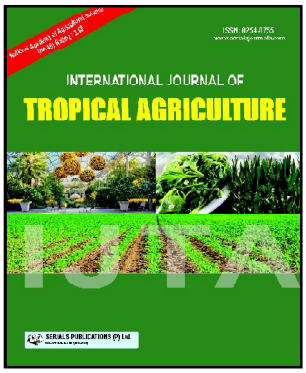

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Multivariate Statistical Analysis of Secondary Metabolites and Total lipid Antioxidants

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Abstract: The leaves of 15 plant species were collected from the catchment areas of river Beas, Punjab, and analyzed for phenols, flavonoids, xanthophylls and total lipid antioxidants. *Polygonum plebeium* contained highest content of phenols (31.56 mg/g dw), whereas highest content of flavonoids was found in *Cannabis sativa* (9.27 mg/g dw). *Polygonum barbatum* was found to contain highest contents of xanthophylls (52.20 mg/g dw) and total lipid antioxidants (16.89 µmole/g fw). Cluster analysis (CA) showed that *Erigeron bonariensis* and *Parthenium hysterophorus* belongs to family Asteraceae and had close proximity to each other. First three components of principal component analysis (PCA) explained 98.57% of the total variance. Factor analysis (FA) explained two factors: factor-1 had maximum loadings on (xanthophylls and flavonoids) and explained for 36.4% of the total variance. Factor-2 had maximum loadings on (phenols and total lipid antioxidants) and accounted for 34.3% of the total variance.

Keywords: Secondary metabolites, antioxidant analyzer, multivariate techniques

INTRODUCTION

Phenolic compounds belongs to a large heterogeneous group of secondary plant metabolites that are present in the plant kingdom and have many applications in food, cosmetic and pharmaceutical industries (1-2). The commercial venture of phenolic compounds with antioxidant activities is now a usage in processed food. Flavonoids are secondary metabolites in the form of flavonols, flavones, isoflavones, flavonones and their major sources include a number of plants such as tea, apple, tomato, cherry, onion, legumes, grapes fruit, lemon etc. (3).

Kumar *et al.* (4-5) studied that these plants are the rich source of polyphenols and amino acids. *Cannabis sativa, Ageratum conyzoides, Parthenium hysterophorus*, *Typha angustata, Chenopodium album, C. ambrosoides, Polygonum barbatum and P. plebeium* are rich source of phytochemicals (6-7). The present study is an attempt to study the secondary metabolites (phenols and flavonoids), xanthophylls and total lipid antioxidant in the leaves of 15 plants.

MATERIALS AND METHOD

Study Area

River Beas originates in the southern side of the Rohtang Pass above Kullu in Beas Kund, in central Himachal Pradesh, India, (31.512 N lat. and 77°052 E long.) and merges into the river Sutlej in the state of Punjab, after traversing a distance of about 470 km. Plant samples were collected from the river bed side between the towns of Beas and Harike. Identification and authentication of the plants were done at Botanical Survey of India, Dehradun, India.

Total lipid antioxidant: Total lipid antioxidants were analyzed by using antioxidant analyzer (Analytic Jena, Photochem). 0.5 g of fresh plant sample was crushed in 1.5 ml of methanol. 10 µl plant samples were injected into the instrument.

Total Phenolic content: Total phenols were determined by the method given by Singleton and Rossi (8).

Total flavonoids: Flavonoid content was determined by the method given by Zhishen et al. (9).

Xanthophyll content: Xanthophylls were determined by using the AOAC method given by Lawrence (10).

Statistical Analysis

Analysis was done in triplicates and results were expressed in mean and standard deviation. The data was analyzed by using cluster analysis (CA), principal component analysis (PCA) and factor analysis (FA) (11-12).

