



Determination and assessment of essential elements in *Acacia Nilotica*-a medicinal and folk uses by different analytical methods

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Abstract

Acacia Nilotica is a good source of several nutrient elements essential for some metabolic processes. The pods of *Acacia Nilotica* collected from different areas in Sudan have been analysed for 6 minor (Li, Na, K, Mg, Ca, P) and 3 trace (Fe, Zn, Mn) elements by flame photometry and atomic absorption spectrophotometry using the standard addition and calibration curve methods, respectively. The phosphorous, however, was determined U.V. spectrophotometrically using the standard addition method for measurement of the absorbance, at 410 nm of the complex produced by the coupling of meta-molybdate ammonium vanadate with phosphorous. The ash content, density, colour and pH were also determined by standard methods. The results obtained showed that *acacia nilotica* samples have been found to be especially rich in several nutrient elements such as, K, Fe and P. Concentration of elements are discussed vis-à-vis their medicinal/therapeutic uses. Several statistics like PCA and correlation matrix between elements and pH were performed to the dataset in order to distinguish between geogenic enrichment, lithiogenic, and anthropogenic pollution elements found in the pods. The results suggested anthropogenic and geogenic origin of Na, Ca, P, Fe, Zn, Mn, while Li, K, Mg showed lithiogenic and/or geogenic origin and was associated and controlled by mixed origin.

Keywords: *Acacia nilotica*; minor and macro nutrient elements; photometric and AAS analysis; anthropotic and statistical analysis.

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1. INTRODUCTION

There are an increasing public interest and acceptance of natural therapies in developing and developed countries[1]. The world health organization (WHO) has declared that about 80% of the world's population, especially in developing countries, uses herbal medicine as their source of primary healthcare [2].The use of medicinal plants always plays an important role in African life where it constitutes a major source of therapy and healthcare needs in Africa. The traditions of collecting plants, processing herbal remedies and applying them have been handed down from generation to generation. African traditional medicine is the oldest and perhaps the most diverse of all medicine systems[3]. *Acacia nilotica* (Sunut in Arabic) is a member of subfamily mimosoideae of leguminous trees. The tree is readily distinguished by long white spines, yellow head inflorescence and grey necklace-like pods. It is a medium to large tree that can reach a height of 10 m, with an average of 4-7 m in height. It is widely distributed in Africa from Egypt and Mauritania to south Africa especially in Sudan "Table 1",some sub species are wide spread in Asia as far east as Burma [4] "Fig 1".It is considered as a very important economic plant since early times as a source of tannins, gums, timber, fuel, fodder and medicines.*Acacia nilotica* pods are used in treatment of wound, malaria, sore throat (aerial part) and toothache (bark)[5-8],while Gum Arabic is applied for kidney diseases treatment[9]. Most of the acacias produce tannins while *acacia nilotica* produces more than 20% tannins, especially the inner bark which is used commercially for tanning and dyeing leather black in Sudan and Egypt [10]. Recently, *acacia nilotica* has been shown to affect central nervous system activities, in addition to their known antioxidant and anti-inflammatory activities these plants could provide novel poly-pharmacological leads of potential benefit to the treatment of Alzheimer's disease [11].The *acacia nilotica* pods are very characteristic, resembling a beaded necklace which are flat, straight or slightly curved, and fleshy when young with reddish hairs, becoming dark blackish when mature "Table 2". The pods are sweetly scented when crushed and contain a sticky fluid[12]. They have many biological activities including antihypertensive, antispasmodic[13]and molluscicidal properties [14]. Also, *acacia nilotica* pods are reported to be rich in polyphenols which decreases the blood glucose levels[15]and rich in tannins such as tannic acid which stimulates the transport of glucose and inhibits adipocyte differentiation [16,17]. Moreover, Egyptian Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods [18]. Extracts from pods have demonstrated growth inhibition of at least four species of pathogenic fungi [19].*Acacia nilotica* is also a multipurpose legume which used for rehabilitation of dry land increases soil organic carbon, total and available forms of nitrogen and phosphorus under its canopy so it can be used in soil amelioration [20]. The quality of many medicinal plants depends on the concentration and type of minerals that they contain as play a very significant role, considering their curative properties against a variety of degenerative diseases. Several authors all across the world reported many studies on the important of elemental constituents of the herbal drug plants which enhances the awareness about trace elements in these plants [21].Because *acacia nilotica* globule heads are widely used as herbal medicine and its extract powder as smoke, a lot of data is available concerning its organic constituents [22],very little data is known about its inorganic components.The present study was carried out on *acacia nilotica* pods. The aim of this study was to establish detailed findings on the alkaline earth metals (Li, Na and K), Alkali metals (calcium and magnesium), heavy metals (iron, manganese and zinc) and phosphorus in the pods selected in five different zones in Sudan with specific variation in soil. *Acacia nilotica* pods used for this study was based on their extensive use in traditional medicinal system of Sudan.

