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Full Length Research Paper

Assessment of herbal products and their composite medicinal plants through proximate and micronutrients analyses

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Fourteen medicinal plant species, growing in different parts of Pakistan, have been used to prepare two herbal products Mussafeen and Itreeful ustokhudus by the Qarshi Industry Pvt Ltd. These products were selected to compare the proximate parameters and micronutrients composition with medicinal plants used therein. In proximate analysis carbohydrate, protein, fiber, fat, ash and energy values while in case of micronutrients; Cu, Ni, Pb, Co, Zn, Fe and Cd were assayed. The proximate parameters of each medicinal plants used in herbal products were different, however, *Fumaria offcinalis* has higher to moderate values of ash, fat, carbohydrates and protein contents compared to other species used in the herbal formulations. Looking at the herbal product's proximate analysis, Mussafeen had highest percentage of fats, ash, proteins, and fibers compared to Itreeful ustokhudus herbal products. In micronutrient's analysis, *Sphaeranthus hiritus*, *Fumaria offcinalis* and *Cuscuta reflexa had* higher concentration of Co, Zn, Fe, Cd, Ni and Pb compared to other composite materials of the herbal products. In case of herbal formulations, Itreeful ustokhudus has the highest concentrations of almost all nutrients, that is, Cu, Zn and Cd, while Mussafeen has highest concentrations of Fe, Pb and Ni. However, the concentrations were found well below the threshold levels of the standards mentioned by the World Health Organizations.

Key words: Herbal drugs, standardization, proximate and micro-nutrient analysis, medicinal plants, Pakistan.

INTRODUCTION

The use of traditional medicines is increasing and getting popularity throughout the developed and developing world (Jia and Zhang, 2005). Herbal medicines are the finished labeled medicinal product that contains active ingredients, aerial or underground parts of the plant or other plant material or combinations (Chakravarthy, 1993; Shinwari, 1996; Chaudhari, 1996; Ritch, 2000). About 80% of the marginal people in developing countries rely on traditional medicine for their primary health care (Latif et al., 2004). With the increase in people's preference and demand, worth of herbal product industry is increasing day by day (Shinwari and Shoukat, 2003; Shinwari et al., 2006; Shinwari et al., 2003).

Since many of these herbal products are used orally, therefore, to know proximate and nutrient analysis of these products and raw material used therein plays a crucial role in assessing nutritional significance and health effects (Kochhar, 2006; Pandey, 2006; Taiga, 2008). As far as herbal drug's standardization is concerned, WHO has also emphasized on the need and importance of determining proximate and micronutrients analysis. Such

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herbal formulations must pass through standardization processes (Niranjan and Kanaki, 2008; Ojokoh, 2008).

Pakistan bestowed with diverse wealth of plant resources. Almost 1,000 species that is, 12% of the plant's diversity have been reported to carry medicinal values and are used by marginal communities to cure various diseases (Latif et al., 2004; Shinwari and Shoukat, 2003). Besides collection from the wild these medicinal plants species are either cultivated at large level or at garden level. The medicinal plant species are used by the local communities to cure various diseases using their traditional knowledge of herbal drug preparations, that is, ayurvedic medicine.

Qarshi Industries Pvt. Ltd. Pakistan is one the pioneer in manufacturing herbal medicines. Two herbal medicines, that is, Musafeen and Itreeful ustokhudus were selected for standardization. The herbal products, their composite medicinal plants and uses are given in Table 2. These herbal products and the medicinal plants species (Fourteen species) used therein were subjected to proximate and nutrients analysis to compare and assess the various contents and concentrations in the products and with that of the medicinal plants (Table 2). In proximate analysis ash, carbohydrate, protein, fat, moisture and energy values were analyzed for the first time of these species while in case of micronutrient analysis, Cu, Ni, Pb, Co, Fe, Zn and Cd were scrutinized.

MATERIALS AND METHODS

Plants collection

Details of the medicinal plants collected for the analysis are given in the Table 1. 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.

Herbal products

Two herbal products Mussafeen and Itreeful ustokhudus were selected to compare the proximate and nutrients in the products and with that of the medicinal plants used in those products. Details of the herbal products are given in Table 2.

Sample preparation

The study involved destructive sampling technique. The samples were washed under running water and blotted dry. The moisture content of the leaf samples was determined at 60ºC (AOAC, 1990). The dried matter obtained was ground to a fine powder and stored at 5^{o}C in air-tight containers prior to further analysis. For carbohydrate and energy values, AOAC (1990) method was followed.

