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Determination of Mineral Elements and Crude Protein of Eight Medicinal Plants from Central Côte D'ivoire

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Abstract: An ethnopharmacological survey carried out in the central region of Côte d'Ivoire enabled us select eight medicinal plants used to treat various human diseases. They are :*Anthocleista djalonensis, Crossopteryx febrifuga, Harungana madagascariensis, Mangifera indica, Margaritaria discoidea, Pericopsis laxiflora, Spathodea campanulata and Terminalia glaucescens*. The purpose of this study is to analyze the elemental content and crude protein and possibly evaluate the link to their therapeutic activities. The analysis by atomic absorption spectrophotometer was used to determine sodium, potassium, iron, magnesium, phosphorus, calcium and zinc contents. The crude proteins were determined using the Kjeldahl method. The values of the mineral elements obtained in the different plants are as follows: Fe (237.46-383.1 ppm) Zn (3.43-33.76 ppm), Ca (17.63-129.05 ppm) P (23.63-36.06 ppm), Na (103.16-492.63 ppm) K (468.3-754.3 ppm) and Mg (736.3-743.79 ppm). The proportion of the crude proteins obtained was in the range of 5.46 to 12.38%. The possible relationship between these minerals elements found in these plants and its' use as remedies against certain diseases is discussed.

Keywords : Central Côte d'Ivoire, crude protein, elemental contents, medicinal plants.

Introduction

In Côte d'Ivoire (West Africa) many indigenous plants are used as spices, food or medicine. A great number of these plants are traditionally noted for their medical properties (**Aké-Assi, 1979a**). It has the advantage of being readily available to the people at a considerable lower cost (**Cunningham, 1993**). The dependence on indigenous medicinal plants for treatment is particularly observed in developing countries where modern medicine is often too expensive therefore out of reach of the majority of the population (**Carillon, 2009**). The results from several studies showed that native plants are used in the treatment of coughs, hypertension, rheumatism, joint pain, diarrhea, dysentery, stomach problems, diabetes, migraine, hemorrhoids, malaria, snake bites, bleeding, convulsions, schistosomiasis, menstrual pain, filariasis, gonorrhea, infertility, epilepsy and even cancer (**Ake-Assi, 1979b**). In the course of our study, we carried out survey involving nine traditional healers, in the central region of Cote d Ivoire ,where we have been able to identify several medicinal recipes, but 8 in particular have attracted our attention due to their utilization in the treatment of malaria (**Bla et al., 2015**). They are: *Anthocleista djalonensis, Crossopteryx febrifuga, Harungana madagascariensis, Mangifera indica, Margaritaria discoidea, Pericopsis laxiflora, Spathodea campanulata and Terminalia glaucescens*. Most of these plants have antiparasitic properties in general and antimalaria in particular (**Mustafa et al., 2000, Koné et al., 2005, Traoré et al., 2013**). Entire parts of these plants are used by the population to treat to treat several diseases (**N'Guessan et al., 2009, Koné et al. 2002**). Thus, bark, roots, stems, leaves, leafy branches and fruits are regularly used for medicinal preparations. The therapeutic actions of these preparations total or totum have shown positive

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results in many ways thanks to the combined action of the active ingredient, mineral elements and vitamins (**Girre, 1997**). However among these substances, it is often difficult to highlight the most essential element for treating a given disease. The feature that stands out is that each active ingredient is accompanied by its precursors and metabolites, which may also be active. In addition, an active principle coexists with other active substances as well as mineral elements which are indispensable, for instance by taking part in the enzymatic reactions. Medicinal plants efficacy would therefore be the result of several substances present in varying amounts in the organism each playing a specific role. In this study, we carried out the determination of total protein and mineral elements such as iron, magnesium, zinc, calcium, phosphorus, sodium and potassium. Their choice is justified by their essential biological role played in the body. These minerals have been recognized as indispensable to human life by WHO (**Picard, 1975**). While proteins are known to play immunostimulatory role (**Ezekwe et al., 2001**). The aim of our study is to determine in these plants, the levels of crude protein and essential minerals and possibly look for the link between these elements and, thus the active substances and the pharmacological effects observed.

