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Effects on Plant Metabolites of *Bauhinia Purpurea Linn*

Ashish Sarkar*[1], LP Singh[1], VD Tripathi[2] and Garima Mishra[3]

1Institute of Pharmacy, HCPG College, India

2Sagar Institute of Technology, India

3Department of Pharmaceutical science, Shri Guru Ram Rai University, India

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***Corresponding author:** Ashish Sarkar, Institute of Pharmacy, HCPG College, Varanasi, Uttar Pradesh, India, Tel: 7525885588; Email: sarkar969696@gmail.com

Abstract

Bauhinia purpurea Linn is an ornamental plant found throughout the globe known as khairwal in Hindi having high therapeutic value in Ayurvedic preparations [1]. Numerous types of biological activities are attributed to bauhinia species. *B. Purpurea Linn* is an important species used to treat many ailments in traditional system of medicine [2]. The plant is useful in diarrhoea, pain, rheumatism, inflammation, cancer and many more medicinal perpuse [3,4]. Since the plant is used in various formulations commonly, its quality and quantity is a prime importance. Current study deals with the changes of chemical constituents due to different factors. The findings of the study suggested that Hydroalcoholic extracts contains strong phytochemical activity while changing different parameters.

Keywords: *Bauhinia Purpurea Linn*; Hydro Alcoholic; Chemical Constituents; Antioxidant Activity

Introduction

The plant is popular in India, bark was reported as anti mycobacterial, antimalarial, antifungal, cytotoxic and anti-inflammatory activities. The leaves were reported to possess antinociceptive, anti inflammatory and antipyretic properties, while the stem was found to have anti-diabetic and adrenergic properties, *Bauhinia statins,* isolated from leaves and bark was reported to inhibit human cancer cell [5-8]. *Bauhinia Purpurea linn* widely distributed in Himalayan region of India [9-13]. Traditionally *Bauhinia Purpurea linn* are intended to use for the treatment of numerous activity namely diarrhea, ulcers, enlarge cervical glands, goiter, scrofulous tumors etc. *Bauhinia Purpurea linn* extracts was scientifically documented for its antinociceptive, antidiarrhoeal, anti-inflammatory, analgesic, anticancer, antipyretic, antimalarial, antimycobacterial, antifungal, anti-diabetics and anti-diarrheal activity etc [14-17].

Inflammatory and arthritis activity of ethanolic extract of *Bauhinia Purpurea* bark was previously reported. *Bauhinia Purpurea linn* contains various types of phytochemicals like polyphenols and flavanoids which area responsible for anti-inflammatory and antiarthritis activity. The constituents of a various medicinal plants may fluctuate while changing in atmosphere and time of collection. Because the plant extracts are used all seasons, it was intended to study changes of chemical constituents if any found while changing season and reason. The samples for this study were collected in summer, monsoon and winter during 2013. Similarly for observing changes in geographical conditions plants samples are collected from Madhya Pradesh, and Himachal Pradesh [18-20].

Materials and Methods

For present study the fresh leaves of *Bauhinia Purpurea linn* was selected. The plant was authenticated by Dr. NK Dubey, Department of Botany, Banaras Hindu University, and Varanasi (U.P.). The dried leaves were reduced to powder and stored moisture free environment till use.

Preparation of Extracts

Approximately 1 Kg of *Bauhinia Purpurea linn* leaves of the plant were packed in soxhlet apparatus and successively extracted with petroleum ether, hydroalcohol and water separately, till the completion of the extraction. The extract was filtered on hot condition further distillation is carried out for removal of solvent completely; samples are dried in a desiccator for removal of moisture and further use.

Determination of Total Phenol Content

Polyphenol content was determined spectrophotometrically. 1.0 ml of prepared extract were reacted with Folin- Ciocalteu reagent for oxidation, during reaction 1.8 ml of sodium

carbonate solution was added. Samples are incubated for 2hrs in room temperature and absorbance was taken at 760 nm. The amount was calculated using gallic acid calibration method [21]. For estimation of flavonol contents 9.8 ml of the prepared extract was added with a 10% aluminum chloride solution. Absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. Results were expressed in quercetin equivalent (QE) mg per 100 ml of the sample.

