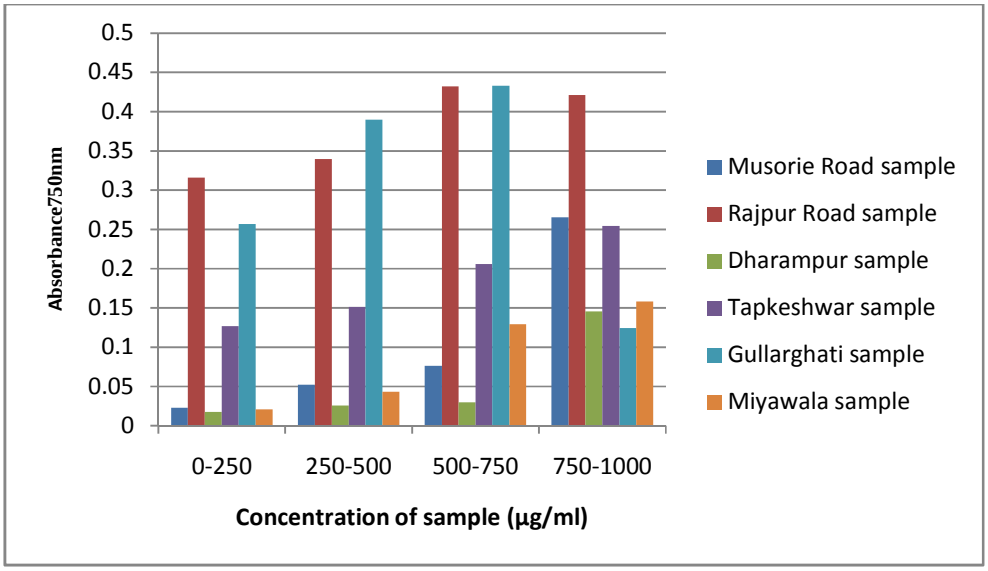
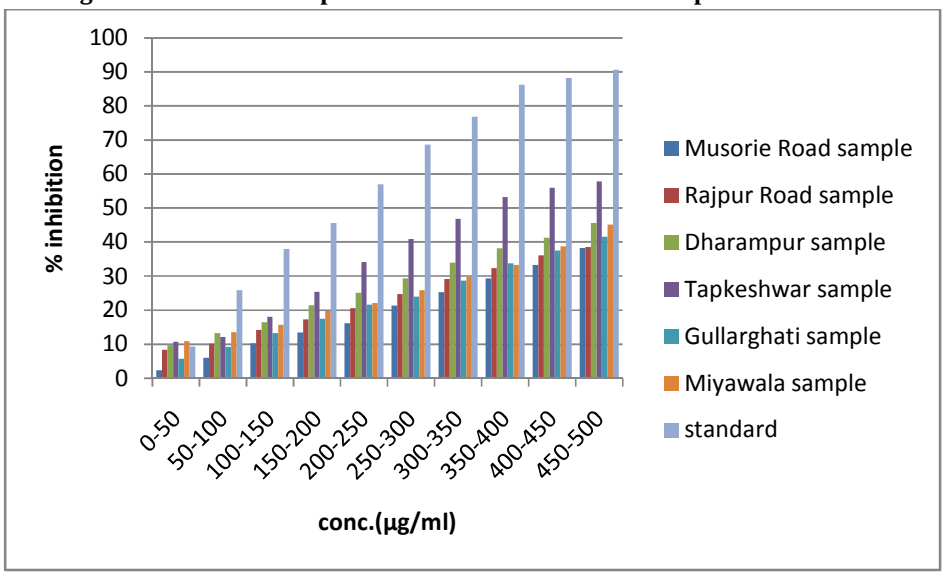



**Fig.6: % inhibition compare of different *Pteris vittata* samples and standard**



## CONCLUSION

The present study comprises of six samples of *Pteris vittata* collected from different places of Dehradun revealed that the percentage yield of crude extract of *Pteris vittata* ranges from 20-50 %. Phytochemical studies showed that protein, alkaloids, amino acid, carbohydrates, flavonoids, tannins, phenolics, and triterpenoids were present whereas glycosides were absent in all samples. The study was also conducted with the reference of reducing power activity and DPPH scavenging activity.Thus, these findings suggest that *Pteris vittata* cultivation should be promoted as a medicinal plant for the development of health care products for aging and chronic disease as they are rich in phenolic and flavonoid which are good source of antioxidants.

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