2. Material and methods

2.1. Study area

Five sites were chosen as source of samples, Elobied lies on the western part of Sudan (latitude 13°N and longitude 30°E) with rainfall of about 600 mm/year and generally sandy clay loam soil, Gadarif state lies on the eastern part of Sudan, adjacent to Ethiopia, between latitude 12.4-15.4° and longitudes 33.3-30.3° E, the soils are deep dark colours, high clay content and strong vitriolic properties, Ad Damazim lies on the southern part of Sudan (Latitudes11.7891° N and Longitudes 34.359° E), the soil consists of dark cracking clay (Vertisol) which extends to at least 15 m in depth, Abu Hamed lies on the northern part of Sudan (Latitudes 19°30' N, and Longitudes 33°20' E) between the 3rd and 5th cataracts in the River Nile and El Gazera state which lies on the central part of Sudan, The soil consists of dark cracking clay (Vertisol) which extends to at least 15 m in depth.

2.2. Sample Digestion

An accurate weight of dried, ground, fine powder pods was incinerated in a platinum crucible over the burner. The charred material was heated in muffle furnace for four hours and digested in (5:1) mixture of nitric acid and perchloric acid[21]. The solution was filtered over Whatman filter paper 40 and the volume was adjusted up to 100 ml using de-ionized water. For lithium, sodium and potassium 1000 ppm stock solution were prepared; 100 ppm of lithium, sodium and potassium solutions were prepared from stock solution. After that the solutions were prepared in 50 ml volumetric flask then emissions of the solutions were measured. The determination of magnesium and calcium was done by preparing 1,2 and 3 ppm solution from 100 ppm solution and diluted in 50 ml volumetric flask then the emissions were read of standard solution and samples. Colorimetric procedures for the determination of phosphate utilizing the stable yellow colour which is developed when an excess of molybdate is added to an acidified solution of orthophosphate and vandate have been described. The method is quite free of interference. Possible interference from soluble silicate was studied at the concentration of nitric acid present during colour development, silica interference is negligible. The optical density at 430 nm of a solution containing 2 mg of soluble silica per 100 ml was studied with varying amounts of nitric acid added to it. 25 ml of the molybdivanadate reagent was added in each case.



2.3. Physico-chemical analysis

It includes number of parameters like colour, pH and ash content. The colour differences of the pods is due to the differences in the tannin's contents of the pods according to different sites and this in accordance with Dilleman [23] which might be attributed to environmental modifications. The soil pH may influence nutrient absorption and plant growth in two ways either by direct effect of the hydrogen ion or indirect through its influence on nutrient availability. Measurements of pH were carried out on a Jenway 3505 pH-meter using a combined glass electrode. Solutions for measuring pH were prepared by weighing 0.1 g of dried, ground pods which was dissolved in a 100 ml volumetric flask using double de-ionized water, filtered after 24 hours, then pH was measured. For ash content the charred material was heated in muffle furnace for four hours at 260 °C and the crucible was cooled at room temperature in desiccator, weighted without delays to determine the ash content percentage.

