Evaluation of *Viola betonicifolia* for its nutrition value

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Abstract: In the present study, *Viola betonicifolia* (whole plant) and solvent fractions obtained thereof were evaluated for various micronutrients such as lead, copper, chromium, iron, nickel, zinc, cadmium, cobalt and macronutrients such as sodium, potassium and calcium using atomic absorption spectrophotometer and flame photometry respectively. It was observed that cobalt and cadmium were not detectible in tested samples while remaining nutrients were present in variable concentrations. The concentrations of all metals were compared with the recommended limits for plants and the daily consumption of all the nutrients were calculated on the basis of dose (15 g/70 kg/day or 214 mg/Kg body weight) prescribed by hakims in their practice. The concentration of chromium was far beyond the recommended limits in almost all the samples. Proximate analysis was carried out in the powdered form of the plant. *Viola betonicifolia* appeared as a good source of life essential nutrients like fats (18.70%), proteins (15.70%), carbohydrates (21.42%), fiber (39.01%) and vitamin C (150 mg/100 g). In conclusion, *Viola betonicifolia* is an excellent source of various micro and macronutrients for human being and can be used safely as a nutritional supplement.

Keywords: *Viola betonicifolia*, macro and micro nutrients, vitamin C and proximate analysis

INTRODUCTION

Like other developing countries of the world, practice of herbal drugs is very common in Pakistan (Saeed *et al*., 2010). Acceptance of herbal drugs in the country can be judged from the fact that the government has allowed the practice of herbal medicines in the Public Hospitals besides thousands of private herbal practitioners. These drugs have substantial share in drug market due to affordable prices and easy access. The most appealing aspect of herbal products is the belief that they are safe and harmless because of their natural origin. In addition to the use of herbal products as therapeutic agents a large number of populations consume the herbs as a food supplement/nutritional supplement. So screening of herbs for their nutrients contents can help the public as well as the herbal medicine practitioners in selection of plants with rich nutrition values.

Viola betonicifolia (Banafsha or Banosha) belongs to the genus viola and family Violaceace. It is widely distributed in Pakistan, Australia, Sri Lanka and India. In Pakistan it is mostly found in Swat, Hazara, and Dir. In the traditional system of medicine, it is mostly used as astringent, diaphoretic, antipyretic, anticancer, purgative, also used for cough, epilepsy, nervous disorders, sinusitis, skin and blood disorders and pharyngitis (lyas and Iqbal 2005, Tiwari *et al.*, 2010, Husain *et al*., 2008, Bhatt and Negi 2006). There is no scientific study on nutritional values of *Viola betonicifolia*. Therefore we focused on the micro and macronutrients analysis and the proximate composition to evaluate the nutritional potentials of the plant in relation to its folk uses.

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MATERIALS AND METHODS

Chemicals and instruments

Nitric acid (Sigma), perchloric acid (Sigma), Deionizer (Elga, B 114), Whatman 42 filter paper, glass wares, Hotplate (Lab. Tech), Fuming hood, transparent bottles, Flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi 2000) and flame photometer (Jenway PFP7, UK). All chemicals used in the study were of analytical reagent grade.

Plant materials

Whole plant of *V. betonicifolia* (VB) was collected from Swat, Khyber Pakhtunkhawa in April 2010. Plant specimen was identified by Prof. Dr. Muhammad Ibrar, Department of Botany, University of Peshawar and specimen was deposited there in the herbarium under voucher number 6410/Bot. The collected whole plant (12 kg) was air dried and powdered. The powder was extracted by maceration with methanol at room temperature for 14 days with occasional shaking. The methanolic extract was filtered and concentrated by rotary evaporator at low temperature (45^{o}C). The methanolic extract was dissolved in distilled water and further fractioned with chloroform, *n*-hexane, ethyl acetate, butanol and aqueous fractions.

Sample preparations for metal analysis

One gram sample was taken from each plant part/extract and 10 ml concentrated HNO_3 (67%) was added. All the solutions were kept overnight. After over night soaking 4 ml of $HClO_4$ (67%) was added and the solution was heated on hot plate at 60^{o}C until a small suspension remained in the flask. Deionized water was added and filtered using Whatman (# 42). The final volume was

made up to 100 ml (Saeed *et al.*, 2010a, Saeed *et al.*, 2010b). The samples were then analyzed in triplicate using flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi 2000) for determination of micronutrients and flame photometer (Jenway PFP7, UK) for determination of macronutrients.

