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Heavy Metals Analysis of Some anti-oxidation Medicinal Plants in Côte d'Ivoire using Energy Dispersive X-ray Fluorescence (EDXRF) Technique

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Abstract

Trace elements concentrations in seven plants with anti-oxidation potency in Côte d'Ivoire were studied using Energy-Dispersive X ray Fluorescence (EDXFR) technique. The aim of the study is to determine qualitatively and quantitatively trace elements in these plants and their medicinal roles in the human body. Leaves were analyzed for their heavy metal contents. Plants samples were found to contain trace elements such as Zn, Fe, Cu, Mn and Se which are well known for their important roles in anti-oxidation preparations (herbal drugs). Medicinal plants were found to be rich more than one of the essential elements under study. All elemental concentrations in leaves of medicinal plants and their biological effects are discussed.

Keywords: Anti-oxidation, Energy-Dispersive X-ray Fluorescence technique, medicinal plants, trace elements

Introduction

It is considered anti-oxidant, a substance able to neutralize or reduce the damage caused by oxygen free radicals in the body. These free oxygen radicals are particularly responsible for the oxidation of cells, an important phenomenon in the aging process of cells or tissues of the body. Excess free radicals not neutralized by the defense is detrimental to the essential macrobiomolecules, resulting in abnormalities in gene expression and membrane receptors, proliferation or cell death, immune disorders and protein deposits [8] One theory to explain the aging of cells or tissues relies on oxygen free radicals. Their action is limited by enzymatic anti-radical defenses (superoxide dismutase cytosolic Cu-ZnSOD and mitochondrial MnSOD, catalase, glutathione peroxidase, thioredoxin reductase) and non-enzymatic (tocopherol or vitamin E, glutathione, uric acid, sulfur-containing amino acids and melanin). This defense system is however not perfect especially on skin that suffers, frontline environmental stress (UV, pollution) that may increase the production of free radicals. Some anti-oxidative actions (diet) or topical recommended.

Diseases associated with aging of body cells, which are caused by oxygen free radicals are multiple and we can cite among other

cancer, eye diseases (cataract and macular degeneration) and disease Alzheimer. Free radicals involved in complications of certain diseases such as AIDS, diabetes, Parkinson's disease and renal failure [8].

In the present study we exclusively interested in seven medicinal plants used in Côte d'Ivoire to fight against the oxidation of cells and tissues of the body by free radicals. These plants shown in Table 1 were analyzed using the technique of X-ray fluorescence energy dispersive photons. These plants contain heavy metals which scavenge (prevent) free radicals damaging tissues of cells of the human body.

The organs of plants consist mainly of leaves. This is *Cassia alata* (PT01), *Manihot esculenta* (PT09), *Mareya micrantha* (PT29), *Ocimum gratissimum* (PT18), *Psidium guajava* (PT06), *Chromolaena odorata* (PT08) and *Azadirachta indica* (PT34).

Materials and methods

Sample collection and preparation

Plants species shown in Table 1 were collected from July 2007 to July 2008 at AGBAN-Bingerville, a village of Bingerville, a town, situated at about 20 km from Abidjan, (Figure 1) in forest zone. They were made up of the leaves. They were gently and thoroughly washed with distilled water do away with surface contamination. They were then dried at ambient laboratory temperatures in the range of 20°-30°C and then grinded into fine powder. About 300 mg of each sample were pelletized using a SPECAC press with a pressure of 2 tons/cm^2 to produce an intermediate thick pellet sample. The pellets produced were kept in a desiccator for at least 24 hours to get rid of the moisture contents. Olive leaves (BCR No 62) was used as the standard reference materials for the validation of the analytical results.

Table 1: Some anti-oxidant plants used in Côte d'Ivoire.

Scientific names	Familynames	Parts of plants	Code
Azadirachta indica	Meliaceae	Leaves	PT34
Cassia alata	Caesalpiniaceae	Leaves	PT01
Chromolaena odorata	Asteraceae	Leaves	PT08
Manihot esculenta	Euphorbiaceae	Leaves	PT09
Mareya micrantha	Euphorbiaceae	Leaves	PT29
Ocimumu gratissimum	Lamiaceae	Leaves	PT18
Psidium guajava	Myrtaceae	Leaves	PT06

Figure 1: Map showing the sampling site.

