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Studies on the mineral composition of the leaves of Graviola plant, *Annona Muricata*, from some selected municipalities in Ghana by activation analysis

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ABSTRACT

Graviola, *Annona Muricata*, leaves is known for its efficacy in the treatment and management of complex disorders in man. Most research work available however targets on their organic contents, viz. essential oils, glycosides, vitamins, alkaloids and other active components. This research work aimed at investigating their elemental composition which is most often overlooked, though they play important roles in the fight against diseases. In order to determine the presence of these elements, even at trace levels, and to minimize chemical contamination during sample preparation, the sensitive analytical technique (which is free from the use of chemicals), instrumental neutron activation analysis (INAA) was used. Potassium, Ca, Cl, Mg, Fe, Na, Br, Mn, Cu, Cr and Co were present in the leaves at detectable concentrations. The concentrations of the elements Cu, Fe and Mn were found to be below their tolerable upper intake levels. The presence of these elements in the leaves of *Annona Muricata* indicates its potential for use in medicinal preparations for the management and treatment of certain diseases.

Introduction

The use of herbal drugs as remedies for various diseases across the world dated from ancient time. In recent years, increasing interest has been focused on phytomedicines as safer and more congenial to the human body. Herbs have been used as principal source of raw material for non herbal medicines (Mohanta *et al.*, 2003). Most African countries have also employed herbs for the management and treatment of many diseases. This is largely due to the high cost of pharmaceutical drugs, low doctor to patient ratio coupled with inadequate health posts. A combination of herbal drugs and orthodox medicine for the health needs has therefore become indispensable since orthodox medicine alone cannot satisfy this goal.

The graviola plant, *Annona Muricata*, is an important medicinal plant. All parts of the graviola tree are used in natural medicine in the tropics, including the bark, leaves, roots, fruit, and seeds, with each part having different properties and uses. The bark, leaves, and roots are considered sedative, antispasmodic, hypotensive, and nervinec, and a tea is typically made for various disorders toward those effects (Feng *et al*, 1962; Carbajal, D., *et al*, 1991). Studies on such medicinal plants pertain to their organic contents, viz. essential oils, glycosides, vitamins, alkaloids and other active components and their Pharmacological or therapeutic effects. Not much however, is done on mineral content, though they are known to play a vital role in the general well-being of humans as well as in the cure of diseases (Underwood, 1977; Prasad, 1993). Many scientists claim the regular intake of protective elements supplements in appropriate doses and correct proportions may one day be recognized as an important measure in the maintenance of health and preventive diseases (Sahito *et al*, 2003).

Considering the importance of trace elements in various human metabolic processes and their curative properties, in the present studies, instrumental neutron activation analysis have been applied as a sensitive analytical techniques to assess the essential elemental content in the leaves of Graviola, *Annona Muricata*. The overall impact of these essential trace elements on human health is also discussed. Even though the direct link between the essential elemental content and their curative capacity is not yet established, the experimental data of the present work will be of immense importance in the use of Graviola leaves as a herbal drug and a food supplement.

Materials and methods

Sample preparation

Graviola leaves were randomly sampled from some selected metropolis and municipalities within the Greater Accra and Ashanti regions of Ghana for analysis from September to December 2010. The samples were placed in pre - cleaned polyethylene bags and transported to the laboratory.

In the laboratory, ten sub-samples from each town were selected and thoroughly washed with doubly distilled water. The cleaned and dried leaves were frozen at -20°C and lyophilized (Christ Gamma 1-16) for 72 hrs at -30°C (corresponding to a vapour pressure of 0.370 mbar). This drying method was employed to ensure preservation of initial sample texture and to facilitate sample milling (Hoeing, 2001). Using a commercial blender with stainless steel blades, the freeze dried leaves were then homogenized.

About 200 mg each of the pulverized leaves were weighed, wrapped and heat sealed (using soldering rod) in ultra-clean polyethylene films. Five replicate sub-samples were prepared for each sample. For short lived radioisotopes, the wrapped films were packaged into a 7.0 ml polyethylene vial (i.e. one wrapped

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film to one polyethylene vial), which were in turn heat-sealed for irradiation. Standard reference materials namely IAEA-V-10 (Hay powder) and NIST–SRM 1572 (Citrus Leaves) were prepared and packed similarly as the leaf samples. However, for the medium and long lived radioisotopes, the standard reference materials were sandwiched between four wrapped samples and together, packaged into one polyethylene vial for irradiation.