Proximate composition

The proximate composition i.e. moisture content, ash, crude protein, fat and crude fiber were determined by the standard methods of AOAC (2003) carbohydrates were determined by difference method and vitamin C by titration method.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Graph pad prism 5 to find the mean and standard deviation (SD).

RESULTS

Micronutrients

Lead was found with the permissible limit in all solvent fractions and powdered plant parts. The highest concentration was found in butanol (0.75 µg/g) followed by *n*-hexane fraction (0.55 µg/g) as shown in table 1, while the whole plant powder accumulated 7.23 µg/g lead as shown in table 3. Copper was present in solvent fraction in concentration range of 7.00 - 47.25 µg/g and in the plant parts it was observed in the concentration range of 15.56 -80.45 µg/g. Chromium was found in the concentration range of 34.50 - 151.00 µg/g in solvent fractions and 20.00 - 67.00 µg/g in plant parts as shown in table 1 and table 3 respectively. The highest concentration of chromium was found in *n*-hexane fraction (151.00µg/g) and root (67.00µg/g) of the plant. The maximum concentration of iron was found in *n*-hexane fraction (500.00 µg/g) followed by chloroform (288 µg/g) and ethyl acetate fractions (280.75 µg/g) while in case of plant parts the highest amount was observed in petioles (340 µg/g) followed by leaves (320 µg/g) and roots (295 µg/g) and in the literature it has been reported that iron is mostly present in the aerial parts of the plants like leaves (Maier and Cattani 1995). Manganese was found in the concentration range of 4.00-54.25 µg/g in solvents fractions and 3-60 µg/g in case of plant parts, the recommended limit of manganese for herbs is 200 µg/g (Srivastava *et al.*, 2006). The permissible limit of nickel for herbs is 1.5 µg/g (Srivastava *et al.*, 2006). Nickel was present in solvent fractions in the range of 0.34-1.15 µg/g and 0.50-1.20 µg/g in plants parts, and was not detectible in *n*-hexane, ethyl acetate fractions, leaves and roots. While in remaining samples it was present in recommended limit. The concentration range of zinc was 12-37µg/g in solvent fractions and 10-50 µg/g in plant parts. The permissible limit of zinc was 50 µg/g for plants (Srivastava *et al.*, 2006). So almost all samples were within permissible limits.

Macronutrients

The results of macronutrients in solvent fractions as well as the plant parts are depicted in tables 1 and 3. Sodium was present in concentration range of 57.50-267.50 µg/g in solvent fractions and 124-723 µg/g in plant parts. The highest concentration was observed in the whole powdered plant (723 µg/g) exceeding the permissible limit i.e. 44-614 µg/g (Ajasa *et al*., 2004). Potassium was present in the maximum concentration among all the nutrients i.e. in the concentration range of 237.50-3295 µg/g in solvent fractions and 170-890 µg/g in plants parts. Calcium was found in the concentration range of 34-200 µg/g in solvent fractions and 132-500 µg/g in plants parts as shown in tables 1 and 3 respectively.

Proximate analysis

Proximate analysis was carried out in the powdered form of the plant. *Viola betonicifolia* appeared as a good source of life essential nutrients like fats (18.70%), proteins (15.70%), carbohydrates (21.42%), fiber (39.01%) and vitamin C (150 mg/100 g).

DISCUSSION

Micronutrients

In the present study various micro nutrients such as lead, copper, chromium, iron, nickel, zinc, cadmium, cobalt were analysed in all the plant parts and all the solvent fractions. Cobalt and cadmium were not detectible in plant parts as well as in solvent fractions. Local hakims use *Viola betonicifolia* at the dose of 15 g/70 kg/day or 214 mg/kg body weight/day, therefore we calculated the daily consumption of all the nutrients on the basis of the above conventional adult dose. The daily consumption of all macro and micronutrients are given in table 2 and table 4. The plant is safe for consumption as we have tested it in mice at high dose of 2 g/kg body weight.

Lead status

Lead is one of the most toxic metals among micronutrients. It is ingested by human beings by various means like contaminated food, use of lead contaminated plants and from environment. The ingestion of lead in high concentration causes its accumulation in body producing various toxic effects like renal, digestive and cardiovascular problems (Jalili and Azizkhani, 2009) but it has no known biological function in the body (Venkatesh, 2004). The permissible limit of lead for herbal material according to WHO is 10 µg/g (WHO, 1999) while the daily recommended consumption of lead for 70kg adult is 250 µg/day (FAO/WHO, 1993). Butanol fraction provides 11.25µg/day and the whole plant powder providing 108.45 µg/day lead at the above mention dose. Both of these concentrations are non toxic in comparison with the recommended limit and the plant can be used safely.

