Analytical Investigation of Soil, Bark and Flowers of *Acacia nilotica* Grown in Desert Environment

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Summary: The hot, arid and sandy Cholistan desert is well known for its medicinal plants. Keeping in view the medicinal importance of *Acacia nilotica*, the levels of pH, moisture contents, organic contents, soluble salts, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, CO_3^{2-}, SO_4^{2-} and PO_4^{3-} in soil and plant samples were studied and a strong positive correlation was observed for certain pairs of parameters. The results were expressed on X±SD basis and showed varied levels of selected nutrients in plant and soil samples. In case of soils the observed order was < Cl^-< SO_4^{2-}<Fe^{2+}< Ca^{2+}< PO_4^{3-} the maximum concentration was exhibited by SO_4^{-2} i.e. 266.88±0.28 ppm and minimum by Mg^{2+} i.e. 0.3±0.02 ppm. The plant is rich in PO_4^{3-} i.e. 248.32±0.16 ppm. Some abnormal behavior was observed for Cl^- and CO_3^{2-}. This base line data generated will be helpful for herbal preparations in Eastern System of Medicines (ESM).

Introduction

Ailments and medicinal plants vary in the world and their nature, frequency and methods of administration can change in relation to geography, time and knowledge. Indigenously different plants have been used to cure a disease or several diseases at a time, but towards the middle of 20th century the contribution of medicinal plants to medicine was reduced by approximately one fourth as research and development favored the use of synthetic chemicals. Now this trend is reversing once again in favor of plants, as they have been discovered to possess natural products that are chemicaly-balanced, effective, least injurious with none or much reduced side effects of synthetic chemicals. Research shows that organic plant derived trace minerals actually replace the heavy metals deposited in the body. Therefore, herbal cures and medicines have an attraction, particularly for those who get fed up or disappointed with other methods of treatments. Natural herbal treatments are also appealing to all the tiers of society simply, because they are far cheaper than the synthetics [1-5].

Cholistan desert is a uniquely located wildland of its own kind with scarcity of endemic flora, counting only 115 species belonging to 28 families. This crippling scarcity of plants, which may get further reduced during prolonged droughts, compelled the desert dwellers to discover in detail medicinally each and every plant growing around them. Therefore, there is no plant in Cholistan desert whose economic, food and medicinal values are not known to the inhabitants. The specialized medicinal knowledge of plants is not common, but 'confined' to the local specialist/ herbalists called as "hakims". They offer human and animal treatments, by providing herbs as decoctions, pellets, fresh, crushed or dried. The properties of all herbs is a trade secret, but amazingly the medicinal properties of some of these plants are well known within communities where this knowledge can be expertly used for themselves and their animal's treatments [6,7].

Acacia nilotica is a common plant of Cholistan desert. Due to medicinal and economical values, it was selected for chemical analysis and to manifest the physiological effect on the human beings by knowing the exact chemical contents in plant with relation to soil nutrients [8-11].

Pharmacological Importance

Acacia nilotica is medium sized evergreen tree with bright yellow flowers, which grow in

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clusters. The maturity of pods varies from one geographical location to another. The tree is native to India, Pakistan, Arabia and different parts of Africa [8-12]. *Acacia nilotica* has been reported to contain l-arabinose, catechol, galactan, galactoaraban, galactose, N-acetyldjenkolic acid, *N*-acetyldjenkolic acid, sulphoxides pentosan, saponin, and tannin. Seeds contain crude protein 18.6 %, ether extract 4.4 %, fiber 10.1 %, nitrogen-free extract 61.2 %, ash 5.7 %, and silica 0.44 %. Phosphorus 0.29 % and calcium 0.90 % of DM. When bullocks were given the seeds and bran (2:1) with dry pasture grass daily DM intakes were 1.82, 0.91, and 5.35 kg respectively. Total DM intake/100 kg bodyweight was 1.40 kg. The animals retained 20.8 g N and 7.4 g Ca daily but the P balance was slightly negative. CP content of the browse at 12.9%, the crude fiber at 15.2 % [5, 13-15].

