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Proximate analysis and Metal analysis of medicinal plants (*Inula crithmoides* and *Heliotropium eichwaldii)* from district Karak, Khyber-Pakhtunkhwa, Pakistan

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Abstract
Medicinal plants are major source of drugs used for the treatment of various diseases. In the present study *Inula Crithomoides* and *Heliotropium eichwaldii* have been analyzed due to its wide application in the indigenous medicinal system and the importance of its chemical constituents. The concentration of ash and fats were determined using Association of Official Analytical Chemists (AOAC) method and soxhlet apparatus respectively. Heavy metals were analyzed by using atomic absorption spectrometer and flame photometer. Heavy metals like Pb, Fe, Ni, Cd, Zn, Cu, Ca, Na and K were detected in all samples of both plant species in different concentration range. In some of the samples the concentration found was higher than the allowed limit while the highest concentration of ash and fat were 21.11% and18.05% respectively.

Keywords: *Inula crithmoides, Heliotropium eichwaldii, heavy metals, phytochemistry*

Introduction
Medicinal plants have been used for healthcare since immemorial times and still used due to their therapeutic properties [1-2]. The secondary metabolites such as alkaloids, flavonoids, terpenes, phenolics present in these plants have been used to prepare different medicines [3]. Most of allopathic drugs are either extracts of these medicinal plants or the compounds present in these extracts, are synthesized. The use of herbal medicines has significantly increased in the past decades especially in the developing countries due to their cheap availability, lesser side effects, and also helping to overcome the malnutrition.

Some species of *Inula* have been used, in traditional Chinese medicine, for abdominal pain, acute enteritis, and bacillary dysentery. *Heliotropium eichwaldii* powder of the shade dried plant is applied on wounds while decoction of the plant is taken to cure hepatic damage [4]. Field of ethno-medicine is still needs exploration and scientific validation in Pakistan. Huge reservoirs of medicinal plants comprising of 5,700 species is present in Pakistan, of which 372 are rare and 456 are used for the treatment of various ailments either separately or in form of recipes. The recipes used by the local practitioner (Hakeem) are playing important role in human health care. However, the plants used in these recipes should be studied in the laboratory in the controlled environment and investigated for their properties, safety and efficiency [5-6]. Metal analysis of these medicinal plants is very important as it helps in evaluating their toxicity.

Plants may absorb these metals from soil, water or air or these herbs may be easily contaminated during growing and processing. The ability of plants to selectively accumulate essential elements is different for different species and is subjected to certain geochemical characteristics depending on the type of soil [7] Although metals found in all living organisms play different roles in structural and control mechanisms of body but excess accumulation can lead to severe detrimental effects [8-10], or redox systems and become significant health hazard [11-12]. Therefore the metal analyses of the selected plants were carried out to know the possible side effect of the recipes beforehand.

Experimental

Chemical and standards

Deionized water used for preparing reagents and standards throughout this work. Hexane (≥ 95%) and porcelain chips were purchased from Merck Pakistan. Whatman ≠ 42 Filter papers were purchased from Syner Tech Pakistan. Elemental standards were purchased from LGC standards China.

Sample Collection: Samples of two different medicinal plants including *Inula Crithomoides* and *Heliotropium eichwaldii* were collected from plain area of district Karak, Khyber Pakhtunkhwa Pakistan. The plants were identified by plant taxonomist, Department of Botany, Kohat University of Science & Technology, Kohat, Pakistan.

Sample preparation: The samples were washed thoroughly with tap water to eliminate dirt, dust and other possible parasites followed by another washed with deionized water. The washed plants were dried under shade at 25-30 ^{0}C. The dried samples were powdered by grinder and then stored in clean, dried plastic bottles for further processing.

Proximate analysis: The proximate analysis for determination of moisture and total ash of the samples were carry out using AOAC methods [13]. This method was based on the measuring of weight before and after the removal of moisture and ash contents in the samples. The lipid content of the samples was determined through Soxhlet apparatus and direct solvent extraction method [14]. All the proximate values are reported in percentage. Each experiment was repeated three times and values expressed are means ± standard deviation.

Fat content determination

Accurately weighted 6 g of the sample was taken and grinded it into a fine powder, using a mechanical blender. Then the sample was transferred into a moisture dish and dried the sample at 125 °C) for 2 -3 hours. A pouch (3x10 cm) (centimeter) of Watmann # 42 filter paper was made. Moisture free sample (5 g) was put inside the pouch and tighter it firmly. Soxhlet extraction apparatus was assembled on heating plate and the pouch containing sample was placed in Soxhlet extraction tube. Hexane (160-180 mL) along with few porcelain chips was poured into extraction flask. The flow of solvent on the sample containing pouch was carefully maintained at 160-180 drops/minute. After 6-8 hours extraction process the sample was cool to room temperature. The contents of flask were transferred (with 2 or 3 repeated washings with hexane) into a clean, dried and pre weighed 250 mL (milliliters) glass beaker (preferably, in two or three equal aliquots). Then the beaker was placed on a hot water bath and the solvent was evaporated in a fume hood. The residue was dried in a hot air oven at 105°C (Degree centigrade) for 30 minutes to remove traces of solvent and moisture. The beaker was cooled in a desiccators, the beaker was cooled and contents were recorded after weighing carefully. The percent oil content of the sample was calculated by the following formula cv [11].