Instrumentation and sample analysis

The irradiation was done using an EDXRF spectrometer at the XRF laboratory of Ghana Atomic Energy Commission (GAEC). Tube excited X-Ray photons from a Mo-anode in a Mo secondary target excitation arrangement was used. The tube was operated at 45 kV/5 mA. A 30 mm^2 active area Si (Li) detector with an energy resolution (FWHM) of 165 eV at 5.9 keV Mn Kα, placed at 45° to the sample surface area was used for detection of characteristic photons. An ortec maestro multichannel analyser programme was employed for the data collection (peak collection).

Three irradiations were made for each sample, being the intermediate thick sample, multi-element target and Sample + Target for a spectrum collection life time of 1500 s. Linear least squares fitting of the AXIL software programme was used for the spectrum deconvolution [10]. Emission-Transmission method in QXAS package was used to convert spectrum peak areas to concentrations.

Results and discussion

The results of the elemental analysis of the selected anti-oxidation medicinal plants presented in Table 2 *show nineteen elements. The Recommended daily* Dietary Allowance (RDA) and the Tolerable Upper Intake Level (UL) values of some of these elements for adults are also reported in the table. The major elements detected were potassium and calcium. Vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, bromine, rubidium and strontium, were present in trace quantities. An element is considered toxic if its concentration exceeds the tolerable upper intake level (UL) [6]. The Recommended Daily Dietary Allowance (RDA) per day for some detected elements for adults is given in Table 3. The elemental concentrations were determined to verify the biological role of trace elements in anti-oxidation medicinal plants. The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatological conditions [5].

Seven medicinal plants previously presented anti-oxidant activity were analyzed using the technique of X-ray fluorescence. The parts of plants used are essentially leaves. This is *Cassia alata* (PT01), *Manihot esculenta* (PT09), *Mareya micrantha* (PT29), *Ocimum gratissimum* (PT18), *Psidium guajava* (PT06), *Chromolaena Odorata* (PT08) and *Azadirachta indica* (PT34).The analysis of these plants by the technique of X-ray fluorescence allowed us to determine the concentrations (in ppm) minerals and trace elements contained in these plants. The results obtained are shown in Table 2.The anti-oxidant plants studied in this work contains trace elements such as Fe, Cu, Zn, Mn in various concentrations. Only the leaves of *Cassia alata*, *Manihot esculenta* and *Ocimum gratissimum* studied contain selenium (Se) and more. After these initial findings, we looked on the essential trace elements that have an effect in the treatment of diseases affecting the aging of cells and tissues. These are indeed Cu and Zn whose association gives Cu-Zn SOD is an enzyme that prevents the formation of superoxide anions that may cause the formation of free radicals. Mn also synthesizing Mn-SOD's role is to prevent the formation of hydrogen peroxide precursor of free radicals. Finally, the catalase and tocopherol catalyzed by Fe and Se are two substances which neutralize hydroxyl radicals responsible eventually cancer [8]. These trace elements, which are essential in this type of pathology, Cu, Zn, Fe, Mn and Se are summarized in Table 1 and concentrations in the plants studied anti-oxidant. The analysis of these plants by the technique of X-ray fluorescence allowed us to determine the concentrations (in ppm) minerals and heavy metals contained in these plants. The results obtained are shown in Table 2.

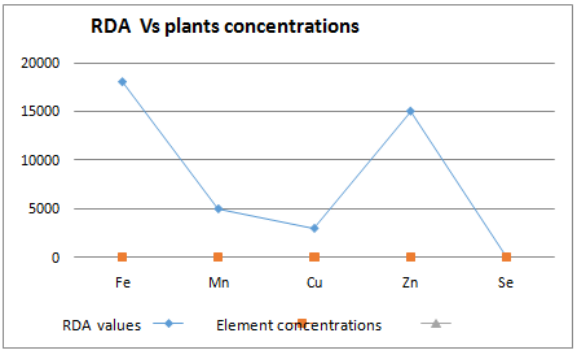

Figure 3: RDA values compared to concentrations of elements from plants studied.