The bark is collected from seven years old or more wild or cultivated trees and is allowed to mature for a year before being used medicinally. Acacia bark and seeds contain 24 % to 42 % tannin and are used for dyeing and tanning leather and also gallic acid. The tannin also contributes to its medicinal use as a powerful astringent. It is also a powerful molluscicide and algicide. Fruits added to ponds in Sudan kill snail species, which carry schistosomiasis without affecting the fish. Bark of Acacia nilotica has been used for treating haemorrhages, colds, diarrhoea, dysentery, tuberculosis, leprosy, as stomachic and as astringent, while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions. The bark is also used for treating depression and to cure asthma [16-20]. Bruised leaves are applied to eyesores in children. The decoction of boiled thorns is taken against pains in the joints and against heavy sweating. The tree also produces gum that is used mainly in the printing industry. The gum is used in paints and medicines and has been collected for millennia. It has similar properties to gum arabic from *A. senegal* and is frequently used in calico printing in India [21].

Pods are used as powerful expectorants. The aqueous extract of the fruit, rich in tannin (18–23 %) has shown algicidal activity against *Chroccoccus, Closteruim, Coelastrum, Cosmarium, Cyclotella, Euglena, Microcystis, Oscillatoria, Pediastrum, Rivularia, Spirogyra*, and *Spirulina* [22]. Pods soaked in cow's milk and dried in shade, ground and mixed with sugar are given in sexual impotency [23, 24]. The pods and leaves are used as astringent in diarrhoea and bark as an astringent lotion. A mixed powder of leaves, flowers, bark, fruit and roots together is used as aphrodasiac. Many *Acacia* species contain some psychoactive alkaloids of which Dimethyltryptamine and *N, N*-dimethyltryptamine, *N*-methyltryptamine are the most prominent and useful. The leaves, stems and/or roots can be made into a brew together with some Monoamine oxidase inhibitors containing plant to obtain an effect when taken orally [25-33].

Results and Discussion

Average level of Na^+ (Table-1) in soil of Cholistan desert is 40 ± 20 ppm which is higher than found in bark i.e. 28 ± 12 ppm, parallel that for highest sodium intake in flowers i.e. 47 ± 3 ppm. Sodium is the major component of the cation of extra cellular fluid. 139 meq / liter of sodium is present in blood plasma of human beings and controls the osmotic pressure including water retention. High concen-tration of sodium leads to hypertension [28, 35].

Level of K^+ in acacia bark is 1200 ± 693 ppm and in flowers is 2167±289 ppm, which are quite high than its level found in periphery soil i.e. 43 ± 27 ppm. This mean that nature has designed plant cells in such a fashion that they have a tendency to pick up more K^+ than Na^+. K^+ is the major component of the cation of intracellular fluid. 5 meq / liter of K^+ is present in blood plasma of human beings and controls the osmotic pressure including water retention. High concentration of K^+ leads to the dilation of arteries and normalize the blood pressure. Excessive amount of K^+ leads to the failure of heart [28, 35].

High concentration of Ca^{2+} in soil samples (Table-1) is averaged to 87 ± 50 ppm (144 ppm in site B soil) than both bark and flowers i.e. 29 ppm. Most soils contain enough Ca^{2+} for adequate plant growth, but acidic soils, where high rainfall occurs; the soils are deficient of Ca^{2+}. The Ca^{2+} is used in the synthesis of new plant cell walls. A protein found in Cytosol, to form calcium-calmodulin complex might be involved in regulation of many metabolic processes. 5 meq / liter of calcium is present in blood plasma of humans [36, 37].

Levels of Mg^{2+} are in the order of flowers < bark < soil and are low in concentration than the

Table-1: Comparative levels of nutritive elements studied in soil, bark and flowers of *Acacia nilotica*.