RESULTS AND DISCUSSIONS

The means and standard deviation of secondary metabolites, total lipid antioxidants and xanthophylls are given in table (1). *Cannabis sativa* contained the highest flavonoids content, whereas lowest content of flavonoids was recorded from *Tamarix dioica*. Xanthophyll content was recorded maximum from *Polygonum barbatum*, and the lowest xanthophylls content was found in *Tamarix dioica*. The highest content of phenol was found in *P. plebeium*, and lowest phenol content was found in *Rumex dentatus*. *Ranunculus sceleratus, Fumaria parviflora, Chenopodium ambrosioides, Polygonum plebeium, Tamarix dioica* and *Typha angustata* showed the same trend for secondary metabolites and xanthophylls i.e. flavonoids > xanthophylls > phenols. *Polygonum barbatum* recorded maximum content of total lipid antioxidant, whereas lowest content of total lipid antioxidant were found in *C. sativa*. The present results find support from Ira et al. (13). They analyzed flavonoids content (2.41 mg/g dw) in *Plumbago zeylanica*. Stankovic (14) reported phenolic content (27.44 mg/g dw) and flavonoids content (18.72 mg/g dw) in *Marrubium peregrinum*. Ibrahim et al. (15) studied flavonoids content (0.317 mg/g dw) and phenolic content (0.581 mg/g dw) in *Labisia pumila*. Agbo et al. (16) analyzed phenolic contents in (11.18 mg/g dw) *Baphia nitida, Mussaenda afzelii* (11.67 mg/g dw), *Crotolaria retusa* (15 mg/g dw) and *Craterosiphon scandens* (16.43). They also analyzed flavonoids contents in *Crotolaria retusa* (10.33 mg/g dw), *Mussaenda afzelii* (3.67 mg/g dw), and *Craterosiphon scandens* (7.00). Agbo et al. (16) studied the ratio of flavonoid to phenolic, i.e., *Luchnera rosea* (1.68), *Crotolaria retusa* (0.69), *Mussaenda afzelii* (0.32) and *Craterosiphon scandens* (0.43). Prasad et al. (17) analyzed xanthophylls content in *Melia azedarach* (1.24 mg/g dw), *Asplenium dalhousiae* (1.36 mg/g dw) and *Osmanthus fragrans* (6.78 mg/g dw).

Table 1
Mean and standard deviation (n=3) of secondary metabolites, xanthophylls and total lipid antioxidants in the leaves of plants

Plant species	*Flavonoid (mg /g dw)*	*Phenols (mg /g dw)*	*Ratio of Flavonoid/Phenols*	*Xanthophylls (mg/g dw)*	*Total lipid antioxidants (µmole/g fw)*
Ranunculus sceleratus L.	1.40±0.14	6.37±0.07	0.220	4.85±0.35	8.47±0.65
Argemone mexicana L.	3.36±0.07	8.51±0.06	0.395	9.26±0.80	8.36±0.11
Fumaria parviflora Lam.	4.39±0.08	6.32±0.04	0.695	4.68±0.35	7.57±0.25
Oxalis corniculata L.	6.64±0.36	4.73±0.25	1.404	11.75±0.86	6.58±0.17
Ageratum conyzoides L.	3.36±0.05	6.11±0.22	0.550	11.92±0.70	9.92±0.64
Erigeron bonariensis L.	3.09±0.21	4.26±0.05	0.725	21.18±0.77	5.63±0.07
Parthenium hysterophorus L.	0.77±0.28	7.32±0.29	0.106	14.51±0.68	6.10±2.25
Chenopodium album L.	3.40±0.10	6.31±0.14	0.539	47.62±0.33	7.52±0.08
Chenopodium ambrosioides L.	8.36±0.21	14.44±0.07	0.579	10.84±0.77	6.17±1.99
Polygonum barbatum L.	7.77±0.66	24.68±0.59	0.315	52.20±0.61	16.89±0.7
Polygonum plebeium R.Br.	3.11±0.24	31.56±0.50	0.099	6.67±0.93	9.17±0.04
Rumex dentatus L.	1.44±0.09	3.76±0.07	0.383	5.81±0.51	8.56±0.09
Tamarix dioica Roxb. ex Roth	0.11±0.25	25.63±1.49	0.005	1.07±0.35	6.12±0.29
Cannabis sativa L.	9.27±1.66	10.95±2.08	0.847	3.72±0.50	4.01±1.02
Typha angustata Chamb.	1.69±0.24	9.34±0.04	0.181	4.01±0.59	4.96±0.90

