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Full Length Research Paper

Characterization of element and mineral content in *Artemisia annua* and *Camellia sinensis* leaves by handheld X-ray fluorescence

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Tea infusion is the most frequently worldwide consumed beverage next to water, with about 20 billion cups consumed daily. *Artemisia annua* leaves contain comparable levels of nutrients and mineral elements (dry matter basis) to many marketed tea (*Camellia sinensis*) leading us to suspect that this crop could also serve as an alternative source of nutrients for humans. Analyzer moveable X-ray fluorescence is used to evaluate the content of major, minor and toxic elements in *A. annua* from two different countries compared to six marketed tea in Senegal. To ensure qualified results, certified reference materials were used to perform the calibration. The very low and often negligible levels of inherent elements in the leaves, which are far below recommended toxic levels, establishes *A. annua* and selected marketed tea as a good reservoir of elements that might favour its use as a potential herbal tonic by humans. The mineral elements are present in different kinds of herbal leaves in various proportions depending on soil composition and the climate in which the plant grows.

Key words: X-ray fluorescence (XRF), *Artemisia annua*, *Camellia sinensis*, elements, leaves, medicinal plant.

INTRODUCTION

Herbal teas and infusions are traditionally used by several people in West Africa for the treatment of malaria and protozoa infections. Although several phytochemical and pharmacological investigations have been done, *Artemisia annua* was incorporated to assess the efficacy for the treatment of malaria and other protozoa diseases taking into consideration the fact that it is an exotic plant species growing in different soil and weather conditions (Malebo et al., 2009). *A. annua* (Asteraceae) is originated from China, where it is known as qinghao (green herb) and has been used for over 2,000 years to treat symptoms associated with fever and malaria. In 1969, the Chinese screened their medicinal plants in search of an effective antimalarial. A diethyl ether extract of *A. annua* was found to be effective against *Plasmodium* sp., and in 1972 the active ingredient, artemisinin, was isolated and identified by the Chinese. Among approximately 400 species of Artemisia, *A. annua* is the main source of artemisinin. Artemisinin has received considerable attention as a promising and powerful antimalarial drug for its stage specificity, its rather low toxicity, effectiveness against drug resistant Plasmodium species and activity against cerebral malaria (Ferreira et al., 1995; Shen et al., 2008).

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Abbreviations: XRF, X-ray fluorescence; **ALP,** alkaline phosphatase; **A. annua,** Artemisia annua; **C. sinensis,** Camellia sinensis.

There are at least six varieties of tea (*Camellia sinensis*): white, yellow, green, oolong, black and puerh. The three most popular types of tea (green, oolong and black) are distinguished on the basis of their degree of fermentation (Powell et al., 1998), but only the green tea was carried out in this paper. However as the standard, the leaves of green tea are dried and roasted whereas black tea is obtained after a fermentation process. Linking soil physico-chemical properties with their effects on biological systems require extensive sampling and a large number of elements to be analyzed coming from the soil to the plants uptake. Thus it is important to assess that the contents of essential and non essential elements, whether required by plants, are present sufficiently in plant-available concentrations, particularly in the tea leaves to ensure the numerous beneficial effects on health. However the presence of some potential toxic elements in tea leaves as lead (Pb), arsenic (As), chromium (Cr), cadmium (Cd) or aluminium (Al) constitutes a reason for concern. In the case of Cadmium we pay attention to the overlapping spectra between cadmium (Cd-L) and potassium (K) lines. For this purpose, the sandwich of molybdenum (Mo), Al and titanium (Ti) as filter was selected for Cd excitation and these additional filters with Ag was performed to improve the signal-to-noise ratio by reducing the measured continuum. On the other hand, Mo filter is the best choice, instead of silver direct excitation source alone, to attain larger signal-to-noise ratios.