Sample Code	Soil/Plant Samples	Na^+ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	Fe^{2+} (ppm)	Cl^- (ppm)	CO_3^{2-} (ppm)	HCO_3^- (ppm)	PO_4^{3-} (ppm)	SO_4^{2-} (ppm)
A	Soil	60	15	56	19	67	213	2	10	16	251
	Bark	35	1600	32	29	670	426	6	14	62	551
	Flower	50	2500	24	10	335	994	10	13	71	628
B	***Soil***	20	70	60	21	300	1065	6	7	45	242
	Bark	15	1600	40	15	167	994	11	16	22	394
	Flower	45	2000	24	9	703	780	5	18	56	677
C	Soil	40	44	144	62	100	710	5	6	85	763
	Bark	35	400	16	34	300	710	4	6	34	782
	Flower	45	2000	40	10	67	1065	9	13	76	820
*Soil	Range	20 - 60	15 - 70	56 - 144	19 - 62	67 - 300	213 - 1065	2 - 6	6 - 10	16 - 85	251 - 763
	Average	40	43	87	34	156	663	4	8	49	419
	SD	±20	±27	±50	±24	±126	±428	±2	±2	±35	±298
*Bark	Range	15 - 35	400 - 1600	16 - 40	15 - 34	167 - 670	426 - 994	4 - 11	6 - 16	22 - 62	394 - 782
	Average	28	1200	29	26	379	710	7	12	39	576
	SD	±12	±693	±12	±10	±261	±248	±4	±5	±21	±195
*Flower	Range	45 - 50	2000 - 2500	24 - 40	9 - 10	67 - 703	780 - 1065	5 - 10	13 - 18	56 - 76	628 - 820
	Average	47	2167	29	10	368	946	8	15	68	708
	SD	±3	±289	±9	±1	±319	±148	±3	±3	±10	±100

*Range, average and standard deviation of samples collected from four different sites of Cholistan desert.

Ca^{2+}. Soil of site C has high concentration of Mg^{2+} i.e. 62 ppm. Mg^{2+} is almost never limiting to the plant growth in soils. In plant cells, Mg^{2+} has a specific role in the activation of enzymes involved in respiration, photosynthesis and synthesis of DNA and RNA. Mg^{2+} is also a part of the porphyrin component of the chlorophyll. It combines with ATP and allows it to function [36, 37].

Plant bark (379 ±2 6162 ppm) and flowers (368 ± 31962 ppm) are rich in Fe^{2+} contents than the soil in which the plant is being growing. Fe^{2+} is the central metal of hemoglobin. Most of iron in food occurs in Fe^{3+} like spinach etc. The use of bark and flowers as traditional medicine may cure for anemia, diarrhea, dysentery and jaundice [28].

Chloride levels are found in order of flowers > bark > soil. Abnormalities of Na^+ metabolism are generally accompanied by abnormalities in Cl^- metabolism because concentration of Na^+ and Cl^- is equal in blood plasma of humans. So bark (710 ± 248 ppm) and flowers (946 ± 148 ppm) containing higher concentration of Cl^- are used in medicine as remedy for diarrhea. Cl^- are also essential in water balance, osmotic pressure regulation as well as acid base equilibrium. Mostly it may be required for cell division in leaves and roots of the plants [38].

Levels of CO_3^{2-} observed in soil, bark and flower samples are lower than the levels of HCO_3^-. Carbonic anhydrase, an enzyme found in mammalian tissues, produces and uses protons and bicarbonate ions and plays a key role in the regulation of pH and fluid balance in different parts of human body. The transport of the protons and bicarbonate ions produced in kidney and eyes influence the water content of the cells at these locations. Absence or malfunction of this enzyme can lead to diseased states, ranging from the loss of acid production in the stomach to kidney failure [38].

PO_4^{3-} level in flowers i.e. 68 ± 10 ppm is higher than observed in adjoining soil i.e. 49 ± 35 ppm, which is higher than found in bark i.e. 39 ± 21 ppm. Phosphorus, as phosphate, is an integral component of a number of important compounds present in plant cells. It act as limiting element in soils and absorbed primarily as monovalent phosphate anion-H_2PO_4. The ability of these two forms of ions is controlled by soil pH i.e. monovalent is favored by pH below 7 and divalent form by pH above 7. Phosphorus remains as free phosphate or bound to organic compounds as esters in plants [37].