Sample irradiation and counting

The irradiation and counting of samples have been described previously by Serfor-Armah, 2006. The prepared samples, standards and empty polyethylene vials were all irradiated in the Ghana Research Reactor-1(GHARR-1) facility at the Ghana Atomic Energy Commission, Kwabenya, operating at 15 KW at a thermal neutron flux of $5x10^{11}$ $ncm^{-2}s^{-1}$. Samples were transferred into the irradiation sites via pneumatic transfer system at a pressure of 0.6 Mpa. The categorization of irradiation was done based on the half-life of the elements of interest. Irradiation time (ti), decay time (td) and counting time (tc) for short-lived radionuclides with half-life less than few hours (i.e. ^{80}Br, ^{49}Ca, ^{38}Cl, ^{66}Cu, ^{56}Mn and ^{27}Mg) were 2 minutes, 1-10 minutes and 10 minutes respectively. For medium-lived radionuclides with half-life of several hours (42K and ^{24}Na), the irradiation time used was one hour, decay time twenty-four hours and a counting time of one hour. The long lived radionuclides with half-life in days and years (i.e. 60Co, ^{51}Cr and ^{59}Fe) were irradiated for four hours and decayed for two weeks with ten hours counting. In short irradiation, each of the sealed samples in the polyethylene capsules were sent for irradiation one after the other in one of the inner irradiation channels. Table 1 describes the nuclear data for the elements of interest.

Evaluation of peak area of γ-spectrum

The counting of the induced radioactivity was performed by a PC- based γ-ray spectrometry. It consists of an n-type high purity Germanium (HPGe) detector (model GR2518) coupled to a computer-based Multichannel Analyzer via electronic modules and a spectroscopy amplifier (model 2020, Canberra Industries Incorporated). The relative efficiency of the detector is 25% with an energy resolution of 1.8 KeV at γ-ray energy of 1332 KeV of ^{60}Co.

The γ-ray product radionuclides were qualitatively identified by the energies emitted and the quantitative analysis was done by converting the counts as area under the photo peaks by the comparator method. Through appropriate choice of cooling time, the detector's dead time was controlled to be less than 10 %. Both qualitative and quantitative analyses were done using the γ-ray spectrum analysis software, MAESTRO-32.

Data analysis

The results represented mean ± standard error (SE) of five replicated determinations. An ANOVA test (SPSS 7.0 statistical software, SPSS Inc.) was used to compare the mean value of each treatment. Significant differences between the means were determined by using the Tukey HSD test ($P<0.05$).

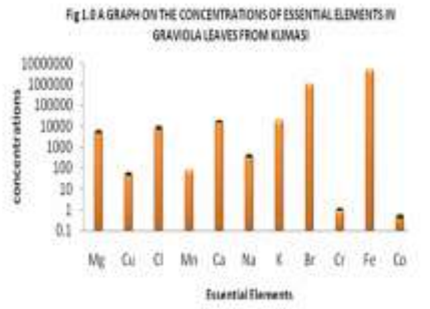
Results and discussion

The SRM of biological origin, NIST–SRM 1572 (Citrus Leaves) and IAEA-V-10 (Hay powder), were irradiated, counted and analyzed for quality control and method validation. It was observed that the concentrations of most elemental contents are within ± 6% of the certified values. Standard deviations were also relatively small. The values listed in the Table 2 (which are averages of three independent measurements) had a precision of ± 4%. From these observations, it can be concluded that a good agreement existed between the results from this work and that of the reported values from the issuing agencies.

The results of elemental analysis obtained by comparator method of INAA techniques are shown in

Table 3 and fig 1-4 in µg/g dry weight (unless indicated otherwise) of the samples. The Recommended daily Dietary Allowance (RDA) and the Tolerable Upper Intake Level (UL) values of some of these elements for adults (DRI, 2001) are also reported in the table.