Materials and Methods

Plant Materials

The experimental species were collected from Moronou Village in Toumodi area, Central Côte d'Ivoire. The plant materials (leaves and bark) were identified and authenticated by Mr. Assi Jean of National of Floristic Centre of University of Felix Houphouet Boigny (Abidjan). The samples were air-dried for 15 days and milled into powder with the aid of an electrical grinder and finally stored in airtight bottles before analysis.

Mineral Analysis and Determination of Crude Proteins

The major elements, comprising calcium, phosphorus, sodium, potassium, magnesium and trace elements (iron and zinc) were determined according to the method of **Shahidi et al. (1999)**. The ground plant samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550 °C in a muffle furnace. The resultant ash was dissolved in 5 mL of $HNO_3/HCl/H_2O$ (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a whatman filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm-long cell was used and concentration of each element in the sample was calculated on percentage of dry matter. Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri (1975). To 0.5 mL of the diluted digest, 4 mL of demineralised water, 3 ml of 0.75M H_2SO_4, 0.4 mL of 10% $(NH_4)_6MO_7O_{24}.4H_2O$ and 0.4 mL of 2% (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and absorbance readings were recorded at 660 nm. The content of phosphorus in the extract was determined. The Kjeldahl method was used to estimate crude protein in plant materials (**Allen, 1989**).

Statistical Analysis

All the analyses were performed in triplicate and data were analyzed using EXCELL 2007. Data were expressed as mean ± standard deviation (SD).

Results

Results of mineral content in eight medicinal plants are given in table 1 and shown in figure 1 to 7, while the results of crude protein summarized in table 2.

Discussion

Iron

The range of Fe in the studied plants was high with a minimum of 237.46 ppm in *M. discoidea* and maximum of 383.1ppm in *C. febrifuga* (Table 1 and Figure 1). The maximum tolerable level for cattle was suggested as 1000 ppm by **National Research Council (1984).** The permissible limit set by **FAO/WHO (1984)** in edible plants was 20 ppm. After comparison, metal limit in the studied medicinal plants with those proposed by **FAO/WHO (1984)** it is found that all plants accumulate Fe above this limit. However, for medicinal plants the **WHO (2005)** limits not yet been established for Fe. **Sheded et al. (2006)** reported that the range of Fe in their study was between 261 ppm to 1239 ppm in selective medicinal plants of Egypt. Fe is necessary for the formation haemoglobin and also plays an important role in oxygen and electron transfer in human body **(Kaya and Incekara, 2000).**

Magnesium

Magnesium deficiency in humans caused muscle spasms, and has been associated with a high blood pressure, many cardiovascular diseases, diabetes and osteoporosis. The necessary daily intake is 350 mg/day for men and 300 mg/day for women **(Mogos, 1997).** The values obtained for Magnesium in analyzed medicinal plants ranged between 0.718 – 0.107%. The highest content was measured in *M. discoidea* (743.79 ppm) and the smallest in *S. campanulata* (73.63 ppm) (Table 1 and Figure 2).

Zinc

The content of Zn ranged between 3.43 ppm in *H. madagascariensis* and 33.76 ppm in *A. djalonensis* (Table 1 and Figure 3). The maximum tolerable zinc level has been set at 500 ppm for cattle and 300 ppm for sheep **(National Research Council, 1984).** The permissible limit set by **FAO/WHO (1984)** in edible plants was 27.4 ppm. After comparison, metal limit in the studied medicinal plants with those proposed by **FAO/WHO (1984)** it is found that all studied plants are below this limit excepted *A. djalonensis.* The zinc content could mean that the plants can play a valuable role in management of diabetes, which results for including insulin malfunctioning. Zinc is essential for the production of insulin **(Okwu and Morah, 2004).**

Calcium

Calcium deficiency for a long time is responsible for osteoporosis, when the bones are deteriorated and increased the risk of fractures. The necessary daily intake is between 350 and 1100 mg/day **(Mogos, 1997).** In analyzed medicinal plants, the calcium contents varied between 17.63-129.05 ppm. The highest calcium content was obtained for *M. discoidea* (129.05 ppm), followed by *H. madagascariensis* (85.76 ppm) and *P. laxiflora* (74.54 ppm). The smaller calcium concentration was determinate for *T. glaucescens* (74.54 ppm) (Table 1 and Figure 4).