Macro and micronutrient analysis: In our experiments the estimation of heavy metals like Zn, Fe, Cu, Ni, Cr, Pb and Cd were carried out on Flame Atomic Absorption Spectrophotometer (Parkinelmer 400), USA while using a working standard of 1000 ppm (dilution according the requirements) for each of the species. Ca, Na and K were carried out on Flame photometer (Parkinelmer, USA).

Results and Discussion

Ash, Fat and Moisture contents

Determination of the ash content of foods is important for a number of reasons. The percentage of ash, fat and moisture contents of different parts of the two plants are given in the table 1. The highest ash content in *Inula Crithomoides* leaves was found to be 21.11% while roots having 17.4% ash contents. On the other hand *Heliotropium eichwaldii* leaves having 17.5% ash content but small amount of ash contents were found in *Heliotropium eichwaldii root* that is 6.0%.

The results of fat analysis showed that *Heliotropium eichwaldii* leaves contained high amount of fats contents that were about 18.05% and small amount of fat contents were found in *Heliotropium eichwaldii* stem that were about 1.97%.

The results of moisture analysis showed that *Heliotropium eichwaldii* stem contained large amount of moisture contents that were about 27.4% and small amount of moisture contents were found in *Inula Crithomoides* flower that were about 07.66%.

Table 1: Percentage of ash, fat and moisture content. Mean (SD), n = 3

Sample name	Ash content%	Fat content%	Moisture content%
Inula Crithomoides root	17.40 ± 0.14	3.16 ± 0.21	11.50 ± 0.50
Inula Crithomoides flower	15.01 ± 1.30	5.05 ± 0.10	7.66 ± 0.12
Inula Crithomoides leaves	21.11 ± 0.20	13.26 ± 1.00	15.01 ± 0.01
Inula Crithomoides stem	7.27 ± 1.00	3.38 ± 0.15	13.04 ± 0.21
Heliotropium eichwaldii root	6.00 ± 0.12	2.49 ± 0.17	17.03 ± 0.01
Heliotropium eichwaldii flower	10.25 ± 1.21	8.01 ± 0.30	13.10 ± 0.21
Heliotropium eichwaldii leaves	17.50 ± 0.50	18.05 ± 2.10	21.10 ± 0.32
Heliotropium eichwaldii stem	7.55 ± 1.30	1.97 ± 0.01	27.04 ± 0.10

Metal analysis

Mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl. Determination of the mineral content of foods is important for a number of reasons. The quality and microbiological stability of many medicinal depends on the concentration and type of minerals they contain. Some minerals are essential to a healthy diet (e.g., calcium, phosphorous, potassium and sodium) whereas others can be toxic (e.g., lead, mercury, cadmium and aluminum). High mineral contents are sometimes used to retard the growth of certain microorganisms. Metal analysis of different parts of both the medicinal plants showed significant variation in concentration of different metals given in the table 2. Lead which is nonessential and carcinogenic element mainly comes from anthropogenic activities was found in the range 19.97 - 163.35 mg/kg. The highest concentration of Pb was found in the leaves of *Heliotropium eichwaldii* which is much above

its recommended level in medicinal plant (10 mg/kg) [15]. In comparison to the WHO limit, the entire samples showed exceeded concentration of iron (47.77 - 403.25 mg/kg). The concentration of copper was ranging from 0 to 25.95 mg/kg while concentration of zinc (18.32 - 48.02 mg/kg) was within the recommended level of WHO [16-17]. The concentration of nickel in both the plant samples was in the range 0.075 - 1.4 mg/kg which is within the permissible limit (1.5 mg/kg) according to United State Environmental protection Agency. The concentration of Cd in analyzed plant samples was in the range 0 - 3.6 mg/kg. Cadmium is non-essential element and not required to human or plants. However according to WHO the limit of Cadmium in Medicinal plant is 0.3 mg/kg [18].