Potassium recorded the highest concentration in the leaves from all the three sampling stations (i.e 1.91±0.03%, 2.59±0.39% and 2.34±0.03% for Kumasi Metropolis, Ga East Municipality and Ga West Municipality respectively). The concentration of elements in the leaves from the Kumasi Municipality is shown in fig 1. The increasing order of the observed elements was K > Ca > Cl > Mg >Fe >Na >Br >Mn>Cu >Cr >Co whereas that in the leaves from the Ga East Municipality was K > Ca > Cl > Mg >Fe >Na >Br >Mn >Cu >Cr >Co (this is shown in fig 2). The order of elemental concentration in the leaves from the Kumasi Metropolis and that of Ga East Municipality were the same though the concentration values varied significantly ($P<0.05$). Within the Ga West Municipality however, the concentration of the elements were of the order K > Ca > Cl > Mg >Br >Na >Cu >Fe>Mn >Cr >Co. Though Na recorded higher concentration than Br in the leaves from the Kumasi Metropolis and Ga East Municipality, the opposite was seen in that from Ga West Municipality. From fig 4, it can be seen that the same elements were observed in the sampes from the three sampling stations. For all the elements detected in the samples from the three sampling stations, majority of the elements in the leaves from Kumasi recorded highest concentrations (i.e. from fig 4.0). In all three sampling areas, Co recorded the lowest concentration of 0.48±0.07 µg/g, 0.02±0.003 µg/g and 0.05±0.008 µg/g in the leaves analysed. From Tukey HSD, the concentrations of the various elements from the three sampling areas varied significantly ($P<0.05$) from one another. This variation in elemental concentration is mainly attributed to the differences in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for a variation in the elemental content are use of fertilizers, irrigation water and climatological conditions (Rajurkar, 1997).



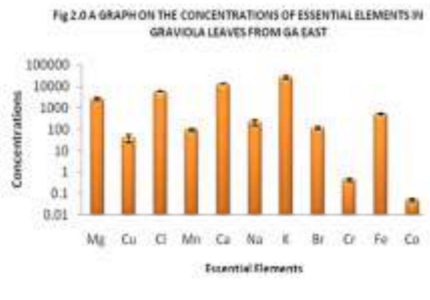


Fig 3.0 A GRAPH ON THE CONCENTRATIONS OF ESSENTIAL ELEMENTS IN GRVIOLA LEAVES FROM GA WEST MUNICIPALITY

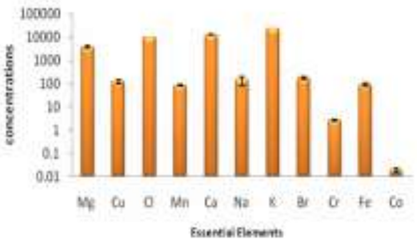
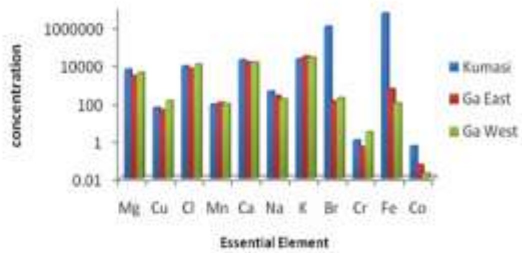


Fig 4 A GRAPH OF CONCENTRATION AGAINST ESSENTIAL ELEMENT CONTENT IN GRAVIOLA LEAVES



Potassium is accumulated within human cells by the action of the Na, K- ATPase (sodium pump) and it is an activator of some enzymes; in particular co-enzyme for normal growth and muscle function (Birch and Padgham, 1994). Potassium deficiency causes nervous disorder, diabetes, and poor muscular control resulting in paralysis. The high content of K in the leaves of *Annona Muricata* may be useful as a medicinal plant for the management and treatment of these disorders.

Other major elements recorded in the leaves were Ca% (1.70±0.11, 1.31±0.05 and 1.29±0.04 in the Kumasi Metropolis, Ga East Municipality and Ga West Municipality respectively), Cl% (which ranged from 0.59±0.008, in the Ga East Municipality, to 0.99±0.008, in Ga West Municipality) and Mg% (0.53±0.04 in the Kumasi Metropolis; 0.37±0.04 in the Ga West Municipality and 0.25±0.04 in the Ga East Municipality). Calcium is essential for healthy bones, teeth and blood. The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, for the activation of enzymes such as the pancreatic lipase. Deficiency of calcium causes rickets, osteomalacia and scurvy (Charles, 1992 and Hughes, 1972).

The concentration of Mg was highest in the Kumasi Metropolis (i.e 0.53±0.04%) followed by 0.37±0.04% in the Ga West Municipality. The minimum concentration of 0.25±0.04% was recorded at Ga East Municipality. Magnesium (Mg) improves insulin sensitivity, protect against diabetes and its complications and reduce blood pressure.

