Chemical constituents of *Ajuga bracteosa* an indigenous medicinal herb of Khyber Pakhtunkhwa

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ABSTRACT

The samples (whole plants) of *Ajuga bracteosa* were collected from their natural habitat in the Khanpur Valley in the sub-Himalayan Mountains of Pakistan. Chemical analysis was carried out at the University of Agriculture, Peshawar. Significant effects were observed at different seasons and sites on various bio-chemical substances. *Ajuga bracteosa* revealed higher significant values for ash (34.13%) at Jabri during summer, crude proteins (15.35%) at Dam during summer, crude fats (10.30%) at Jabri during summer, essential oil (2.48%) at Mang during summer, NFES (46.17%) at Dabola during winter, NFEE (186.28%) at Dam during summer, sodium (6.96mg/100g) at Mang during winter, potassium (559.67mg/100g) at Jabri during winter, calcium (254.20mg/100g) at Jabri during summer, magnesium (264.31mg/100g) at Mang during winter; and zinc (6.53mg/100g) at Dam during winter. Ajuga bracteosa gave higher values for bio-chemicals during the last week of October.

Key Words: *Ajuga Bracteosa, habitat, potassium, bio-chemical.*

INTRODUCTION

An interest has been developed in wild species for their possible medicinal values in diets. Wild plant species provide minerals, fibers, vitamins and essential fatty acids and enhance taste and colour in diets. In addition, they have anti-bacterial, hepatoprotective and anti-carcinogenic properties, and therefore, have medicinal value (M Mates *et al*., 2011). The leaves and stems of most of the plants were rich in alkaloids, flavonoids, tannins and phenolic compounds. They had already been examined to show medicinal activity as well as exhibiting physiological activity (Okigbo *et al.,* 2009). Valleys in Pakistan are full of medicinal herbs and could be analyzed proximately and biochemically to make a more modern drug from a traditional product. It is an established fact that traditional medicinal plants are a good source of protein, minerals, crude fiber, fat and energy (Haq *et al.* 2011).

Northern Pakistan is very rich in indigenous medicinal herbs and its people take a keen interest in the processing of natural herbs and their consumption for medical care (Shinwari, 2010). The Khanpur valley in the sub-Himalayan Mountains of Pakistan was taken as an opportunity to study the nutritional traits of Ajuga bracteosa with a focus on the following objectives: To find out the comparative suitability of seasons and sites for the best harvest of bio-chemical attributes of *Ajuga* bracteosa of the Khanpur valley and to elaborate on the bio-chemical prospects of *Ajuga bracteosa* for possible use as a source of food supplement.

METHODOLOGY

The impact of different seasons and sites on the nutritional and bio-chemical attributes of *Ajuga bracteosa* of the Khanpur valley was conducted. Mature leaves of *Ajuga bracteosa* were collected from their natural habitat at all the four sites and two seasons. Plant material was collected in the summer during the last week of July and in the winter during the last week of October.

Through the quadrate transact method, three transacts (replications) were taken, and in every transact, the material was collected from different available plants and fresh weights were recorded. The samples were brought to the laboratory of Agricultural Chemistry, The University of Agriculture Peshawar. The sample was thoroughly cleaned manually and then made into powder

by a laboratory grinder. The samples in the grinded form were then analyzed for moisture content, dry matter, ash/minerals, crude fibre, crude protein, fat content, essential oil, NFES, NFEE, Na, K, Ca, P, Mg, Cu, Fe, Mn, and Zn, using standard procedures.

RESULTS AND DISCUSSIONS

Proximate Analysis

Moisture (%)

Table 1 displays the mean moisture percentage data for various seasons and locations. The results revealed that the effect of seasons on moisture % was significant while the effect of sites and their interactions were non-significant. Summer had the highest moisture percentage (89.27%), while winter had the lowest (86.80%).

