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Analysis of Sudanese *Cassia senna:* Some Parameters for Chemical Quality Control

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Abstract

The quality control determination on senna pods (prepared from *Cassia senna* L., which grows wildly in Central Sudan), have been conducted. Moisture, ash, crude fibre water and ethanol soluble extractive and total hydroxyanthracene glycosides (calculated as sennoside B) content have been carried out using C. *senna* pods collected from three different populations. Concentrations of K, Ca, Ti , Cr , Mn , Fe, Cu, Zn , Pb, Br, Rb, Sr, Zr and Nb in senna pod were also determined by X-Ray fluorescence. The mean content of sennoside B of Sudanese senna pods ranges between 4.5% and 6.0% of dry pod weight. Thus, the investigated pod samples could be considered as official. Within the three localities of the study, variations exist in relation to the mineral content of the pod.

Keywords: *Cassia senna;* sennoside B; Quality control; metal content; Sudan.

Introduction

Growing world-wide interest is stimulating ever-increasing and diverse studies of the properties and uses of medicinal plant materials and raised legitimate inquiries about their quality, safety and efficacy. Scientifically sound data are lacking for many medicinal plant materials, extracts and active ingredients, and in most tropical countries, including Sudan the herbal medicine market lacks regulations; some products may be neither registered nor controlled.
In continuation of our work on chemistry of Sudanese medicinal and aromatic plants [1]-[3]. We have taken into consideration the quality control determination on senna pods prepared from *Cassia senna L.* (Family Caesalpiniaceae), growing wildly in three different localities in central Sudan, namely, Ed-Damer, Tendelti and El-Obeid.
Cassia senna is undoubtedly is the most reputed indigenous medicinal plants of the Sudan. Pods are a rich source of pharmaceutically active anthraquinones, which is in high demand by international companies and are widely used as laxative and described in most pharmacopoeias [4].
Quality and purity of this important herbal drug has now become a key issue in industrialized and

developed countries.

Materials and Methods

Plant Materials: The samples of *C. senna* pods were collected from three localities in Central Sudan, namely, Ed-Damer (around 17^0 34′ N Lat. And 33^0 56′ E Long.) Tendelti (around 13^0 02′ N Lat. and 31^0 55′ E Long.) and Elobeid (around 13^0 10′ N Lat. and 30^0 14′ E Long.) in September, 2003). The pods were dried for 2 weeks at room temperature before analysis.

Properties of senna pods. The moisture, ash, acid-insoluble ash, sulfated ash, crude fibre, alcohol- and water- soluble extractive of senna pod samples were determined according to standard procedure [5-6].

Moisture content:

The moisture content was determined according to the A.O.A.C method (1980). Six random lots of two-gram samples of each ground plant material were accurately weighed and placed in separate crucibles. The samples were left in an oven at 150 ^{0}C for three hours then transferred to a desiccator for one hour to cool. The samples were finally weighed and moisture percentage was calculated.

Total ash:

Total ash was determined according to standard procedure (Zhi-cen,1980). Six random lots each of two-grams of ground material were separately placed in different crucibles. Each was pre-ignited and weighed. The crucibles were placed in a muffle furnace at 450 ^{0}C until free from carbon. Each crucible was cooled in desiccators and weighed and the weight was calculated in g of ash per 100g of air-dried material. The determination of ash is a method used to measure the amount of the residual substance not volatilized when the plant sample is ignited by the above method described.

Acid-insoluble ash content:

Acid-insoluble ash content was determined according to standard method (Zhi-cen, 1980). The pre-weighed ash was dissolved in 25 ml HCL (70 g/L) in the crucible covered with a watch-glass, boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and rinsing were added to the crucible. The insoluble matter was collected on an ashless filter paper and then washed with hot water until the filtrate is neutral.

The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and then ignited to constant weight and the content was calculated in g of acid-insoluble ash per 100g of air –dried material. The acid-insoluble ash is the residue obtained by boiling the total ash with diluted HCl, collecting the insoluble matter in a filter, washing and igniting. The determination of acid-insoluble ash is a method intended to measure the amount of silica, especially sand and siliceous earth, present in the plant.

Sulfated ash content:

Sulfated ash content was determined according to standard procedure (Zhi-cen, 1980). Two-grams of each of six random lots of ground material were separately placed in different crucibles and 2 ml of sulphuric acid (1760 g/l) was added, heated at first on a hot plate until the sample was carbonized and then incinerated carefully to about 800 ^{0} C unit carbon-free. Each crucible was cooled and then a few drops of sulphuric acid (1760 g/L) was added to moisten the residue, heated on a hot plate and ignited as before. A small amount of ammonium carbonate was added and then ignited to constant weight. The

content was calculated in g of sulfated ash per 100g of air-dried material.
The sulfated ash is the ignited with concentrated sulphuric acid. The determination of sulfated ash is a method intended for determining the amount of inorganic substances contained as impurities in an organic substance contained as components in an organic substance, or the amount of impurities contained in a heat volatile inorganic substance.

