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Morphological and Anatomical Characteristics of the Raw Materials Slimming Herbal Preparation (Royal Regime Tea)

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Abstract: ***This paper deals with the morphological and anatomical features of Cassia sennaleaf , Chicoriumintybus leaf and F. vulgare fruits. The aim of this research was to define concrete diagnostic parameters permitting detection of adulterants in commercial samples of a slimming herbal preparation (Royal Regime Tea, RRT). A Leica microscope was used for the identification of the different diagnostic characters of the ingredients and the preparation. Anatomical study of each ingredient of RRT and quantitative microscopy of C.sennaleaf, C.intybus leaf and F. vulgare fruits was performed for the authentication of the three plant tissues. The physical constant values was determined for RRT and the ingredients and compared with the known standard values in the literature. Moisture, dry matter, crude protein, ether extract, crude fiber, ash values and mineral contents passed the pharmacopeia standard for ingredients.***

Keywords: Cassia senna, C.intybus leaf and F. vulgare fruits, leaves, anatomy, morphology

1. Introduction

The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al., 2007). Sudan has a rich heritage of indigenous herbal practices that have helped to sustain the health of most of rural people.

According to the World Health Organization (WHO, 2002) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which,' are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous, 2005). Also, WHO (2011) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo*et al.,* 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (Mahmood *et al.,* 2012). Modern drugs still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and wellbeing and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The on-going growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala, 2000). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Cowman, 1999 and Adesokan et al., 2008). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000).

Herbal medicines continue to be a popular healthcare choice with the general public not only for health maintenance and well-being, minor ailments, chronic conditions and serious chronic diseases, but also for 'enhancement' of functions or processes, such as the use of Ginkgo biloba products for memory enhancement. There is an increasing number of products that respond to the public demand for “lifestyle” medicines, for example, herbal alternatives to 'slimming/weight-loss' preparations and breast-enlargement products. Typically, these types of products are sold over the internet without any assurance of their quality, safety and efficacy.

The last decade has seen several important developments with respect to herbal medicines. The most significant of these has been the introduction of a new regulatory framework for traditional herbal medicines worldwide. The regulatory landscape for herbal medicines has changed substantially. Issues that are related to the quality, safety and efficacy of herbal medicines are likely to continue. Therefore, with respect to quality, consumers and healthcare professionals should be aware that the labels of unlicensed herbal medicines may not reflect their actual contents, and that the precise constituents of herbal medicines containing

the same herbal ingredients but produced by different manufacturers are likely to differ. The quality of herbal medicines (i.e. uniformity of dose) is important for their efficacy: clinical trial results for a particular herbal medicinal product cannot necessarily be extrapolated to other products containing the same ingredients, since their precise contents may differ. There is an increasing amount of research comprising qualitative (i.e. the profile of chemical constituents) and quantitative (quantity of chemical constituents) analysis of herbal medicines which showed variations in the contents of the different manufacturers' products. The quality of herbal medicines is also important in regards to their safety, and safety concerns with herbal medicines, including intrinsic toxicity as well as problems due to adulteration and contamination, continue to arise.

Phytotherapeutic products are standardized herbal preparations consisting of mixtures of one or more plants which contain as active ingredients plant parts or plant material in the crude or processed state. Insufficient data exist for most herbs to guarantee their quality, efficacy and safety. The quality control determination on the Royal Regime tea which is prepared from a mixture of *Cassia sennaL., Foeniculum vulgare L. and Foeniculum vulgare* Mill. is demanded by international companies. The tea is widely used as laxative and described in most pharmacopoeias (WHO,1999). Scientifically sound data for this product are lacking in Sudan, where the herbal medicine market lacks regulations and some products are not registered nor controlled. Thus, the present study was undertaken to deal with the macroscopic (morphological) and microscopic (anatomical) characteristics of the *Cassiasenna* leaf ,*Chicoriumintybus* leaf ,*F. vulgare* fruits and a slimming herbal preparation (Royal Regime Tea). Traits of importancefor their differentiation were established for an easier and more accurate control of commercial samples of these raw materials and for the detection of the presence of prohibited adulterants and falsifications.

2. Material and Methods

Materials

Plant material:
The tested materials consist of the herbal tea (royal regime tea, as well as its ingredients *Cassia senna* L. leaf, *Cichoriumintybus* L. leaf and *Foeniculum vulgar* fruits. All tested materials were brought from the local markets .

The crude ingredients were authenticated by medicinal and aromatic plants research institute (MAPR), Khartoum, Sudan.

Methods

The four tested samples were subjected to phytochemical screening using the methods described by Edeoga et al (2005).