Arsenic is strongly associated with human lung and skin cancer, and may cause other internal cancers as well. Skin lesions, peripheral neuropathy, and anaemia are consequences of chronic arsenic ingestion. Arsenic is the major risk factor for black food disease. The contribution of tea drinking to mineral absorption is not certain, as the bioavailability of many of these elements with tea is not known (Brisibe, 2006; Ferreira et al., 2006; Hayat et al., 2009). *A. annua* is a vigorous growing annual weedy herb, usually single-stemmed, reaching up to 2 to 3 m in height. The plant produces a beautiful portfolio of bioactive compounds. Thus far, the most important of the sesquiterpenoids seems to be artemisinin. Artemisinin is a cadinane-type sesquiterpene lactone with an endoperoxide bridge which is presently the most potent and efficacious compound against the late-stage ring parasites and trophozoites of Plasmodium falciparum (Rajurkar et al., 1998; Shen, 2008). For millennia in Asia, the infusions of green tea (*C. sinensis*) are valued for their taste and medicinal properties. Green tea is not fermented, but used almost in its natural state. The leaves are dried immediately to fresh air immediately after harvest. Some varieties are roasted. This preparation allows leaves to retain their color, but also the valuable substances that make the tea more than a drink: it has many medicinal properties.

The tea leaf contains not less than 350 components, 40% carbohydrate, 20% protein, 2% fat, 9% minerals (including Manganese, Potassium, Magnesium, Fluor), many B vitamins (B1, B2, B3), vitamin C (in green tea), and vitamin D to promote the elasticity of blood vessels (Poblete, 1995).

The six types of *C. sinensis* leaves are imported from China. The TEA 5 and 6 are marketed particularly in Mauritania in Northern Senegal. The data of macro, micro and trace elemental content in tea (*C. senensis*) leaves is of vital importance to understand the pharmacological actions of these kinds of tea compared to *A. annua*; a medicinal plants, which are also tea, consuming for the treatment of malaria among others as throat infections, diarrhea, intestinal cramps, weight loss, etc (Mueller et al., 2000). It is also interesting to note that comparison of the elemental contents of these six *C. sinensis* marketed to *TEA* A (0.1% of artemisinin content) and *TEA* B (1.2% of artemisinin content) shows the difficulty to assess elemental composition in medicinal plant and green tea depending on the soil and the climatic conditions of the growing area.

This study used an analyzer portable X-ray fluorescence for the evaluation of the major, minor and toxic elements in two different kinds of *A. annua* from Cameroun and Luxembourg and in six *C. sinensis* marketed in Senegal, western Africa. According to the Department of Statistics Senegal annually imports different varieties of green tea from china an average for a $ 6 million (2008). The Energy Dispersive X-ray Fluorescence is a simultaneous, reliable, sensitive, quantitative multi-elemental and non-destructive technique, suitable for routine analysis because of minimal sample preparation (Kitsa et al., 1992; Marques et al., 1993; Vincze et al., 1993, Calliari et al., 1995; Ostachowicz et al., 1995). The elements are found to be present in the different kinds of *C. sinensis* in various proportions depending on soil composition and climate in which the plant grows.

The purposes of this study were: to analyze the macro, micro and potential toxic elements composition of these two kinds of *A. annua* from Luxembourg and Cameroon, and others selected *C. sinensis* marketed in Senegal.

MATERIALS AND METHODS

Samples were dried-out in an oven at 80°C until constant mass was obtained. To be ready for EDXRF analysis, the tea was ground to 120 μm using an agate mortar. But for homogenization a vibrating Planetary mill ("Pulverisette 5"), and to exclude any grain size effect in the analysis quantitative, was used to reduce grain size < 70 μm. The samples were prepared as a mixture of tea and Ethylene bis-Stearamide (known as Licowax or Hoechtwax HWC) used as binder agent, in a proportion of 1 g of tea sample to 10% of HWC of the mass of the sample. The mixture was thoroughly homogenized once more in a planetary mill and pressed (under 5 tones) into a disc pellet of 32 mm diameter. The pellets were measured using an EDXRF portable analyzer spectrometer (Niton XL3t900s Gold Air) with a 50 KV and 40 μA operating spectrometer with Au-anode excitation source and several filters as secondary targets for excitation.

