Chemical constituents of *Euphorbia hirta* an indigenous medicinal herb of Khyber Pakhtunkhwa

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ABSTRACT

The samples (leaves) were collected from *Euphorbia hirta* found at their natural habitat of 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 upon various biochemical substances. *Euphorbia hirta* gave higher significant contents for ash (17.95%) at Jabri during summer, crude proteins (15.42%) at Jabri during summer, crude fibers (12.78%) at Mang during winter, crude fats (10.64%) at Jabri during summer, essential oil (1.94%) at Dabola during summer, NFES (59.49%) at Dam during winter, NFEE (198.69%) at Jabri during summer, calcium (199.17mg/100g) at Dam during summer, phosphorus (185.37mg/100g) at Jabri during winter. *Euphorbia hirta* gave higher values for biochemical during the last week of October.

Key words: *Euphorbia hirta* , Himalayan Mountains, Khanpur Valley, biochemical, Jabri

INTRODUCTION

Medicinal plants are staging a comeback and an herbal 'renaissance' is happening worldwide (Chatterjee et al., 2020). The herbal medicines of today symbolize safety in contrast to the synthetics that are considered unsafe for humans and the environment (Fortunate et al., 2019). Plants are natural resources that contribute significantly to human nutrition and medicine (Pieroni et al., 2021). Historically, plants have been harvested or collected from nature by men and women since the birth of life, for their wellbeing (Monocyte et al., 2020). There are plant species which are used in the preparation of functional food, which serves both the purposes of nutritional as well as medicinal requirements (Waisundara, 2020). Such dual-purpose species are of immense importance and need comprehensive investigation in the natural flora of ecologically diverse habitats for their existence (Deák et al., 2021).

Phyto-medicines are also being used on a large scale in Western Europe (Jain and Mathur, 2020). Recently, the US government has established the "Office of Alternative Medicine" at the National Institute of Health at Bethesda. Ayurveda, a system of herbal medicine in India, Sri Lanka, and South-East Asia, has more than 8000 traditional uses while 35,000-70,000 plant species are medicinal (Selvadurai, 2011). China has demonstrated the best use of traditional medicine in providing health care (Boukhatem and Setzer, 2020). China has pharmacologically validated and improved many phyto-pharmaceuticals and eventually integrated them into modern medicines (Salem et al, 2021).

Human body needs essential nutrients, biochemical compounds (proteins, fats, carbohydrates, etc), major minerals (Ca, P, K, Na, Mg) and trace elements (Fe, Zn, Cu, Mn, and others), which are essential for general health, growth and reproduction (Freisinge and Sigel, 2019). We must bear in mind that the consequences of essential trace mineral deficiency may be just as severe as those of a deficiency of a major essential mineral. Many elements are associated with one another in maintaining our normal growth and health. The medicinal plants may prove to be an effective treatment for a variety of common and complex ailments, as well as a source of some of the essential nutrients required for human nutrition (Kumar et al., 2021).

Pakistan is very rich in species diversity and its various ecological zones are unique in their ethnobotanical and traditional healthcare systems. The Khanpur valley in the sub-Himalayan mountains of Pakistan was selected to study the biochemical attributes of *Adhatoda vasica* with the aim of achieving the following objectives: To find out the comparative suitability of seasons and sites for the best harvest of biochemical attributes of *Adhatoda vasica* of Khanpur valley and to elaborate on the biochemical prospects of *Adhatoda vasica*, for possible use as a source of food supplement.

MATERIAL AND METHODS

An impact of different seasons and sites on the nutritional and biochemical attributes of *Adhatoda vasica* of the Khanpur valley was conducted during 2012-2013. Mature leaves of *Adhatoda vasica* 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 December.

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 laboratory grinder. The samples in the grinded form were then analyzed for the following biochemical attributes, 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 and sites on moisture percentage was significant while the effect of their interactions was non-significant. Summer had the highest moisture percentage

(87.31%), while winter had the lowest (85.40%). Similarly, the Dam site had the highest moisture percentage (87.97%), while the Mang site had the lowest (83.94%).

Dry Matter (%)

Table 1 displays the mean dry matter percentage data for various seasons and locations. The results revealed that the effect of seasons and sites on dry matter % was significant while the effect of their interactions was non-significant. Winter had the highest dry matter percent (14.60%) and summer had the lowest (12.69%). Similarly, the Mang site had the highest moisture percent (16.06%), while the Dam site had the lowest (12.02%).