Morphological and anatomical characteristics
Commercial herbal tea(20% *Chicoriumintybus* L.,30% *Cassia senna* L. and 50% *Foeniculum vulgare* L.) samples were collected from random shops in local markets. The standard mixtures were then prepared from this collection. The samples were used for isolation of standard anthraquinone derivatives.

Plant samples of *C. intybus* and *C. senna*were collected from different locations in the Central region of Sudan. The leaves of the two plant species were used for anatomical investigation. The taxonomical description of the plants was carried out according to Davis (1975). Samples were fixed in 70% alcohol for anatomical studies. Their photographs were taken with a Nikon FDX-35 microscope. The length and width of the stoma were measured by an ocular micrometer using the surface section from the upper and lower parts of the leaf epidermis. The stomatal index was calculated according to the method described by Meidner and Mansfield (1968).

The *F. vulgare* L. fruits material was bought from local markets. The plants samples were properly washed in tap water and then rinsed in distilled water. The rinsed fruits were dried in an oven at a temperature of 35-40°C for 3 days. The dried plant samples were pulverized, using a sterile electric blender, to obtain a powered form. The powdered form of these samples were stored in airtight glass containers, protected from sunlight and used for analysis.

Proximate analysis
Proximate composition of the various plant samples was determined as described by Antia et al. (2006).

Moisture content
Moisture content determination was done by IR moisture balance. In this method sufficient amount of powdered plant samples is spread in the evaporating plate of IR moisture balance till the pointer touches zero. Then temperature is maintained at 105°C for 30 minutes, after that moisture content was measured directly in percent.

Ash content
Ash determination involved the incineration of each sample in a muffle furnace (Naber Industries of enbau, Bremen, Germany) at 550°C for 12 h.

Ether extract
Ether extract determination was achieved by exhaustively extracting the samples with diethyl ether.

Crude fiber
Crude fiber was estimated from the loss in weight of the crucible and its contents on ignition after ashing, following the sequential extraction of the samples with 1.25% sulphuric acid and 1.25% sodium hydroxide.

Protein content
Protein was determined using the microkjeldal nitrogen method which involved the digestion of 0.5 g of sample with sulphuric acid and a catalyst followed by calorimetric determination of nitrogen. The value of nitrogen was multiplied by 6.25 to obtain percentage crude protein. The carbohydrate content was obtained by subtracting the values of total ash, crude fiber, lipid and protein from the total dry matter (Antiaet al., 2006).

Dry matter

Dry matter in the samples was determined by the Association of Official Analytical Chemists method (A.O.A.C., 2000).

Mineral analysis

All plants material was first cleaned dried and then powdered using an electric blender. Samples in powder form were used for Atomic Absorption Spectrophotometer (AAS). Each plant material (0.25 g) were taken in 50 ml flask and add 6.5 ml of mixed acid solution that is, Nitric acid (HNO_3), Sulfuric acid (H_2SO_4) and Perchloric acid ($HClO_4$) (5:1:0.5). The samples were boiled in acid solution in fume hood on hot plate (model VWR VELP Scientifica, Germany) till the digestion has been completed which was indicated by white fumes coming out from the flask. Thereafter, few drops of distilled water were added and allowed to cool. Then these digested samples were transferred in 50 ml volumetric flasks and the volume was made up to 50 ml by adding distilled water. Then the extract was filtered with filter paper (Whatmann No. 42) and filtrate were collected in labeled plastic bottles. The solutions were analyzed for the different minerals utilizing atomic absorption spectrometer Shimadzu AA-670 with suitable hollow cathode lamps. The contents of different elements in these samples were determined by the corresponding standard calibration curves obtained by using standard AR grade solutions of the minerals that is Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, P, Fe^{2+}, Mn^{2+}, Cu^{3+}, Zn^{2+} and Pb^{4+}.

3. Results and Discussion

Microscopic screening

***C. Senna* leaf**

A transverse section of the lamina(Fig. 2) of *C. senna* L. indicated that the upper and lower epidermis was covered withcuticle. The number of epidermal cells were 129 and 140 cells for the upper and lower epidermis, respectively (Table 1). There were multilayered glandular hairs and littleglandular hairs on the upper and lower epidermis. The mesophyll contained three layers of parenchyma cells with densechloroplast on the upper and lower epidermis and 6layersof isodiametric spongy parenchyma cells. The midrib region was triangular. There were three large vascular bundles. A layer of collenchyma cells was located at both the upper and lower epidermis (Fig. 2). Leaf was equifacial. The stomata type was anomocytic. Stomata were present on both epidermalsurfaces; however stomata cells were more common on the lower epidermis. The number of stomata was 35/mm2 on the upper epidermis and 54 /mm2 on the lower epidermis of the leaf. The stomataindex was 0.96.