Crude fibre content:

The crude fire determination method (A.O.A.C, 1975) was used. The six random lots of two grams of dried sample were used in the ether extract procedure. Each was transferred carefully and completely from the wrapping paper to a 600 ml Berzelius beaker. Two hundred ml of boiling 1.25% sulphuric acid were added carefully down the side of the beaker was placed on a preheated crude fibre apparatus and boiled for 30 minutes, after which it was lowered and the condensate rinsed with acid from a wash bottle. The beaker was removed and filtered at once through linen cloth on a 3 inch Hirsh funnel using vacuum. The residue rinsed with a stream of distilled water. The filter residue was were then washed three times with distilled water. The cloth and the residue was removed from the funnel and draped across the top of the beaker until it was ready for boiling with sodium hydroxide. After the sample was transferred completely from the filter cloth to the beaker, the volume was made up to 200 ml with 1.25% boiling sodium hydroxide with addition of several drops of antifoam. The beaker was placed on the crude fibre apparatus to boil for 30 minutes, after which the content was filtered through a filter crucible while washing with distilled water four times. The crucible was then dried for 15 hours at 100^0 C, cooled to room temperature in a desiccator and weighed. Then the crucible and the fibre were a shed at 550^0 C cooled in a desiccator to room temperature and weighed.
Calculation:
% Crude fibe =

Determination of ethanol-soluble and water-soluble extractive:

Ethanol-soluble and water-soluble extractives were determined according to standard procedure (Zhi-cen, 1980). Six lots of four-grams each consisted of ground plant sample were weighed accurately into a coppered conical flask. 100 ml of ethanol or water was added, and the flask was weighed, shaken well and then allowed to stand for 1 hour. A reflux condenser was attached to the flask and was re-adjusted with the specified solvent. The liquid was shaken vigorously and filtered rapidly through a dry filter into a dry container. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed dish on a water-bath and then dried at 105^0 C for 6 hours, cooled in a desiccator for 30 minutes and weighed rapidly. The content of extractives was calculated in g per 100g of the air-dried material.
Mineral content. Senna pod samples were analyzed by X-Ray Fluorescence (XRF) spectroscopy for concentrations of potassium (K), calcium (Ca) , titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), lead (Pb) , bromine (Br), rubidium (Rb), strontium (Sr), zirconium (Zr) and nobium (Nb).

Determinations of major and trace constituent elements of senna samples:

X-Ray Fluorescence (XRF) spectroscopic analysis has used extensively in quantitative and qualitative analysis of major and trace constituent elements. In this study, the XRF technique was applied for each

of random lots of plant samples (Table 1), collected from different locations in central Sudan to know the major and trace elements and their concentrations. Plant samples that had been collected were analyzed for concentrations of potassium (K), calcium (Ca), titanium (Ti) , chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), lead (Pb), bromine (Br), rubidium (Rb), strontium (Sr), zirconium (Zr) and niobium (Nb).
The plant samples were first crushed into fine powder and then they were pressed into pellet from using a 15 ton pressing machine. The diameter of each pellet was abound 2.5 cm and the mass about 1 gram. The pellets were presented to the XRF spectrometer system, where each of them was measured for 2000 sec. The spectra were first analyzed using program called Analysis of X-Ray Spectra by Iterative least Square filling (AXIL), which is a FORTRAN program. The AXIL software is able to separate overlapping peaks, and in this way to identify the elements and determine the net area of the peaks. The net area will be proportional to the concentration of the element in the sample. A plant standard was used to ensure reliability of the results (Hay Standard was obtained from the International Atomic Energy Agency (IAEA, Vienna).

Quantitative analyses of Hydroxyanthracene glycoside (calculated as sennoside B) of *senna* pods:
Quantitative analyses of sennoside B was performed by spectrophotometry according to standard procedure (BP, 1988) [6]. Six random lots of 0.15g of material were weighed accurately, and 30 ml of distilled water was added and then weighed again. It was then heated in a boiling water-bath for 15 minutes under reflux, cooled and weighed. The weight was adjusted with water and then centrifuged. 20 ml of supernatant liquid was transferred to a separating funnel. After adding 0.1 ml of 2M HCL, fraction was shaked with three quantities, each of 15 ml chloroform. The layers were allowed to separate and then the chloroform layer was discarded. After adding 0.1g sodium hydrogen carbonate, aqueous layer was shaken for three min., centrifuged and then the supernatant liquid was transferred to flask. 20 ml of 10.5% w/v solution of iron (III) chloride hexahydrate was added, mixed and heated in a water-bath for 20 min. under reflux. After adding 1 ml HCL, heating was continued for 20 min., with frequent shaking, until the precipitate was dissolved. The mixture was cooled and transferred to a separating funnel and then was shaked with three quantities, each of 25 ml of ether. The ether extracts were combined, washed with two quantities, each of 15 ml of water. This extract was diluted to 100 ml with ether. 10 ml of ether extract was evaporated to dryness, and the residue was dissolved in 10 ml of MKOH. The absorbance of the resulting solution was immediately measured by spectrophotometer at 515 nm.
Calculation:
Percentage content of sennoside B was calculated from expression:

i.e taking the specific absorbance to be 240
A = Absorbance at 515 nm
M= Mass of the substance to be examined in grams size used.