This configuration allows the attainment of improved sensitivity and signal to noise ratio by sequentially selecting appropriate

Table 1. *Artemisia a.* and *Camellia s.* leaves samples selected in this study and their provenance.

Sample	Plant species	Label	Provenance	Commercial area
TEA A	Artemisia annua	Artemisia an	Luxembourg	Luxembourg
TEA B	Artemisia annua	Artemisia an	Cameroon	Cameroon
TEA 1	Camellia sinensi	Flecha	China	Senegal
TEA 2	Camellia sinensi	Cheval	China	Senegal
TEA 3	Camellia sinensi	Thé la force	China	Senegal
TEA 4	Camellia sinensi	Forte	China	Senegal
TEA 5	Camellia sinensi	The Sava	China	Mauritania
TEA 6	Camellia sinensi	El Hella	China	Mauritania

Table 2a. Spectrometer specifications and operating conditions.

Spectrometer specification	Operating condition
Resolution	162.15 eV (910 cps Mn Kα)
Window Thickness	12,7 µm Be
Rating	50KV, 40µA maximum power of the tube 2 W
Diameter of the beam	7 mm
Filters	List of Element interest
Ag excitation source	Sb, Sn, Cd, Pd, Ag, Mo, Nb, Zr, Sr, Rh, Bi, As, Se, Au, Pb, W, Zn, Cu, Re, Ta, Hf, Ni, Co, Fe, Mn, Cr, V, Ti
Sandwich of	
Al, Ti and Mo	Ba, Sb, Sn, Cd, Pd, Ag
Cu	Cr, V, Ti, Ca, K
No filter	Al, P, Si, Cl, S, Mg

combinations of filters as secondary targets and different groups of elements were carried out depending on element of interest. These pellets were (three replicate measurements for each tea sample were performed) placed within the stand lid system. The use of portable analyzer for excitation and the characteristic X-rays emitted by the constituents of the samples were carried out for a period of 200 s. The XRF spectra deconvolution and the quantification were performed using Niton Data Transfer software (NDT) with internal algorithm.

The precision of the method was verified by using three different replicate samples due to the fact that measurements have been made in reproducible conditions. Table 2b shows the results of precision attained after the analysis of the NIST 2780.

The accuracy of the instrument was verified by analyzing five different materials: CCRMP TILL-4 180 to 601, NIST 2780 180 to 625, SiO_2 99.995% 180 to 472, RCRA 180 to 436, NIST 2709a 180 to 469a. For the detection of present elements, X-ray fluorescence has been performed to evaluate environmental contamination of tea leave and medicinal plants and the verification of the bio-accumulation potential. In this study enhancement effects and/or absorption of characteristic X-ray emitted by the sample constituents make the quantification of the detected elements difficult. On the other hand, the use on thin sample for analyze was carried out by assessing the proportion between area density and attenuation factor and assuming monochromatic excitation source (Karimi et al., 2008; Markowick et al., 1993; Salvador et al., 2002; Matsumoto et al., 1976).

RESULTS AND DISCUSSION

The average values for each element were calculated only for those elements for which results had very high concentrations of interest. The values of Se, W, Ni and Co are below the detection limit. The cases presenting missing or wrong values are excluded. The list of determined element includes macro, micro, toxic and trace elements (Cu, Zn, P, Si, Fe, S, Ti, Mn, Mg, As, Pb, Sr, Rb, Al and Cl) in six *C. sinensis* marketed leaves coupled with two kinds of *A. annua* plants. The concentrations of rare earth elements (Dy, Eu, Gd, Sm, Tb and Yb) were not detected in any tea leaves samples. The whole data from Tables 3 to 5 show that the different kinds of tea contain the elements in various and no proportional value. The arithmetical mean and standard deviation were carried out for elements which stand for a concern.