Antioxidant Activity

After phytochemical investigation hydroalcoholic and aqueous extract of *Bauhinia Purpurea linn* were selected for further in vitro antioxidant activity. Antioxidant ability of *Bauhinia Purpurea linn* extract was assessed by reducing power assay models, hydrogen-donating activity, and total polyphenol content estimation. DPPH reacts with reducing agents, then decreasing color stoichometrically as the number of electrons depleted. Depleted electrons are measured spectrophotometricallty at 517 nm. Findings of results shown in Table 1. A hydroalcoholic and aqueous extracts of *Bauhinia Purpurea linn* strongly scavenged DPPH with IC50 being 111.81 and 153.03 µg/ml. Scavenging effect was dose dependent. Ascorbic acid which is used as standard scavenged DPPH radical was reported 93.42. The findings of the study suggested that hydroalcoholic extracts exhibited higher scavenging effect compared to water extracts.

Table 1: Determination of total polyphenol content of *Bauhinia purpurea Linn*.

		Winter	Monsoon	Summer		Winter	Monsoon	Summer
MP	Hydro Alcoholic	91.36±1.11	89.11±1.59	81.98±1.32	**HP**	93.38±1.39	90.88±1.23	90.54 +_1.51
	Aqueous	51.29±1.18	42.22±1.88	34.21±1.11		52.22±1.98	46.22±1.32	38.21±1.85

Data expressed as Gallic acid equivalent (GAE) mg per gm of the extract.

Table 2: Total flavonol content (QE mg/gm).

		Winter	Monsoon	Summer		Winter	Monsoon	Summer
MP	Hydro Alcoholic	40.21±0.25	32.91±0.44	22.90±0.55	**HP**	64.11±0.24	62.41±0.64	42.90±1.75
	Aqueous	24.54±1.88	18.90±1.75	14.60±1.55		52.22±1.98	42.14±1.78	35.44±1.43

Total Flavonol Content

Flavonoid content in both extracts i.e. hydroalcoholic and aqueous extract of *Bauhinia Purpurea linn* were determined spectrophotometrically using aluminum chloride. The content was expressed in quercetin equivalents. Standard curve of quercetin was plotted .Using Beer's law regression coefficient is calculated. Flavonoid contents are listed in Table 2. The flavonoid content in hydroalcoholic and aqueous extract of *Bauhinia Purpurea linn* was found to be 62.41 and 45.37 QE mg/gm, respectively. The findings of the current study suggested that hydroalcoholic extracts exhibited highest amount of flavonoids compared to aqueous extracts. It has been reported that flavonoids, which contain hydroxyl, are responsible for the radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. The results of the study suggest that total polyphenol and flavonol content of hydroalcoholic and aqueous extract of *Bauhinia Purpurea linn* supports the study of DPPH scavenging.

Result

In order to determine seasonal variation of the concentration of phenolic compound, flavanoids, antioxidant activity the analysis of plant material during three different seasons and geographical condition collection of plant material was performed. Two deferent extracts were used hydroalchoholic and aqueous.

Total Phenolic Content

For investigation of phenolic content of *Bauhinia Purpurea linn* a standard curve was plotted using gallic acid. Hydroalcoholic and water extracts are expressed in terms of galiic acid equivalents. Results of plant collected from H.P.total phenolic content of hydro alcoholic extract was found to be 93.38±1.39 in winter where as 90.88±1.23 in monsoon it was also reported 83.54±1.51 in summer apart from that plants collected from M.P. shows 91.36±1.11, 89.61±1.59 , 81.98±1.32 in winter, monsoon and summer respectively. The results of the study shows that hydro alcoholic extracts exhibits highest amount of total phenolic content in phase 2 i.e. in winter season. Findings of the study also suggested that geographical distribution having small change in metabolite content. Study suggested that phenolic contents are present in highest amount on winter season whereas lowest amount are recorded in summer but in rainy season it have marginal increase in content compare to winter.