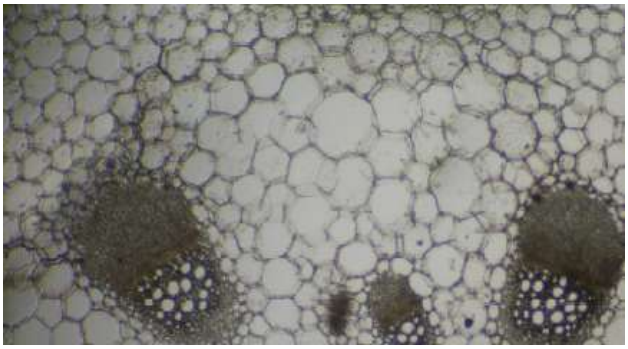

Figure 6: Transverse section of the *C. senna* leaf

Also the microscopic examination of the transverse section of senna leaf revealed isobilateral structures. Nonlignified unicellular trichomes with warty walls and fibrovascular bundles lined with abundant prisms of calcium oxalate are characteristic features of senna leaf.

Table 1: Microscopic screening of *C. senna* and *C. intybus*leaves herbal plants used in the study

Plants	Epidermis	No. Epidermal cell	Stomata		Trichomes	
			No.	Index	Present	Absent
C. senna	Upper	129	35	-	-	-
	Lower	140	54	0.96	-	-
C. intybus	Upper	157	52	-	-	-
	Lower	158	48	1.15	+	-

***C. intybus* leaf**

Capitula was 2.5- 3.5 cm broad, axillary. Outerphyllaries were ovate, inner phyllaries were lanceolate (Fig. 4).The number of cells of the upper epidermis was 157 cells, while that of the lower epidermis was 158 cells. Stomata were present on both surfaces; however stomata cells were more common on the upper epidermis. The number of stomata was 52/ mm2 on the upperepidermis and 48 /mm2 on the lower epidermis of the leaf. The stomataindex was 1.15 (Table 1).Similar anatomical characteristics of *C. intybus* plants were recorded by Esau (1977) andMetcalfe and Chalk (1979).According to Metcalfe and Chalk (1979)anatomical diversity is commonly observed in the structure ofleaves of plants that belong to the Asteraceae family. They also pointed out that there were homogeneous and equifacial mesophyll in the family Asteraceae.

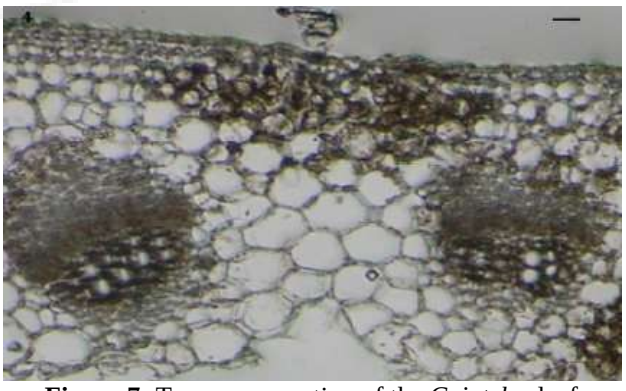

Figure 7: Transverse section of the *C. intybus*leaf

***F. vulgare* Fruits**.

In the powder tracheids, vittae, endosperm, endocarp, mesocarp were seen during the microscopical study (Fig.7).

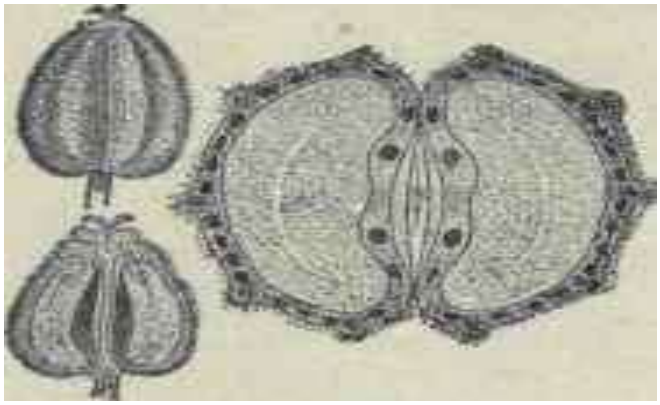

Figure 8: Transverse section of the *F. vulgare* Fruits

Royal Regime Tea

Microscopic examination of the tea essentially revealed the presence of stomata, the polygonal shape of the epidermal cells, trichomes as isolated or attached to fragments of epidermis and calcium oxalate crystals which are frequently visible in the powder. Cluster crystals appear isolated or in fragments of parenchyma (Fig.9).