Table 1: Micronutrients (µg/g) and macronutrients (µg/g) in various solvent fractions of VB

Micronutrients							
Solvent fraction	Pb	Cu	Cr	Fe	Mn	Ni	Zn
Methanolic	0.25±0.22	17.25±0.20	52.00±0.03	154.5±13	13.00±0.22	1.15±0.22	20.00±0.12
Butanol	0.75±0.07	8.25±0.22	50.75±0.21	221.25 ±19	4.00±0.32	1.10±0.11	34.70±0.22
Chloroform	0.50±0.11	16.00±0.11	55.25±0.34	288.00±22	5.00±0.22	0.34±0.55	12.40±0.14
Aqueous	0.25±0.12	47.25±0.32	34.50±0.12	216.25±11	25.00±0.34	1.45±0.16	37.00±15
n-hexane	0.55±0.22	17.75 ±0.10	151.00±0.20	500.00±21	54.25±0.12	-	23.60±0.13
Ethylacetate	0.20±0.23	7.00±0.02	52.75±0.14	280.75±10	5.00±0.22	-	26.00±0.16

Macronutrients			
Solvent fraction	Na	K	Ca
Methanolic	175.00±0.22	1005.00±0.01	200.00±0.17
Butanol	267.50±0.21	647.50±0.11	150.00±0.15
Chloroform	210.00±0.10	1261.00±0.10	123.00±0.12
Aqueous	125.00±0.14	3295.00±0.19	35.00± 15
n-hexane	57.50±0.22	237.50±0.02	-
Ethyl acetate	160.00±0.14	620.00±0.23	34.00±0.14

Data are expressed as the Mean ± SD (n = 3)

Table 2: Daily consumption of micronutrients and macronutrients in µg at the dose of 15 g/70 kg body weight/day

Micronutrients							
Solvent fraction	Pb	Cu	Cr	Fe	Mn	Ni	Zn
Methanolic	3.75	258.75	780.00	2317.50	195.00	17.25	300.00
Butanol	11.25	123.75	761.25	3318.75	60.00	25.50	520.50
Chloroform	7.50	240.00	828.75	4320.00	75.00	5.10	186.00
Aqueous	3.75	708.75	517.50	3243.75	375.00	21.75	555.00
Hexane	8.25	266.25	2265.00	7500.00	813.75	-	354.00
Ethylacetate	3.00	105.00	791.25	4211.25	75.00	-	390.00

Macronutrients			
Solvent fraction	Na	K	Ca
Methanolic	2625.00	15075.00	3000.00
Butanol	4012.50	9712.50	2250.00
Chloroform	3150.00	18915.00	1845.00
Aqueous	1875.00	49425.00	525.00
n-hexane	862.50	3562.5.00	-
Ethyl acetate	2400.00	9300.00	510.00

Copper status

Copper is an essential trace metal found in humans and animals. It is involved in oxidation-reduction reactions, energy production, connective tissue formation, iron metabolism, neurotransmitter synthesis and metabolism of neurotransmitters (Amin *et al.,* 2003). The quantity of copper in most of the samples exceeded its permissible limit i.e. 10 µg/g (Srivastava *et al.,* 2006). The highest concentration was observed in the aqueous fraction (47.25µg/g) and in whole powdered plant (80.45µg/g). The recommended daily dose of copper is 900µg/day (IOM, 2001, ATSDR, 2008). It is clear from table 2 and table 4 that almost all the samples are within permissible limit except the whole plant powder.

Chromium status

The permissible limit of chromium for the herbal materials is 1.5µg/g (Srivastava *et al*., 2006), while the daily recommended intake limit of chromium is 11-25 µg/day for child and 30-35 µg/day for adult (IOM, 2001, ATSDR, 2008). The concentration of chromium was found to be far beyond the recommended limits. The possible reason of the high concentration of the chromium accumulations is the use of fertilizers in the crops which were grown near to this plant. The presence of fertilizer containing phosphorus makes easier the accumulation of chromium easier in the plants. It has been reported that chromium primarily moves in the xylem of the plants (Golovatyj *et al.,* 1999) and maximum amount of chromium is always contained in roots and a minimum in