Proximate analysis

For proximate analysis, standard techniques of AOAC (1990) and AOCS (2000) were followed. The proximate analyses (moisture, fiber, ash, fats, proteins and carbohydrates) of all the samples were determined in three replications. Briefly, 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, involving digestions, distillation and finally titration of the sample (Pearson, 1976). The nitrogen value was converted to protein by multiplying a factor of 6.25. Carbohydrate was determined by difference method. All the proximate values are presented in percentage (AOAC, 1990; AOCS, 2000).

Micronutrient analysis

Micronutrients in the medicinal plants and its herbal products were analyzed following previous method of Hussain et al. (2009). The samples were digested by a mixture of concentrated nitric acid and perchloric acid mixed in 1:1 v/v ratio. The micronutrients concentration of Cu, Ni, Zn, Pb, Co, Cd and Fe of the species and products was done using atomic absorption spectrometer (Perkin Elmer AA Analyst 700). The results were obtained while using a working standard of 1000 ppm for each of the species and replicated thrice.

RESULTS AND DISCUSSION

Proximate Analysis of Medicinal Plants

The result of proximate analysis shows variant concentration/proportions of biochemicals and other contents. The moisture contents of each species were found different. Looking at the overall percentage of moisture composition, it was highest in *Vitis venifera* (large) followed by *Vitis venifera* (small), while other plants had comparatively lesser composition (Table 3). In case of ash contents, it was highest in *Fumaria offcinalis* compared to rest of the species (Table 3).

According to the results revealed, *Artemisia vulgaris* followed by *Terminalia chebula* had highest and significant level of energy values (Table 3) while rest of the other plant species had minor values. Looking at the results obtained from carbohydrate analysis, *Terminalia belerica*, followed by *T. chebula* and *Zizyphus vulgaris* had prominent levels compared to other species (Table 3).

While analyzing the protein contents in the medicinal plant species used in herbal products, the results showed that *Tinospora cordifolia* had highest concentration of protein as compared to other species (Table 3). The results of fat analysis showed that *F. offcinalis* has higher concentration compared to other species (Table 3). Looking at the resulted achieved from fiber analysis, it was high in *A. vulgaris* and *Melia azadirachta* compared to other species (Table 3). The difference found in the proportion of proximate parameter of these medicinal plants might be attributed to the conditions on which the plant species are harvested along with environmental parameters (Nordeide et al. 1996; Kutbay and Ok, 2001).

Table 1. Details of medicinal plants collected for the proximate and micronutrient analysis.

Plants	Place of collection	Season of collection	Voucher number
Vitis venifera (small)	Qarshi Herb Garden, Hattar	April, 2009	501
Artemisa vulgaris	Industrial Estate of Hattar, NWFP	March, 2008	461
Terminalia chebula	Qarshi Herb Garden, Hattar	April, 2009	507
Tinospora cordifolia	Qarshi Herb Garden, Hattar	April, 2009	511
Swertia chirata	Industrial Estate of Hattar, NWFP	April, 2009	505
Fumaira officinale	Qarshi Herb Garden, Hattar	May, 2003	41
Polypodium vulgare	Qarshi Herb Garden, Hattar	September, 2008	480
Vitis venifera (large)	Qarshi Herb Garden, Hattar	April, 2009	509
Melia azadirachta	Qarshi Herb Garden, Hattar	April, 2009	540
Terminalia belerica	Qarshi Herb Garden, Hattar	April, 2009	523
Cuscuta reflexa	Qarshi Herb Garden, Hattar	April, 2009	531
Zizyphus vulgaris	Industrial Estate of Hattar	September, 2003	281
Tephrosia purpurea	Industrial Estate of Hattar	April, 2009	500
Sphaeranthus hiritus	Industrial Estate of Hattar	October, 2003	325

Table 2. Herbal drugs and their medicinal composition.

Product	Composite plant species	Quantity (mg) *	Herbal drug uses
Itreeful ustokhudus (500 mg)	*Terminalia chebula* Retz.	290	Headache, migraine, chronic catarrh, epilepsy, amnesia (forgetfulness), expectorates and cures constipation.
	Cuscuta reflexa Roxb.	145	
	Polypodium vulgare	145	
	Terminalia belerica Roxb.	145	
	Vitis venifera Linn. (large)	145	
	Vitis venifera Linn. (small)	145	
Mussffeen (500 mg)	*Artemisia vulgaris*	57.14	Blood purifying, spots and specks on the face, and prickles, boils and eruption, scabies, itching, leucoderma, syphilis, ulcers and fistulas on the skin.
	Fumaria offcinalis	57.14	
	Melia azadirachta Linn.	57.14	
	Sphaeranthus hirtus	57.14	
	Swertia chirata	57.14	
	Tephrosia purpurea	57.14	
	Terminalia chebula	57.14	
	Tinospora cordifolia	57.14	
	Zizyphus vulgaris	57.14	

*The quantity of medicinal plant species used in respective product: adopted from Qarshi.

