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Endogenous transitional metal and proximate analysis of selected medicinal plants from Pakistan

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Transitional metal and proximate analysis plays an important role in assessing the appropriateness of medicinal plants or their extracts orally taken by the marginal communities. To acquaint, various proximate parameters like ash, carbohydrate, protein, fiber, fat and moisture (both dry and wet) and endogenous transition metals like copper, magnesium, manganese, lead, cadmium, iron, chromium and sodium; four medicinal plant species, viz. *Rhazya stricta*, *Dalbergia sisso*, *Phlomis cashmeriana* and *Phlomis bracteosa* were assayed while using standard techniques of AOAC. The results showed that *Phlomis bracteosa* has highest percentage of fiber, fat and energy values compared to other species. However, the concentration of magnesium, manganese, chromium and sodium were found highest in *Dalbergia sisso* compared to the other three species. The study reveals that all the values of metals concentration are in accordance with WHO standards.

Key words: Proximate contents, metals analysis, medicinal plants, Pakistan.

INTRODUCTION

Medicinal plants or their composite extracts are unique type of natural product requiring special considerations due to their potential impact on people's health. Medicinal plants provide accessible and culturally relevant sources of primary health care to a majority of the world's human population (Ji et al., 2009). Medicinal plants offer a rich source of structural biodiversity in the form of a variety of bioactive natural products, which has played pivotal role in drug discovery process (Cragg et al., 1997). Almost 80% of the people of marginal communities rely on medicinal plants for their various diseases (Latif et al., 2004). In Pakistan, more than 60% of the population lives in rural areas, where the generation old indigenous knowledge of medicinal plant used against many day to day diseases, still exist and practiced (Shinwari et al., 2006). Such a high dependency on medicinal plants or their herbal formulations put forth a need to analyze their metal composition and proximate analysis (Hussain et al., 2009).

For this very reason, four plants species *Rhazya stricta*,

Phlomis cashmeriana, *Phlomis bracteosa* and *Dalbergia sisso* were subjected to analysis. Besides collection from the wild, at minor spaces these are cultivated either at large level or at garden levels. *Phlomis* is a genus of the family Lamiaceae (Labiatae) consisting of 100 species of herbs or shrubs, many of which are highly variable. Some *Phlomis* species are used as tonics and stimulants in Anatolian folk medicine. However, no phytochemical study has so far been carried out on *P. bracteosa* and *P. chashmarina* previously (Kyriakopoulou et al., 2001; Calis and Kirmizibekmez, 2004). *R. stricta* is a stiff-growing plant with erect stems, 2 - 3 feet high and upright thickish smooth leaves placed close together on the stem. Its infusion is a good tonic with peculiar bitter taste. Phytochemical analysis of the plant revealed more than 100 alkaloids. These alkaloids have several pharmacological properties. It is also used for throat sour, in fever, general debility and as curative for chronic rheumatism and tumor. *R. stricta* is used traditionally in Asia for the treatment of different types of diseases such as skin diseases, stomach diseases and antihypertensive. The leaves, flowers and fruit are also used in joint infections and for cancer (Khan and Khan, 2007).

Dalbergia sisso has many reputed medicinal properties

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and have been used culturally for a variety of ailment including skin diseases, blood diseases, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, aphrodisiac, expectorant (Kirtikar and Baso, 1975). Since various medicinal plant species are also used as food along with their medicinal benefits, evaluating their metal content can help to understand the suitability of these plants species (Pandey et al., 2006; Taiga et al., 2008; Hussain et al., 2009). In the present study, proximate parameters such as ash, carbohydrate, protein, fat, moisture and energy values were analyzed for the first time of these species while in case of metal analysis Cu, Na, Pb, Co, Cr, Fe, and Cd were assayed.

MATERIALS AND METHODS

Plants collection

The medicinal plant species *P. bracteosa* and *P. cashmeriana* were collected from shentoi, Parachinar Kurrum agency and *R. stricta*, *D. sisso* were collected from KDA Kohat of NWFP Pakistan in July 2008. The collected plants were packed in the Kraft paper and herbarium sheets were prepared. These plants were identified by a plant taxonomist of Botany Department, Kohat University of Science and Technology, Kohat. The details of each plant species, in respect of their local names, part used and collection areas are elaborated in Table 1.

