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MINERAL PROFILE OF DIFFERENT MEDICINAL PLANTS AND THEIR QUANTITATIVE ANALYSES COLLECTED FROM NORTH WEST OF PAKISTAN

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ABSTRACT

Wild plants mainly used for medicinal purpose were collected from different areas of North West of Pakistan. Acorus calamus, Plantago sp., Psyllium ovata, Mentha longifolia, Taraxacum officinale, Bistata implexical, Sonchus sp., and Foeniculum vulgare were analyzed for proximate composition and mineral content to investigate their medicinal properties with their chemical composition. The crude protein content of wild medicinal plants ranged from 1.7 to 2.7%. The minimum protein content was observed in B. implexical (1.8%) while maximum protein content was found in M. longifolia and F. vulgare which was 2.7% for each. The ash content of the various plants ranged from 1.3 to 3.3%. Foeniculum vulgare contained the least while T. officinale contained the highest ash content. Moisture ranged from 82 to 93%. Acorus calamus and P. ovata contained the lowest amount of moisture (82%) while Sonchus sp. contained the highest amount of moisture content (93%). The fiber content of the plants was observed 3.2-7.3%. The least amount (32%) of fiber was present in M. longifolia and the highest amount of fiber was possessed by P. ovata which contained 7.3% fiber. Mineral content showed that all the plants contained lower amount of Na and Ni. However, all other mineral were present in considerable amount. Na content ranged in 1.1-8.0ug/g with highest amount observed in Sonchus sp. (8.0 ug/g Na), Ca content ranged in 234.0-408.4, Mg, Cr, Mn, Fe, Ni and Zn in 72.5-95.0, 2.0-14.0, 4.85-90.45, 1.20-209.25, 18.8-81.2, 0.8-5.7, and 6.60-24.2ug/g, respectively. Higher Fe and Cu contents showed that plants might be helpful in restoring the Fe and Cu deficiency in the body when consumed regularly. The appreciable amount of Zn, Mg and Mn showed that the plants curing effect might be related to their mineral composition. However, the presence of heavy metals like Cr and Ni in large amounts might be dangerous if used in regular diets.

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INTRODUCTION

Medicinal plants are the gift of Allah for human being. Fortunately Pakistan, with diverse climatic and phytogeographic ranges, contains flora of 6000 species, where 10% of this flora is used for medicinal purposes by the local communities in Pakistan. More than 4000 plant species grow in mountainous regions of Khyber Pakhtunkhwa Province (KPK) and northern region of Hindukush – Himalayas (Shinwari *et al*., 2002). District Swat at far northern part of KPK province contains a rich flora with many endemic species. Stewart (1967) reported 1550 species as total estimated species in Swat.

Traditionally forests and rangelands are the main sources of medicinal plants in Swat. The lack of knowledge about the part used and time of collection leads to misuse of the species. Presently a number of barriers exist to the sustainable cultivation, gathering and use of medicinal plants. These include lack of clear resource tenure and custodianships, little understanding of sustainable management parameters and knowledge of market requirement (Sher and Khan, 2006).

Traditional medicine (TM) has been defined by the WHO as "referring to health practices, approaches, knowledge, and beliefs incorporating plant-, animal- and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being"(WHO, 2003).

Ethnobotanically, the valley is rich in medicinal plants and most of the people are using these plants as a primary source of health care. Annually a large number of medicinal plants are harvested and bought by the local shopkeepers of the valley. Among these medicinal plants *Acorus calamus* used as stimulant, emetic, carminative and as expectorant. Similarly, *Adhatoda vasica, Dioscorea deltoidea and Xanthoxylum armatum* are used for different diseases (Ur-Rahman, 2001).The above mentioned plants contain carotenoids such as β-carotene, α-carotene, γ-carotene and β-cryptoxanthin. These compounds have provitamin-A activity and are potent antioxidants and modulate the pathogenesis of several chronic degenerative diseases (Niizuand Rodriguez-Amaya, 2005).

Although the efficacy of herbs for curative purposes is often accounted for in terms of its organic constituents, trace elements play a very important role in the formation of the active chemical constituents present in medicinal plants and are there responsible for their medicinal as well as toxic proprieties. Medicinal uptakes are of greater interest from the toxicological and nutritional points of view. Thus, a quantitative estimation of various essential or toxic element concentrations is necessary for determining the effectiveness of the medicinal plants in treating various diseases and also to understand their pharmacological action (Dim*et al.*, 2004).