Tables 1 to 3 show the concentrations of elements K, Mg, Ca, P and S considered as essential in the maintenance of equilibrium in the organism. Along these macro elements, the concentration of K is higher in *TEA B* of *A. annua*, Cameroon; while only in *THE B* is detected the presence of Mg. The concentration of Ca is maximum in *TEA 3*, “Thé la force”, Senegal. P is mainly present in *TEA A* of *A. annua*, Luxembourg. S is detected with a maximum concentration in *TEA 6*, “El Hella”, and Mauritania (Figure 1). Fe, Mn, Cu and Zn were also detected in the different varieties of *C. sinensis* and A. annua at varied concentrations. The elements Fe, Mn, Cu and Zn are essential trace elements (micronutrients) for living

Table 2b. Result of average value and certified value NIST 2780 (µg/kg).

Element	Certified value ± Unc	$\overline{X}$	σ_X	$(\frac{\sigma_X}{\overline{X}})*100$
Al	88700 ± 3300	46007.23	790.64	1.7
Cd	12.1 ± 0.24	11.98	3.18	26.5
Pb	5770 ± 410	5136.42	69.84	1.3
As	48.8 ± 3.3	80.06	10.03	12.5
Fe	27840 ± 800	27235.71	263.07	0.9
K	33800 ± 2600	34545.16	3096.47	8.9
Ca	1950 ± 200	2052.61	45.92	2.2
P	427 ± 40	3034.37	136.19	4.4
Mn	462 ± 21	449.38	21.12	4.7
S	12630 ± 420	9877.18	203.46	2.1
Sr	217 ± 18	219.85	2.82	1.3
Rb	175	182.49	0.30	0.2
Cu	215.5 ± 7.8	337.63	1.3	0.4
Zn	2570 ± 160	2069.4	13.92	0.7
Mg	5330 ± 200	N.D.	-	-

$\overline{X}$ is the average value for three replicate measurements, σ_X the standard deviation, $\frac{\sigma_X}{\overline{X}}$ the relative standard deviation.
N.D. (Not Detected) due to the limit of detection for Mg (L.O.D = 9864.39 (µg/kg)).

Table 3. Concentration (µg/kg $_{dry}$) of essential macroelements in the studied tea leaves samples.

Sample	K	Mg	Ca	P	S
TEA A	219770 ± 1640	<88140	93540 ± 1040	18970 ± 530	24820 ± 430
TEA B	298740 ± 2110	89690 ± 43680	64850 ± 890	15180 ± 450	21910 ± 380
TEA 1	134040 ± 1160	< 65250	35480 ± 490	14360 ± 490	25940 ± 450
TEA 2	119270 ± 1030	< 70010	90710 ± 890	11880 ± 460	19950 ± 390
TEA 3	116970 ± 990	< 73970	121480 ± 1110	13520 ± 460	26110 ± 420
TEA 4	141540 ± 1240	< 80010	87340 ± 950	15610 ± 530	25180 ± 460
TEA 5	147450 ± 1250	< 69050	45220 ± 580	15490 ± 510	25890 ± 450
TEA 6	163410 ± 1430	< 79960	50550 ± 670	14780 ± 540	27060 ± 490
Mean value	167648.7	77010	734646.2	14973.7	24607.5
Standard deviation	62161	31710	29369.6	2027.8	2421
Min. value	117000	-	45220	11880	19950
Max. value	298740	-	121480	18970	27060

organisms and well known for their role against parasitic diseases (Wong et al., 1998; Zhou et al., 1996). Mn is a component of several enzymes including manganese-specific glycosyl transferase and phosphoenolpyruvate carboxykinase and essential for normal bone structure. Mn deficiency can manifest as transient dermatitis, hypercholesterolemia and increased ALP level.