Proximate analysis of herbal products

Looking at the results shown in Table 3 for proximate analysis of herbal products, it is revealed that Musfeen has the higher protein, fat, ash and fiber contents compared to the other product. Itreeful ustokhudus has highest energy values and carbohydrate and moisture contents (Table 3). In overall all comparison, the Musfeen having higher concentrations of most of the proximate parameters compared to Itreeful ustokhudus.

Micronutrient analysis

The micronutrients analysis of the medicinal plant species showed significant variation among different micronutrients (Table 4). In case of Cu, it was highest in *Sphaeranthus hiritus* followed by *Vitis venifera* (large). Increased level of Fe was revealed in *S. hiritus* however, considerable amount has also been found in *F. offcinalis.* The level of Zn was highest in *Cuscuta reflexa* followed by *Swertia chirata* and *S. hiritus*. The concentration on

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

S/No	Specie name	Energy value	Carbohydrate	Protein	Fiber	Fat	Ash	Moisture
1	*Artemisa vulgaris*	362.89 ± 0.08	81.09 ± 0.1	3.72 ± 0.34	32.62 ± 0.11	2.62 ± 0.02	5.78 ± 0.023	6.76 ± 0.1
2	*Fumaria offcinalis* Linn.	316.87 ± 0.08	55.81 ± 0.091	10.43 ± 0.076	18.56 ± 0.34	5.76 ± 0.09	18.56 ± 0.10	9.42 ± 0.05
3	*Melia azadirachta*	358.36 ± 0.09	75.39 ± 0.23	5.60 ± 0.34	30.43 ± 0.37	3.82 ± 0.1	4.778 ± 0.08	10.45 ± 0.03
4	*Swertia chirata*	349.05 ± 0.34	73.84 ± 0.1	8.65 ± 0.33	27.67 ± 0.09	2.10 ± 0.08	7.73 ± 0.13	7.43 ± 0.07
5	*Tephrosia purpurea*	357.41 ± 0.08	69.60 ± 0.32	10.58 ± 0.32	24.65 ± 0.04	4.073 ± 0.09	8.66 ± 0.2	7.37 ± 0.09
6	*Terminalia chebula*	361.89 ± 0.16	83.43 ± 0.09	3.77 ± 0.1	19.33 ± 0.034	1.45 ± 0.1	2.68 ± 0.17	8.65 ± 0.1
7	*Tinospora cordifolia*	350.45 ± 0.08	69.80 ± 0.31	13.32 ± 0.098	23.30 ± 0.1	1.99 ± 0.16	9.24 ± 0.12	5.63 ± 0.06
8	*Vitis venifera* Linn. (large *)*	325.78 ± 0.08	69.49 ± 0.30	4.85 ± 0.1	1.22 ± 0.34	3.10 ± 0.17	3.30 ± 0.11	19.20 ± 0.03
9	*Vitis venifera* Linn. (small)	316.87 ± 0.31	75.45 ± 0.1	2.89 ± 0.09	1.20 ± 0.034	1.64 ± 0.01	2.45 ± 0.1	17.42 ± 0.09
10	*Terminalia belerica*	358.75 ± 0.25	84.80 ± 0.1	3.27 ± 0.31	2.95 ± 0.098	0.71 ± 0.1	2.64 ± 0.13	8.58 ± 0.09
11	*Cuscuta reflexa*	347.42 ± 0.09	72.32 ± 0.076	10.13 ± 0.14	22.5 ± 0.34	1.96 ± 0.09	7.79 ± 0.07	7.78 ± 0.02
12	*Polypodium vulgare*	356.64 ± 0.12	78.44 ± 0.26	5.66 ± 0.09	13.30 ± 0.29	2.27 ± 0.04	5.22 ± 0.07	8.45 ± 0.01
13	*Zizyphus vulgaris*	343.87 ± 0.15	83.12 ± 0.00	1.81 ± 0.01	3.45 ± 0.00	0.41 ± 0.00	2.84 ± 0.00	11.84 ± 0.01
14	*Sphaeranthus hirtus*	377.40 ± 0.01	78.42 ± 0.05	7.41 ± 0.03	4.39 ± 0.08	3.68 ± 0.00	6.54 ± 0.00	3.71 ± 0.00
				Herbal products				
1	Musafeen	326.4 ± 0.21	75.96 ± 0.1	2.35 ± 0.023	4.03 ± 0.08	1.4 ± 0.01	17.86 ± 0.03	2.30 ± 0.04
2	Itreeful ustokhudus	337.4 ± 0.20	82.7 ± 0.045	0.75 ± 0.06	2.4 ± 0.2	0.37 ± 0.01	1.32 ± 0.02	14.7 ± 0.03