2.4. Quality control analysis

Chemicals used for samples and standard solutions preparations were purchased from LOBA CHEMYE and Scott science. Standard solutions were prepared in double de-ionized water and used to calibrate the instruments. Precision and accuracy of analysis were checked through repeated analysis against NITS Standard Reference Material 1570A. A SHIMADZU U.V1800 ING200V UV-Visible double-beam spectrophotometer with matched 1-cm quartz cuvettes and a fixed slit width (2nm) was used for all the absorbance measurements and treatment of data. The atomic absorption spectroscopy (AAS) measurements were determined by an air-acetylene flame atomic absorption spectrometer (Perkin Elmer 3110). Metal hollow cathode lamps specific for each metal, were used as radiation/sources and a JENWAY Clinical PFP7 flame photometer.

2.5. Statistical analysis

2.5.1. Principal component analysis (PCA)

PCA is a powerful analytical technique for pattern recognition that is designed to transform the original variables into new, uncorrelated variables (axes), called the principal components, which are linear combinations of the original variables. It extracts the eigenvalues and eigenvectors from the covariance matrix of original variables. The eigenvalues of the PCs are the measure of their associated variance, the participation of the original variables in the PCs is given by the loadings, and the coordinates of the objects are called scores [24-26]. PCA has been widely used to deliver more information on links among sampling sites, pollutant concentrations, correlation patterns, and latent factors responsible for the data-set structure in the environmental studies [27-30]. Principal component analysis (PCA), is generally employed to reduce the dimensionality of a data set while attempting to preserve the relationships present in the original data [31].

2.5.2. Correlation Matrix

Correlation can tell you something about the relationship between variables. It is used to understand whether the relationship is positive or negative and the strength of this relationship. Correlation is a powerful tool that provides these vital pieces of information. It is measured by what is called coefficient of correlation (r). Its numerical value ranges from +1.0 to -1.0. It gives us an indication of the strength of relationship. In general, $r > 0$ indicates positive relationship, $r < 0$ indicates negative relationship while $r = 0$ indicates no relationship (or that the variables are independent and not related). Here $r = +1.0$ describes a perfect positive correlation and $r = -1.0$ describes a perfect negative correlation. Closer the coefficients are to +1.0 and -1.0, greater is the strength of the relationship between the variables.

2.5.3. Cluster analysis (CA)

The purpose of cluster analysis is to identify groups or clusters of similar sites on the basis of similarities within a class and dissimilarities between different classes [32]. Hierarchical cluster analysis (HCA) examines distances between samples. The most similar points are grouped forming one cluster and the process is repeated until all points belong to one cluster [33,34]. The result obtained can be presented in a two-dimensional plot called dendrogram.

All multivariate statistical analyses, including principal component analysis (PCA) and correlation matrix (CM) were conducted using SPSS 16.0 for windows. For comparison of the heavy metal and pods data, statistical significance was computed by ANOVA. Differences are considered to be significant if $P < 0.05$ [35,36]. In order to look for a possible linear dependence between the different heavy metal homologues in pods samples, obtained heavy metals were tested for correlations between them using Pearson's correlation coefficient based on the assumption that the data were normally distributed.

