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POLYPHENOL PROFILING IN THE LEAVES OF PLANTS FROM THE CATCHMENT AREA OF RIVER BEAS, INDIA

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ABSTRACT

A total of 11 polyphenols viz. gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, quercetin, kaempferol and ellagic acid were analyzed in the leaves of 16 plants by using UHPLC (Ultra high performance liquid chromatography). *Polygonum barbatum* (1756.42 µg/g dw) had the highest, whereas *Cannabis sativa* (18.01 µg/g dw) had the lowest content of polyphenols. Highest positive Pearson's correlation was found between the umbelliferone and kaempferol. Cluster analysis revealed that all the members of family asteraceae and chenopodiaceae were included in the same cluster and had close proximity on the basis of polyphenol content. The total variance of 65.89% was explained by first three components of PCA (principal component analysis). In factor analysis factor-1, factor-2 and factor-3 accounted 25.3%, 24.4% and 16.2% of the total variance respectively.

KEYWORDS: **River Beas, polyphenols, UHPLC, multivariate techniques**

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INTRODUCTION

Phenolic compounds belong to a large heterogeneous group of secondary plant metabolites and are the most important category of water soluble antioxidants. They have many applications in food, cosmetic and pharmaceutical industries[4]. They occur almost in every plant as tannins, lignans and flavonoids. Flavonoids are the most studied compounds, and were synthesized by the phenylpropanoid pathway. Flavonoids represent more than 9000 structurally different compounds. Catechins and other flavanols may act as defense chemicals to protect the plants from predators and insects. They also scavenge ROS formed from the photosynthetic electron transport system in plant cells[10]. Commercially, phenolic compounds with antioxidant activities are used in processed food. Flavonoids are secondary metabolites in the form of flavonols, flavones, isoflavones, flavonones, and their major sources include plants e.g., tea, apple, tomato, cherry, onion, legumes, grapes fruit, lemon etc.[2] Catechin, rutin and quercetin have multiple biological activities such as cardioprotective, antiviral, antibacterial, anti-inflammatory and anti-carcinogenic[5,12]. Polyphenol contents have been reported in many plants such as *Myrtus communis*, *Rhododendron arboretum, Punica granatum, Eucalyptus globules, Origanum vulgare, Mellisa officinalis, Ocimum sanctum, Sideritis cretica* etc.[3,11,1,6,9] A survey was conducted around the catchment areas of river Beas and following plants were collected for the analysis of polyphenols: *Sida acuta, Cannabis sativa, Debregeasia longifolia, Sesbania bispinosa, Ageratum conyzoides, Erigeron bonariensis, Lantana camara, Alternanthera philoxeroides, Achyranthus aspera, Chenopodium ambrosioides, C. album, Polygonum barbatum, P. lanigerum, Rumex dentatus* and *Typha angustata*.

MATERIALS AND METHODS

Study area

Plant samples were collected from the catchment areas of river Beas, India (31.51 ′N lat. and 77°05 ′E long.). Identification and authentication of plants were done from the Botanical Survey of India, Dehradun.

UHPLC (Ultra high performance liquid chromatography)

100 ml methanolic extract was prepared from 1 g of oven dried leaves of each of the plant species and the extracts were dried in rotary vacuum evaporator. To the dried extract, 4 ml of methanol was added which was used for the analysis of different polyphenols. 5 µl of sample was injected into the system. Polyphenolic compounds were analyzed by using 130 MPa Shimadzu UHPLC system (Nexera) attached with DGU-20As prominence degasser, LC-30 AD liquid chromatography, SIL-30Ac autosampler, CTO 10AS VP column oven and SPD-M20A photodiode array detector (PDA). Flow rate of 1 ml/min at 280 nm was used for analysis. C-18 column with 150 and 4.6 mm dimensions and 5 µm pore size was used. Solvent system composed of solvent A: 0.01% acetic acid in water, and solvent B: 100% methanol.

Statistical analysis

The results were analyzed by using Pearson's correlation matrix, Cluster analysis (CA), factor analysis (FA) and principal component analysis (PCA). The software used was PAST, Statistica-12, Minitab 14 and MS-Excel.