Dry Matter (%)

Table 1 displays the mean dry matter percentage data for various seasons and locations. The results revealed that the effect of seasons on dry matter in % was significant while the effect of sites and their interactions were non-significant. Winter had the highest dry matter percent (13.21%), while summer had the lowest (10.73%).

Ash / Minerals (%)

Table 1 displays the mean ash and mineral percent data for various seasons and sites, while Figure 1 depicts their interaction. The results revealed that the effect of seasons, sites, and their interactions on ash and minerals was significant. Summer had the highest ash/minerals% (30.00%) and winter had the lowest (22.84%). Similarly, the Jabri site had the highest ash and mineral content (29.75%), while the Mang site had the lowest (23.97%). In the case of interactions, maximum ash % was recorded at the Jabri site during summer (34.13%), while a minimum of it was observed at the Dabola site during winter (21.69%).

Crude Proteins (%)

The mean data of crude protein percent for various seasons and sites is shown in Table 1, while their interaction is shown in Figure-2. The results revealed that the effect of seasons and their interactions on crude protein content was significant while the effect of sites was non-significant. Summer had the highest crude protein percentage (14.20%), while winter had the lowest (11.83%). In the case of interactions, the maximum crude protein percent was recorded at Dam site during summer (15.35%), followed by the Dabola site during summer (14.73%), while the minimum was observed at the Dam site during winter (11.25%).

Crude Fibres (%)

The mean data of crude fibres % for various seasons and sites is shown in Table 1, while their interaction is shown in Figure 3. The results revealed that the effect of seasons and sites on crude fibres was significant while that of interactions was non-significant. Winter had the highest crude fibre percentage (12.64%), while summer had the lowest (9.44%). Similarly, the Jabri site had the highest crude fibre percentage (11.65%) and the lowest (10.48%).

Crude Fats (%)

Table 1 displays the mean crude fat percent data for various seasons and sites, while Figure 4 depicts their interaction. The results revealed that the effect of seasons and their interactions on crude fat content was significant, while the effect of sites is non-significant. Summer saw the highest crude fat% (9.65%) and winter saw the lowest (7.83%). In the case of interactions, the highest crude fat percent was observed at the Jabri site during the summer (10.30%), followed by the Dam site during the summer (10.11%), and the lowest was observed at the Jabri site during the winter (6.93%).

Essential Oils (%)

The mean data of essential oil content for various seasons and sites is shown in Table 1, while their interaction is shown in Figure 5. The results revealed that the effect of seasons on essential oil production was non-significant while the effect of sites and interactions was significant. The Mang site had the highest essential oil content (2.33%), while the Jabri site had the lowest (0. 91%).In case of interactions, the maximum essential oil percent was recorded at the

Mang site during summer (2.48%), followed by the Mang site during winter (2.18%), while the minimum of it was observed at Jabri site during summer (0.40%).

Nitrogen Free Extractable Substances (NFES) (%)

The mean data of NFES % for various seasons and sites is shown in Table 1, while their interaction is shown in Figure 6. The results revealed that the effect of seasons, sites, and their interactions on NFES % was significant. Winter had the highest NFES% (44.87%) and summer had the lowest (36.70%). Similarly, the highest NFES% was found at the Mang site (44.35%), while the lowest was found at the Jabri site (37.22%). In the case of interactions, the maximum NFES % was recorded at the Dabola site during winter (46.17%), followed by the Dam site during winter (45.87%), while the minimum of it was observed at the Jabri site during summer (31.53%).

Net Free Energy Estimation (NFEE)

The mean data of NFEE for various seasons and sites is shown in Table 1, while their interaction is shown in Figure 7. The results revealed that the effect of seasons, sites, and their interactions on NFEE was significant. Summer had the highest NFEE (177.49) and winter had the lowest (160.29). Similarly, the highest NFEE was measured at the Dam site (173.95), while the lowest was measured at the Jabri site (163.24). In the case of interactions, the highest NFEE was observed at the Dam site during the summer (186.28), followed by the Jabri site during the summer (176.54), and the lowest was observed at the Jabri site during the winter (149.94).

