

EVALUATION OF METAL IONS CONCENTRATIONS, TOTAL FLAVONOID CONTENT AND DPPH FREE RADICALS SCAVENGING IN THE FRUITS OF *TETRAPLEURA TETRAPTERA* (PREKESE) FROM GHANA

*Eva Tabuaa Gyamfi¹, Michael Ackah¹ Alfred Kwablah Anim¹, Nafisatu Zakaria¹, Lebene Kpattah¹, Sheriff Enti-Brown¹, Esther Saah Nyarko¹, John Hanson¹, Delali Tulasi¹, Juliet Osei¹, Akwasi Akomea Agyekum²

¹Ghana Atomic Energy Commission. National Nuclear Research Institute.
Nuclear Chemistry and Environmental Research Centre. P. O. BOX LG80. Legon-Accra, Ghana

²Ghana Atomic Energy Commission. Radiation and Medical Sciences Research Institute.
Applied Radiation Biology Centre P. O. BOX LG80. Legon-Accra, Ghana

*E-mail:evagymfi@gmail.com

Abstract

Tetrapleura tetraptera, widely known in Ghana as “Prekese” is one of the widely used medicinal plants in West Africa. Its fruits possess considerable quantities of mineral elements and some phytochemicals. It is highly imperative to know some of its unique trace element and phytochemical constituents to standardize its use as potential raw materials.. Flame atomic absorption spectrometry, after microwave-assisted acid digestion was employed to determine the metal ion contents in dried fruits (pulp) of *Tetrapleura tetraptera* in eight (8) major markets in Ghana. DPPH radical scavenging assay was used to evaluate the radical scavenging activity of extracts of the dried fruit while the aluminium chloride assay method using quercetin as a standard was used to determine the total flavonoids in the dried pulp. Iron was the most dominant mineral element while Nickel was the least dominant mineral element. The concentrations, based on three replicate measurements of the essential metal ions were observed in the range of 4.11 ± 1.41 - 54.08 ± 0.00 , 3.12 ± 0.35 - 5.27 ± 0.40 , 0.003 ± 0.00 - 0.97 ± 0.25 , 0.31 ± 0.15 - 2.15 ± 1.95 , 0.32 ± 0.23 - 1.06 ± 0.08 mg/Kg (milligrams per kilograms) for Fe, Mn, Cu, Zn, and Ni respectively. On the other hand, the concentration of toxic lead (Pb) was in the range of 1.44 ± 1.11 - 10.59 ± 8.49 mg/Kg. The total flavonoid contents in the fruits ranged between 19.042 to 83.713 QE/g.

Keywords: Atomic absorption spectrometry, metals, total flavonoids, DPPH free radical scavenging assay

Submitted: 04.11.2015

Reviewed: 23.02.2016

Accepted: 30.03.2016

1. INTRODUCTION

Minerals are essential to life. Some minerals are required in macronutrients amounts to perform essential functions of life (Saracoglu et al. 2009). These include Ca, Mg, Na, K, P and S. Others are required in smaller quantities and are referred to as trace minerals (Fe, Cu, Mn, and Zn). The other elements required are Li, B, F, Si, V, Cr, Mn, Co, Ni, As, Se. Studies have shown that high intake of elements beyond recommended limits can lead to metal poisoning whereas low intake levels can lead to deficiency effects. One major route of entry for essential and toxic metals into living organisms is via the food chain. Due to the health hazards that minerals may pose when taken in excess, the World Health Organization and other

international bodies have set standards relating to daily allowances or tolerable intake of elements. Therefore all mineral elements entering the human body via foodstuffs need to be monitored and evaluated to be sure their amounts are within recommended limits (Dermelj et al., 1996). The composition of minerals in plants plays a crucial role in the medicinal values of plants and their therapeutic effects on health and diseases. (Kaneez et al. 2001). As a result of increased awareness of the vital role of minerals in human health, there has been a revival of interest in the use of plants as a source of conventional and complementary therapies (Chouddhary and Rehman, 2002). A lot of nutritionally important elements and their presence in plants have been the subject of many studies (Sena et al. 1998).