Table 1
Concentration of different polyphenols

Plant species	Gallic acid µg/g dw	Catechin µg/g dw	Chlorogenic acid µg/g dw	Epicatechin µg/g dw	Caffeic acid µg/g dw	Umbelliferone µg/g dw	Coumaric acid µg/g dw	Rutin µg/g dw	Quercetin µg/g dw	Kaempferol µg/g dw	Ellagic acid µg/g dw	Total
Sida acuta Burm.f.	2.90	0.18	2.22	118.98	1.16	668.70	-	0.19	19.62	733.62	-	1547.57
Cannabis sativa L.	3.32	0.19	3.27	1.30	0.37	0.60	2.04	-	1.31	1.89	3.73	18.01
Debregeasia longifolia (Burm.f.)Wedd	0.02	0.39	6.29	1.99	3.09	0.80	0.29	46.91	-	16.07	0.93	76.79
Sesbania bispinosa (Jacq.)W.F.Wight	8.30	74.93	1.52	-	49.98	44.34	0.69	27.73	-	12.38	70.01	289.88
Ageratum conyzoides L.	0.95	127.56	0.04	46.26	0.38	0.17	0.60	-	2.28	-	7.07	185.31
Erigeron bonariensis L.	0.26	19.68	0.00	9.41	0.04	34.24	0.72	0.78	4.13	38.64	34.30	142.19
Parthenium hysterophorus L.	2.68	25.37	4.25	49.47	0.05	2.83	1.53	-	0.00	-	3.72	89.90
Lantana camara L.	3.25	0.00	0.98	38.66	-	12.38	1.05	-	591.76	21.23	1.88	671.18
Alternanthera philoxeroides (Mart.)Grisb.	-	-	2.72	2.01	0.27	7.45	-	0.16	18.86	9.12	0.00	40.59
Achyranthes aspera L.	0.13	0.08	0.97	0.41	1.46	0.85	3.12	-	0.00	4.92	38.18	50.13
Chenopodium ambrosioides L.	-	13.84	0.57	1.09	0.27	0.13	0.23	0.15	10.28	-	-	26.55
Chenopodium album L.	-	0.69	5.23	0.58	1.88	1.69	0.03	-	1.29	16.45	-	27.84
Polygonum barbatum L.	676.06	166.71	152.87	3.66	0.68	36.54	0.26	-	155.86	403.67	160.09	1756.42
Polygonum lanigerum R.Br.	34.38	7.74	210.56	0.77	-	-	0.01	-	628.94	-	169.16	1051.55
Rumex dentatus L.	2.70	8.83	1.79	57.61	1.00	1.16	0.21	-	-	-	-	73.30
Typha angustata Chamb.	0.07	60.49	2.27	1.69	0.48	0.92	0.16	-	2.21	-	289.18	357.46

'-'not detected

Table 2
Polyphenols with significant Pearson's correlation

Catechin- gallic acid	0.707**
Chlorogenic acid- gallic acid	0.589*
Quercetin- chlorogenic acid	0.629**
Ellagic acid- chlorogenic acid	0.527*
Kaempferol- epicatechin	0.634**
Umbelliferone- epicatechin	0.770***
Rutin- caffeic acid	0.508*
Kaempferol- umbelliferone	0.890***

Significant at *P <0.05 **P <0.01 ***P <0.001

Table 3
Weights of PCA for different polyphenols

Variables	PC 1	PC 2	PC 3
Gallic acid	0.824	-0.073	0.177
Catechin	0.672	0.044	0.447
Chlorogenic acid	0.847	0.087	-0.270
Epicatechin	-0.178	-0.884	-0.067
Caffeic acid	-0.061	0.204	0.762
Umbelliferone	-0.036	-0.939	0.132
Coumaric acid	-0.356	0.263	-0.176
Rutin	-0.183	0.237	0.680
Quercetin	0.443	0.087	-0.530
Kaempferol	0.325	-0.889	0.174
Ellagic acid	0.706	0.232	0.038

Table 4
Factor analysis of different polyphenols

Variables	Factor-1	Factor-2	Factor-3	Communality
Gallic acid	0.837	-0.119	-0.046	0.716
Catechin	0.763	-0.036	0.267	0.654
Chlorogenic acid	0.770	0.099	-0.419	0.779
Epicatechin	-0.243	-0.860	-0.141	0.818
Caffeic acid	0.138	0.091	0.774	0.627
Umbelliferone	-0.061	-0.948	0.008	0.902
Coumaric acid	-0.374	0.295	-0.045	0.229
Rutin	0.003	0.139	0.731	0.554
Quercetin	0.305	0.154	-0.607	0.485
Kaempferol	0.303	-0.914	-0.036	0.928
Ellagic acid	0.708	0.207	-0.108	0.556
Variance	2.78	2.67	1.78	7.24
% Var	25.3%	24.4%	16.2%	65.9%