The results revealed that the effect of seasons on all parameters was highly significant. In summer, maximum values were found for moisture, crude protein, crude fats, minerals/ashes, essential oils, and NFEE, while in winter, maximum values were found for dry matter, crude fibres, and NFES. This seasonal effect was reasonably due to the plant's growth habits and environmental conduciveness. Ajuga sprouts during summer and during the last week of July, which is the metabolically most active period of its growth (Yadav et al., 2011); most of the compounds were in high concentration. Another reason for high moisture, essential nutrients, and minerals during summer was high soil moisture content (Fekete et al., 2011) due to more rain fall and a high rate of transpiration due to high temperatures (Francini et al., 2011). So, when more growth took place, more dry biomass was also produced. Ajuga plants sprout naturally in May-July and mature in

September-December. The rapid growth stage of the plant, as well as the availability of more moisture during the summer in most of the sites, could explain the higher percentages of moisture, CP, CFts, and NFEE at these sites (Gamon et al., 2013). Higher winter values for dry matter, crude fibres, and NFES are undoubtedly related to low moisture and other nutrient accumulation in plant tissues as a result of environmental and physiological stresses (Gruda, 2005).

The effect of sites on moisture, dry matter, crude proteins, and crude fats was non-significant. Maximum values were found for NFEE at the Dam site, while maximum values for essential oil and NFES were found at the Mang site. At Jabri, maximum values were recorded for minerals, and crude fibres. High values of ashes and crude fibres at Jabri might be due to favourable conditions of the soil, as most of the plant species contain these compounds at higher levels at maturity (Sher and Aldosari, 2010).

Table-1. Effect of Different Seasons and Sites on Elemental analysis of *Ajuga bracteosa*, indigenous to Khanpur Valley, in sub-Himalayan Mountains of Pakistan.

Seasons	Moisture%	Dry Matter %	Ash/minerals %	Crude Proteins %	Crude Fibers%	Fats%	Essential Oils%	NFES %	NFEE
Summer	89.27a	10.73b	30.00a	14.20a	9.44b	9.65a	1.38	36.70b	177.49a
Winter	86.80b	13.21a	22.84b	11.83b	12.64a	7.83b	1.55	44.87a	160.29b
LSD at α 0.05	0.826	0.826	0.586	0.338	0.386	0.521	ns	0.791	4.805
Sites									
Dam	86.99	13.01	24.84c	13.29	11.32ab	9.12	1.46b	41.42b	173.95a
Dabola	88.75	11.25	27.10b	13.28	10.70bc	8.75	1.15bc	40.16b	169.42ab
Jabri	87.84	12.16	29.75a	12.76	11.65a	8.62	0.91c	37.22c	163.24b
Mang	88.55	11.45	23.97c	12.72	10.48c	8.67	2.33a	44.35a	168.96ab
LSD at α 0.05	Ns	Ns	1.712	Ns	0.863	ns	0.539	2.351	6.386
Interactions									
Seasons*Sites	ns	Ns	*	*	ns	*	*	*	*

Means followed by similar letter(s) in column do not differ significantly.
Ns = Non Significant.and * = Significant at 5 % level of probability.

Elementology

Sodium (Na) mg/100g:

The mean data of Na for various seasons and sites is shown in Table-3.8, while their interaction is shown in Figure-3.39. The results revealed that the effect of seasons, sites and their interaction on Na was significant. Maximum Na was observed at summer (5.85 mg/100g) while minimum at winter (4.24 mg/100g). Similarly maximum Na was recorded at Mang site (6.60 mg/100g) while minimum at Dabola site (4.18 mg/100g). In case of interactions maximum Na was observed at Mang site during winter (6.96 mg/100g) followed by Jabri site during summer (6.45 mg/100g). While minimum Na was observed at Dam site during winter (2.74 mg/100g).