Results and Discussion

In the present work properties and mineral content of senna pods collected from three different localities in central Sudan was examined. The results are shown in Tables 1 & 2. According to the

results obtained this work (Table 1), certain properties such as total ash and acid-insoluble ash contents of *C.senna* pods collected from Ed-Damer, Tendelti and Elobeid regions are in agreement with those values reported in literature, WHO, 1999[5]. The water soluble extractive and moisture contents showed variation for different regions, and these values also were different with those values by WHO, 1999, sporadic stands of *C.senna* species occur on shallow desert wadies, on the sites of drainage ditches, in depressions, seasonal water runnels, on seriously eroded pavements and on un-cultivated river banks. These habitats belong sites of varying moisture levels.

It can be seen from Table 1, that the mean content of sennoside B ranged between 4.5% and 6.0% of dry pod weight. Ed-Damer region sample of *C,senna* pods yielded relatively large amounts of sennoside B. the reason for reporting sennoside B content is that the British Pharmacopoeia has a minimum requirement for it, and our results are of economic importance. Thus the investigated sample plant could be considered as official. In general, the amount of secondary metabolites in a plant can very with the season, type of the soil [9-10]. This can explain the different localities and times.

All the investigated sample show high levels of K in comparison with the rest of the elements. This is not surprising, because high K concentration is needed for purpose of activation of numerous enzymes [11]. Tendelti sample was found to have considerably higher levels of fourteen elements investigated (Table 2). Elobeid sample presents a slightly higher content of Ti , Mn , Fe, Cu, Zn, Pb, Zr and Nb compared with Ed-Damer sample. The difference for the three samples reflect the different presence of some organic compounds having a ligand character.

As would be expected, collection of crude medicinal herbs in the wild cannot guarantee a high and constant quality over a longer period. Very heterogeneous amounts of raw material was found on the market. Furthermore, trained collectors are rare and, for this reason, the identify of the plant material cannot always be guaranteed either. The collection, trade and supply of numerous medicinal herbs in Sudan is not restricted by authorized/legislative regulations concerning endangered species and conservation of plant diversity. It is therefore vital that systematic and rationally managed cultivation of the mot important medicinal herbs should be started simultaneously in rural areas in order to conserve the biodiversity and protect endangered species and also opening up of additional jobs and better income opportunities for the poor people.

The sale herbal products has increased considerably over the last few decades in the Sudan. This growing trend to use herbal products to tread a wide range of ailments has been promoted by the development of new diseases, with severe complications, and the belief that herbal products are safer and hence pose lesser health problems than synthetic drugs.

There are many constraints for Sudanese *Senna* to be competitive in the world market. Some of the problems associated with that are: (1) poor raw materials due to indiscriminate harvesting and poor post-harvest treatment and storage , (2) lack of commitment and support from government, (3) lack of financial resources, loans and credit facilities, and (4) difficulties in marketing (lack of access to market information and contacts).

Despite the present abundance of *Cassia* in many parts of Sudan, the ecological shrinkage of favorable habitats of *C.senna* in favor of extension of other cash crops undetermined the much need conservation of bio-diversity. Localized stands of other important medicinal herbs in some locations are threatened by continued degradation. Special attention should be paid to protect these important species.

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Table 1. Properties of *Cassia senna* pods collected in three different localities.

Property	**Localities**		
	Ed-Damer	**Tendelti**	**Elobeid**
Moisture %	7.2	10.7	8.3

Total ash%	2.5	5.0	5.0
Acid-soluble ash%	1.5	2.1	1.5
Sulfated ash%	2.1	0.7	1.4
Crude fibre%	9.6	10.0	17.7
Water soluble extractive%	15.0	2.5	5.0
Ethanol soluble extractive%	2.5	1.0	4.5
Sennoside B	6.0	5.5	4.5

* two determinations were carried out for each specimen

Table 2. Content of minerals in the *Cassia senna* pods.

Element (µg/g)	**Localities**		
	Ed-Damer	**Tendelti**	**Elobeid**
K	7500	10300	7180
Ca	2760	5420	2900
Ti	163	262	179
Cr	39.4	81.8	38.7
Mn	22.0	36.7	32.6
Fe	215	345	253
Cu	6.0	15.7	9.9
Zn	77.2	86.7	825
Pb	3.0	6.0	5.1
Br	11.1	19.6	4.8
Rb	17.0	14.8	8.2
Sr	35.0	83.2	30.5
Zr	1.0	6.0	2.5
Nb	0.4	0.8	0.6