Copper recorded a concentration of 112.26±22.24 µg/g in the Ga West Municipality which is the highest concentration recorded from the three sampling stations. This was followed by a concentration of 51.45±3.86 in the Kumasi Metropolis with Ga East Municipality recording the least concentration of 39.61±14.89 µg/g. Copper is necessary for normal biological activities of amino-oxides and tyrosinase enzymes. Tyrosinase is required for the catalytic conversion of tyrosine to melanin, the vital pigment located beneath the skin, which protects the skin from dangerous radiation (Durdana *et al*, 2007).

Manganese (Mn) concentrations ranged from 93.76±10.91 µg/g in the Ga East Municipality to 77.21±1.74 µg/g in the Kumasi Metropolis with those in the Ga West Municipality recording a concentration of 83.17±5.74 µg/g. Deficiency of Mn in the body may lead to central nervous system (CNS) disorders (Torente et al. 2002), and low manganese level may reduce manganese dependent superoxide dismutase activity which can increase cancer susceptibility (Finlay and Davis 1999).

On the other hand, Fe concentrations ranged from 505.13±24.39 µg/g to 90.54±13.58 µg/g in the Kumasi Metropolis and Ga West Municipality respectively. Iron deficiency is the most prevalent nutritional deficiency in humans and is commonly caused by insufficient dietary intake and excessive menstrual flow. Such situation usually results in an anemia (Reddy, 1987). General tonic preparation from the leaves may be advised to compensate for iron deficiency in man.

The Br content in the leaves varied from 97.84±15.33 µg/g (in Kumasi Metropolis) to175.29±17.13 µg/g (in Ga West Municipality). In experiments conducted by Anke *et al*, 1988, with growing, gravid and lactating goats, a poor Br-nutrition (<1 mg/g dry matter) led to a significantly reduced growth, a worse conception rate, reduced milk fat production and decreased hemoglobin content. The high concentration of Br along with Fe in *Annona Muricata* leaves, suggests their possible use in the preparation of drugs for curing natural diuretic, phlegm eliminating and stomach invigorating diseases. It is also needed for purifying breast milk (Chen *et al*, 1998 and Anke *et al*, 1988). The possible source of Bromine to these leaves however is agricultural chemicals such as methyl bromide as fumigant.

The concentration of Cr was observed to be highest (i.e 2.73±0.21 µg/g) in the Ga West Municipality and lowest (i.e 0.99±0.05 µg/g) in the Kumasi Metropolis though its concentration in the Ga East Municipality was 0.99±0.06 µg/g. Deficiency of chromium causes glucose intolerance. It plays an important role in diabetes treatment. The function of chromium is directly related to the function of insulin, which plays a very important role in diabetes. Chromium is found in the pancreas, which produces insulin. Due to the presence of Cr in the leaves, the leaves of *Annona Muricata* may be advised for the treatment and control of diabetics.

In the leaves of *Annona Muricata*, the concentration of Co was highest in the Kumasi Metropolis (0.48±0.07 µg/g) and least in the Ga West Municipality (0.02±0.003 µg/g). Cobalt is known to play an important role in thyroid metabolism in humans.

Sodium involves in the production of energy, transport of amino acids and glucose into the body cells. The concentration of Na in leaves ranged from 145.09±58.84 µg/g (in the Ga West Municipality) to 377.99±44.75 µg/g (in the Kumasi Metropolis).

The highest concentrations recorded for Cu (112.26±22.24 µg/g), Fe (505.13±37.88 µg/g) and Mn (93.76±10.91µg/g) were all below the tolerable upper intake levels of 10mg, 45mg and 11mg respectively.

Conclusions

This study presents a preliminary observation on concentrations of some essential elements in the leaves of the Graviola plant, *Annona Muricata*, cultivated in some communities in Ghana. The emphasis was on the medicinal values of the elemental contents. The study revealed the presence of eleven elements (i.e K, Ca, Cl, Mg, Fe, Na, Br, Mn, Cu, Cr and Co) in fair quantities. Majority of the elements in the leaves from Kumasi recorded highest concentrations. The presence of these elements in the leaves of *Annona Muricata* indicates its potential for use in medicinal preparation of general (e.g. heart and blood) tonics, treatment and control of diabetics and supplements for deficiency of some of the observed elements in the human body. The concentrations observed for elements such as Cu, Fe and Mn which are all known for their essentiality were below the tolerable upper intake level hence eliminating concerns for toxicity.