3. Results and discussion

3.1. Macronutrients (Li, Na, K, Mg, Ca, and P)

A bar plot of macronutrients (Li, Na, K, Mg, Ca and P) at mg/g concentration in "Figure 3" shows little variations in elemental concentration except for K, which vary in a wide range. The K concentration of all pods collected from different sites is much higher than that of all elements. Lithium is not known to be an essential plant nutrient but there is some evidence that Li^+ can affect plant growth and development. Due to lithium's small size and high charge density, it shares some properties with Na^+ [37]. Most metals interact with soil particles via cation exchange and possibly specific adsorption depending on their chemical properties. The biochemical mechanisms of action of lithium appear to be multifactorial and are intercorrelated with the functions of several enzymes, hormones and vitamins, as well as with growth and transforming



factors[38].Li⁺ concentration varied from 0.143 mg/g for pods collected from the northern site to 0.966 mg/g for the pods collected from the eastern site. The highest mean Li content found in the Rosaceae family plants was about 2.9 mg/kg[39]. The US Environmental Protection Agency (EPA) in 1985 estimated the daily Li intake of a 70 kg adult to range from 650 to 3100 µg [40]. Na⁺ concentration ranged from 0.081 mg/g in the pods collected from the northern site to 0.322 mg/g in those collected from the western site. Potassium in plants functions in protein synthesis, activation of enzymes, major solute functioning in water balance and thus affecting osmosis, operation of stomata. In animal, K has a diuretic nature while Na plays an important role in the transport of metabolites. The K⁺ concentration ranged from 1.962 mg/g in the pods collected from the central site to 6.321 mg/g in those collected from the southern site. The ration of K/Na in any food is an important factor in prevention of hypertension arteriosclerosis with K depresses and Na enhances blood pressure. A plot of K/Na ratio showed that the highest K/Na ratio was in pods collected from the southern site,39.25, followed by those collected from central site, 28.43, then those collected from the northern site,19.91, followed by those collected in the eastern site, 15.5, and the pods collected from southern site showed the lowest value 11.79. Results confirm that the consumption of *Acacia nilotica* pods cultivated in different Sudan sites, have contributed to the near complete absence of cases of high blood pressure. Magnesium plays an important role in plants, particularly into the seeds. It is also said to promote formation of oils and fats, possibly by increasing photosynthetic activity in the leaves. Mg⁺⁺ in animal is required in the plasma and extra cellular fluid where it helps in maintaining osmotic equilibrium in the body. Mg concentration varied from 0.870 mg/g for pods collected from northern site to 0.340 mg/g for pods collected from southern site.

Calcium is essential in plants for the formation and stability of cell walls and maintenance of membrane structure and permeability, activates some enzymes, regulate many responses of cells to stimuli. Calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin. "Table 3" shows that calcium concentration varied from 0.19 mg/g for pods collected from the southern site to 0.535 mg/g for the pods collected from the central site. Phosphorus is generally available in the soil as phosphate, an anion that is not bindable by the cation exchange complex and thus can be easily leached from the soil by rain or runoff [41]. Phosphorus is located in every cell of the body and is vitally concerned with many metabolic processes, including those involving the buffers fluids. Phosphorus concentrations varied from 1.555 mg/g for the pods collected from the northern sites to 2.442 mg/g for the pods collected from the central site. This may be due to the nature of the soil of central and northern sites that contain large amounts of clay which sorb more P than those containing small amounts [42]. There is definite relationship between calcium and phosphorus in the formation of bones and teeth and as the major structural elements of the skeletal tissue. Under conditions of salt restriction, calcium appears highly important in helping to maintain the potassium content of tissue [43]. A bar plot of Ca/P ratio is shown in "Figure3 " the highest Ca/P ratio was in pods collected from the northern site 0.232, followed by those collected from central site, 0.219, then those collected from the eastern site,0.183, followed by those collected in the western site, 0.172, and the pods collected from southern site showed the lowest value, 0.082 mg/g.