Sample preparation

The study involved destructive sampling. The samples were washed under running water and blotted dry. The moisture content of the leaf samples was determined at 60 °C. The dried matter obtained was ground to a fine powder and stored at 5°C in air-tight containers prior to further analysis. For carbohydrate and energy values, AOAC (1990) method was followed while for other proximate parameters the quantities used are given in Table 2.

Proximate analysis

The proximate analyses (moisture, fiber, ash, crude fats, proteins and carbohydrates) of all the samples were determined. The moisture and ash were determined using weight difference method. Fiber content was estimated from the loss in weight of the crucible and its content on ignition. Carbohydrate was determined when the sum of the percentages of moisture, ash, crude protein, either extract and crude fiber were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method described by Pearson (1976), involving digestions, distillation and finally titration of the sample. The nitrogen value was converted to protein by multiplying a factor of 6.25. Carbohydrate was determined by difference method. All the proximate values were reported in percentage (AOAC, 1990).

Digestion of plants

0.5 g of the dried plant material was taken and transferred to a digestion flask. To this 0.5 g of the digestion mixture (Copper sulphate: Potassium sulphate: Ferrous sulphate; 5:94:1 ml (w/w/w)) was added and about 20 ml of concentrated sulphuric acid was added. The solution was heated until the solution became clear and frothing ceased. Then it was boiled briskly for another 2 h by adding

20 ml mixture of perchloric acid and nitric acid; 10:10 mL (v/v), cooled and to the digest about 50 ml water was added in 5 ml portions with mixing. The digest was transferred to a 100 ml volumetric flask and made the volume up to the mark.

Metals analysis

The concentration of Pb, Na, Cd, Cr, Mg, Mn, Cu, and Fe of each species was done using atomic absorption spectrometer mineral (Perkin Elmer AA Analyst 700). The results were obtained while using a working standard of 1000 ppm for each of the species (Hussain et al., 2009).

Statistical analysis

Each experiment was repeated three times. The results are presented with their means, standard deviation and standard error.

RESULTS AND DISCUSSIONS

Proximate analysis of medicinal plants

The results of proximate analysis showed difference in biochemicals and other contents of each species. However, the results showed that *P. bracteosa* has highest percentage of fiber, fat and energy values compared to other species. In case of individual parameters, the moisture contents of each species were different. It was highest in *R. stricta* followed by *D. sisso* while in other species it had comparatively lesser proportion (Table 3). The ash contents were found highest in *P. cashmeriana* and *D. sisso* (Table 3). In case of energy values, significant amount was revealed in *P. bracteosa* and *D. sisso* (Table 3), however, the other plant species had minor energy values. Looking at the results obtained from carbohydrate analysis, *D. sisso* and *P. cashmeriana* had prominent levels compared to other species (Table 3).

While analyzing the protein contents in the medicinal plant species, the results showed that *D. sisso* and *Phlomis bracteosa* had highest concentration of protein as compared to other species (Table 3). The results of fat analysis presented that *P. bracteosa* and *R. stricta* has significant concentration as compared to other species. Looking at the result achieved from fiber analysis, it was high in *P. bracteosa* and *P. cashmeriana* compared to other species.

Looking at the correlation analysis of the selected parameters, it was found that similar parameter has highly significant correlation while among parameters the correlation is either non-significant or less significant or moderate relation (Table 4). Ash and fat showed negative or non-significant correlation while ash and protein showed moderate correlation (Table 4). Fats and carbohydrate showed significant correlation with energy values, however, the rest of the parameters showed non-significant or no correlation.

Table 1. Medicinal plants collected for the study and pattern of local use.