This has increased the need to study the elemental composition of many edible plants which could be used as an important source of elements.

Ghana is situated on the West Coast of Africa with a total land area of 238,540 square kilometres and an estimated population of about 24 million (Ghana Statistical Service, 2000). Ghana abounds in important medicinal plants. However, a few of them have been analysed for their mineral composition. One such important plant is *Tetrapleura tetraptera* which is locally known as Prəkese in the Twi dialect of Ghana. This plant is native to fringes of the West African rainforest belt and mostly found in Ghana (Wikipedia 2015). The species is found throughout the high forest zone, in riverine forest, in the southern savannah-woodland and in the forest outliers in the African plains and are native to Benin, Burkina Faso, Cambodia, Chad, Cote d'Ivoire, Gambia, Ghana, Guinea, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, Togo and Uganda. Its use as traditional medicine is widespread (where the leaves, bark, roots and the kernels are used) and in Ghana, particularly the fruit and flowers are used as perfumes and in pomades prepared from palm oil. It has also been widely reported that *Tetrapleura tetraptera* has been utilized in Tropical African traditional medicine for the management and/or control of an array of human ailments, including arthritis and other inflammatory conditions, asthma, ulcer, diabetes mellitus, hypertension, epilepsy, schistosomiasis and *Staphylococcus aureus* (Aladesanmi, 2007; Ojewole and Adewunmi, 2004). Its sweet fragrance is highly valued, its fruit is used to spice dishes, and its bark is used for medicinal purposes (Kyei and Allman, 2001; Cole and Ross, 1977). In Ghana, there is a move to increase the usage of the fruit, and it's being added to chocolate, made into jam and used as flavouring for biscuits and some alcoholic beverages. Dried fruits are used in flavoring soups, particularly the traditional pepper soup, a delicacy consumed by mothers from the first day of delivery to prevent postpartum contractions, as a lactation aid (Enwere, 1998;

Nwaiwu and Akali 1986) and for gastrointestinal disorders, especially stomach ulceration (Noamesi et al., (1992). Some of the authenticated biological activities of this plant include its usage as a molluscicide (to control slugs and snails), in schistosomiasis, in gastrointestinal ulcer, as antimicrobial (especially against *Staphylococcus aureus*), anticonvulsant, in hypertension (to lower blood pressure), and as contraceptive. Recent studies in Nigeria have shown that the fruit extract of *Tetrapleura tetraptera* (in alcohol) possesses significant anti-malarial, analgesic and anticonvulsant activities. *Tetrapleura tetraptera*, like other medicinal plants, has ability to produce and store a wide range of chemical substances (Mbotto et al. 2013). Most of these substances are secondary metabolites general referred as phytochemicals, which though have no apparent function in the primary metabolism of the plant; serve to defend the plant against attacks from microorganisms and other predators (Udobi and Onaolapo, 2009).

It has become imperative to standardize its use in traditional medicine thereby justifying detailed studies of the fruits of *Tetrapleura tetraptera* to quantify the crude chemicals and bioactive constituents present therein (Uyoh et al. 2013). Although much nutritional evaluations on the dried fruits of *T. tetraptera* have focused mainly on the nutrients, phytochemistry and mineral contents including metals and vitamins (Adebayo et al. 2000; Essien et al. 1994; Okwu 2003, Akin-Idowu et al. 2011), studies conducted on the fruits in Ghana is relatively few. This study sought to determine trace metals, and some phytochemicals especially the flavonoids, and DPPH free radicals of *T. tetraptera* fruits from Ghana. This study is particularly useful when drawing up an abatement program to curtail effects of toxic metals in edible fruits in the face of rapid urbanization with attendant threats of environmental pollution in developing countries. It is expected that the study will also contribute to our understanding of the scientific basis of traditional uses of the *Tetrapleura tetraptera* fruit.