However, in order to develop a stronger basis for appreciating the curative effects of *Annona Muricata* leaves, there is a need to study the effect of soil and climatic conditions on the elemental contents of the Graviola plant leaves.

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Table 1: Nuclear data used for determination of elemental concentrations (IAEA TECDOC-564)

Element	Nuclear Reaction	Half life	Gamma ray energy (Kev)
Br	$^{79}Br(n, \gamma)^{80}Br$	17.7 min	616.2
Ca	$^{48}Ca(n, \gamma)^{49}Ca$	8.7 min	3084.4
Cl	$^{37}Cl(n, \gamma)^{38}Cl$	37.3 min	1642.4, 2167.5
Cr	$^{50}Cr(n, \gamma)^{51}Cr$	27.72 d	320.0
Co	$^{59}Co(n, \gamma)^{60}Co$	5.27 y	1173.2, 1332.4
Cu	$^{65}Cu(n, \gamma)^{66}Cu$	5.1 min	1039.4
Fe	$^{58}Fe(n, \gamma)^{59}Fe$	44.5 d	1099.2, 1291.6
K	$^{41}K(n, \gamma)^{42}K$	12.36 h	1524.7
Mg	$^{26}Mg(n, \gamma)^{27}Mg$	9.46 min	843.8, 1014.4
Mn	$^{55}Mn(n, \gamma)^{56}Mn$	2.58 h	846.7, 1810.7, 2112
Na	$^{23}Na(n, \gamma)^{24}Na$	15.02 h	1368.6, 2754.1

Table 2: Comparison of elemental concentrations in reference materials analyzed by INAA with certified/recommended values, n = 5

Elements	NIST–SRM 1572 (Citrus Leaves) Concentration (µg/g unless indicated otherwise)		IAEA-V-10 (Hay powder) Concentration (µg/g unless indicated otherwise)	
	This work	Certified value	This work	Certified value
Br			7.54±1.71	8.0±1.20
Ca%	2.91±0.06	3.15± 0.10	2.15±0.32	2.16±0.32
Cl	414.01±31.05	414.00*		
Cr	0.82±0.14	0.80±0.2	6.29±0.09	6.50±0.90
Co			0.14±0.04	0.13±0.002
Cu	17.0±0.82	16.50±1.0	9.00±0.62	9.40±3.05
Fe	90.00±11.01	90.00±10.00	190.21±17.11	186.00±18.51
K%	1.81±0.02	1.82 ± 0.06	2.00±0.39	2.10±0.02
Mg%	0.57±0.04	0.58 ± 0.03	0.12±0.05	0.13±0.02
Mn	23.50±3.21	23.00±2.00	46.26±4.89	47±2.11
Na	160.10±18.49	160.00±20.00	50.20±7.53	50.00±4.52

*Recommended value or uncertified

Table 3: Essential elements content (in µg/g unless otherwise indicated) in the leaves of graviola, *Annona Muricata*

Elements	Kumasi Metropolis	Ga East Municipality	Ga West Municipality	RDA (per day) adults.	UL (per day) adult
Br	97.84±15.33[a]	115.36±16.39[b]	175.29±17.14[c]	1.5-2.5 mg	
Ca%	1.70±0.11[b]	1.31±0.05[a]	1.29±0.04[a]	800-1200 mg	
Cl%	0.81±0.12[b]	0.59±0.008[a]	0.99±0.008[c]		
Cr	0.99±0.05[b]	0.43±0.06[a]	2.73±0.21[c]		
Co	0.48±0.07[c]	0.05±0.008[b]	0.02±0.003[a]		
Cu	51.45±3.85[b]	39.61±14.89[a]	112.26±22.24[c]	0.9 mg	10mg
Fe	487.97±24.39[b]	505.13±37.88[c]	90.54±13.58[a]	8-18 mg	45mg
K%	1.91±0.03[a]	2.59±0.39[c]	2.34±0.03[b]	1525-5625 mg	
Mg%	0.53±0.04[c]	0.25 ±0.04[a]	0.37±0.04[b]		
Mn	77.21±1.74[a]	93.76±10.91[c]	83.17±5.74[b]	1.0-5.0 mg	11mg
Na	377.99±44.75[c]	221.12±62.79[b]	145.09±58.84[a]		

Different superscripts (i.e. a-c) in a row show significant difference ($P<0.05$), based on the Tukey HSD test following one-way ANOVA.