Table 2: Heavy Metals Concentration (mg kg^{-1}) in *Inula Crithomoides* and *Heliotropium eichwaldii.* Mean (SD), n = 3

S. No.	Sample Name	Pb	Fe	Ni	Cd	Zn	Cu
1	Inula Crithomoides root	29.32 ± 0.17	179.57 ± 0.12	0.63 ± 0.01	ND	27.72 ± 0.07	ND
2	Inula Crithomoides Stem	19.97 ± 0.43	403.25 ± 0.05	0.50 ± 0.01	ND	18.32 ± 0.01	ND
3	Inula Crithomoides Leaves	29.27 ± 0.33	113.32 ± 0.04	0.90 ± 0.02	0.38 ± 0.01	33.20 ±0.01	ND
4	Inula Crithomoides Flower	33.05 ± 0.35	203.12 ± 0.17	0.60 ± 0.02	ND	23.35 ± 0.01	ND
5	Heliotropium eichwaldii root	37.47 ± 0.41	312.00 ± 0.17	0.08 ± 0.03	ND	32.50 ± 0.02	25.95 ± 1.12
6	Heliotropium eichwaldii stem	27.72 ± 0.29	47.77 ± 0.03	0.30 ± 0.01	2.60 ± 0.02	37.52 ± 0.01	ND
7	Heliotropium eichwaldii Leaves	163.35 ± 0.49	123.10 ± 0.11	1.40 ± 0.01	3.60 ± 0.04	48.02 ± 0.01	ND
8	Heliotropium eichwaldii Flower	4.12 ± 0.03	85.97 ± 0.12	0.20 ± 0.02	ND	38.50 ± 0.02	ND
9	Permissible level	10	1	1.5	0.3	50	20 - 150

The analysis of different parts of the two medicinal plants for calcium, potassium and sodium are given in the table 3. The analysis of different parts of both the medicinal plants revealed that Ca (68 - 837 mg/kg) and K (165 - 439 mg/kg) are well below the permissible level while Na (34 - 74 mg/kg) is also lower than the WHO permissible level (2g) [19]. Considering the usefulness of these minerals in the body, these results become very important. Sodium, calcium and potassium maintain the ionic balance of the human body, growth of the bones and regularize the muscular moments [20]. However, the lower concentration of sodium have an extra advantage as it can cause hypertension in human when taken in excess [21].

Table 3: Calcium, Sodium and potassium Concentration (mg kg^{-1}) in *Inula Crithomoides*, and *Heliotropium eichwaldii*. Mean (SD), n = 3

S. No	Sample Name	Ca	Na	K
1	*Inula Crithomoides root*	089 ± 0.20	034 ± 0.12	345 ± 0.001
2	*Inula Crithomoides stem*	068 ± 0.17	070 ± 0.001	430 ± 0.21
3	*Inula Crithomoides leaves*	274 ± 0.30	056 ± 0.20	326 ± 0.11
4	*Inula Crithomoides flower*	153 ± 0.02	064 ± 0.13	274 ± 0.21
5	*Heliotropium eichwaldii root*	085 ± 0.21	058 ± 0.17	391 ± 0.13
6	*Heliotropium eichwaldii stem*	330 ± 0.15	053 ± 0.21	229 ± 0.13
7	*Heliotropium eichwaldii leaves*	770 ± 0.20	074 ± 0.13	439 ± 0.002
8	*Heliotropium eichwaldii flower*	837 ± 0.10	040 ± 0.002	165 ± 0.10
9	WHO recommended level [12]	1000 mg/day	2 g sodium/day	3510 mg/day

This study concluded that different parts of the two medicinal plants i.e. *Inula Crithomoides* and *Heliotropium eichwaldii* accumulates significant amount of Na, K, Ca, and Fe. It is observed that there is large variation in the concentration of heavy metals in deferent parts of the two plants in comparison to the international safety standards prescribed for medicinal plants, which can cause metal poisoning in humans.

The data obtained in present study will be helpful in the synthesis of new modern drugs with various combinations of plants. The metal analysis shows that these medicinal plants can be used for medical purposes, but one can be sure to use the suitable amount of the plants extract not to exceed the permissible level. The plants and their recipes were analyses for the heavy metals and the results of the plants and recipes are given in the figure 1.

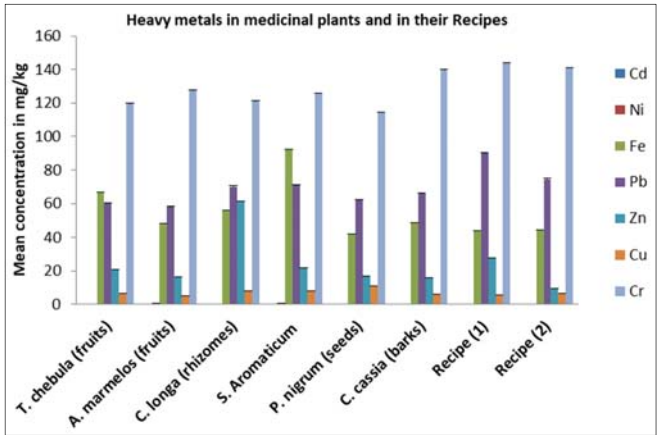

Fig 1: Concentration of Heavy metals in Plants and their recipes. Mean (SD), n = 3

The level was found below the permissible level according to the international standards set by different protection agencies, hence it is safe to use these recipes as for as the concentration of the metals is concerned.

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