3.2. Micronutrients (Fe, Zn and Mn)

Bar plot of micronutrients (Fe, Zn and Mn) in "Figure 3" shows little variation in elemental concentration except for Fe which vary in a wide range. Iron is a component of cytochromes, electron transport, activates some enzymes, plays a role in chlorophyll synthesis, where, it functions as haemoglobin in the transport of oxygen, in cellular respiration. Iron concentration varied from 2.988 mg/g for the pods collected from northern to 0.659 mg/g for the pods collected from the eastern site. The permissible limit of FAO/WHO [44, 45] in edible plants was 20 mg/kg. Zinc is active in formation of chlorophyll, activates some enzymes, plays a role in formation of auxin, chloroplasts, and starch. Zn concentrations vary from 0.946 mg/kg for the pods collected from the eastern site to 0.893 mg/kg, for the pods collected from the southern. "Table 3" shows that the differences in concentrations of zinc for the pods collected from different sites was very small compared to 27.4 ppm permissible limit set by FAO/WHO [45] in edible plants and this could be attributed that Zn has low mobility in soils [46] and has a tendency of being adsorbed on clay size particles[47]. Manganese is active in formation of amino acids, activates some enzymes, coenzyme activity, required for water-splitting step of photosynthesis, chlorophyll synthesis. Mn concentration varied from 0.418 mg/g in pods collected from the central site to 0.192 mg/g in pods collected from the eastern site. Mn was found below the permissible limit of 6.61 ppm as suggested by WHO [44]. It is clear from the results at hand that the micronutrients iron, zinc and manganese concentrations in the *acacia nilotica* pods collected from the five sites of Sudan is below permissible limit set by FAO/WHO.

3.3. Statistical data treatment

3.3.1. Correlation matrix analysis

Correlation analysis of the total element contents and pH "Table 4" showed weak negative and positive correlation among pH and all the elements. Li showed moderate positive correlation among Zn ($r = 0.637$) and P ($r = 0.762$) and negative correlation among Na ($r = - 0.671$) and K ($r = - 0.564$), respectively. Na showed moderate correlation among K ($r = 0.572$) and Mg ($r = 0.664$) and negative correlation among Fe ($r = - 0.628$) and Zn ($r = - 0.731$), respectively. Ca moderately and positively correlated with Fe ($r = 0.714$) and Zn ($r = 0.786$) and strongly and negatively with Mn ($r = - 0.930$). The relationships between the elements in the soils in the different area appear complex and difficult to explain individually. These strong correlations among metal-metal pair may be an indication of common sources of these metals as well as similar geochemical characteristics [48]. Other elements did not show any significant correlation among each other in the pods matrix suggesting different origin or dissimilar sediment-logical properties. All elements contents in pods do not correlate with pH, owing to the narrow range of pH (3.6- 5.1) measured. This means that pH has limited importance on the elements mobility and distribution [49].The correlation analysis showed significant differences in elements between sites



for all pods samples which confirm their probable common natural or anthropogenic origin. More explanation can be enabled by PCA.

3.3.2. PCA

It was applied to the whole set of data, the concentration of each elements in the five studied area were treated as collective variables. PCA yielded three principal components with eigenvalues > 1 , they accounted for more than 83.939 % of the total variance. The components were rotated using Varimax rotation. "Table 5" gives the rotated loadings and communalities. The loadings having a greater than 0.5 are marked bold in the table. Factor analysis was used to give information about the distribution and source identification of element pollution based on eigenvalues [50,51]. The scores and loadings plots of PCA are presented in "Figure 4" in order to have clear visualization of the data trends. Factor 1 explaining 47.852% of the total variance, was moderately and positively related to Li (0.637), strongly and positively related to Ca (0.879), Fe (0.810) and Zn (0.987), moderately and negatively related to Na (-0.932), Mg (-0.734) and to Mn, (-0.793), (Table 5). The relation of these metals with PC1 can be indicated by their different natural sources lithogenic and /or geogenic, where Ca, Fe, Zn and Mn are well known to be geogenic [51-54]. Non-existence of any correlation of P "Table 4" with other metals except Li ($r = 0.595$) and its lower loading in PC1 "Table 5" indicates its lithogenic origin. The high negative correlation of Mn with Ca ($r = -0.930$), Fe ($r = -0.786$) and Zn ($r = -0.750$) and the negative loading in PC1 can be explained by its different source and its chemical behaviour in soils, notably the formation of oxides onto which other elements are adsorbed [48]. The high loading of Zn on PC1 (0.987) indicates industrial or urban contribution (anthropogenic) and/or from parental rocks (geogenic). Factor 2 and 3 explaining 19.821 and 16.267 % of the total variance respectively showed highly positive factor loading on K (0.845) and moderate negative loading on Li (-0.644) which may indicate the lithogenic and/or geogenic source, although these two factors are insignificant and represent the physicochemical source of the variability [49].