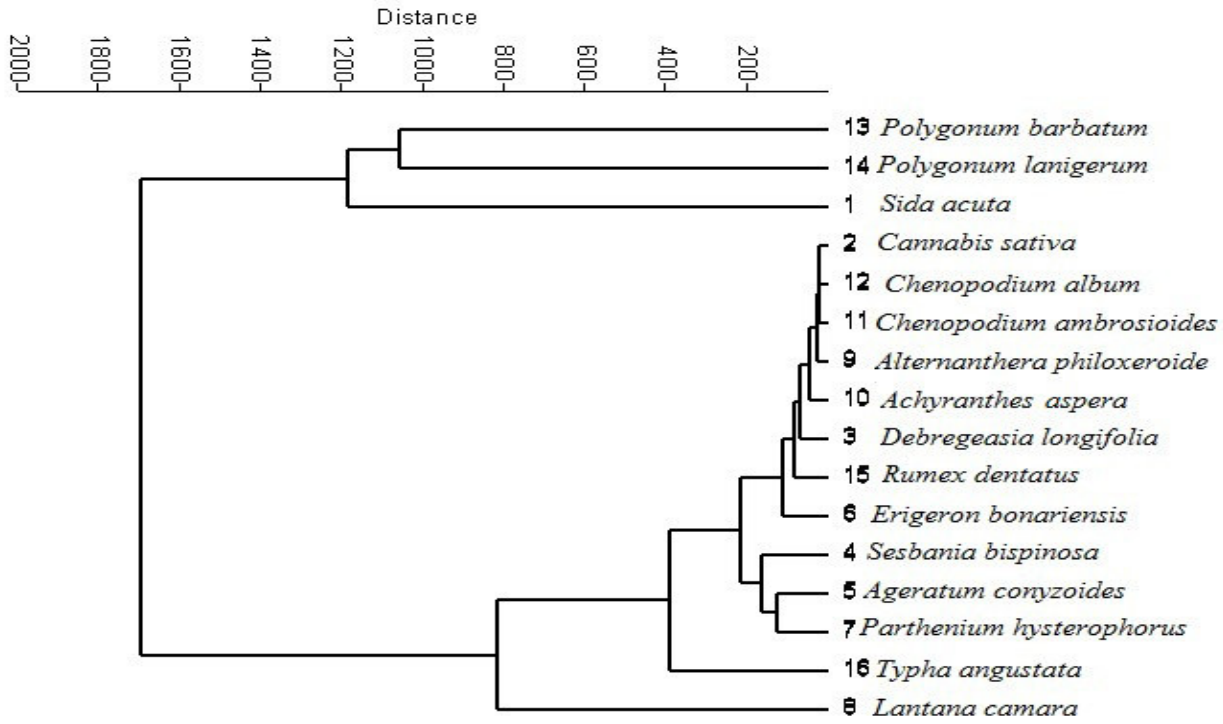

Figure 1
Cluster analysis for different concentrations of polyphenols

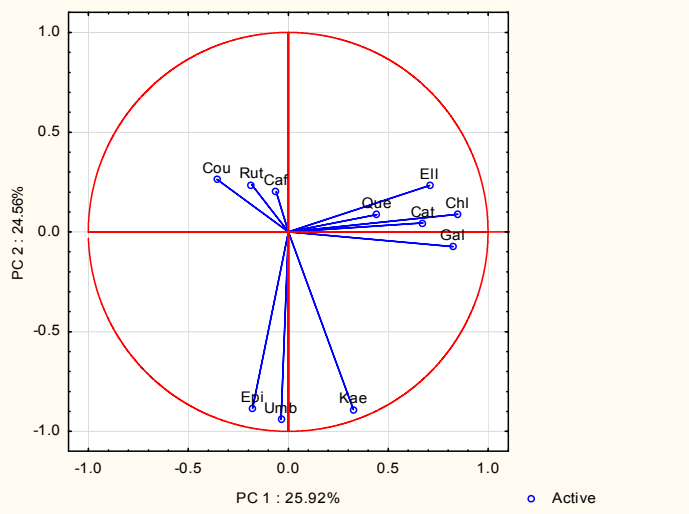

Figure 2(a)
PCA for different polyphenols

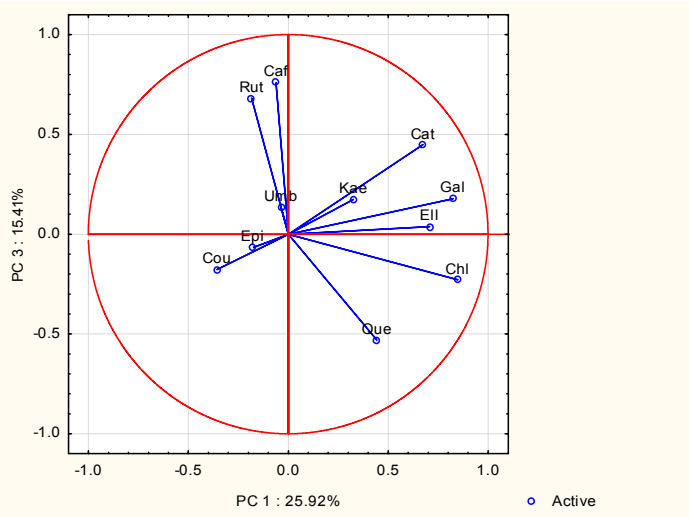

Figure 2(b)
PCA for different polyphenols

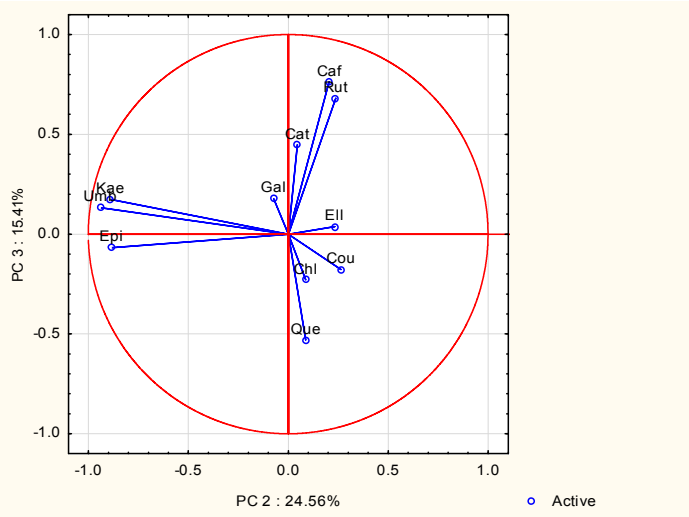

Figure 2(c)
PCA for different polyphenols

RESULTS AND DISCUSSION

A total of eleven polyphenols such as gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, quercetin, kaempferol and ellagic acid were determined in dry plant samples by using UHPLC. The concentrations of different polyphenols are given in the table (1). *Polygonum barbatum* contained highest total polyphenols content, whereas *Cannabis sativa* contained low content of polyphenols. Panwar et al. (8) reported the presence of coumaric acid and gallic acid in *Parthenium hysterophorus.* Catechin, rutin, ellagic acid and quercetin were reported in *Alternanthera sessilis* by Mondal et al. (7). Pearson's correlation among different concentrations of polyphenols was applied. Table (2) gives the pairs of polyphenols for which correlations were statistically significant at p≤ 0.05. Highest correlation existed between kaempferol and umbelliferone, and kaempferol and epicatechin. Umbelliferone also highly correlated with epicatechin. Gallic acid also shows high correlation with catechin. CA was applied to the

contents of different polyphenols (Figure 1). *Polygonum barbatum* and *P. lanigerum* belong to the family polygonaceae and had close proximity. Members of chenopodiaceae and asteraceae family had high similarity on the basis of polyphenols content. *Chenopodium ambrosioides, C. album, Alternanthera philoxeroide* and *Achyranthes aspera* belong to the order Caryophyllales and included in the same group. PCA was also applied to the polyphenols content. The first three components of PCA explained 65.89% of the