Phosphorus

Table shows that the concentration of phosphorus ranged between 23.63 ppm in *P. laxiflora* and 36.06 ppm in *T. glaucescens* (Table 1 and Figure 5). All examined samples were above the deficiency level of phosphorus which is below 1.5 ppm **(Allen, 1989; Al-Jaloud et al., 1994).** Phosphorous maintain blood sugar level, normal heart contraction dependent on phosphorous also important for normal cell growth and repair, needed for bone growth, kidney function and cell growth. It plays important role in maintaining body's acid-alkaline balance **(Johns and Duquette, 1991).**

Sodium

The lowest content of Na that is 103.16 ppm was in *T. glaucescens* and maximum concentration was estimated as 492.63 ppm in *M. indima* (Table 1 and Figure 6). All plants showed low accumulation. Sodium is a major cation of extracellular fluid and therefore plays a central role in the maintenance of water balance and osmotic pressure in various fluid compartments (**Tietz et al., 2001**). **Ikekpeazu et al. (2010)** have shown that malaria infection leads to a reduction in the levels of both serum Na^+ and K^+.

Potassium

The range of K varied between 468,3 ppm in *S. campanulata* and 754,3 ppm in *T. glaucescens* (Table 1 and Figure 7). All the studied plant show high K contents and our results coincided with the previous studies on medicinal herbs **(Badri and Hamed, 2000; Ozcan and Akbulut, 2007).** Contrarily, K^+ is a major intracellular cation, having an average cellular concentration in tissue cells of 150 mM **(Kaplan et al., 1995).** Besides water balance, these electrolytes play an important role in maintenance of pH, regulation of heart and muscle function, electron transfer reactions as well as serving as cofactors for enzymes **(Tietz et al., 2001).**

Crude protein

The minimum crude protein was 5.46 % in *M. indica* and the maximum was 12.38 % in *P. laxiflora* (Table 2). Our concentration values are relatively low compared to **Atangwho et al. (2009)**, where concentrations were found in an amount ranging from 13.42 to 25.55 % in *Azadirachta indica*, *Vernonia amygdalina and Gongronema latifolium*. All these three plants have anti-diabetic properties (**Nimenibo-Uadia, 2003**).

Conclusion

The mineralization by calcination in atomic mass spectrometry method has helped to identified the content level of Na, K, Fe, Mg, P, Ca and Zn in eight medicinal plants used in traditional medicine to treat malaria in Côte d'Ivoire. Among these minerals, macronutrients such as Mg, Ca and K are present in good quantity in most plants. The presence of these essential mineral elements coupled with phytochemicals (steroids, terpenes and flavonoids) in these plants may have contributed to their potential usage as medicinal plants highly esteemed in some parts of the tropical world for malarial treatment.

Acknowledgement

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Conflict of interest

The authors have none to declare.

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Table 1: Concentration (ppm®) of major and trace elements of studies plants