Figure 9: Macroscopic features of Royal Regime Tea

The proximate analysis

The proximate composition of the herbal samples are presented in Table 2. The tested parameters varied between the three samples. High levels of dry matter (96.3 % - 97.3 %) were recorded in the dried herbal samples. The total ash ,crude protein and ether extract were highest in *C. intybus* dried leaves, while, crude fiber content was highest in *F. vulgare* fruit samples. Each value represents the mean + SD of six determinations. Similar levels were reported by Edeoga and Eriata (2001), Jerez (1991) and Lioveras (1990).

The royal regime tea sample showed 97.0% dry matter as ground sample, 7.95% ash, 9.9% crude protein, 18.2% crude fiber, 1.89% ether extract and 2.9% moisture (Table 2). The leaves of *C. senna* showed 97.3% dry matter as ground sample, 9.63% ash, 7.37% crude protein, 12.5% crude fiber, 1.06% ether extract and 2.7% moisture (Table 2).

The fruits of *F. vulgare* showed 96.3% dry matter as ground sample, 8.54% ash, 1.79 % crude protein, 20.1% crude fiber, 2.25% ether extract and 3.7% moisture (Table 2).The leaves of *C. intybus* showed 96.7% dry matter as ground sample, 11.6% ash, 14.7% crude protein, 16.7% crude fiber, 1.96% ether extract and 3.3% moisture (Table 2). An analysis of F. vulgare showed it contains moisture 6.3%,crude protein 9.5%, ether extract 10%, ash 13.4%, crude fiber 18.5% and carbohydrates 42.3%. F. vulgare volatile oil is a mixture of at least a dozen of different chemicals and the main ingredients are: anethole (40 - 70%), fenchone (1 - 20%) and estragole (2 - 9%) (WHO,1999).

Table 2: Proximate analysis of royal tea and herbal plants used in the study (Mean ± Standard deviation)

Minerals		*Royal regime tea*	*C. senna*	*F. vulgare*	*C. intybus*
Moisture	% dry weight	2.9±0.15	2.7±0.15	3.7±0.31	3.3±0.02
Crude Protein		9.9±0.23	7.37±0.93	1.79±0.19	14.7±0.19
Ether extract		1.89±0.42	1.06±0.55	2.25±0.30	1.96±0.01
Ash		7.95±0.51	9.63±0.13	8.54±0.96	11.6±0.55
Dry matter		97.0±2.11	97.3±2.35	96.3±0.64	96.7±2.64
Crude fiber		18.2	12.5	20.1	16.7

Jerez et al. (1991) reported that *C. intybus* contains 3.74% crude protein, 29.83% crude fiber, 2.01% ether extract and 60.0% sugar while *T. repens*contains 3.35% crude protein, 27.5% crude fibre, 1.98% ether extract and 59.3% sugar and concluded that the *C. intybus* is of high nutritive value. Lioveras (1990) reported the nutritive value of *C. intybus*as 5% crude protein, 27% crude fiber, 1.5% ether extract, 60.80% sugar and 7.5% ash. Saeed (1972) reported the nutritive composition of fresh and dried *C. intybus*, which had 75.6% water, 10% nitrogenous matter, 0.94% ether extract, 3.44% sugar, 17.62% nitrogenous free extract, 0.97% cellulose and 0.78% ash in case of fresh *C. intybus* while dry *C. intybus* had 12.16% water, 6.09% nitrogenous matter, 2.055 ether extract,15.87% sugar, 46.71% nitrogen free extract, 11.0% cellulose and 6.12% ash. All these findings supported the present results except with slight differences in values.

The mineral content

The concentrations of ten minerals were determined in *C. senna,F. vulgare*, *C. intybus* and Royal tea. The concentrations of the minerals determined in each of these plant samples and Royal tea are collectively listed in Table 3. It was observed that all medicinal plants contain significant amounts of the minerals and the mineral content in the samples presented a wide variability.