Currently there is little evidence of the conservation of indigenous medicinal plant species in the study area. However, little information is available on the allelopathic potential of medicinal plants. The area is known for its importance for biodiversity of traditional system of medicine. Some of the important medicinal plants were collected and tested for their chemical compositions in order to select the potential allelopathic species.

MATERIALS AND METHODS

Sample collection

The research work was carried out in the department of Agricultural Chemistry, The University of Agriculture Peshawar, Pakistan during 2010. Nine medicinal plants i.e. *A. calamus, Psyllium* sp.*, P. Ovata, T. officinale, B. implexical, Sonchus* sp.*, M. longifolia* and *F. vulgare* were collected from different areas of north west of Pakistan. All the samples were collected as a whole plant body including root, stem and leaves and stored at room temperature.

Sample preparation

The whole plant body was clean from dirt with water and then all the samples were dried and ground into fine particles through grinder in laboratory for chemical analysis.

Proximate composition

Samples were analyzed for moisture, ash, crude protein and fiber in accordance with the standard method of A.O.A.C. (2000).

Moisture

One gram of sample was accurately weight in a clean pre-weighed Petri-dish (W1). Petri-dish was placed, partially covered, in the oven at 105^{o}C for 6 hrs. Petri dish was removed and covered fully with its lid and placed in desiccator for 30 minutes in order to cool it. After cooling, the dish (W2) was weighed out again and percent moisture was calculated as follows:

$$\% \text{ Moisture} = \frac{W_2\text{-}W_1 \text{ X } 100}{\text{Wt of sample}}$$

Ash Content

Clean empty crucibles were placed in a muffle furnace at 660 ^{o}C for an hour, cooled in desiccator and then weight of empty crucibles was noted (W1). 1 gm of sample was placed in each crucible (W2) in triplicate. The sample was charred over the burner with the help of blowpipe. The crucibles were then placed in a muffle furnace at 550-600 ^{o}C for 3-5 hours. After the complete ignition the furnace was turned off. The crucibles were cooled and weighed (W3). Percent ash was calculated as follows:

$$\%Ash = \frac{\text{Weight of ash x 100}}{\text{Weight of sample}}$$

Crude protein

The percent crude protein (N x 5.70) in each sample was determined by Kjeltech apparatus. The process involved the following steps:

Digestion

Powdered sample (1g) was taken in the digestion tubes. Four grams of the digestion mixture (CuSO4 + K2SO4, 1:8) and 12 ml of the conc. H2SO4 were added to the digestion tubes. The content of the flask was mixed and heated till the mixture became clear. The digestion was completed in about 2 to 3 hours and the organic matters were completely oxidized.

Distillation

The digestion flask with digested sample was transferred into the distillation assembly of Kjeltech. 50 ml of H2O and 50 ml NaOH (40%) were added gradually. Three minutes of distillation time was set and the mixture was heated by steam. The digest was distilled and the distillate was collected in a conical flask containing 20 ml of 4% boric acid solution with modified methyl red indicator. The original pinkish color of the solution was changed into yellow orange during distillation.

Titration

The distillate with yellow orange color was titrated against standard 0.05 N HCl solutions till the appearance of pinkish color. A blank titration was also practiced and the volume of HCl used was noted.

Calculation

Crude protein % was calculated as follow:

$$\%\text{Crude protein} = \frac{(S-B) \times N \times D \times 0.014 \times 100}{\text{Wt. of sample x Aliquot taken}}$$

Where

B = blank titration reading D = sample dilution with distilled water

Crude fiber content

Crude fiber (CF) is defined as an organic matter that remain after digested first with a weak H_2SO_4 solution and then with a weak solution of NaOH solution. The residue that collected after digestion is dried and ashed in furnace .The loss in weight is recorded as CF.

Reagents

H_2SO_4 .0.1M solution (21 ml H_2SO_4 (1.84g/ml) is completed to 2.5 liter with distilled water; NaOH 1.5 M (155 g NaOH /2.5 liter distilled water); HCl acid 0.3 M solution (50 ml HCL is completed to 2 liter with distilled water); Na_2 EDTA (Di sodium di amine tetra acetate, dehydrate crystals) Acetone.

