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PRELIMINARY COMPARATIVE ANALYSIS OF FOUR BOTANICALS USED IN THE TRADITIONAL MEDICINES OF PAKISTAN

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Abstract

In present study, the proximate parameters and nutrient profile of four medicinal plants viz., *Datura innoxia, Adhotoda visica, Solanum surattens,* and *Acacia nilotica* (leaves and roots) were determined through standard procedures of AOAC and Inductively Coupled Plasma Emission Spectrometer (ICP-OES DV 7300, Perkin Elmer, USA). In proximate parameters, significantly higher ($p \leq 0.05$) ash content and crude fats was observed in *Datura innoxia* as compared to other medicinal plants. The concentration of fiber was highest in *Adhotoda visica* (42.186%) and lowest in *Solanum surattens* (18%). The highest energy value was recorded (329.11 Kcal/100g) for *Acacia nilotica* (roots), while protein and carbohydrates were significant in *S. surattens*. The concentration of the macronutrients ranged from 54.242 to 97.468 ng/g for calcium, 27.552-97.468 ng/g for potassium, 3.457-21.707 ng/g for magnesium, 6.546-9.136 ng/g for sodium, 2.617-8.976 ng/g for potassium and 0.047-0.74 ng/g for sulfur. In conclusion, *D. innoxia* and *S. surattens* were found to be a good source of ash, fats, fiber, carbohydrates and macronutrients (Ca, K, Mg, Na, P, and Si), which can be considered for healthy life besides their medicinal values.

Introduction

From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action (Abbasi *et al*., 2009). Medicinal plants serve as an indispensable constituent of human diet supplying the body with minerals salts, vitamins and certain hormone precursors, in addition to protein and energy (Amaechi, 2009). Medicinal plants contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1982).

The major source of food, nutrition, and health care for both humans and animals are plants and they have provided a source of inspiration for novel drug compounds; as plant derived medicines have made significant contributions towards human health (Shinwari, 2010). Phytomedicines can be used for the treatment of diseases as is done in case of Ayurvedic and Unanii medicine system or it can be the basis for the development of medicines (Ashish *et al*., 2009).

In addition to the therapeutic importance, the search for high-quality but cheap sources of protein and energy has continued to be a major concern of government agencies in charged with the responsibility for food and nutrition in many parts of the developing world. There is a need for a constant search of new resource in developing countries to alleviate hunger, which arises from increasing population, shortage of fertile land and non mechanization of the farming system. Forest products are important for the security of food, health, social and economic welfare of rural communities. Predictions of the future needs, based on the current rates of increasing population and food production, emphasize the seriousness of this problem (Akoja & Amoo, 2011). In the current study, it was aimed to determine the proximate composition of the selected medicinal plants growing in various parts of Pakistan and is important for local communities (Table 1). Besides that, we assessed the nutrient profile of essential and non-essential elements of these species to know whether the intake of these medicinal plants is not harmful.

Material and Methods

Sample collection: Four selected traditionally used botanicals were collected from different areas of Islamabad, Pakistan and were identified by the plant taxonomist. The woody parts and dirt were removed prior to analysis.

Sample preparation: The plants selected for analysis were washed manually with distilled water and ground into a fine powder with the help of grinder (IKA, MF 10) using standard sieve of 1 mm size. Each sample obtained was wrapped in a black polyethylene bag and stored in an air-tight sample bottle in a refrigerator (4°C) for 7 days before analyses.

Table 1. Plant samples collected for the study and pattern of parts used and voucher number.

Specie name	Family	Parts used	Voucher number	Collection areas
Datura innoxia	Solanaceae	Leaves	126866	Quaid-i-Azam University (Campus)
Adhotoda visica	Acanthaceae	Leaves	126871	Bharakau, Islamabad
Solanum surattens	Solanaceae	Leaves	126867	Malpur, Islamabad
Acacia nilotica (roots)	*Fabaceae*	Roots	126875	Shahdarah, Islamabad
Acacia nilotica (leaves)	*Fabaceae*	Leaves	126876	Shahdarah, Islamabad