CA was applied to the content of secondary metabolites by using Ward's method and Euclidean distance as a measure of similarity (Fig. 1). *Polygonum plebeium* and *Tamarix dioica* belongs to order Caryophyllales and are include in the same cluster. *Erigeron bonariensis* and *Parthenium hysterophorus* belongs to family Asteraceae and had close proximity to each other. *Chenopodium album* and *Polygonum barbatum* belongs to order Caryophyllales and are included in the same cluster. *Ranunculus sceleratus* and *Fumaria parviflora* had close proximities to each other and belongs to order Ranunculales.

First three components of PCA explained 98.57% (73.12%, 23.18 and 2.27% respectively) of the total variance. Factor analysis was applied to the secondary metabolites (Table 2).

Table 2
Factor analysis of secondary metabolites and total lipid antioxidants

Variables	*Factor-1*	*Factor-2*	*Communality*
Flavonoid	**0.741**	0.134	0.567
Phenols	-0.100	**-0.864**	0.757
Xanthophylls	**0.794**	-0.318	0.731
Total lipid antioxidants	0.518	**-0.713**	0.776
%Var	36.4%	34.3%	0.707

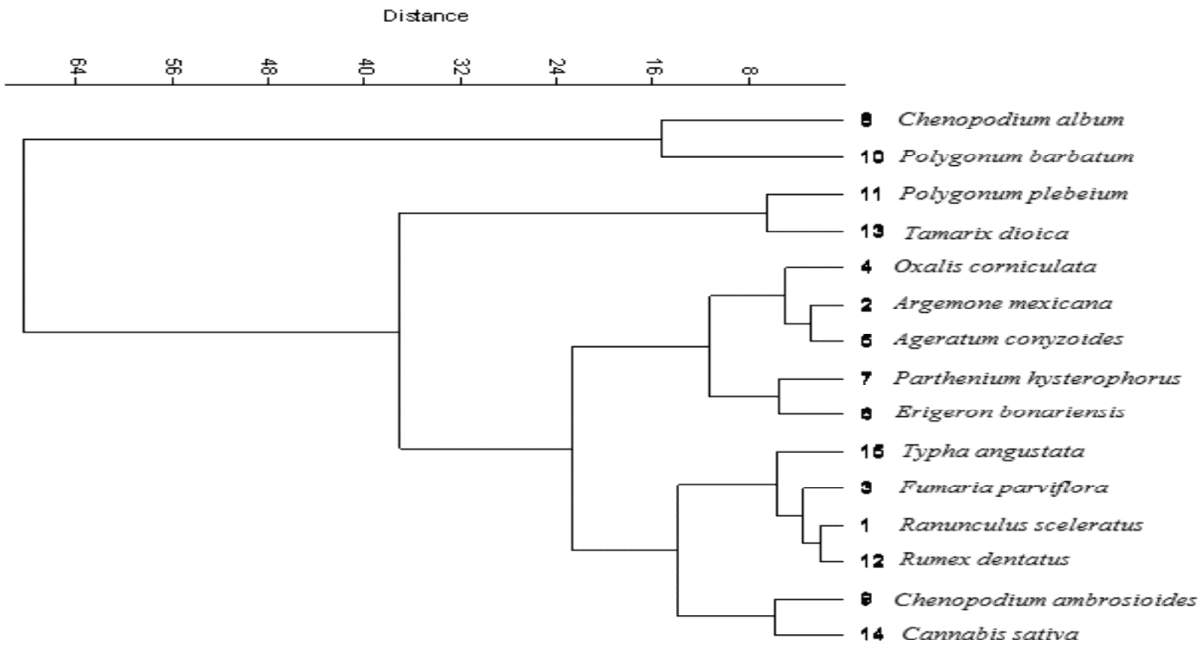

Figure 1: Cluster analysis of different plant species

Factor-1 accounted for 36.4% of the total variance and had maximum loadings on flavonoids and xanthophylls. Factor-2 had maximum loadings on phenols and total lipid antioxidants and explained 34.3% of the total variance (Fig. 2).