N.B. RDA: Recommended Dietary Allowance.

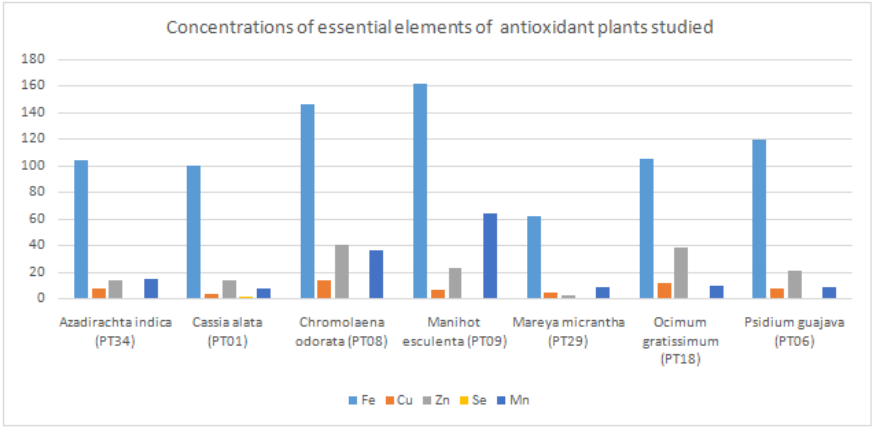

Figure 2: Concentrations of essential elements of anti-oxidant plants studied.

Table 2 : Concentrations ofessential elements of anti-oxidant plants studied.

	Concentrations of essential elements of the anti-oxidant plants (mg/kg or ppm)				
Anti-oxidant plants	Fe	Cu	Zn	Se	Mn
Azadirachta indica(PT34)	104,4±70,6	7,3±4,5	13,6±7,8	-	14,5±10,8
Cassia alata(PT01)	100,0±5,9	2,7±0,7	13,2±1,0	1,4±0,3	7,0±2,1
Chromolaena odorata(PT08)	145,6±7,1	13,7±1,1	40,1±2,0	-	36,0±4,7
Manihot esculenta(PT09)	161,1±8,5	5,7±0,7	22,6±1,3	0,3±0,2	63,3±4,4
Mareya micrantha(PT29)	61,4±4,4	3,7±0,8	2,0±0,5	-	7,8±2,9
Ocimum gratissimum(PT18)	104,5±76,9	11,6± 8,0	38,4±2,5	0,4±0,3	9 ,0±7,6
Psidium guajava(PT06)	119,4±6,6	7,6±0,8	21,0±1,2	-	7,9±2,4

Anti-oxidant plants studied in this work contains trace elements such as Fe, Cu, Zn, Mn in various concentrations. Only leaves of *Cassia alata*, *Manihot esculenta* and *Ocimum gratissimum* studied contain selenium (Se) and more. After these initial findings, we looked on essential trace elements that have an effect in the treatment of diseases affecting the aging of cells and tissues. These are indeed Cu and Zn whose association gives Cu-Zn SOD is an enzyme that prevents the formation of superoxide anions that may cause the formation of free radicals. Mn also synthesizing Mn-SOD's role is to prevent the formation of hydrogen peroxide precursor of free radicals. Finally, the catalase and tocopherol catalyzed by Fe and Se are two substances which neutralize hydroxyl radicals responsible eventually cancer [8].

These metals, which are essential in this type of pathology and concentrations in the plants studied anti-oxidant are summarized in Table 2.

Table 3: Various plant parts analysed and their elemental concentrations.