Plant	Fe	Mg	Zn	Ca	P	Na	K
A. djalonensis	350,43±8,70	194,21±3,26	33,76±2,25	20,03±2,88	31,6±0,20	173,73±0,68	668,14±1,80
C. febrifuga	383,10±6,48	244,81±0,90	18,83±0,47	38,07±1,69	29,73±0,45	175±2,78	627,64±1,32
H. madagascariensis	241,46±5,12	408,21±1,66	3,43±0,32	85,76±2,77	25,63±0,28	285±2,29	667,46±1,75
M. indica	248,33±5,93	105,88±2,78	5,43±0,75	39,54±4,69	30,76±0,40	492,63±0,70	648,97±0,76
M. discoidea	237,46±5,49	743,79±4,30	12,33±0,66	129,05±5,61	27,96±0,90	297,43±1,50	546,30±3,32
P. laxiflora	251,73±5,39	704,13±4,58	13,56±0,97	74,54±2,40	23,63±0,60	153,16±2,02	743,40±1,91
S. campanulata	277,10±4,55	73,63±1,98	17,8±0,65	71,6±1,22	33,56±0,35	141,83±1,89	468,30±0,76
T. glaucescens	344,93±6,10	154,46±2,15	12,16±0,50	17,63±1,60	36,06±1,50	103,16±3,32	754,30±4,01

® : Average concentration of element ± standard deviation (n=3) (mg/Kg)

Table 2: Concentration of crude protein of studied plants

Plant	Crude proteins (%)
Anthocleista djalonensis	10.72
Crossopteryx febrifuga	9.46
Harungana madagascariensis	7.43
Mangifera indica	5.46
Margaritaria discoidea	ND
Pericopsis laxiflora	12.38
Spathodea campanulata	ND
Terminalia glaucescens	10.15