Proximate analysis: For moisture determination, 3 g of dried sample was taken in the oven (WiseVen, WON-50, Korea) at 105°C until constant weight was reached. Ash content was determined according to the reported method (AOAC, 1990). To remove carbon, about 2 g powdered sample was ignited in a porcelain container and incinerated in a Muffle Furnace (Wise Therm, FHP-03, Korea) at about 550°C for 3 h. Lipid extraction was carried out with a soxhlet extractor with 250 ml of petroleum ether and then the solvent was removed by evaporation. The alcohol soluble extractives were extracted with 90% ethanol (100 mL) by the reported method (Falodun & Irabor, 2008). Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25% NaOH (w/v) solutions (Hussain *et al.,* 2010). Total energy values were calculated by multiplying the amount of proteins and carbohydrates by a factor of 4 and lipid by a factor of 9 kcal/100g (Al-Farsi *et al.,* 2007; Hussain *et al*., 2010). The carbohydrate content was determined by subtracting the protein, fiber, moisture, ash content and fat from 100 (Hussain *et al*., 2011; Al-Harrasi *et al*., 2012). The proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25 (Al-Harrasi *et al*., 2012). All the analyses were done in triplicate.

Macro and micro nutrients analysis: The dried homogenized sample (0.5 g) was taken in Kjeldahl tube (250 mL) and digested with 20 mL of 98% Sulfuric acid (Sigma Aldrich) at 370°C to a colorless liquid. The resultant liquid was diluted with distilled water up to 100 mL and filtered using whatman-42 filter paper. The elements of the four selected medicinal plants were analyzed using Inductively Coupled Plasma Emission Spectrometer (ICP-OES DV 7300, Perkin Elmer, USA) equipped with Perkin Auto-Sampler was used with following parameters: Plasma Flow Rate (15 L/min), Nebulizer Flow Rate (0.8 L/min), RF Power (1500 Watts), Auxilliary Flow Rate (0.2 L/min), Sample Flow Rate (1.25-2.5 L/min), Torch position (-3) for aqueous samples and 15 Sec equilibration.

Results and Discussion

The proximate compositions of *D. innoxia, A. visica, S. surattens,* and *A. nilotica* (leaves and roots) are presented in Table 2. The moisture content in the leaves of *A. nilotica* (7.1%) was highest, while *A. visica* had the lowest (0.3%) in comparison with other medicinal plants. A significant difference ($p \leq 0.05$) was observed in the moisture contents of the plant species (Table 2). According to National Research Council, moisture content of 5-20% in dry matter is regarded as high. Moisture content of *A. nilotica* (both roots and leaves) and *D. innoxia* were found in the recommended range, while other plant species were lower. The results of *D. innoxia* and *A. nilotica* were relative to the moisture contents of *Gliricidia sepium* (6.8%), *Albizu zygia* (7.8%), *Doneillia ogea* (9.9%) and *D. mespiliformis* (9.0%) reported by Ezeagu *et al*., (1996). Similarly, Ayuba *et al*., (2011) showed that the different organs (leaf, seed, stem, pod and roots) of *D. innoxia* have moisture content in the range of 3.50% in roots to 15% in stem. However, the inner water status mainly depends on plant physiology and surrounding environmental conditions.

The ash content, which is an index of mineral contents in plants, was highest in *D. innoxia* (13.6%) while it was lowest in the *A. nilotica* (roots) (3.6%). The *A. visica*, *S. surattens* and *A. nilotica* (leaves) had 9.9, 11.8 and 5.1% ash contents respectively (Table 2). Onwugbuta (2004) reported 12.7, 8.1 and 6.7% ash contents of cow pea seedlings grown under flood and draught conditions showed similarity with the present results. Results of *D. innoxia* resembled to the same Nigerian plant (*D. innoxia*) reported by Ayuba *et al*., (2011). Statistical analysis showed a significant difference ($p<0.05$) in the ash contents of the plant species. Lockeett *et al*., (2000) had also reported high ash content in some greens used by the lactating mother such as bitter leaves, *Veronia colorate* (15.9%) and *Moringa oleifera* (15.1%). This indicated *D. innoxia* could be good source of mineral elements.

The crude protein of the *D. innoxia, A. visica, S. surattens, A. nilotica* (roots) and *A. nilotica* (leaves) were found to be 0.7, 0.5, 0.9, 0.2 and 0.7%, respectively (Table 2). There was significant difference ($p<0.05$) in the protein contents of the four botanicals. According to the National Research Council, USA, crude protein of less than 20% indicates low protein content of that feed stuff. Results of the crude protein were found lower when compared with the result of some tropical plant seeds analyzed by Ezeagu *et al*., (1996), who reported that *Diospyno mespiliformis* and *Entandrophragma angolense* had crude protein contents of 3.5 and 12.3%, respectively. The daily recommended proteins for men and women are from 14.5 to 53.3% of ration. Based on this fact, the samples were considered as poor sources of proteins.