2. MATERIALS AND METHODS

Sampling

The fruits of *T. tetraptera*, locally known as “prekese” were obtained from open markets in seven selected towns in seven geographical regions in Ghana. Two samples were selected from each region. The samples were transported in Ziploc bags and transported to laboratories of the Ghana Atomic Energy Commission. In the laboratory, the samples were manually washed with distilled water and air-dried for 3 days. The samples were ground into fine powder, after air drying. The powdered samples were subsequently stored in labelled airtight plastic bags, ready for chemical determinations.

Reagents

Analytical reagent-grade chemicals were employed in the preparation of all solutions. Double distilled water was used in this study for the preparation of all reagents and for washing. The HCL, HNO₃ and H₂O₂ were sourced from (AnalaR NORMAPUR, France). All the plastic and glassware were cleaned by soaking in dilute nitric acid (10%) and were rinsed with double distilled water prior to use. The standard solutions of investigated analytes namely Fe, Mn, Cu, Zn, Ni and Pb for calibration procedure were produced by diluting a stock solution of 1000mg L⁻¹ of the investigated element supplied by Sigma – Aldrich Chemie, GmbH (Germany).

Apparatus and Experimental procedure

The Association of Official Analytical Chemists (AOAC) methods were employed in this study (AOAC, 2003; Jorhem, 1993). Metal ion (Fe, Mn, Cu, Zn, Ni, and Pb) composition was determined by wet acid digestion of the dried and pulverized samples of *Tetrapleura tetraptera* leaves using Milestone laboratory protocol (1996-2000). About 6 mL of HNO₃ (65%) and 1mL of H₂O₂ (30%) were added to 0.50g of the *Tetrapleura tetraptera* sample. The sample and acid mixture was kept in a programmed microwave oven to achieve the desired digestion. After digestion, the remaining digestate was allowed to cool, and subsequently, the digestate was transferred into a 20mL volumetric flask.

The digested sample was made up to 20 mL using distilled water. The metal ion compositions of the standard and sample solutions were determined using flame atomic absorption spectrometry (FAAS) in an air-acetylene flame using a fast sequential Atomic Absorption Spectrometer (Varian AA240 FS) at the Inorganic Laboratory of the Nuclear Chemistry and Environmental Research Centre of Ghana Atomic Energy Commission. A calibration curve showing a plot of the absorbance of each element versus the element concentration was utilized to determine the concentration of each element in the *Tetrapleura tetraptera* samples. The analytical conditions is as shown in Table 1.

Table 1. Analytical Conditions for Atomic absorption spectrometry

Parameter	Fe	Mn	Cu	Zn	Ni	Pb
Wavelength(nm)	248.3	279.5	324.8	213.9	232.0	244.8
Slit width(nm)	0.2	0.2	0.5	1.0	0.2	0.2
Lamp current(mA)	5.0	5.0	4.0	5.0	4.0	5.0
Background correction	OFF	OFF	OFF	ON	ON	ON
Acetylene flow(L/min)	2	2	2	2	2	2
Airflow (L/min)	13.5	13.5	13.5	13.5	13.5	13.5

Phytochemical Analysis

Chemical Reagents

All chemicals used were of analytical grade. Folin-Ciocalteu phenol reagent (FCR), Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH•), quercetin and ascorbic acid (vitamin C) were obtained from Sigma Aldrich, Germany. AlCl₃, Na₂CO₃, CH₃COOK, were obtained from Merck (Darmstadt, Germany). Ethanol and methanol were obtained from Jansen Chimica (Beerse, Belgium).

Preparation of aqueous and methanol Extract,

The dried plant materials were frozen under liquid nitrogen and freeze dried using an Eyela Freeze drier (Tokyo Rikakikai Co. Ltd., Tokyo Japan). Lyophilized samples were pulverized, vacuumized, and stored at 4°C till required. 2.5 grams of the pulverized sample was extracted in 40 ml of 96% methanol (cold percolation) for 24 hours. The extracts were centrifuged at 8500 rpm for 10 min and supernatants recovered. Additional 20 ml of methanol was used to re-extract the plant residue and the supernatants pooled. The procedure was repeated using distilled water instead of methanol as solvent. The MeOH (MEL) and aqueous (WEL) extracts of samples were stored in 50ml polypropylene tubes at 4 °C until needed. Extraction yields were established for each solvent by evaporating off two (2) ml of the extract to dryness and measuring the solid residue to establish the amount of extractable solids (extraction yield).