Elément	*Azadirachtaindica (PT34)*	*Cassia* alata (PT01)	*Chromolaenaodorata* (PT08)	*Manihotesculenta* (PT09)	*Mareyamicrantha* (PT29)	*Ocimumgratissimum* (PT18)	*Psidiumguajava* (PT06)	RDA (per day) adults. mg	[UL per day]
S	-	-	1260,3±803,4	-	-	-	-	-	-
Cl	-	-	824,9±357,0	-	-	-	-	1525-5625mg	-
K	4105,9±2886,7	2585,5±179,4	3724,3±215,0	3010,5±182,7	1553,3±145,0	8781,3±6561,1	4426,1±255,7	1525-5625mg	-
Ca	14500,0±10100,0	3160,5±193,6	4647,7±226,6	2651,1±145,7	14000,0±60 ,0	7262,8±5422,1	2372,3±139,3	800-1200mg	-
Ti	-	-	-	-	-	-	11,4±10,2	-	-
V	-	-	-	11,6±5,1	-	-	-	<1,8mg	1,8mg
Cr	-	-	5,4±3,5	-	5,0±4,0	-	-	-	-
Mn	14,5±10,8	7,0±2,1	36,0±4,7	63,3±4,4	7,8±2,9	9,0±7,6	7,9±2,4	1,0-5,0	11mg
Fe	104,4±70,6	100,0±5,9	145,6±7,1	161,1±8.5	61,4±4,3	104,5±76,9	119,4±6,6	8-18 mg	45mg
Co	-	-	1,7±1,2	-	2,3±1,3	-	2,2±1,4	-	-
Ni	4,1±2,8	2,4±0,7	2,2±0,9	-	2,4±0,9	3,0±2,4	2,5±0,8	0,13-0,4mg	1.000 µg
Cu	7,3±4,5	2,7±0,7	13,7±1,1	5,7±0,7	3,7±0,8	11,6±8,0	7,6±0,8	1,0-3,0 mg	10mg
Zn	13,6±7,8	13,2±1 ,0	40,1±2,0	22,6±1,3	2,0±0,5	38,4±25,0	21,0±1,2	15 mg	40mg
Se	-	1,4±0,3	-	0,3±0,2	-	0,4±0,3	-	55 µg	400µg
Br	13,0±4,9	6,2±0,4	14,1±0,7	5,7±0,4	4,2±0,3	14,0±6,0	11,6±0,7	1,5-2,5mg	-
Rb	15,6±4.8	20,2±1,0	25,9±1,2	9,7±0,5	9,4±0,5	27,9±9,5	23,9±0,8		-
Sr	51,5±14,3	22,9±1,1	21,0±1,0	18,5±0,9	85,3±3,7	35,9±11,0	14,7±0,8		-
Pb	1,6±1,4	1,6±0,7	1,8±1,0	1,7±0,7	1,5±0,8	1,3±1,1	2,4±1,1		-

RDA: Recommended Dietary Allowance
Concentrations of Elements are in ppm or mg/kg
UL: Tolerable Upper Intake Levels

Leaves of *Cassia alata* (PT01) [14], *Manihot esculenta* (PT09) [5], of *Mare*ya *micrantha* (PT29) [9], *Ocimum gratissimum* (PT18) [2], of *Psidium guajava* (PT06) [17] of *Chromolaena odorata* (PT08) (Srinivasa et al. 2010), and those of *Azadirachta indica* (PT34) [12] showed some anti-oxidant properties. These plants therefore contain molecules capable of removing free radicals. The study that we conducted allowed us to identify these plants in the concentrations of trace elements which have a role in eliminating free radicals. The results presented in Table 2 and illustrated in Figure 2 allow us to do as before the following plant by plant analysis.

***Azadirachta indica* A. Juss.** (Meliaceae); [1].

Leaves of *Azadirachta indica* (PT34) were an high iron concentration (104.4 ppm), an average copper concentration (13.7 ppm), a low concentration of manganese (14.5 ppm) and zinc (13.6 ppm) and a zero concentration in selenium. The leaves of *Azadirachta indica* contain essential elements except selenium. The leaves of this plant can therefore contribute to the elimination of free radicals. This plant is used against malaria, jaundice and general fatigue [1].

***Cassia alata* L.** (Caesalpiniaceae); [4]. Leaves of *Cassia alata* (PT01) are richest in selenium (1.4 pp), riches in iron (100 ppm) and poor in zinc (13.2 ppm), copper (2.7 ppm) and manganese (7.0 ppm).These leaves have, at various levels, trace elements that fight against the aging of cells and tissues. We therefore believe that such items catalase, Cu-Zn SOD, MnSOD from essential heavy metals and Se, can allow this plant to fight against the oxidation of cells and tissues. In traditional medicine *Cassia alata* cures herpes [1].