Concentration of SO_4^{2-} is higher i.e., 763 ppm at site C, than the observed levels for PO_4^{3-}. Sulpher in plants, absorbed as divalent Sulphate ions-SO_4^{2-}, occur in proteins especially in amino acids cystein and methionine that are building blocks of proteins. It is an essential component of vitamins, biotin and co-enzyme [37].

The linear correlation between pairs of selected nutritive parameters studied in soils adjacent to roots of acacia nilotica is shown in Table 2. The statistical linear correlation study at p <0.01 (Table-2) shows strong positive correlations for Na^+-HCO_3^- (r = 0.721). K^+ has strong + correlation with Fe^{2+}, Cl^- and CO_3^{2-}. Ca^{2+} and Mg^{2+} have positive correlation with PO_4^{3-} and SO_4^{2-}. Fe^{2+} shows correlation with Cl^-

Table-2: Linear correlation coefficient matrix* for selected nutritive parameters studied in soil adjacent to *Acacia nilotica* (n = 3).

	Sodium	Potassium	Calcium	Magnesium	Iron	Chloride	Carbonate	Bicarbonate	Phosphate
Potassium	-1.000								
Calcium	-0.040	0.072							
Magnesium	-0.041	0.073	1.000						
Iron	-0.924	0.912	-0.345	-0.344					
Chloride	-0.995	0.998	0.136	0.137	0.883				
Carbonate	-0.961	0.969	0.316	0.317	0.782	0.983			
Bicarbonate	0.721	-0.742	-0.722	-0.722	-0.401	-0.784	-0.885		
Phosphate	-0.419	0.447	0.924	0.925	0.039	0.504	0.654	-0.931	
Sulphate	0.015	0.016	0.998	0.998	-0.396	0.081	0.263	-0.682	0.902

*Values>0.3 or < -0.3 are significant at P< 0.01

Table-3: Linear correlation coefficient matrix* for selected nutritive parameters studied in bark of *Acacia nilotica* (n = 3).

	Sodium	Potassium	Calcium	Magnesium	Iron	Chloride	Carbonate	Bicarbonate	Phosphate
Potassium	-0.500								
Calcium	-0.756	0.945							
Magnesium	0.967	-0.703	-0.897						
Iron	0.704	0.262	-0.068	0.501					
Chloride	-0.866	0.000	0.327	-0.711	-0.965				
Carbonate	-0.961	0.721	0.908	-1.000	-0.480	0.693			
Bicarbonate	-0.655	0.982	0.990	-0.825	0.075	0.189	0.839		
Phosphate	0.731	0.225	-0.106	0.534	0.999	-0.974	-0.513	0.037	
Sulphate	0.806	-0.916	-0.997	0.930	0.148	-0.402	-0.939	-0.975	0.186

*Values>0.3 or < -0.3 are significant at P< 0.01

and CO_3^{2-}. Cl^- is positively correlated with CO_3^{2-} and PO_4^{3-}. CO_3^{2-} and PO_4^{3-} are positively correlated to PO_4^{3-} and SO_4^{2-} respectively [38].

Tables-3 and 4 shows differences in correlation pairs for plant bark and flowers indicating the selectivity of soil nutrient absorption by different plant cell of acacia niolotica. This correlation data is very useful to find out the medicinal uses of different parts of the plant. However, this needs proper source identification in order to know which sources contribute to a significant correlation of these parameters in plants and adjacent soils [38].

Experimental

During this study, 6 samples (2 from each site, mixed together to give single representative sample of each site) of *Acacia nilotica* and adjoining soil near the plant roots were collected during September 2000 to January 2001 from three different areas of Cholistan desert as given in Table-5. The samples were then analysed for different cations and anions like Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cl^-, CO_3^{2-}, HCO_3^-, PO_4^{3-} and SO_4^{2-} using different laboratory methods [37-39].