Determination of Total Flavonoid Content

The aluminum chloride colorimetric assay method (Olajire and Azeez, 2011) was employed to evaluate total flavonoid content (TFC) in the sample using quercetin as standard. 500µL of the extract was mixed with 1500µL of 99.9% ethanol (EtOH), 100µL of 1 M potassium acetate, 100µL of 10% aluminum chloride and 3000µL of distilled water. The resulting mixture was incubated for 30 minutes at room temperature and corresponding absorbance measured at 415 nm. All determinations were carried out in triplicates. A

standard calibration curve was constructed using quercetin standard solutions of 12.5µg/mL, 25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL. About 500µL of each standard was treated in the same manner as the samples and calibration linear regression equation ($y=0.062x + 0.0268$, $r^2 = 0.9638$) generated. Flavonoid content of each extract were determined from the curve and the final results recalculated and expressed as microgram quercetin equivalent per gram of dry sample (µg QE)/gdw).

DPPH Free Radical Scavenging Activity

The free radical scavenging assay has been used widely to evaluate the radical scavenging activity of the different types of antioxidant substances (Cotelle et al., 1996). The color changes will allow the detection of the scavenging activity at 517nm (Mahmoudi et al. 2015). The free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Blois, 1958 as modified by Brand-williams et al. 1995 and Yen and Chen, 1995. About 200µL of each extracts was added to 3800µL of 0.004% DPPH in methanol. After 60 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm. A blank sample containing only methanol was used to zero the Shimadzu UV-VIS spectrophotometer (Kyoto, Japan). Ascorbic acid (Vitamin C) was used for comparison. Each experiment was performed in triplicate. Radical scavenging activity (I %) was calculated as follows:
$$\left(\frac{Abs_0 - Abs_1}{Abs_0} \right) * 100\%$$

Where Abs0 = absorbance of 0.004% DPPH without analyte and Abs1 = absorbance of 0.004% DPPH plus the test compound

Analytical Quality Assurance

Appropriate quality assurance procedures were employed in this study to ensure the reliability of the results. The samples were carefully handled to avoid contamination. Glassware was properly washed and all chemicals used were of analytical grade. Double distilled deionized water was used for the preparation of all standard solutions and for washing purposes.

Reagent blank readings were done to correct errors due to instrument readings. Recovery studies was conducted for the metal ion determination by spiking and homogenizing pre-analyzed samples with varied amounts of standard solutions of the metals. The average recoveries obtained were 93.0-99.0, 99.8-100.2, 95.1-99.5, 92.0-102.0, 97.8-101.5, 98.6-103.0 for Fe, Mn, Cu, Zn, Ni and Pb respectively.

3. RESULTS AND DISCUSSION

The mean concentrations of metal ion concentrations were in this order; Fe>Pb>Mn>Zn>Ni>Cu. The highest concentration of Iron was found in *Tetrapleura tetraptera* sample from Sunyani Market, while the least concentration of Iron was detected in *Tetrapleura tetraptera* samples from Somanya. The concentration range with the respective standard deviations of three replicates of metal ion determinations in of the prekesse samples from major markets is shown in Table 2.

The concentration of Iron measured for *Tetrapleura tetraptera* in this study ranged from 4.11 to 54.08 mg/Kg. This result was comparable to a study conducted in Nigeria (Akin-Idowu et al 2011), who recorded Iron concentration range of 29.69-65.06 mg/Kg. The recommended dietary allowance (RDA) of Iron for male adolescents (14 - 18 years of age) is 11 mg/day; that of female adolescents (14 - 18 years of age) is 15 mg/day. On the other hand, Fe RDA for male adults (19-50 years old) and female adults (19-50 years old) is 8 mg/day and 18 mg/day respectively. Iron in the fruits of *Tetrapleura tetraptera* could serve as a

rich source of iron to help boost the immune system of individuals. Iron is also very important in reducing the incidence of anaemia and it is an important component of hemoglobin, the protein that is utilized for the transportation of oxygen through the blood to the lungs and tissues. The consumption of the fruits of *Tetrapleura tetraptera* by lactating mothers has been linked to its high Fe content (Abii and Amarachi, 2007).