total variance (25.92%, 24.56% and 15.41% respectively). Loadings of PCA are given in the table (3). Epicatechin, catechin, ellagic acid, chlorogenic acid and quercetin had maximum loadings on PC1. PC2 had maximum loading on coumaric acid. Rutin and caffeic acid had maximum loadings on PC3. Projections of PCs for unit circle factor plane are given in the Figures 2 a, b and c. The projection of unit circle represents the similarity of groups of polyphenols. FA was also applied to the concentrations of different polyphenols (Table 4). Three factors were mainly responsible for the polyphenols. Factor-1 accounted for 25.3% of the total variance and had positive loadings on gallic acid, catechin, chlorogenic acid, quercetin, kaempferol and ellagic acid. Quercetin and kaempferol are flavonols and both are included in the same factor. Gallic acid and ellagic acid are derived from cinnamic/ benzoic acid and are included in the same factor. Factor-2 explained 24.4% of the total variance and had maximum positive loadings on coumaric acid. Rutin and caffeic acid are influenced by factor-3 and explained 16.2% of the total variance.

CONCLUSION

From the present study, it was established that *Polygonum barbatum* contained highest content of polyphenols, and lowest amount of polyphenols found in *Cannabis sativa*. Highest Pearson's correlation of umbelliferone found with kaempferol and epicatechin. Similarity in plant species on the basis of polyphenols content has been revealed by cluster analysis. Chenopodiaceae and asteraceae family members have high similarity in polyphenol contents. First three components of PCA explained 65.89% of the total variance. FA showed that factor-1 influences gallic acid, catechin, chlorogenic acid, quercetin, kaempferol and ellagic acid. Factor-2 influence coumaric acid. Rutin and caffeic acid are influenced by factor-3.

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