3.3.3. Metals and pH grouping using hierarchical cluster analysis (HCA)

Dendrogram is a branching diagram that represents the relationships of similarity among a group of entities. We can use a dendrogram to represent the relationships between any kinds of entities as long as we can measure their similarity to each other. In this dendrogram, "Figure 5", we have cut a text into 15 chunks (15 segments). The arrangement of the chunks or segments tells us which chunks or segments are most similar to each other. The length of the branch points indicates how similar or different they are from each other: The greater the branch, the greater the difference. There are two ways to interpret this dendrogram: in terms of large-scale groups or in terms of similarities among individual chunks. Results of HCA performed on the elements found in the studied areas are depicted in "Fig.5". To identify large-scale groups, we start reading from right to left, finding the branch points that are at middle levels in the structure. In this dendrogram Chunks Li, K and Mg are more similar to each other in origin than they are to Mn, P, Zn, Ca, Fe and Na. Going more from the middle level to the left, chunks pH is linked with all elements. If we are trying to identify which individual segments are most similar to each other, we read the dendrogram from left to right (bottom of the dendrogram), identifying the first clades to join together as we move from left to right. The connection between Li and K, Mg and the southern site is the closest link to the bottom of the diagram. Therefore they are most similar and join together first in the branching diagram and similarly clustered than they are to any other chunks. Moreover, the cluster comprised of western and central sites is linked with the first cluster. In the third cluster pH, northern and eastern are very well linked. The fourth cluster comprised of Mn, P and Zn but is also linked with Ca, Fe and Na. The longer of the horizontal lines indicates the degree of difference between branches, the longer the line, the greater the difference. The branch points that are at high levels in the structure indicate that chunks Li, K, Mg, southern, western and central areas are not associated with Mn, P, Zn, Ca, Fe and Na. The elements Li, K, Mg clusters may be lithogenic and/or anthropogenic, while the elements Mn, P, Zn, Ca, Fe and Na clusters may represent the mixed origin (anthropogenic and geogenic) and/or be present under natural condition. Correlation analysis mentioned earlier supports the results of cluster analysis. From the results, a strong evidence of mutual dependence of these elements in the acacia nilotica system is anticipated.

4. Conclusion

The concentrations of 6 minor and three trace elements were determined in *acacia nilotica* by flame photometry and atomic absorption spectrophotometry, respectively using the standard addition and calibration curve methods. The ranges of elemental concentrations have been found to vary in a wide range of mg/g in all the samples except K. It has been observed that *acacia nilotica* is enriched in K, especially grown in southern area (6.321 mg/g), Fe, especially grown in southern and northern areas (2.988 mg/g) and P, especially grown in southern area (2.442 mg/g). Further, the highest K/Na ratio changes in a wide range 20.6 (central site) to 4.0 (southern site). The highest Ca/P ratio ranged from 0.239 (northern site) to 0.103 (southern site). The statistical data obtained revealed that the concentrations of the elements in the pods are strongly affected by the characteristics of the soil in the studied areas. The results also suggest anthropogenic and/or geogenic origin of Na, Ca, P, Fe, Zn and Mn, while lithogenic and geogenic of Li, K and Mg. When compared to estimated daily intake values, the elements concentrations did not exceed the limits in all of the studied areas. This means that the daily intake of these elements from the pods and their possible role in the body system are safe.



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