The mean value of lead ion in the dried fruit was 5.26 mg/Kg. Lead is known to have no beneficial role in human metabolism, rather merely causing harm after uptake from food, air or water. Lead accumulation ultimately causes toxic effects on humans by affecting food quality of crops (Kawada and Suzuki, 1998) and animal products as well as drinking water quality (De Vries et al. 2007) and animal health by affecting fodder quality and by direct intake of contaminated soil (Adriano, 2001). Lead can enter the human body through uptake of food (65%), water (20%) and air (15%). Foods such as fruit, vegetables, meats, grains, seafood, soft drinks and wine may contain significant amounts of lead. Lead can enter (drinking) water through corrosion of pipes. Lead can cause several unwanted effects, such as: the disruption of the biosynthesis of hemoglobin, anaemia, rise in blood pressure, kidney damage, miscarriages and subtle abortions. The other toxic effects of lead are the disruption of nervous systems, brain damage, declined fertility of men through sperm damage, diminished learning abilities of children, behavioural disruptions of children, such as aggression, impulsive behavior and hyperactivity.

Table 2 Mean concentrations of trace elements in dried fruit samples of *Tetrapleura tetraptera*

Markets/Trace element	Fe(mg/Kg) (mean±SD)	Mn(mg/Kg) (mean±SD)	Cu(mg/Kg) (mean±SD)	Zn(mg/Kg) (mean±SD)	Ni(mg/Kg) (mean±SD)	Pb(mg/Kg) (mean±SD)
Madina	18.71±2.41	3.89±0.55	0.56±0.14	0.65±0.17	0.61±0.28	3.19±1.36
Sefwi-Bekwai	5.93±2.09	3.33±0.34	0.003±0.00	0.40±0.14	0.32±0.23	10.59±8.49
Techiman	13.36±4.41	3.84±0.37	0.17±0.02	0.61±0.27	0.87±0.54	5.83±1.01
Sunyani	54.08±0.00	5.27±0.40	0.03±0.00	0.55±0.10	0.52±0.23	1.44±1.11
Kumasi	17.11±3.52	3.86±0.08	0.97±0.25	1.16±0.28	0.78±0.08	4.78±0.99
Dome	19.36±2.18	4.43±0.57	0.69±0.07	2.15±1.95	0.64±0.45	4.60±1.13
Winneba	7.32±4.11	3.12±0.35	0.03±0.00	0.31±0.15	1.06±0.08	4.29±1.47
Somanya	4.11±1.41	5.09±0.30	0.14±0.02	0.79±0.20	0.91±0.74	7.39±0.24
Conc. Range	4.11-54.08	3.12-5.27	0.003-0.97	0.31-2.15	0.32-1.06	1.44-10.59

Lead can enter a foetus through the placenta of the mother and it subsequently could cause serious damage to the nervous system and the brains of unborn children.

Manganese concentrations in the samples ranged from 3.12 to 5.27 mg/Kg. This was lower than the manganese concentration range recorded for a similar study conducted in Nigeria by Akin-Idowu et al 2011, in which the manganese concentration range of 16.23-178.91 mg/Kg was recorded. The recommended daily intake for manganese is 5mg (RDI, 2009). Manganese is needed by the body to sustain certain functions including carbohydrate and protein metabolism, connective tissue, joint fluid production, nerve tissue and Vitamin B1 utilization.

Copper concentrations in the samples ranged from 0.003 to 0.970 mg/Kg. Copper is an essential component of about 13 different enzymes (Saracoglu et al 2009). Copper is important for iron metabolism, elastic tissue formation, skin and hair pigmentation function, and many other functions. Copper determined in the dried fruits of *Tetrapleura tetraptera* were found to be lower than Copper measured in a similar study in Nigeria, where Akin-Idowu et al.2011 recorded mean Cu content of 4.0mg/Kg.