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 / minerals was significant. Summer had the highest ash/minerals% (15.99%), and winter had the lowest (14.55%). Similarly, maximum ash/mineral content was recorded at the Jabri site (16.62%) while the minimum was at the Dam site (13.84%). In the case of interactions, the maximum ash content was recorded at the Jabri site during summer (17.95%) while the minimum was observed at the Dam site during winter (13.16%).

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, sites, and their interactions on crude proteins was significant. Summer had the highest crude protein percentage (13.11%), while winter had the lowest (11.18%). Similarly, the Jabri site had the highest crude protein percent (13.89%), while the Mang site had the lowest (10.40%). In the case of interactions, the maximum crude protein percentage was recorded at the Jabri site during summer (15.42%), followed by the Dam site during summer (15.12%), while the minimum was observed at the Mang site during winter (10.037%).

Crude Fibers (%)

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, sites, and their interactions on crude fibres was significant. Summer had the highest crude fibers (10.90%), while winter had the lowest (8.61%). Similarly, the highest crude fibre percent was found at the Jabri site (10.92%), while the lowest was found at the Dam site (8.44%). In the case of interactions, the maximum crude fibres % was recorded at the Mang site during summer (12.78%), followed by the Jabri site during summer (11.55%), while the minimum of it was observed at the Dam site during winter (6.51%).

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, sites, and their interactions on crude fat content was significant. Summer had the highest crude fat% (9.14%) and winter had the lowest (7.98%). Similarly, the highest crude fat% was found at the Jabri site (9.71%), while the lowest was found at the Dabola site (7.45%). In the case of interactions, the maximum crude fat percentage was recorded at the Jabri site during summer (10.64%), followed by the Dam site during summer (10.12%), while the minimum was observed at the Mang site during winter (7.15%).

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 sites on essential oil production was non-significant while the effect of seasons and interactions was significant. Summer had the highest essential oil content (1.80%), while winter had the lowest (1.55%). In the case of interactions, the maximum essential oil content was recorded at the Dabola site during summer (1.94%), followed by the Dam site during summer (1.89%), while a minimum of it was observed at the Mang site during winter (1.48%).

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% (57.68%) and summer had the lowest (50.86%). Similarly, the maximum NFES % was recorded at the Dabola site (58.55%) while the minimum was at the Jabri site (49.15%). In the case of interactions, the maximum NFES % was recorded at Dam site during winter (59.49%), followed by Dabola site during winter (59.30%), while the minimum was observed at Jabri site during summer (44.44%).

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 (182.85), while winter had the lowest (171.79). Similarly, the highest NFEE was measured at the Dam site (190.83), while the lowest was measured at the Dabola site (168.09). In the case of interactions, the highest NFEE was observed at the Jabri site during the summer (198.69), followed by the Dam site during the summer (198.38), and the lowest at the Mang site during the winter (160.44).

Table-1. Effect of Different Seasons and Sites on Proximate analysis of *Euphorbia hirta*, 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 **Winter**	87.31a 85.40b	12.69b 14.60a	15.99a 14.55b	13.11a 11.18b	10.90a 8.61b	9.14a 7.98b	1.80a 1.55b	50.86b 57.68a	182.85a 171.79b
LSD at α 0.05	0.992	0.992	0.459	0.456	0.576	0.329	0.097	1.018	3.368
Sites									
Dam	87.97a	12.02c	13.84b	13.68a	8.44b	9.36a	1.71	54.68b	190.83a
Dabola	85.88b	14.12b	14.08b	10.61b	9.04b	7.71b	1.73	58.55a	168.09b
Jabri	87.62ab	12.38bc	16.62a	13.89a	10.62a	9.71a	1.67	49.15c	189.26a
Mang	83.94c	16.06a	16.55a	10.40b	10.92a	7.45b	1.59	54.69b	161.10c
LSD at α 0.05	1.760	1.760	1.197	0.793	1.320	0.670	ns	2.710	5.301
Interactions									
Seasons*Sites	ns	Ns	*	*	*	*	*	*	*

Means followed by similar letter(s) in column do not differ significantly.

ns = Non Significant.

* = Significant at 5 % level of probability.