ND : undetermined data

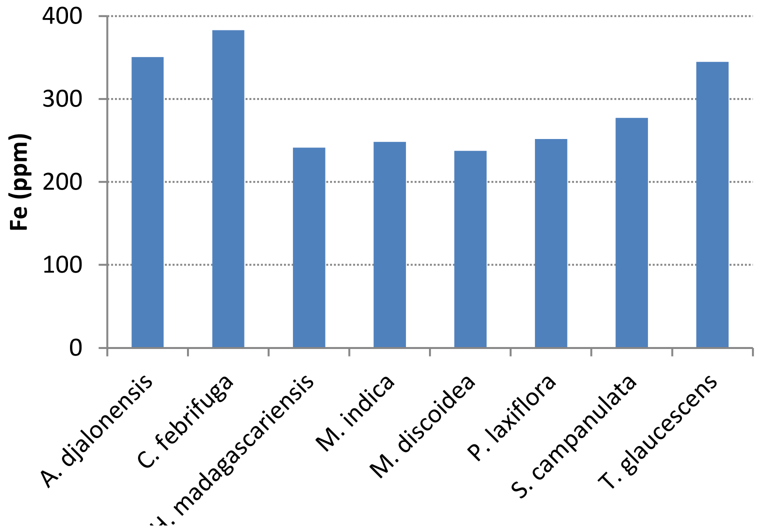

Figure 1: Concentration of iron in studied plants

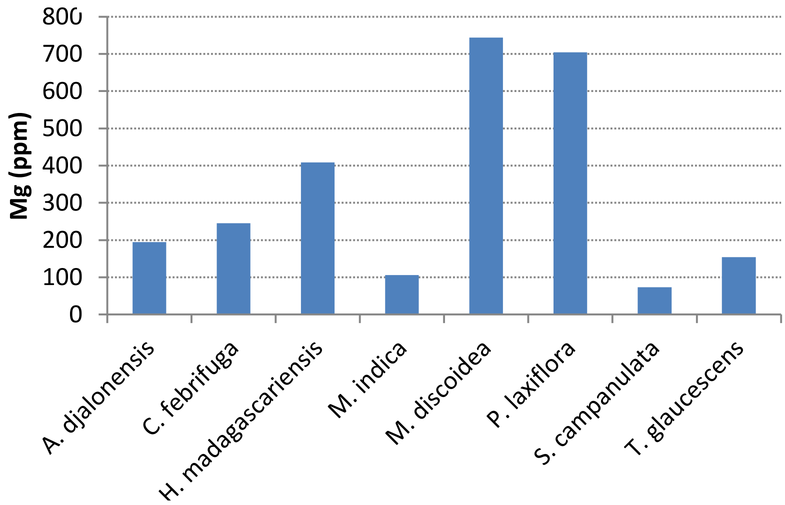

Figure 2: Concentration of magnesium in studied plants

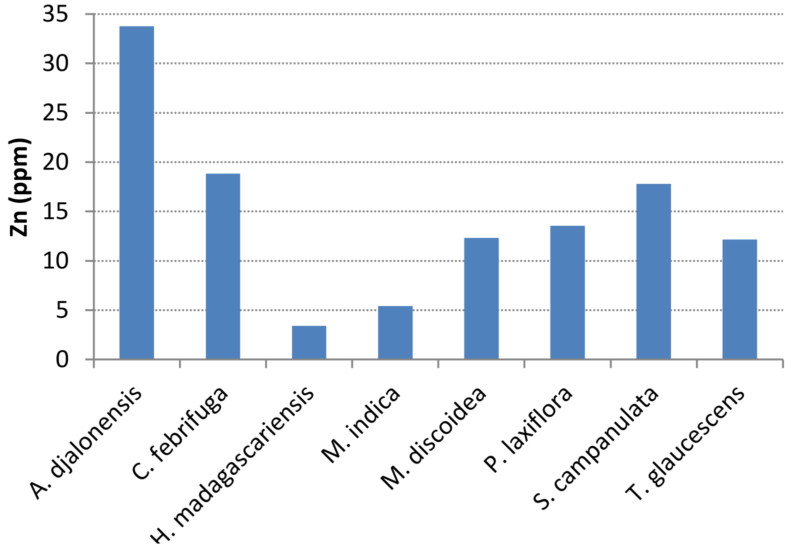

Figure 3: Concentration of zinc in studied plants

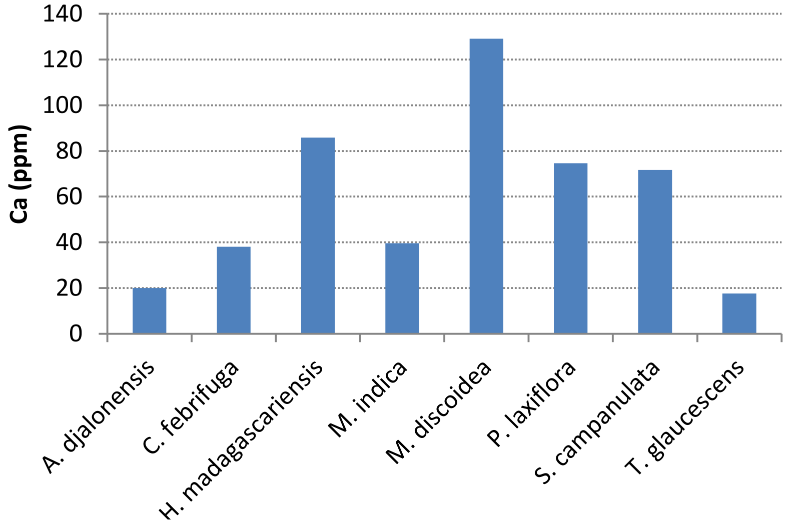