The crude lipid content followed the same pattern with the *A. nilotica* (roots) having the lowest lipid content of 0.9% while the *A. nilotica* (leaves) had the highest (3.2%). *D. innoxia, A. visica,* and *S. surattens* had 3.2, 2.2, and 2.6%, respectively. The result of the lipid content of *D. innoxia* was low as compared with the findings of Ayuba *et al*., (2011), who worked on four different parts of *D. innoxia* belonging to Nigeria with the range of 6-15%.

Crude fiber, which measures the fibrous component (cellulose, hemicellulose and lignin), was highest in the *A. visica* 42.2% followed by roots of *A. nilotica* (37.3%), leaves of *A. nilotica* (37.2%), *D. innoxia* (31.4%) and *S. surattens* (18.3%), respectively. The recommended dietary allowance (RDA) of fibers for children, adults, pregnant and lactating mothers are 19-25%, 21-38%, 28% and 29% respectively. In the current study, the crude fiber in medicinal plants is high compared to 8.5-20.9% in some Nigerian vegetables (Ifon & Bassir, 1980). Adequate intake of dietary fiber can lower the serum cholesterol level, risk of coronary heart disease, constipation, hypertension, diabetes, colon and breast cancer (Ishida *et al*., 2000; Jimoh *et al*., 2010).

Looking at the results of carbohydrates, it was highest in *S. surattens* (66.3%) and lowest in *A. visica* with 45.0% (Table 2). The contribution of the carbohydrates to the energy in food additives recommended is from 55 to 75%. The carbohydrate values of selected samples fall in the acceptable range set by World Health Organization. Thus these medicinal plants can be used as a source of energy contribution in a food ration. The results of carbohydrates obtained from this research were similar to the medicinal plants reported by Hussain *et al*., (2009); on the other hand, it also contained same available carbohydrates compared to *Corchorus tridens* (75.0%) and sweet potatoes leaves (82.8%) (Asibey -Berko & Tayie, 1999).

Table 2. Proximate analysis of the selected medicinal plants. Each value in the table is obtained by calculating the average of three analyses ± standard deviation (percentage).

Plant species	Moisture	Ash	Fiber	Fats	ASE	Proteins	CHO	EV
D. innoxia	6.473 ±0.028	13.58 ± 0.050	31.423 ±0.110	3.242 ±0.08	8.276 ±0.059	0.744 ±0.09	51.007 ±0.150	236.13 ±0.651
A. visica	0.290 ±0.003	9.903 ±0.041	42.168 ±0.191	2.240 ± 0.03	13.322 ±0.081	0.472 ±0.05	44.977 ±0.223	201.96 ±0.917
S. surattens	0.858 ±0.004	11.831 ±0.042	18.275 ±0.052	2.611 ± 0.02	7.991 ±0.002	0.958 ±0.01	66.310 ±0.019	292.58 ±0.117
A. nilotica (roots)	5.657 ±0.008	3.562 ±0.003	37.354 ±0.073	0.852 ±0.03	16.515 ±0.153	0.232 ±0.023	57.998 ±0.07	329.11 ±28.39
A. nilotica (leaves)	7.082 ±0.043	5.125 ±0.041	37.210 ±0.073	3.230 ±0.005	11.339 ±0.026	0.724 ±0.003	53.44 ±0.030	245.86 ±0.085

ASE = Alcohol Soluble Extractives, CHO = Carbohydrates, EV = Energy Value (Kcal/100g)

Table 3. Correlation matrix of proximate parameters.

	Moisture	Ash	Fiber	Fats	ASE	Proteins	CHO	EV
Moisture	1							
Ash	-0.390	1						
Fiber	0.238	-0.506	1					
Fats	0.157	0.558	-0.252	1				
ASE	0.088	-0.806	0.717	-0.825	1			
Proteins	-0.146	0.644	-0.755	0.805	-0.954	1		
CHO	-0.077	-0.042	-0.832	-0.166	-0.260	0.390	1	
E.V	0.192	-0.452	-0.399	-0.625	0.288	-0.209	0.799	1

ASE = Alcohol Soluble Extractives, CHO = Carbohydrates, EV = Energy Value

The extractive potency of the selected samples in alcohol was also determined. Low level of extractive potency was observed in *S. surattens*, while *A. nilotica* (roots) was found to be significantly higher in extractive potency than the remaining samples ($p \leq 0.05$). Significant difference was ($p \leq 0.05$) observed in the alcohol soluble extractives of plant samples.