***Chromolaena odorata* (L.)** R. M. King and Robins. (Asteraceae) ; [13]

Leaves of *Chromolaena odorata* (PT08) were a very high iron content (145.6 ppm), copper (13.7 ppm) and zinc (40.1 ppm). An average manganese content (36.0 ppm) and zero selenium content. These leaves contain all elements except selenium. The leaves of this plant can help fight against free radicals that are responsible for diseases such as cancer, Parkinson's and Alzheimer's diseases. Traditional healers use the plant against headache [13]

***Manihot esculenta* Crantz** (Euphorbiaceae); [1]. Leaves of *Manihot esculenta* (PT09) were a very high iron concentration (161.1 ppm) and manganese (63.3 ppm). An average copper concentration (5.7 ppm) and zinc (22.6 ppm). A low concentration of selenium (0.3 ppm). Leaves of *Manihot esculenta* may be prescribed to fight against free radicals because they contain elements that contribute once again to the formation of enzymes such as catalase, superoxide dismutase manganese (MnSOD), superoxide dismutase copper-zinc (Cu-Zn SOD) and selenium. This plant is used against colic in traditional medicine [1].

***Mareya micrantha* (Benth.) Muell. Arg.** (Euphorbiaceae); [11].

Leaves of *Mareya micrantha* (PT29) have a iron poor content (61.4 ppm), copper (3.7 ppm), zinc (2.0 ppm) and manganese (7.8 ppm). A zero selenium concentration. *Mareya micrantha* leaves contain elements except selenium. They can be used in the treatment of free radicals but may be less effective given the very low levels obtained for the essential elements. The plant is known laxative in traditional medicine [1].

***Ocimum gratissimum* L.** (Lamiaceae); [15].

A high iron content (104.5 ppm), copper (11.6 ppm) and zinc (38.4 ppm). A selenium content of 0.4 ppm and 9 ppm manganese. Leaves of *Ocimum gratissimum* may be prescribed in the fight against free radicals

because they contain heavy metals needed to eliminate free radicals. The leaves are used to cure acne and diarrhea [1].

***Psidium guajava* L.** (Myrtaceae); [16]. Leaves of *Psidium guajava* (PT06) contain an high rate of iron (119.4 ppm), an average rate of copper (7.6 ppm) and zinc (21.0 ppm) and low levels of manganese (7.9 ppm) and zero rate of selenium. The leaves of *Psidium guajava* contain essential elements except selenium. The leaves of this plant can be used to fight against free radicals. Health traditional healers use it to cure diarrhea and amoebas [15].The presentation of anti-oxidant plants with their respective trace elements allows us to classify them into two categories. The first category includes plants that contain trace elements such as Fe, Cu, Zn, Mn and Se. This category includes leaves of *Cassia alata*, *Manihot esculenta* and those of *Ocimum gratissimum* that will be effective in eliminating free radicals because they contain heavy metal elements suitable for this fight. The second category concerns other plants that contain various concentrations of essential trace elements except selenium.

Conclusion

This study is aimed at verifying the pharmacological action of herbs with emphasis on the medicinal values of the trace element contents. The elemental analysis of some medicinal plants used for the treatment of oxidation diseases by EDXRF technique revealed eighteen elements in varying concentrations. These plants contain appreciable concentration levels of Fe, Cu, Zn, Mn and Se which are elements well established for their pharmacological action in plants. Leaves of *Cassia alata*, *Manihot esculenta* and those of *Ocimum gratissimum* may be prescribed to fight against free radicals because they contain the essentials needed to disposal such as iron (catalase), manganese (MnSOD), the copper and zinc (Cu-Zn SOD) and selenium. Leaves of *Mareya micrantha*, *Chromolaena odorata*, *Azadirachta indica* and those of *Psidium guajava*, despite the lack of selenium can help treat to fight against free radicals but will be less effective in our opinion in the elimination of free radicals if the presence of selenium was needed.

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