Potassium (K) mg/100g

The mean data of K for various seasons and sites is shown in Table-3.8, while their interaction is shown in Figure-3.40. The results revealed that the effect of seasons, sites and their interaction on P was significant. Maximum K was observed at winter (475.25 mg/100g) while minimum at summer (162.79 mg/100g). Similarly maximum K was recorded at Jabri site (377.33 mg/100g) while minimum at Dabola site (244.50 mg/100g). In case of interactions maximum K was observed at Jabri site during winter (559.67 mg/100g) followed by Dam site during winter (485.00 mg/100g). While minimum K was observed at Dam site during summer (83.67 mg/100g).

Calcium (Ca) mg/100g

The mean data of Ca for various seasons and sites is shown in Table-3.8, while their interaction is shown in Figure-3.41. The results revealed that the effect of seasons, sites and their interaction on P was significant. Maximum Ca was observed at summer (194.64 mg/100g) while minimum at winter (127.66 mg/100g). Similarly maximum Ca was recorded at Jabri site (197.95 mg/100g) while minimum at Mang site (120.98 mg/100g). In case of interactions maximum Ca was observed at Jabri site during summer (254.20 mg/100g) followed by Dam site during summer (199.00 mg/100g). While minimum Ca was observed at Mang site during winter (95.10 mg/100g).

Phosphorus (P) mg/100g

The mean data of Phosphorus for various seasons and sites is shown in Table-3.8. The results revealed that the effect of seasons and sites on Fe was significant while the effect of their interactions was non-significant. Maximum P was observed at winter (196.73 mg/100g) while minimum at summer (138.13 mg/100g). Similarly maximum P was recorded at Jabri site (212.78 mg/100g) while minimum at Mang site (98.87 mg/100g).

Magnesium (Mg) mg/100g

The mean data of Mg for various seasons and sites is shown in Table-3.8, while their interaction is shown in Figure-3.42. The results revealed that the effect of seasons, sites and their interaction on P was significant. Maximum Mg was observed at winter (264.31 mg/100g) while minimum at summer (174.94 mg/100g). Similarly maximum Mg was recorded at Mang site (233.48 mg/100g) while minimum at Jabri site (204.42 mg/100g). In case of interactions maximum Mg was observed at Mang site during winter (288.57 mg/100g) followed by Dabola site during winter (263.40 mg/100g). While minimum Mg was observed at Jabri site during summer (152.50 mg/100g).

Copper (Cu) mg/100g

The mean data of Cu for various seasons and sites is shown in Table-3.8, while their interaction is shown in Figure-3.43. The results revealed that the effect of seasons, sites and their interaction on Cu was significant. Maximum Cu was observed at winter (0.92 mg/100g) while minimum at summer (0.56 mg/100g). Similarly maximum Cu was recorded at Dabola site (0.86 mg/100g) while minimum at Dam and Jabri sites (0.69 mg/100g). In case of interactions maximum Cu was observed at Dabola site during winter (1.017 mg/100g) followed by Mang site during winter (0.097 mg/100g). While minimum Cu was observed at Mang site during summer (0.47 mg/100g).

Iron (Fe) mg/100g

The mean data of Fe for various seasons and sites is shown in Table-3.8. The results revealed that the effect of seasons on Fe was significant while the effect of sites and their interactions was non-significant. Maximum Fe was observed at winter (238.88 mg/100g) while minimum at summer (178.13 mg/100g).

Manganese (Mn) mg/100g

The mean data of Mn for various seasons and sites is shown in Table-3.8. The results revealed that the effect of seasons and sites on Mn was significant while the effect of their interactions was non-significant. Maximum Mn was observed at winter (6.03 mg/100g) while minimum at summer (3.58 mg/100g). Similarly maximum Mn was recorded at Jabri site (8.03 mg/100g) while minimum at Dabola site (2.78 mg/100g).