According to the results of the energy calculations, based on the carbohydrates, crude fats, and crude protein, the highest value (329.11 kcal/100g) was found in *A. nilotica* (roots), while *A. visica* was found to contain the lowest energy value (201.96 kcal/100g). The energy value of our tested samples were found in close proximity to the reported values of some Nigerian leafy vegetables (248.8-307.1 kcal/100g) (Isong *et al.,* 1999), some Ghanian green leafy vegetables like *Corchorus tridens* (283.1 kcal/100g), and sweet potato leaves (288.3 kcal/100g) (Asibey-Berko & Toyie, 1999).

Correlation matrix: Looking at the correlation analysis of the selected medicinal plants, highly significant correlation was observed between similar parameters, while among others the correlation is either non-significant or less significant and moderately related. Strong positive correlation was observed between fats and proteins; fiber and alcohol soluble extractives; and carbohydrates and energy value as 0.805, 0.717, and 0.799 respectively. Fiber showed strong inverse correlation with carbohydrates and protien (-0.832 & -0.755); ash and fats with alcohol soluble extractive (-0.806 & -0.954); and alcohol soluble extractives with proteins (Table 3).

Nutrients profile of selected medicinal plants: Nutrients rich foods are vital for proper growth both in adults and children. There are five major mineral elements which are not only important for plant cellular metabolism but also for human physiology as well. These are calcium (Ca), phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg). The concentration of calcium in the leaves of *A. nilotica* and *S. surrattens* was found to be in greater amount (97.4 and 97.3 ng/g respectively), while lesser amount of calcium was found in *D. innoxia* (54.2 ng/g) (Table 4). *D. immoxia* was found to be in greater amount in potassium (153.8 ng/g), and phosphorus (8.9 ng/g) among all the medicinal plants (Table 4). In case of Mg and Na, *S. surrattens* has the highest concentration of 21.7 and 9.1 ng/g respectively.

Calcium content of the leaves of *A. nilotica* and *S. surrattens* is low when compared with the other medicinal plants used in the western part of Nigeria (Abolaji *et al*., 2007) and South-western part of Nigeria (Alabi *et al*., 2005). Calcium and phosphorus play an important role in the development of plants growth, while its intake in human can enhance the development of bones and teeth, helps in normal blood clotting, regulates heart rhythm, maintain proper nerve and muscle functions (Holechek, 1998). Deficiency of calcium may cause poor development of the growth and different abnormalities (Holechek, 1998). The presence of these minerals is advantageous since certain inorganic mineral elements (potassium, zinc, phosphorus, calcium, and sodium etc.) play important roles in the maintenance of normal glucose tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary & Bandyopadhyay, 1999). However, the lower concentration of Na content is an added advantage because of the direct relationship of sodium intake with hypertension in human (Dahl, 1972).

Table 4. Essential nutrient's composition in the selected medicinal plants (ng/g dry weight). Each value in the table is obtained by calculating the average of three analyses ± standard deviation.

Medicinal Plants	Ca	K	Mg	Na	P
D. immoxia	54.242 ± 0.08	153.803 ± 0.01	10.458 ± 0.06	6.546 ± 0.08	8.976 ± 0.18
A. visica	90.332 ± 0.06	61.518 ± 0.07	16.609 ± 0.08	6.687 ± 0.09	5.899 ± 0.05
S. surrattens	97.392 ± 0.07	104.815 ± 0.05	21.707 ± 0.04	9.136 ± 0.11	5.689 ± 0.12
A. nilotica (roots)	72.059 ± 0.19	27.522 ± 0.09	3.457 ± 0.05	6.937 ± 0.21	2.617 ± 0.06
A. nilotica (leaves)	97.468 ± 0.01	37.563 ± 0.09	6.534 ± 0.09	8.921 ± 0.22	6.747 ±0.03

Table 5. Composition of some trace elements in the medicinal plants samples with Standard Error (ng/g dry weight). Each value in the table is obtained by calculating the average of three analyses ± standard deviation.