The results revealed that the effect of seasons on all parameters was highly significant. In summer, maximum values have been found for moisture, crude protein, crude fats, crude fibers, minerals/ashes, essential oils, and NFEE, while in winter, maximum values were found for dry matter and NFES. This seasonal effect was reasonably due to the plant's growth habits and environmental conduciveness (Guo et al., 2020). *E. hirta* sprouts during late spring and summer, which is the metabolically most active period of its growth (Al-Snafi, 2018); so most of the minerals are absorbed during summer. Another reason for high moisture, essential nutrients, and mineral content during summer was high soil moisture content (Säurich et al., 2019) due to more rain fall and a high rate of transpiration due to high temperatures (Slot et al., 2021). So, when more growth took place, more dry biomass was also produced. E. hirta plants sprout naturally in May-July and mature in September-December. Higher percentages of moisture, CP, CF, and NFEE during the summer at these sites could be attributed to the plant's rapid growth stage and the availability of more moisture during the summer at most of the sites (East and Sankey, 2020). Higher winter values for dry matter and NFES are undoubtedly related to low moisture and other nutrient accumulation in plant tissues as a result of environmental and physiological stresses (Toscano et al., 2019).

The effect of sites on all the parameters was significant, except on essential oil. Maximum values were found for moisture and NFEE at the Dam site, while maximum values for NFES were found at the Dabola site. This might be correlated with the availability of high moisture content in the soil and its water holding capacity at the dam site (Hussain et al., 2020). At Jabri, maximum values were recorded for minerals, crude proteins, and crude fats, while at Mang, maximum values were found for dry matter and crude fibers. High proteins might be due to the high nitrogen content in the soil (Visioli et al., 2018) available during summer at the site. High dry matter, ashes, and crude fibres might be due to saline conditions of the soil, as most of the plant species contain these compounds at higher levels at maturity when present in a saline environment (Seleiman and Kheir, 2018). At the Dabola site during the summer, the analysis showed the maximum value for essential oils.

ELEMENTOLOGY

Sodium (Na) mg/100g

The mean data of Na for various seasons and sites is shown in Table-2. The results revealed that the effect of seasons and sites on Na was significant while the effect of their interaction was non-significant. Winter had the highest concentration of Na (2.87 mg/100g) and summer had the lowest (2.61 mg/100g). Similarly, the maximum Na concentration was found at the Dam site (3.34 mg/100g) and the lowest at the Dabola site (2.21 mg/100g).

Potassium (K) mg/100g

The mean data of K for various seasons and sites is shown in Table-2. The results revealed that the effect of sites on K was significant while the effect of seasons and interactions was non-significant. Maximum K was observed at the Dam site (81.74 mg/100g), while minimum K was at the Mang site (68.96 mg/100g).

Calcium (Ca) mg/100g

The mean data of Ca for various seasons and sites is shown in Table 2, while their interaction is shown in Figure 8. The results revealed that the effect of seasons, sites, and their interaction on Ca was significant. Ca levels were highest in winter (152.46 mg/100g) and lowest in summer (126.74 mg/100g). Similarly, maximum Ca was recorded at the Dam site (192.51 mg/100g) while the minimum was at the Jabri site (117.63 mg/100g). In the case of interactions, maximum Ca was observed at the dam site during summer (199.17 mg/100g), followed by the dam site during winter (185.85 mg/100g). While minimum Ca was observed at the Dabola site during the summer (79.17 mg/100g),

Phosphorus (P) mg/100g

Table 2 displays the mean phosphorus data for various seasons and sites, while Figure 9 depicts their interaction. The results revealed that the effect of seasons, sites, and their interaction on P was significant. P levels were highest in the winter (143.74 mg/100g) and summer (89.27 mg/100g). Similarly, maximum P was recorded at the Jabri site (153.92 mg/100g) while the minimum was at the Mang site (59.75 mg/100g). In the case of interactions, maximum P was observed at the Jabri site during winter (185.37 mg/100g), followed by the Dam site during winter (176.69 mg/100g). While minimum P was observed at Mang site during summer (44.07 mg/100g)

Magnesium (Mg) mg/100g

The mean data of Mg for various seasons and sites is shown in Table 2. The results revealed that the effect of seasons and sites on Fe was significant while the effect of their interactions was non-significant. Mg levels were highest in the summer (225.65 mg/100g) and lowest in the winter (188.50 mg/100g). Similarly, maximum Mg was recorded at the Jabri site (282.47 mg/100g) while the minimum was at the Dam site (225.48 mg/100g).