Flavonoid Concentration

Solution of aluminum chloride was used for determination of flavonoid contents in *Bauhinia Purpurea linn* using spectrophotometric method and results are expressed in quercetin equivalents. The Quantities of flavonoid identified in both extracts are shown in Table 3. Samples collected from H.P during summer it was 42.90±1.75 mg RU/g in monsoon 62.41±0.64 mg RU/g .Where as in winter it was reported 64.11±0.24 mg RU/g high. Samples collected from M.P during summer it was

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reported 22.90±0.55 mg RU/g in monsoon 32.91±0.44 mg RU/g. Where as in winter it was reported 40.21±0.25 mg RU/g drastic change was observed that geographical distribution plays major role in changes of flavonoid contents. Samples obtained from Himachal Pradesh shows significant amount of flavonoid content in all seasons where as extracts of M.P. shows relatively lesser amount of flavonoid content.

Table 3: antioxidant activity determination of *bauhinia purpurea linn*.

		Winter	Monsoon	Summer		Winter	Monsoon	Summer
MP	Hydro Alcoholic	142.65	118.23	105.55	**HP**	147.66	122.75	109.09
	Aqueous	117.66	110.76	101.03		121.05	122.53	103.43

Antioxidant Activity

In vitro anti-oxidant activity was performed of both the extracts of *Bauhinia Purpurea linn* by using hydrogen donating assay and reducing power assay. DPPH radical reacts with appropriate reducing agent and developers color at 517nm. Plants collected from H.P. Antioxidants activity shows 147.66, 122.75 and 109.09mg/ml during winter, monsoon and summer respectively. Whereas plants collected from M.P. shows 142.65, 118.23, 105.55 mg/ml in three different seasons respectively. The highest amount of antioxidant activity obtained plants collected from H.P, during winter season and lowest amount was observed in Plants collected from M.P. during summer.

Discussion

Synthesis of secondary metabolites depends on the direction of plant ontogeny. Each plant species enters its specifities into metabolic processes which result in the synthesis of various metabolites. Variations in the concentration of secondary metabolites are the result of both biotic and abiotic factors. Quantitative and qualitative analysis of the dynamics and distribution of secondary metabolites, the impact of environmental factors on their structure as well as biological activity, provide answers to many current issues in several disciplines. Phenols are a large class of secondary metabolite, similar results are not observed in hydroalcoholic and aqueous extracts therefore it is better to go forward with hydroalcoholic extracts. Samples of *Bauhinia Purpurea linn* collected during all the three seasons showed variation in constituents. It clearly revealed that climate does affect the chemical constituents of *Bauhinia Purpurea linn* for the plant.

The advantage of this study is that the plant can be collected at any time of the year but for better result it must be collected during winter season i.e. from month of September to March. The study on geographical variations of *Bauhinia Purpurea* revealed that the results of extracts of the plants collected from Himachal Pradesh and Uttar Pradesh were not similar to each other. The presence of variations in samples collected from the two different regions indicates that the environmental condition does affect the chemical constituents. However variations are minor but still can play vital role in pharmacological activity. The present study sets at rest the speculations on the probable variations in chemical constitutions induced by the seasons and environment. Not every plant can be cultivated everywhere. Even some plant is able to grow in other regions, the phytochemicals of the plant growing in two different regions are found to be different. Thus it is implicit that plants produce various secondary metabolites as a result of their interaction with the environment. It is a very positive result as far as standardization of the plant material is concerned. Similar analysis need to be carried out for all other plants to certify its consistency before they are used to prepare formulations.

Conclusion

Finding of the current study shows minor difference in total phenolic content, flavonoid concentration and antioxidant activity during the three deferent collections time i.e. summer, winter and Monsoon and their compositions also vary due changes in geographical distribution. It also noticed that the highest concentration is obtained in hydroalcoholic solvent. Therefore, present study can be useful to get a higher concentration of these substances since this species has a long history of commercial use. Change of season or region does affect drastically the synthesis of phytochemicals of the plant.

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