Table 3: Micronutrients (µg/g) and macronutrients (µg/g) in various parts of VB

Micronutrients							
Plant part	Pb	Cu	Cr	Fe	Mn	Ni	Zn
Leaves	6.45± 0.21	23.90 ± 0.22	40.00± 0.15	320.00±0.33	14.00±0.11	-	10.00±0.15
Petioles	5.63± 0.23	15.56 ± 0.11	20.00± 0.17	340.00±0.20	9.00 ± 0.22	0.50±0.11	34.00±0.26
Root	-	42.67 ± 0.26	67.00± 0.20	295.00±0.19	3.00 ± 0.02	-	28.00±0.02
Flower	1.34± 0.26	40.89 ± 0.27	50.00± 0.10	220.00±0.31	27.00±0.25	1.00±0.17	36.00±0.22
Whole plant	7.23± 0.32	80.45 ± 0.22	35.00± 0.19	245.00±0.45	60.00±0.22	1.20±0.30	50.00±0.25

Macronutrients			
Plant part	Na	K	Ca
Leaves	156.00 ± 0.12	890.00 ± 0.26	132.00 ± 0.12
Petioles	623.00 ± 0.32	325.00 ± 0.11	256.00 ± 0.24
Root	214.00 ± 0.38	235.00 ± 0.40	134.00 ± 0.11
Flower	124.00 ± 0.44	170.00 ± 0.56	200.00 ± 0.38
Whole plant	723.00 ± 0.51	191.00 ± 0.122	500.00 ± 0.32

Data are expressed as the Mean ± SD (n = 3)

Table 4: Daily consumption of Micronutrients and Macronutrients in µg at the dose of 15 g/70 kg/day.

Micronutrients							
Plant part	Pb	Cu	Cr	Fe	Mn	Ni	Zn
Leaves	96.75	358.5	600.00	4800.00	210.00	-	150.00
Petioles	84.45	233.40	300.00	5100.00	135.00	7.50	510.00
Root	-	640.05	1005.00	4425.00	45.00	-	420.00
Flower	20.10	613.35	750.00	3300.00	405.00	15.00	540.00
Whole plant	108.45	1206.75	2025.00	3675.00	900.00	18.00	750.00

Macronutrients			
Plant part	Na	K	Ca
Leaves	2344.00	13350.00	1980.00
Petioles	9345.00	4875.00	3840.00
Root	3210.00	3525.00	2010.00
Flower	1860.00	2550.00	3000.00
Whole plant	10845.00	2865.00	7500.00

the vegetative and reproductive organs i.e. in beans, only 0.1% of the chromium was found in the seeds and 98% was found in roots (Huffman and Allaway, 1973). The reason of the high accumulation of chromium in roots of the plants could be the immobilization of chromium in the vacuoles of the root cells (Shankar *et al.,* 2004).

Iron status

Iron is the need of human beings at all stages but its need is very high in pregnancy and in menstrual cycle. Therefore, searching for a natural source of iron is the demand of the modern era because most of the synthetic iron has lots of side effects. It is a common perception of the people that natural products are safe with lesser side effects. Therefore, a large population will appreciate the availability of natural iron. The recommended concentration of iron for plants is 36-241 µg/g (Ajasa *et al.*, 2004). The mean dietary intake of iron is 16 to 18 mg/day for men and 12 mg/day for women (IOM, 2001). The highest daily consumption of iron at dose of 15g/70 kg body weight/day was observed in *n*-hexane 7500.00 µg/g and in petioles 5100 µg/day. It means that *viola betonicifolia* is the richest source of iron and can be used safely as iron supplement.

Manganese status

It is clear from tables 1 and 3 that all samples accumulate less concentration of manganese. The daily consumption of manganese for these samples represents that this plant is a poor source of manganese, as the daily recommended allowance for manganese is 2.3 mg/day (IOM, 2001).

Nickel status

Nickel is naturally present in abundance on the earth. Therefore, its nutritional deficiency is rare in all types of food. The toxic effects of nickel include skin allergies, lung fibrosis, kidney and cardiovascular system poisoning (Denkhaus and Salnikow, 2002). Nickel is one of the toxic micronutrients and causes toxic effects at higher concentrations. The recommended daily allowance of

nickel is 35µg/day and all the samples were found to be within permissible limit. The daily consumption of nickel for all the samples was within permissible limit i.e. below 35µg/day. So the plant under investigation is a rich source of nickel with non toxic concentrations.