Medicinal plants	Ag	Al	B	Ba	Cu	Zn	Si	Sr	W	Fe	Li	Mn
D. immoxia	0.027 ±0.00	0.174 ±0.01	0.395 ±0.02	0.0023 ±0.00	0.054 ±0.00	0.163 ±0.01	3.739 ±0.03	0.257 ±0.01	0.161 ±0.01	0.547 ±0.02	0.003 ±0.00	BD
A. visica	0.028 ±0.00	0.156 ±0.01	0.435 ±0.01	0.0024 ±0.00	0.046 ±0.00	0.199 ±0.02	4.683 ±0.04	0.513 ±0.04	0.158 ±0.02	0.524 ±0.04	BD	0.466 ±0.02
S. surrattens	0.025 ±0.00	0.091 ±0.00	0.608 ±0.02	0.0034 ±0.00	0.093 ±0.00	0.227 ±0.02	3.860 ±0.02	0.374 ±0.02	0.163 ±0.01	0.472 ±0.03	0.035 ±0.00	0.161 ±0.01
A. nilotica (roots)	0.027 ±0.00	0.126 ±0.00	0.292 ±0.01	0.0038 ±0.00	0.067 ±0.00	0.078 ±0.00	3.557 ±0.04	0.471 ±0.02	0.161 ±0.00	0.991 ±0.20	BD	BD
A. nilotica (leaves)	0.028 ±0.00	0.106 ±0.00	0.527 ±0.00	0.0028 ±0.00	0.128 ±0.00	0.336 ±0.02	4.280 ±0.05	0.484 ±0.04	0.162 ±0.02	0.627 ±0.02	BD	0.906 ±0.03

BD = Below detection

In case of non-essential elemental compositions of medicinal plants, 29 different elements including silver (Ag), aluminuim (Al), barium (Ba), copper (Cu), Beryllium (Be), bismuth (Bi), arsenic (As), gold (Au), cadmium (Cd), cobalt (Co), chromium (Cr), lithium (Li), nickel (Ni), lead (Pb), plutonium (Pt), Antimony (Sb), silicon (Si), tin (Sn), strontium (Sr), titanium (Ti), selenium (Se), Thallium (Tl), vanadium (V), tungestun (W), iron (Fe), zinc (Zn), manganese (Mn), molybdenum (Mo), and boron (B) were analyzed by ICP-OES. The quantities of these trace and less essential elements are presented in Table 5.

As, Au, Be, Bi, Cd, Co, Cr, Mo, Ni, Pb, Pt, Sb, Se, and Sn were not detected during this analysis. This indicates that these elements are not present in a detectable amount in the plant parts. This is beneficial to consumers, since it has been reported that some of these minerals like lead, cobalt and cadmium are highly toxic even at low concentrations (Asaolu *et al*., 1997). It has also been reported that for many plant species Cr proved to be toxic at 5 mg/L. In this regard, all the studied plants have very lesser concentration of Cr as compared to that of recommended level for toxicity in plants (Adriano, 1986). In case of Pb concentration, the suggested concentration in plant species is 2 to 6 mg/L (Broyer *et al*., 1972), so the analyzed plant species carries very lesser level of Pb, which further clarifies their use as food supplement or their medicinal benefits.

Low concentration of silver (Ag), aluminium (Al), boron (B), barium (Ba), copper (Cu), zinc (Zn), silicon (Si), strontium (Sr), tungestun (W), iron (Fe), lithium (Li) and manganes (Mn) were observed in all the medicinal plant samples. Although low quantities of some heavy metals, such as copper, and zinc, are essential for many organisms, they are potentially toxic and accumulate in soil over long period of time and this result in soil pollution (Ahmad *et al.*, 2008).

Conclusion

The analysis of selected medicinal plants growing in Pakistan showed a wide range of significantly higher proximate and nutrient parameters. Among these medicinal plants analyzed using standard methods of AOAC (1990), *D. innoxia* and *S. surattens* showed the most balanced and adequate amount of nutritional qualities and essential nutrients. In view of its nutrient composition, all the medicinal plants considered as sole source of macro elements and can be used as one of the potential sources of the elements in the diet. All these medicinal plants can be essential for the maintenance of healthy life and normal body functioning when used in the right proportion.

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