Table 3: Mineral contents (mg/kg) of royal tea and herbal plants used in the study (Mean ± Standard deviation)

Minerals		*Royal regime tea*	*C. senna*	*F. vulgare*	*C. intybus*
Mg	------- ---- mg/kg ------- ------- -	3.04 ± 0.48	3.79 ± 0.53	2.77 ± 0.39	0.75 ± 0.03
Ca		4.87± 0.36	4.16 ± 0.25	4.69 ± 0.21	2.53 ± 0.17
Na		7.62 ± 0.88	7.91 ± 1.09	8.34 ± 0.66	9.36 ± 0.06
K		3.79 ± 0.47	3.18 ± 0.09	5.19 ± 0.24	1.17 ± 0.11
P		2.12 ± 0.33	0.96 ± 0.44	2.55 ± 0.07	2.36 ± 0.42
Fe		46.8 ± 3.89	47.8 ± 1.48	58.6 ± 3.57	71.1 ± 2.93
Mn		23.6 ± 1.90	27.7 ± 1.11	22.4 ± 2.90	25.4 ± 1.12

Cu	---	14.4 ± 1.30	15.7 ± 0.35	15.2 ± 1.06	15.2 ± 1.06
Zn	ppm--	24.6 ± 3.10	38.2 ± 2.75	37.9 ± 2.12	57.9 ± 2.03
Pb	--	16.9 ± 2.30	15.8 ± 0.93	20.0 ± 0.57	21.7 ± 0.92

Mg content of the samples ranged from 0.75 mg/kg to 3.04 mg/kg. Ca was in the range of 2.53–4.87 mg/kg. The highest Mg and Ca were present in royal regime tea. Fernandez et al. (2002) determined the Mg and Ca content of black and green teas in the range of 5 and 22 mg/l. Lozaket al. (2002) reported data on Ca and Mg present in row mint leaves as 15,3 mg/kg and 57.8 mg/kg, respectively. Na content of the samples was in the range of 7.62 mg/kg - 9.36 mg/kg. K was in the range of 1.17–5.19 mg/kg. The highest Na content was in *C. intybus* and for K , it was present in the fruit samples of *F.vulgare*. P content of the samples ranged from 0.96 mg/kg to 2.53 mg/kg. The highest P content was present in *C.senna* (Table 3).

Fe content of the samples was in the range of 46.8 –71.1 ppm. Fe concentration found for all of the herbs was higher than those reported by Lozaket al. (2002) who determined Fe content in mint and nettle preparations. Mn in the samples varied in a range of 22.4–27.7 ppm. It was reported by Lozaket al. (2002) that the concentration of Mn is 18.8 ppm in mint leaves. The results of the analysis of all samples indicated that Zn levels of all samples are relatively higher than Cu levels. They were determined in the range of 14.4 - 16.2 ppm and 24.6 - 57.9 ppm, respectively. In *C.intybus*, both have reached the highest value. Lozaket al. (2002) reported 12 mg/kg Cu and 51.0 mg/kg Zn in mint leaves. Pbwas found in the samples in the range of 1508-21.7 ppm.

Previous studies (Hacet al., 1997 and Kuoet al., 2000) have shown that of the 10 elements determined in this study, some are necessary to human health, such as Mg, Ca, K,P and Zn whereas others have been shown to be toxic, such as Pb. The other elements are not toxic to human unless they are present in high concentrations. It must be noted that all of the herbs considered in this study are prepared as hot beverages such as herbal tea.

Beverages of these medicinal herbs that are consumed widely in may contain toxic elements because medicinal herbs may be contaminated easily during growing and / or processing. It is important to have a good quality control for herbal medicines in order to protect consumers from contamination.

The contents of trace elements are found to be within the permissible limit for all the raw materials, oleoresins and finished formulations.

Herb materials were not found to contain any heavy metals such as Cd, As, Pb and Hg (Obiajunwaet al., 2002). The results of this study of mineral contents of medicinal plants show minor differences when compared with literature (Ozcan, 2004). These differences might be due to growth conditions, genetic factors, geographical variations and analytical procedures (Ozcan, 2004).

High amounts of Ca are important because of its role in bones, teeth, muscle system and heart functions (Brody 1994). Iron is an important element for human body and plays a role in oxygen and electron transfer. It is necessary for the formation of haemoglobin (Kaya and Incekara, 2000). Lead cause both acute and chronic poisoning, adverse effects on the kidney, liver, heart, vascular and immune system (Heyes, 1997). Copper and Zinc are required in the diet because they exhibited a wide range of biological functions such as components of enzymatic and redox systems (McLaughlin *et al*., 1999). Decreasing of these toxic element contents is an advantage.

The results also show that these plant materials contain elements of vital importance in man's metabolism and that are needed for growth and developments prevention and healing of diseases (Obiajunwaet al., 2002).

4. Conclusions

Our data show that these plant materials contain elements of vital importance in man's metabolism and that are needed for growth and developments prevention and healing of diseases. A national study is needed to provide more information and representthe actual spectrum of Cassia senna and C.intybus leaves as well as F. vulgare fruits.

5. Conflict of Interest

The authors have no conflict of interest to report.

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