Species name	Family name	Local name	Parts used	Local use	Status
<i>Rhazya stricta</i>	<i>Apocynaceae</i>	Gandarai	Whole plant	Toothache and gum diseases, Diabetes and blood purification	Wild
<i>Dalbergia sisso</i>	<i>Fabaceae</i>	Shawa	Whole plant	Used in leprosy, boils, eruptions, astringent, gonorrhoea and stops vomiting	Wild
<i>Phlomis bracteosa</i>	<i>Labiataeae</i>	Skha botai	Whole plant	Bone fractures, Sinus congestion, lymph fluid disorder, indigestion	Wild
<i>Phlomis cashmeriana</i>	<i>Labiataeae</i>	Darshol	Whole plant	Also used in bone fractures	Wild

Table 2. Quantity of the plant material used for each of the experiments of proximate analysis.

Ser. No.	Parameter	Quantity (in grams)
1	Moisture	3.0
2	Ash	1.0
3	Protein	0.5
4	Fat	3.0
5	Fiber	3.0

Table 3. Proximate analysis of the selected medicinal plant species in their raw form.

Species name	Energy value (KCal/100g)	Carbohydrate (%)	Protein (%)	Fiber (%)	Fat (%)	Ash (%)	Moisture (%)
<i>Phlomis cashmeriana</i>	315.04 ± 0.09	62.85 ± 0.21	9.51 ± 0.07	23.96 ± 0.02	2.84 ± 0.13	17.66 ± 0.17	7.13 ± 0.31
<i>Rhazya stricta</i>	274.86 ± 0.20	50.09 ± 0.11	9.67 ± 0.07	12.85 ± 0.19	3.98 ± 0.14	6.21 ± 0.07	30.50 ± 0.07
<i>Phlomis bracteosa</i>	449.00 ± 0.33	47.09 ± 0.24	10.61 ± 0.12	24.50 ± 0.22	24.24 ± 0.17	10.83 ± 0.22	7.22 ± 0.21
<i>Dalbergia sisso</i>	333.19 ± 0.10	63.64 ± 0.06	12.12 ± 0.20	13.62 ± 0.08	3.35 ± 0.02	12.33 ± 0.06	8.74 ± 0.02

Table 4. Correlation analysis of the various proximate parameters of selected plant species.

	Energy value	Carbohydrate	Protein	Fiber	Fat	Ash	Moisture
Energy value	1.0						
Carbohydrate	0.66	1.0					
Protein	0.24	0.403	1.0				
Fiber	0.46	-0.14	-0.33	1.0			
Fat	0.94	-0.70	0.022	0.59	1.0		
Ash	0.05	0.73	0.05	0.56	-0.21	1.0	
Moisture	-0.62	-0.42	-0.43	-0.65	-0.33	-0.77	1.0

Table 5. Metal concentration in the medicinal plants species.

Specie name	Concentrations (ppm)							
	Fe	Cu	Mg	Mn	Cr	Cd	Na	Pb
<i>Rhazya stricta</i>	1.234	0.284	17.739	0.44	0.064	0.050	10.899	0.608
<i>Phlomis bracteosa</i>	6.125	12.81	10.739	0.263	0.202	0.176	7.015	1.069
<i>Phlomis cashmeriana</i>	15.441	10.42	19.829	0.465	0.299	0.158	6.485	0.09
<i>Dalbergia sisso</i>	3.941	8.29	21.969	0.584	3.759	0.121	18.419	0.726

Metal analysis

The metal analysis of the medicinal plant species showed significant variation among different micronutrients (Table 5). In case of Cu, it was highest in *P. bracteosa* followed by *P. cashmeriana*. Significant concentrations level of Fe was revealed in *Phlomis cashmeriana* however, considerable amount has also been found in *P. bracteosa*.

The Cd concentration was highest in *Phlomis bracteosa* compared to other species (Table 5). The Pb concentration was found high in *P. bracteosa* while in other it was found in significant amount. In case of the Pb concentration, the suggested concentration in plant species is 2 to 6 mg/L (Broyer, 1972; Adriano, 1986). *D. sisso* and *P. cashmeriana* had significant level of Mg and Mn concentrations. It has been reported that for many plant species Cr proved to be toxic at 5 mg/L. Cr and Na concentration in *D. sisso* was highly significant. All the plants had lesser concentration of Cr as compared to that of recommended level for toxicity. However, the plant species under investigation carries very lesser level of Pb, which further clarifies their use as food supplement.

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