1 gram sample was weighed and transferred in to the beaker. 50 ml H_2SO_4 solution was added. The condenser was placed on the top of the beaker and the water flow to condenser. And was heated for 5 minutes strongly and then heat was adjusted for an even boiling. Heating was continued for 30mins with cooling on beakers. 15ml NaOH solution was added quickly and heating was continued for a further 35mins, 0.5g Na_2EDTA was added 5mints before the boiling time is over. The hot solution through the crucible was attached to filtration apparatus Use low vacuum for filtration. Hot water was used for rinsing the beaker. Filtrate was rinsed in the crucible with 15 ml HCL solution and was washed with hot water until it is free of acid. It was then wash with 15 ml acetone two times. The crucible was dried in oven at 100 ^{0} C. It was then cooled in a desiccator and was weighed. . Crucible containing the dried residue was placed in a muffle furnace for two hours at 550 ^{0}C and was cooled in desiccators. The crucible was again weighed.

$$\%CF = \frac{\text{(wt of crucible+dried residue)-(Wt of crucible+ashed residue) x 100}}{\text{(Wt of crucible+Sample) −Wt of empty crucible}}$$

$$\%\ CF\ in\ DM = \frac{\%CF\ in\ sample\ x\ 100}{\%DM\ in\ sample}$$

Mineral analysis

The dried sample was first digested with nitric acid and perchloric acid and then the aliquots were used for the determination of Cu, Cr, Ni, Fe, Zn, Mg, Mn and Ca by Atomic Absorption Spectrophotometer, while Na by Flame Photometer.

Acid digest

Sample (1g) was weighed and placed in digestion tubes. 10 ml of concentrated nitric acid was added to it and kept over night. Next day 4 ml of perchloric acid was added to it. It was then placed on heater and temperature was raised up to 200 º C. Heating was

continued till the white dense fumes of perchloric acid disappeared. After digestion, tubes were cooled till room temperature and the contents were filtered by Wattman filter paper No. 42. The contents were transferred to 100 ml volumetric flask and the volume was made with distilled water. The prepared sample was stored in refrigerator.

Sodium content

Sodium was determined by Flame Photometer (PFP7) method as detailed by Khalil and Saleem (2004).

Preparation of standard curves

Standard solution was diluted to different known concentrations. An aliquot (10 ml) was taken from each of the standard solution and 3 ml LiCl was added to it. Then volume was made up to 50 ml in a volumetric flask. Emission intensity was noted by flame photometer using a sodium filter of 589 nm for NaCl solution. Standard curves of sodium were prepared by plotting emission intensity versus concentrations.

Sample assay

An aliquot (10 ml) was taken from acid digest and placed in 50 ml volumetric flask. LiCl (3 ml) was added to it and volume was made up to the mark. Emission intensity of sodium and potassium was recorded by using their respective filters in flame photometer. Amount of each of the mineral elements was noted from the respective standard curve. Mineral content was calculated as mg kg^{-1} of the sample.

Micro minerals

Micro minerals i.e. Nickel, Chromium, Iron, Copper, Zinc, Manganese, Calcium and Magnesium were determined by Atomic Absorption Spectrophotometer (AAS) by using the standard solutions of the above minerals and their respective cathode lamp (Khalil and Saleem. 2004). Data was calculated by using following formula:

$$\text{Micro mineral (mg/L)} = \frac{\text{ppm from graph x dilution factor}}{\text{Weight of Sample}}$$

RESULTS AND DISCUSSION

Wild plants that are mainly used as medicinal plants were collected from tehsil Matta, district Swat. The *A. calamus, Psyllium ovata, Plantago* sp*., T. officinale*, *B. implexical, Sonchus* sp*., M. longifolia and F. vulgare* were analyzed for proximate composition and mineral content. The data was summarized in the form of following tables.

Table-1 showed the proximate composition of the medicinal plants. The crude protein content of wild medicinal plants ranged from 1.7% to 2.7%. The data indicated that the plants contained less protein content than cultivated vegetables which contain up to 12%

protein content. The minimum protein was observed in *B. implexical* which contained 1.8% of crude protein. The maximum amount of protein was found in *M. longifolia* and *F. vulgare* which was 2.7% each. The low protein content showed that most of the plant material would be made of carbohydrates (Asekun*et al*., 2007).

Table-1. Proximate analysis of medicinal plants

	Protein (%)	Ash (%)	Moisture (%)	Fiber (%)
Acorus calamus	2.0	1.4	82	3.4
Psyllium ovata	2.5	1.4	83	6.6
Mentha longifolia	2.7	2.1	92	3.2
Plantago sp.	2.3	1.4	82	7.3
Taraxacum officinale	2.3	3.3	85	5.6
Bistata implexical	1.8	2.4	87	7.0
Sonchus sp.	2.7	3.3	93	3.8
Foeniculum vulgare	2.3	1.3	86	5.7