The concentration of Zn in *TEA 6*, *TEA 5* and *TEA 3* is the same, as well as it is not possible to assess the source plantation was the same in these three cases. Zinc is an essential component of over 200 enzymes having catalytic and structural roles. Zn deficiency is characterized by recurrent infections, lack of immunity and poor growth. Low intake of zinc may cause coronary artery disease. Clinical materials prove that Zn can have good effect on eliminating ulcer and promoting healing wounds. Following zinc and iron, copper is the third most abundant trace element in the body. Copper is involved in the oxidation of Fe^{2+} to Fe^{3+} during hemoglobin formation. It is an important catalyst for iron absorption. Copper deficiency may be a risk factor for cardiovascular disease. When overt copper deficiency occurs, symptoms include Neutrogena, cardiac disorders, osteoporosis and anemia. Iron is needed for a healthy immune system and for

Table 4. Concentration (µg/kg $_{dry}$) of essential micro and trace elements in the tea leaves samples.

Sample	Fe	Mn	Cu	Zn
TEA A	6280 ± 190	1410 ± 100	250 ± 30	270 ± 20
TEA B	12200 ± 260	1780 ± 110	100 ± 20	280 ± 20
TEA 1	1730 ± 110	7300 ± 220	160 ± 20	130 ± 20
TEA 2	4520 ± 160	9660 ± 260	180 ± 30	140 ± 20
TEA 3	4340 ± 170	8700 ± 260	170 ± 30	150 ± 20
TEA 4	2950 ± 160	11940 ± 320	180 ± 30	140 ± 20
TEA 5	1600 ± 110	9060 ± 250	190 ± 30	150 ± 20
TEA 6	1870 ± 130	10170 ± 290	180 ± 30	150 ± 20
Mean value	4436.2	7502.5	176.2	176.2
Standard deviation	3542	3877.2	41	61
Min. value	1600	1410	100	130
Max. value	12200	11940	250	

Table 5. Concentration (µg/kg $_{dry}$) of non essential or toxic elements in the tea leaves samples.

Sample	Al	As	Pb	Rb	Sr	Cd
TEA A	12830 ± 5780	<20	50 ± 10	50 ± 10	20 ± 10	<20
TEA B	23400 ± 6660	30 ± 10	100 ± 10	100 ± 10	60 ± 10	<20
TEA 1	<8040	<20	<20	100 ± 10	30 ± 10	<20
TEA 2	10830 ± 4370	<20	<20	130 ± 10	40 ± 10	<20
TEA 3	18470 ± 5390	<20	<20	190 ± 10	90 ± 10	<20
TEA 4	10920 ± 5010	<20	<20	150 ± 10	40±10	<20
TEA 5	8950 ± 4350	<20	<20	130 ± 10	40 ± 10	<20
TEA 6	<8040	<20	<20	160 ± 10	40 ± 10	<20
Mean value	14233.33	21.45	75	126.25	45	-
Standard deviation	5557	10.6	37.2	43.1	21.4	-
Min. value	<8040	<20	<20	100	20	-
Max. value	23400	30	100	190	90	-

The concentration of Rb and Sr exhibits the highest value in TEA 3.