concentration on which Zn effect human health ranges from 100 to 500 mg/l (Macnicol and Beckett, 1985). In case of Co concentration, it was highest in *S. hiritus*.

The Cd concentration was highest in *S. hiritus* while in rest of the species it was not detected (Table 3). Cadmium concentration of 0.2 ppm is also not in the limits set by WHO because the daily intake of 0.06 – 0.07 mg/day is permissible (FAO, 1993). The range of Ni obtained in this study was lower than 0.05 – 5 mg/kg reported for plant foods (FAO, 1993). The Ni concentration was very high in *F. offcinalis* while the concentration in other plant species was either not detected or negligible (Table 4).

The Pb concentration was found high in *F. offcinalis* while in other it was negligible/not detected. In case of the Pb concentration, the suggested concentration in plant species is 2 to 6 mg/l, however, the plant species under investigation carries very lesser level of Pb, which further clarify their use as food supplement (Broyer et al., 1972).

Micronutrient analysis of herbal products

Looking at the results, it revealed that Itreeful ustokhudus has the highest concentrations of Cu, Zn and Cd, while Mussafeen has highest concentrations of Fe, Pb and Ni (Table 4). However, the levels of these concentrations were below the level of WHO recommendation, e.g. Pb has 3.8 ppm and its recommended level is 10 ppm (WHO, 2007).

Conclusion

These medicinal plants species are also used by Local Communities of Northern Pakistan to cure similar kind of diseases for which these products are used (Shinwari et al., 2006; Ahmad and Khan, 2007; Inamdar et al., 2007). Both the proximate and nutrients concentrations for herbal products were almost found in accordance with the standard devised by World Health Organizations (2000) (Lavhale and Mishra, 2007). Contamination of herbal materials includes environmental pollution (that is, contaminated emissions from factories and leaded petrol and contaminated water including runoff water which finds its way into rivers, lakes and the sea and some pesticides), soil composition and fertilizers (WHO, 2007).

Table 4. Micronutrients composition in the medicinal plant species and the herbal products.

Species name	Concentration of micronutrients (ppm)						
	Cu	**Ni**	**Zn**	**Pb**	**Co**	**Cd**	**Fe**
Tephrosia purpurea	9.2	ND	33.4	ND	3.6	ND	702.8
Vitis venifera Linn. (small)	15.4	3.4	14.4	ND	ND	ND	235
Artemisa vulgaris	0.4	ND	7.6	ND	ND	ND	195.4
Terminalia chebula	24.2	ND	34.4	ND	6.6	ND	446
Tinospora cordifolia	ND	ND	3.4	ND	ND	ND	37.6
Swertia chirata	9.8	7.8	45.4	ND	ND	ND	
Fumaira officinale	17.2	13.8	42	9	4.4	ND	2034
Polypodium vulgare	5.8	ND	34.2	ND	2.4	ND	199
Vitis venifera Linn. *(large)*	28.6	ND	17.4	8.4	5	ND	388
Melia azadirachta	5.6	8	15.8	ND	ND	ND	121.8
Terminalia belerica	6.2	ND	32.8	ND	ND	ND	221.8
Cuscuta reflexa	21.8	ND	51.4	ND	8.2	0.2	1546.8
Zizyphus vulgaris	0.2	<0.006	14.6	<0.015	<0.009	1	7.6
Sphaeranthus hiritus	47.4	<0.006	45	<0.015	10.4	1.6	4225
	Herbal products						
Mussafeen	9.8	5.8	22.2	3.8	ND	0.4	209.8
Itreeful ustokhudus	16.8	ND	27.6	ND	ND	8.56	ND

ND = Not detected.

However, for some species difference or higher concentration was recorded, which may be due to pre-vailing environmental and soil conditions and the season when the plants were collected for analysis (Patterson, 1996; Kutbay and Ok, 2001; Odebunmi et al., 2009). The in-depth research would be helpful to further investigate the anti-nutritive, enzymatic and molecular effect on human health of both the products and plant species.

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