Figure 4: Concentration of calcium in studied plants

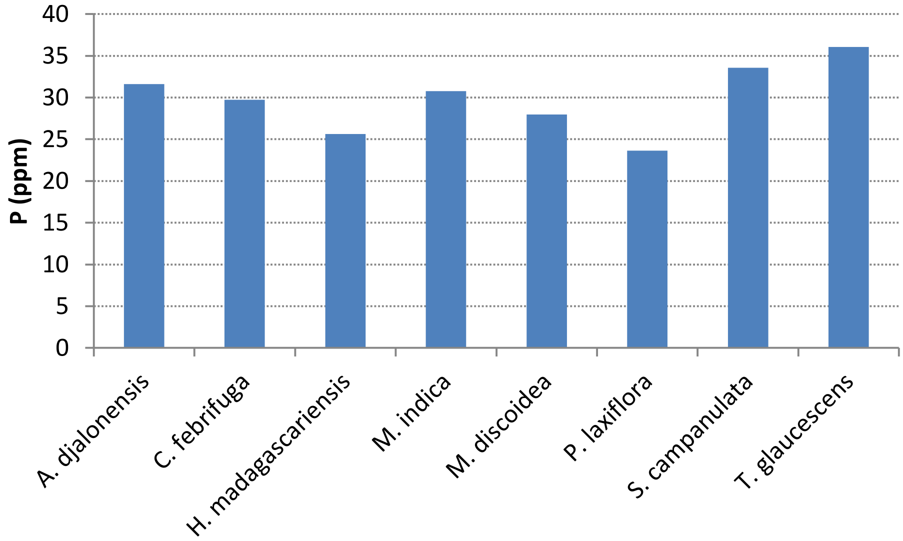

Figure 5: Concentration of phosphorus in studied plants

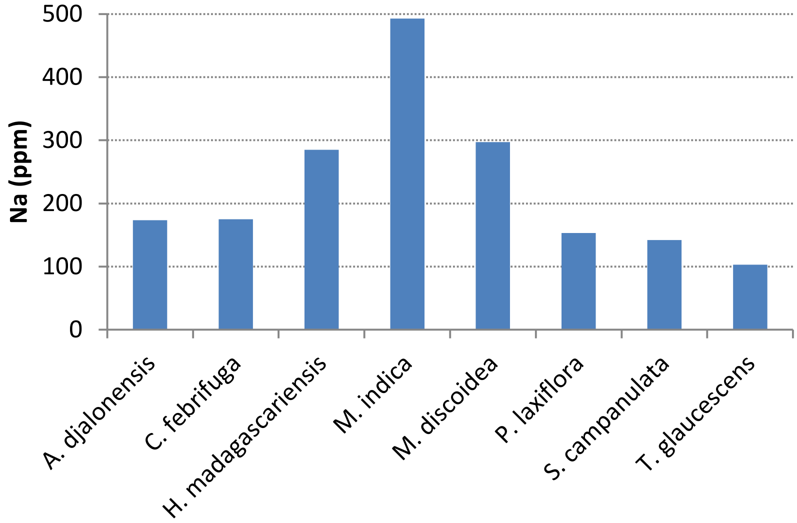

Figure 6: Concentration of sodium in studied plants

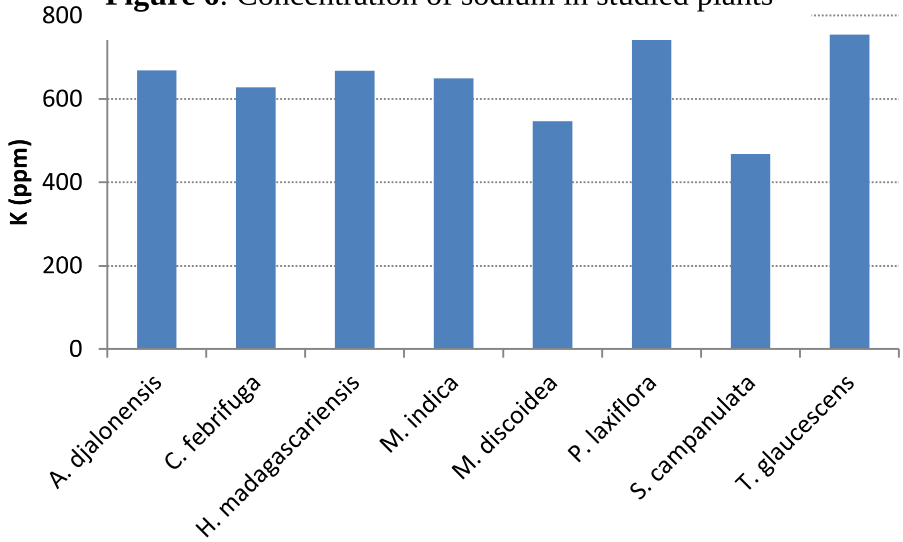

Figure 7: Concentration of potassium in studied plants