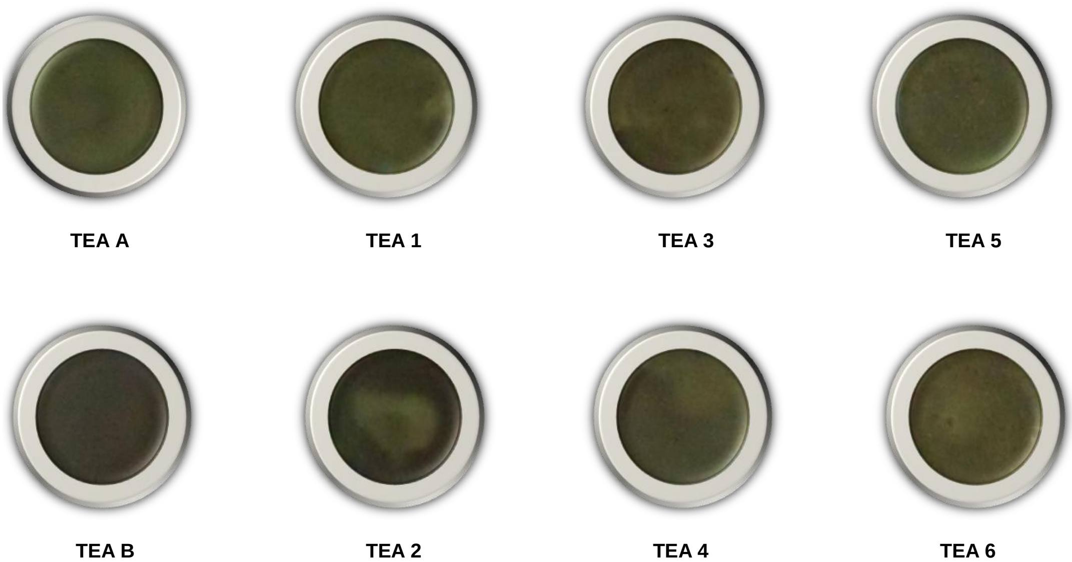

Figure 1. Pellet of tea leaves sample.

energy production. In young children, iron deficiency can manifest in behavioral abnormalities (including reduced attention) reduced cognitive performance and slow growth. In adults, severe iron deficiency anemia impairs physical work capacity.

We found one essential element as halogen Cl at higher concentration in *TEA B*. Ti is present in all analyzed tea samples with a maximum concentration in *TEA 3*. This metal, among others, is employed as fingerprints in the diagnosis of neoplastic tissues (Fung et al., 2003). It tends to form complexes in colon carcinoma with biological molecules such as nucleic acids and proteins. In Table 4, we drew attention for the presence of non-essentials elements, as Al, As, Sr, Rb and Pb in some considerable concentration. The maximum concentration of Al is found in *TEA B* of *A*. *annua* from Cameroon; while As is only present in *TEA B*. The presence of traces of Pb was detected (Foy et al., 1984; Coriat, Gillard, 1986; Koch, 1990; Delhaize, Ryan, 1995; Kochian, 1995; Kidd and Proctor, 2000).

Aluminum toxicity is a major factor limiting crop performance on acid soils that predominate under tropical climate. The primary toxicity symptom observed in plants is inhibition of root growth (Ruan, Wong, 2001; Rao, 1994), followed by less nutrient and water absorption, resulting in poor growth and production. Al interferes with the uptake, transport and utilization of essential nutrients including Ca, Mg, K, P, Cu, Fe, Mn and Zn (Shankar, Prasad, 1998; Black, 2003; Brown et al., 2002; Kordas, Stoltzfus, 2004).

Pollutants may also show high special variability depending on the pattern of emission sources and processes of transportation prevailing in the atmosphere and in deposition. The concentration of Rb and Sr shows the highest value in *TEA 3*. Lead is physiologic and neurological toxin that can affect several organs and organ systems in the human body. It also reduces cognitive development and intellectual performance in children and damage kidneys and the reproductive system (Rajurkar et al., 1998). The present results have to be in compliance with the World Health Organization permissible limits of Cd, As and Pb (Baqui et al., 2003; Chen et al., 2009; Zimmermann et al., 2010).

The high concentrations of Cd, As and Pb, generally in some medicinal plant, shows that the environment in concern is not free of pollution: the nature of soil and immediate surroundings. These chemical constituents present in herbal leaves in general are responsible for their medicinal as well as toxic properties which include vegetables bases comprising alkaloids and amines. For this point of view the consumers of these kinds of herbal leaves have to be careful of possible reason of serious health alterations (Sheded et al., 2006; Koe, Sari, 2009; Ashraf and Mian, 2008; Gjorgieva et al., 2011).