The concentration of nickel (Ni) in the dried *Tetrapleura tetraptera* fruits ranged from 0.32 to 1.36 mg/Kg, with a mean concentration of 0.71mg/kg. This value is higher than the mean Nickel concentration of 0.31 mg/kg recorded for *Tetrapleura tetraptera* by Nkansah and Amoako 2010, studying some heavy metals of common Ghanaian spices. Nickel is a compound that occurs in the environment only at very low levels. Foodstuffs naturally contain small amounts of nickel. Humans may be exposed to nickel by breathing air, drinking water, eating food or smoking. Small quantities of nickel is essential, but when the uptake is too high it can be a danger to human health. An uptake of too large quantities of nickel has the following consequences: Higher chances of development of lung cancer, nose cancer, larynx cancer and prostate cancer, sickness and dizziness after exposure to nickel

gas, lung embolism, respiratory failure, birth defects, asthma and chronic bronchitis, allergic reactions such as skin rashes, mainly from jewelry and heart disorders.

Zinc concentrations in the *Tetrapleura tetraptera* fruit samples ranged from 0.31-2.15 mg/Kg. Zinc is a metal that is considered an essential trace element meaning that it is needed for human health in very small amounts. It is naturally present in soil, rock, water and air, so most animals and plants contain zinc. It is necessary for protein synthesis, carbon dioxide transport, sexual function, insulin storage, carbohydrate metabolism and wound healing (MIT, 2005). The highest concentration is found in muscles (65%), in red and white blood cells, bone, skin, liver, kidneys, pancreas, eye retina, in the male prostate gland and sperm; it helps make cell membranes strong. (MIT, 2005). The RDA of zinc for male adolescents (14-18 years of age) and female adolescents (14-18 years of age) is 11 mg/day and 8 mg/day respectively. On the other hand, the RDA of male adults (19 years and older) and female adults (19 years and older) is 11mg/day and 8mg/day respectively. Zinc content in *Tetrapleura tetraptera* fruits from Nigeria was found to range between 5.35-25.16mg/Kg. Nkansah and Amoako 2010 recorded mean Zn concentrations of 0.692mg/Kg.

Flavonoids

Flavonoids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E. The antioxidant activity of flavonoids depends on their molecular structure. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. Flavonoids have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, anti-tumor and antioxidant activities (Buhler et al, 2000). The total flavonoids contents is shown in Table 3.

Table 3. Total Flavonoids Content

Total Flavonoid content in T. tetraptera from maj. markets	QE/g
Madina	83.713
Sefwi-Bekwai	43.593
Techiman	73.533
Sunyani	42.395
Kumasi	27.425
Dome	57.964
Winneba	19.042
Somanya	54.371

DPPH

DPPH is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the EPR signal of the DPPH (Alger, 1997). The results of the DPPH scavenging activity are displayed in table 4. DPPH scavenging activity at 1% ranged from 0.571-0.957.

Table 4. DPPH

DPPH	Absorbance	1%
Madina	0.184	0.819
Sefwi-Bekwai	0.298	0.708
Techiman	0.315	0.691
Sunyani	0.060	0.941
Kumasi	0.437	0.571
Dome	0.095	0.907
Winneba	0.385	0.623
Somanya	0.044	0.957

4. CONCLUSIONS

This paper documents the range of trace elements and some phytochemicals constituents in the dried fruits of *Tetrapleura tetraptera*. The study showed that Dried fruits contained varying concentrations of trace elements which were comparable to other studies reported in literature. The flavonoids

and free radical scavenging activity of extracts of the dried fruits of *Tetrapleura tetraptera* makes it a potential to be used a raw material in the food and drug sectors of the economy.

5. ACKNOWLEDGEMENTS

The authors are grateful to the Directors of National Nuclear Research Institute and Radiological and Medical Sciences Research Institute for making available the equipment for carrying out the research. We also acknowledge Juliet Osei for proofreading the final article and making useful suggestions.

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