Zinc status

A lot of synthetic zinc supplements are available in the market like Surbex-Z (by Abbott Pvt., Ltd.) but still a large number of populations are seeking for natural sources of zinc supplements. Zinc is a very important micronutrient as more than 300 body enzymes are zinc dependent. The richest source of zinc was the whole powdered plant (50 µg/g) followed by aqueous fraction (37.00 µg/g). Median intake of zinc from food in the United States is approximately 9 mg/day for women and 14 mg/day for men. The rich source of zinc was the whole powdered plant providing 750.00µg/day at above mentioned dose.

Macronutrients

Sodium status

The recommended daily intake of sodium is 1-3.8 mg/day (IOM, 2001). The daily consumption dose range of sodium at mentioned dose is about 862.50-3150.00 µg/day and 1860-10845 µg/day for the solvent fractions and plant parts respectively. It means that the plant is a rich source of sodium.

Potassium status

Potassium is most abundantly present in the plant and its permissible limit is 6380-36600 µg/g (Ajasa *et al*., 2004). The richest source of potassium is the solvent fraction and especially, the aqueous fraction. The average intake of Potassium is 2300 mg/day for adult women and 3100 mg/day for adult men (IOM, 2001)

Calcium status

Calcium is one the most important macronutrients, which plays a very significant role in skeletal mineralization, neuromuscular conduction, blood coagulation, maintenance of normal tone and excitability of skeletal and cardiac muscles, stimulated secretion of exocrine glands and preservation of cell membrane integrity and permeability, particularly in terms of sodium and potassium exchange. The recommended calcium limit in plants is 2610-51340 µg/g (Ajasa *et al.*, 2004). So all the samples had accumulated a poor quantity of calcium. However, the plant parts contain higher amount of calcium than the solvent fractions. The highest concentration was observed in whole powdered plant followed by petioles. According to the Food and Nutrition Board, the recommended daily intake of calcium is 1000 mg/day. The daily consumption of calcium was calculated for all the samples as shown in tables 2 and 3. It was found that *Viola betonicifolia* is a rich source of natural calcium.

Proximate composition

The proximate composition included percent ash value, moisture, fats, proteins, carbohydrates, fibers and vitamin C. Carbohydrates percentage was determined by subtracting the sum of moisture, fats, proteins and fibers from 100. All these parameters are the basic components of a balanced diet. The results of proximate analysis are shown in table 5. Ash value indicates the presence of inorganic components. The moisture content is 5.20% which is very helpful in prolonging the shelf life of the crude drug, as the microbial growth increases with increased moisture content and ultimately causes the destruction of drug. Fats are also known as triglycerides in medical literature. They are found in nuts, seeds and adipose tissues of animals. Fats were observed to be about 18.7% of the proximate composition of *Viola betonicifolia*. A large number cyclopeptides (containing amino acids) have been isolated from the family violaceae (Herrmann *et al.*, 2008). It means that this family is a rich source of proteins and amino acids. About 50% of the cell interior is composed of proteins, showing the importance of proteins in living organisms. The percent amount of fats in our results was 15.70%. Carbohydrates are the most abundant bio-molecules on the earth and in human beings they form about 1% of the body mass. In our results we found 21.42% carbohydrates. Mostly carbohydrates are obtained from plants sources. In addition to serve as an energy source in the form of ATP, they perform a lot of significant vital functions in the human body. Crude fibers are the important component of diet and in our results they were found to be about 39.01%. Fiber absorbs bulky amounts of water in the intestines, and makes stools softer and easier to pass. Anyone taking a high-fiber diet will observe the difference in stool bulk and alleviate constipation and hemorrhoids. The quantity of vitamin C was about 150.12 mg/100g.

Table 5: Proximate composition of VB

Content	Percentage composition
Ash	9.80± 0.32
Moisture	5.20± 0.24
Fats	18.70± 0.19
Proteins	15.75± 0.33
Fiber	39.02± 0.27
Carbohydrate	21.42± 0.14
Vitamin - C	150.12± 0.35 mg/100g

Data are expressed as the Mean ± SD (n = 3)

CONCLUSION

In conclusion, *Viola betonicifolia* is an excellent source of various micro and macronutrients for human being and can be used safely as a nutritional supplement. Besides the micro and macronutrients the plants is also a rich

source of proteins, fibers, carbohydrates and vitamin C. So it is recommended that this plant should be used as a tonic and energizer.

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