Many studies have concluded that *C. sinensis* has numerous beneficial effects on health, including the prevention of many diseases such as skin cancer, Parkinson's disease, myocardial infarction, and coronary artery disease (Ajasa et al., 2004; Maiga et al., 2005). The difference in element of concentrations may therefore be attributed to *C. sinensis* leave products being produced in different fields with varying element of concentrations in the soils resulting in variation in plants elemental uptake. Moreover, differences in methods used in processing and storage could be contributory factors for this difference. Somewhere different results using different techniques and methods such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectroscopy (ICP-MS), energy dispersive polarized X-ray fluorescence (EDPXRF) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) are reported (Powell et al., 1998; Brisibe, 2006; Shen et al., 2008; Zimmermann et al., 2010; Chen et al., 2009; Sheded et al., 2006; Koe, Sari, 2009; Ashraf et al., 2008; Ajasa et al., 2004; Maiga et al., 2005).

Conclusion

Medicinal plants contain essential and trace elements, which can be available to the human body on consumption of herbs. Indeed today many, if not most, classes of drugs include a natural product prototype. The search for active chemicals from plant sources has continued and many compounds have been isolated and introduced into clinical medicine. Modern medicine is now beginning to accept the use of standardized plant extracts. Present study was also conducted to enhance the same knowledge further and is focused to investigate chemical composition including estimation of nutritional value, trace elements and potential toxic elements of *A. annua* and *C. sinensis (*Basgel, Erdemoglu, 2006; Jabeen et al., 2010; Djama et al., 2011*)*.

A total of 35 elements have been determined by analyzer portable X-ray fluorescence spectrometer in two types of *A. annua* and six *C. sinensis* marketed in Senegal. X-ray fluorescence has been applied to the analysis of essential mineral element composition of tea leaves. But the presence of some toxic elements due to fertilizers or soil composition which grow plants that hamper the usefulness of the most and trace elemental content in tea leaves consumed beverage infusion next to water. The differences in the concentration of the various elements within the different tea (Table 5) is attributed to the preferential absorbability of a particular plant for the corresponding element and the mineral composition of the soil in which the plant grows as well as its surrounding climatologically conditions.

We have to draw attention on the presence of non essentials and/or potential toxics elements. However, as tea is an indispensable part of everyday life for many people in Senegal, studies should never be stopped or neglected to ensure that public health is maintained. Interest is the desirability of the high contents of some of the functional properties of the leaves including minerals such as iron, sodium and zinc, which are important nutritional indicators of the usefulness of the plant as a likely feed resource. Thus taken together, the results reported in the current study therefore show that *A. annua*, when used effectively, can assist in meeting some of the important nutrient and mineral requirements of monogastric and polygastric animals. Thus the leaves and inflorescences of *A*. *annua* should be further explored and used fully as a feed supplement in the production of livestock (Zaidi et al., 2004; Kumar et al., 2005).

From recent studies it seems that hemae is primarily involved in the antimalarial activity of the constituents of *A*. *annua* L. Thus the interaction of a mix with hemae may represent a crucial screening test to define its efficacy. The mechanism of the action of artemisinin remains a very complex process, although iron appears to be involved in activating this endoperoxide to generate cytotoxic free radicals. Several candidates have been hypothesized as targets of artemisinin, including hemae (Clarkson et al., 2004; Prentice, 2008; Dellicour et al., 2007). In the other side, there is an urgent need for antimalarial drugs that are affordable, effective against susceptible and multi-drug resistant malaria, and that have low toxicity. It is important to highlight that any use of tisane to treat malaria, even through *A. annua* leaves, must be followed by a physician to prevent the appearance of strains of *Plasmodium falciparum* resistant to this new line of antimalarial drug.

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