Copper (Cu) mg/100g

The mean data of Cu for various seasons and sites is shown in Table-2. The results revealed that the effect of seasons and sites on Fe was significant while the effect of their interactions was non-significant. Cu levels were highest in the winter (2.37 mg/100g) and summer (1.45 mg/100g). Similarly, maximum Cu was recorded at Mang site (2.34 mg/100g) while the minimum was at the Dam site (1.49 mg/100g).

Iron (Fe) mg/100g

The mean data of Fe for various seasons and sites is shown in Table-2. The results revealed that the effect of seasons and sites on Fe was significant while the effect of their interactions was non-significant. Winter had the highest Fe concentration (267.98 mg/100g), while summer had the lowest (206.57 mg/100g). Similarly, maximum Fe was recorded at the Mang site (289.00 mg/100g) and the minimum at the Dabola site (190.64 mg/100g).

Manganese (Mn) mg/100g

The mean data of Mn for various seasons and sites is shown in Table-2. The results revealed that the effect of seasons and sites on Mn was significant while the effect of their interactions was non-significant. Winter had the highest Mn concentration (29.43 mg/100g), while summer had the lowest (20.40 mg/100g). Similarly, maximum Mn was recorded at the Jabri site (27.88 mg/100g), while the minimum was at the Dam site (21.32 mg/100g).

Zinc (Zn) mg/100g

The mean data of Zn for various seasons and sites is shown in Table 2. The results revealed that the effect of seasons on Zn was significant while that of sites and their interactions was non-significant. Winter had the highest concentration of Zn (9.07 mg/100g), while summer had the lowest (5.99 mg/100g).

The results can be summarized as the effect of seasons on these biochemical elements was highly significant. Maximum values were obtained in winter (October-November) and minimum values in summer for all of the tested elements except magnesium. The simplest explanation for this result can be that *E. hirta* matures in the early winter season and hence the minerals accumulate in the plant parts like seed and roots. Lin et al., 2021 discovered the same thing: high moisture contents remain available in the soil in early winter, resulting in higher nutrient uptake.

It is evident from the results that the early winter (last week of October) season is the best season for maximum medicinal and nutritional components in *E. hirta* at Dam and Mang sites, lower Khanpur valley. In the plain, shady areas with a humid climate are the natural habitats for *E. hirta* (Islam et al, 2021), which proved true for Mang and Dam sites.

Table-2. Effect of Different Seasons and Sites on Elemental analysis of *Euphorbia hirta*, 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 **Winter**	2.61b 2.87a	71.23 75.19	126.74 b 152.46a	89.27b 143.74a	225.65a 188.50b	1.45b 2.37a	206.57b 267.98a	20.40b 29.43a	5.99b 9.07a
LSD at α 0.05	0.251	Ns	5.019	8.186	22.092	0.214	17.043	1.744	0.568
Sites									
Dam **Dabola** **Jabri** **Mang**	3.34a 2.21c 3.04b 2.36c	81.70a 70.35b 71.83b 68.96b	192.51a 126.94b 117.63b 121.33b	146.06a 106.31b 153.92a 59.75c	225.48b 255.78ab 282.47a 264.57a	1.49c 1.90b 1.91b 2.34a	232.33b 190.64c 237.12b 289.00a	21.32b 22.74b 27.88a 27.73a	7.61 8.12 7.44 6.95
LSD at α 0.05	0.206	8.075	9.673	9.056	35.291	0.401	27.710	2.519	ns
Interactions									
Seasons*Sites	ns	Ns	*	*	ns	ns	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

The Euphorbia hirta gave higher levels of ash (17.95%), crude proteins (15.42%), crude fibres (12.78%), crude fats (10.64%), essential oil (1.94%), and crude fats (10.64%) at Jabri during summer, as well as NFES (59.49%) at Dam during winter and NFEE (198.69%) at Jabri during summer. At Dam last week in October, the tree also produced higher levels of calcium (199.17mg/100g). Euphorbia hirta produces higher levels of biochemical.

CONFLICT OF INTEREST

The authors have stated that they have no conflicts of interest

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