Chemical Reagents

All the reagents and chemicals used were of AR grade procured from E.Merck and BDH. All the solution of Standards and Samples were prepared in freshly prepared deionised water.

Plant Sample Preparation

The plants were cleaned visually to remove the dust particles and dried at 150 °C to a constant weight. The dried plants were grinded to fine powder and then used for dry ashing. The pre-cleaned silica crucible was heated at 600 °C to a constant weight. The powdered plant material in the crucible was heated in a muffle furnace at 600 °C until there was no evolution of smoke. The crucible containing plant ash was cooled at room temperature and moistened with deionised water to keep it overnight. The undissolved particles were filtered and make up the volume to 1000 ml. This solution was used as sample solution [37].

Soil Sample Preparation

20 grams of dried soil was taken in a 500ml. beaker, stirred it with 100 ml. deionised water for 30

Table-4: Linear correlation coefficient matrix* for selected nutritive parameters studied in flowers of *Acacia nilotica* ($n = 3$).

	Sodium	Potassium	Calcium	Magnesium	Iron	Chloride	Carbonate	Bicarbonate	Phosphate
Potassium	1.000								
Calcium	-0.500	-0.500							
Magnesium	0.500	0.500	0.500						
Iron	-0.090	-0.090	-0.817	-0.908					
Chloride	0.278	0.278	0.693	0.971	-0.982				
Carbonate	0.655	0.655	0.327	0.982	-0.812	0.908			
Bicarbonate	-0.500	-0.500	-0.500	-1.000	0.908	-0.971	-0.982		
Phosphate	0.277	0.277	0.693	0.971	-0.982	1.000	0.908	-0.971	
Sulphate	-0.697	-0.697	0.969	0.272	-0.651	0.494	0.085	-0.272	0.495

*Values>0.3 or < -0.3 are significant at $P< 0.01$

Table-5: Plant Sampling Location of Cholistan desert*.

Sample code	Sampling Areas
A	Plant and soil samples collected near Cholistan institute of desert studies, Baghdad-ul-Jadeed Campus, Islamia University, Bahawalpur.
B	Plant and soil samples collected near Electrical engineering department, Baghdad-ul-Jadeed Campus, Islamia University, Bahawalpur.
C	Plant and soil samples collected from Model Town B, Bahawalpur.

*Sample from each site is the representative mixture of two individual samples of that site.

minutes and filtered through 'Whatman 42' filter paper. The volume of the filtrate was made up to 1000 ml. and is used for quantitative estimation of Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cl^-, CO_3^{2-}, HCO_3^-, PO_4^{3-} and SO_4^{2-} [37].

Methods

Sodium and potassium were determined by flame photometer model 'Corning – 40'. Calcium and magnesium were determined by cmplexometric titration with EDTA. Iron was determined by standard oxidation-reduction titration. Carbonates and bicarbonates were determined by titrimetric method and chlorides were determined by the standard Argentometric method using potassium chromate indicator Phosphates were determined by calorimetric method using 'ammonium dihydrogen phosphate' as standard solution and 'molybodate' as complexing agent. Sulphates were estimated gravimetrically [37, 39].

Statistical analysis

The data was statistically analysed by using SPSS 12 and STATSICA (StatSoft 1999) softwares on P-IV system. The concentrations of elements in canal water samples were correlated by linear correlation coefficient matrix (pearson).

Conclusions

Sophisticated analytical techniques of the present day have given a new impetus for the use of plants within medical research. Active ingredients from plants individually or in combination from different sources, are generating new interest in modem drug therapies and their combination with synthetics has increased. Plant derived organic, ionic minerals naturally chelate or remove the excess inorganic minerals from the body and cannot build up to toxic levels in the soft tissue as well as accelerate the healing process of chronic illness. This data will help in establishing baseline levels on the use of the plant as medicine.Acknowledgements

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