The ash content of the various plants was in the range of 1.3 to 3.3%.*F. vulgare* had the lowest while the *T. officinale* contained the highest ash content. The ash content represents mineral portion of the plants. Its larger amount might be due to the presence of certain minerals in greater quantity, thus fulfilling the need of the body mineral nutrition. Moisture content was observed from 82 to 93%. *A. calamus* and *P. ovata* contained the lowest amount of moisture (82%) while *Sonchus* sp. contained the highest amount of moisture content (93%). The moisture content showed that the absorption of water from the ground was efficient or it might be the reason for succulence in the branches and leaves of the plants, which when edible are easy to eat and taste. The fiber content of the plants ranged from 3.2 to 7.3%. The least amount (32%) of fiber was present in *M. longifolia* and the highest amount of fiber was possessed by *P. ovata* which contained 7.3% fiber. The higher fiber content of the plant might be due to the presence of cellulose in the leaves or succulent branches. High fiber content is beneficial for good absorption in the alimentary canal passage of the food items. The present study was supported by Nivas *et al.* (2010).

Table-2 contains data on the mineral content of the medicinal plants. From the overall data it was obvious that all the plants contained lower amount of Na and Ni. However they contained all other minerals in appreciable amount. Na content was found from 1.1 to 8.0ug/g. The highest amount was observed in S. specie which contained 8.0 ug/g of Na. The values obtained for Ca content were

ranging from 234.0 to 408.4. This showed that plant contained high amount of Ca. The reason for this high Ca contentcan be related to the soil composition there in the valley. Some of the parental rocks contain much Ca and the soil thus has a lot of available Ca that affect the Ca content of the plants. The content of Mg, Cr, Mn, Fe, Ni and Zn ranged from 72.5 to 95.0, 2.0 to 14.0, 4.85 to 90.45, 1.20 to 209.25, 18.8 to 81.2, 0.8 to 5.7 and 6.60 to 24.2ug/g respectively. The higher iron and copper contents showed that plants might be helpful in restoring the Fe and Cu deficiency in the body when consumed regularly. Similar results were reported by Hussain *et al*. (2009) who observed higher levels of iron in *Mentha sylvestris*. The appreciable amount of Zn, Mg and Mn showed that the curing effect of plants might be related to their mineral composition. However the presence of Cr and Ni in higher proportions might endanger their use in regular diets, because of accumulation of these minerals in the body as heavy metals. Similar study was conducted by Anjorin *et al.* (2010).

Table 2. Mineral content (ug/g) the of medicinal plants

Sample/Minerals	Na	Ca	Mg	Cr	Mn	Fe	Zn	Ni	Cu
Acorus calamus	1.1	297.8	91.5	9.5	4.85	7.5	18.8	1.75	24.2
Psyllium ovata	2.1	312.6	86.5	4.0	16.0	23.6	32.1	2.20	16.8
Mentha longifolia	1.0	332.4	95.0	6.0	48.5	19.5	43.6	5.7	26.1
Plantago sp.	1.8	304.3	86.0	4.5	64.5	142.4	34.2	1.65	18.1
Taraxacum officinale	2.5	408.4	72.5	14.0	21.9	158.3	24.6	2.5	9.50
Bistata implexical	1.2	378.0	82.5	11.1	90.45	199.5	44.8	0.8	6.20
Sonchus sp.	8.0	234.0	76.5	2.0	49.05	209.3	60.6	1.5	46.5
Foeniculum vulgare	2.2	362.8	74.5	2.5	61.25	32.70	31.2	1.9	45.0

CONCLUSION

The crude protein content of wild medicinal plant was in the range of 1.7-2.7%. Minimum protein content (1.8%) was there in *B. implexical*and maximum in *M. longifolia* and *F. vulgare*(2.7% each). The ash and moisture content of the various plants rangedbetween 1.3-3.3% and 82-93%, respectively. The fiber content of the plants was least (32%) in *M. longifolia* and highest was possessed by *P. ovata*(7.3%). All the plants contained lower amount of Na and Ni;however they contained all other mineral in considerable or even in greater amount. Sodium content of the plants was present in range of 1.1-8.0ug/g. The range of Ca content was 234-408ug/g. Mg, Cr, Mn,

Fe, Ni and Zn were present in the ranges of 72.5-95, 2-14, 4.85-90.45, 1.2-209.25, 18.8-81.2, 0.8-5.7 and 6.6-24.2ug/g, respectively. The higher Fe and Cu contents showed that plants might be helpful in restoring their deficiency in the body when consumed regularly. The appreciable amount of Zn, Mg and Mn showed that the plant curing effect might be related to their mineral composition. However the presence of Cr and Ni might endanger their used in regular diets, which might cause accumulation of these minerals in the body which are considered as heavy metals when present in large amount.

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