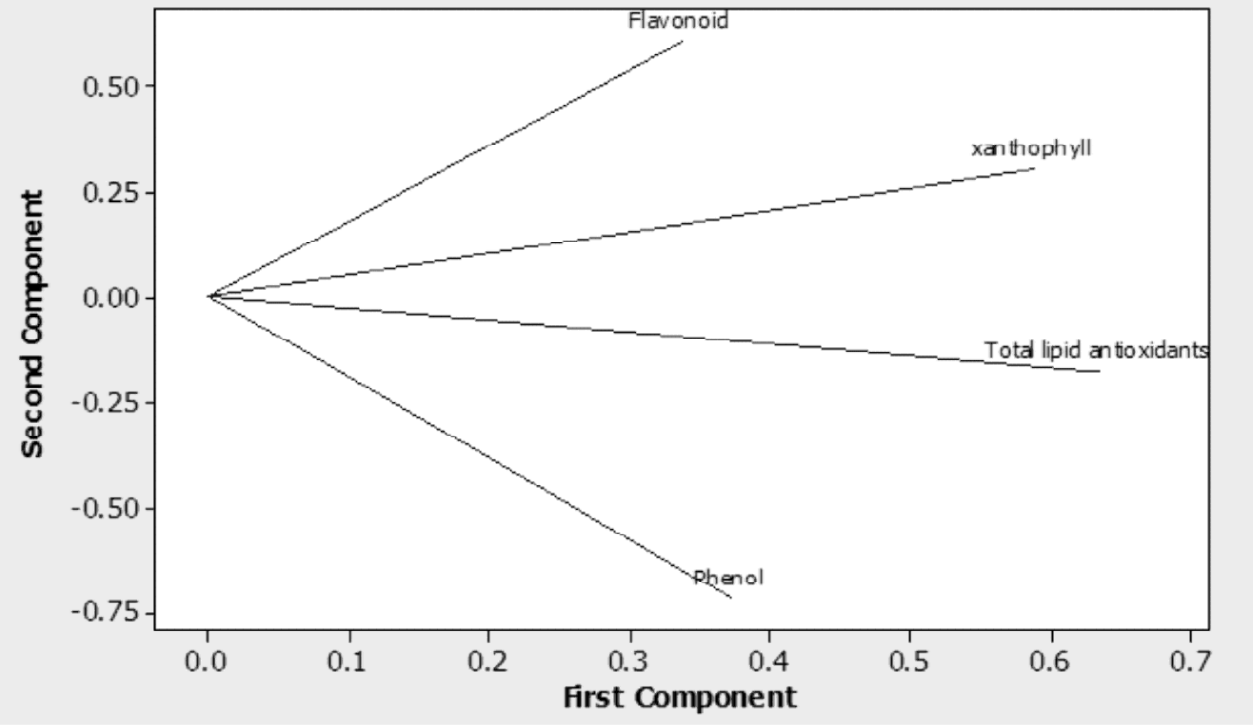

Figure 2: Loading plots of PCA for secondary metabolites

CONCLUSIONS

From the present study, it is found that *R. sceleratus, F. parviflora, C. ambrosioides, P. plebeium, T. dioica* and *T. angustata* showed the same trend for secondary metabolites and xanthophylls i.e. flavonoids > xanthophylls > phenols. CA showed that *P. plebeium* and *T. dioica* belongs to order Caryophyllales and had close proximity to each other. FA explained two factors: factor-1 (36.4%) and factor-2 (34.3%) respectively.

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