Zinc (Zn) mg/100g

The mean data of Zn for various seasons and sites is shown in Table-3.8, while their interaction is shown in Figure-3.44. The results revealed that the effect of seasons, sites and their interaction on Zn was significant. Maximum Zn was observed at inter (5.23 mg/100g) while minimum at summer (3.03 mg/100g). Similarly maximum Zn was recorded at Dam site (4.65 mg/100g) while minimum at Mang site (3.37 mg/100g). In case of interactions maximum Zn was observed at Dam site during winter (6.53 mg/100g) followed by Jabri site during winter (5.73 mg/100g). While minimum Zn was observed at Mang site during summer (2.70 mg/100g). Ajuga starts sprouting or its seed germinate naturally in May-July when conditions become favorable for germination. It initiate flowering in August to October and set seed in October to December. After first week of January no plants are seen in the natural vegetation.

The results can be summarized as the effect of seasons on these bio-chemical elements was highly significant. Maximum values were obtained at winter (October-November) and minimum at summer for all of the tested elements except Sodium and Calcium. The simplest explanation for this result can be that Ajuga matures at early winter season and hence the minerals accumulate in the plant parts like seed and roots. Similarly at early winter high moisture contents remain available

in the soil which causes higher nutrients uptake (Ramirez et al., 2006). The effect on all elements except Iron (Fe) was significant which demonstrate the diversity of soil and climatic condition among various sites of the Khanpur Valley.

It is evident from the results that early winter (last week of October) season is the best season for maximum medicinal and nutritional components in Ajuga at the cooler points of all the four sites of the Khanpur valley. Jabri site showed maximum results for some parameters and so it is pertinent that hilly and moist areas with humid climate are the natural habitats for Ajuga (Abbas, 2003).

Table-2. Effect of Different Seasons and Sites on Elemental analysis of *Ajuga bracteosa,* indigenous to Khanpur Valley, in sub-Himalayan Mountains of Pakistan.

Seasons	Sodium (Na)	Potassium (K)	Calcium (Ca)	Phosphorus (P)	Magnesium(Mg)	Copper (Cu)	Iron (Fe)	Manganese(Mn)	Zinc (Zn)
Summer	5.85a	162.79b	194.64a	138.13b	174.94b	0.56b	178.13b	3.58b	3.03b
Winter	4.24b	475.25a	127.66 b	196.73a	264.31a	0.92a	238.88a	6.03a	5.23a
LSD at α 0.05	0.319	40.153	14.847	7.312	10.535	0.049	12.592	0.524	0.366
Sites									
Dam	4.20c	284.33b	165.87a	171.63c	229.63a	0.69b	206.07	2.90c	4.65a
Dabola	4.18c	244.50b	159.80ab	186.43b	210.97b	0.86a	221.82	2.78c	4.15a
Jabri	5.19b	377.33a	197.95a	212.78a	204.42b	0.69b	208.10	8.03a	4.37a
Mang	6.60a	369.92a	120.98b	98.87d	233.48a	0.72b	198.03	5.52b	3.37b
LSD at α 0.05	0.579	79.277	40.288	12.165	14.843	0.102	ns	0.413	0.690
Interactions									
Seasons*Sites	*	*	*	Ns	*	*	ns	ns	*

Means followed by similar letter(s) in column do not differ significantly.
Ns = Non-Significant.
* = Significant at 5 % level of probability.

CONCLUSION

Four sites and two seasons were used to collect the Ajuga bracteosa samples (whole plants). Ajuga bracteosa revealed considerable effects on numerous bio-chemical compounds at different seasons and locations. During the summer, Ajuga bracteosa showed higher significant values for ash (34.13 percent) at Jabri, crude proteins (15.35 percent) at Dam, crude fats (10.30 percent) at Jabri, essential oil (2.48 percent) at Mang, NFES (46.17 percent) at Dabola during winter, NFES (186.28 percent) at Dam during summer, sodium (6.96mg/100 g) at Mang during winter, potassium (559.67mg/100g). Throughout the last week of October, Ajuga bracteosa gave higher value after biochemical assessment.

CONFLICTS OF INTEREST

All authors have read and approved the final manuscript. The authors declare no conflict of interest in submission of this manuscript.

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