



Determination of Tannin Concentrations in African Indigenous Vegetables, Grains and Cassava Roots from Emuhaya District, Western Kenya

Joseck Olukusi Alwala¹, Francis Ndilu Kiema¹, Wycliffe Wanzala^{2,*}

¹Department of Chemistry, Maseno University, P.O. Box 333-40105, Maseno, Kenya

²Department of Biological Sciences, School of Pure and Applied Sciences, South Eastern Kenya University, P.O. Box 170-9002, Kitui, Kenya

*Corresponding author (Email: Osundwal@yahoo.com)

Abstract - The study evaluated tannin concentrations in four vegetables, three grains and cassava roots as the most common foodstuffs in the diet of local communities from Emuhaya district in western Kenya and their nutritional health implication. Plant specimens were collected from local arable farms and market centres and their identities confirmed at Maseno University herbarium. Tannin content was evaluated using the International Pharmacopoeia Method. Percent composition of tannins in the sampled specimens were: 9.49 ± 0.6 for rattle pod (*Crotalaria brevidens* (Emiroo); Family, Fabaceae), 8.38 ± 0.3 for narrow-leaf bitter-pea (*Daviesia leptophylla* (Omurere); Family, Fabaceae), 2.49 ± 0.2 for amaranth (*Amaranthus hybridus* (Tsimboka); Family, Amaranthaceae), 0.42 ± 0.04 for black nightshade (*Solanum nigrum* (Lisutsa); Family, Solanaceae), 9.21 ± 0.4 for peanut/groundnut (*Arachis hypogaea* (Tsinjugu); Family, Fabaceae), 8.24 ± 0.4 for sesame (*Sesamum indicum* (Tsinuni); Family, Pedaliaceae), 8.11 ± 0.2 for finger millet (*Eleusine coracana* (Obulee); Family, Poaceae) and 6.99 ± 0.5 for cassava (*Manihot esculenta* (Emioko); Family, Euphorbiaceae). *Solanum nigrum* had significantly the lowest tannin composition profile followed by *A. hybridus* and *M. esculenta* in that order ($P < 0.05$). Interestingly, these three plants are preferred in the diet to the rest of the plants. The results provide scientific rationale for dietary exploitation of these plants by the local communities and may lay down some groundwork for exploiting partially refined products such as peanut butter and a wide range of grades of flour for human consumption and livestock feeds. However, consumption of tannins in the diet may sometimes be necessary for optimal health but caution needs to be taken for their conflicting adverse medical-based nutritional and physiological effects.

Keywords - Tannins, Composition profiles, Human diet and health, Food vegetables, Grains and cassava roots

1. Introduction

Tannins (also called tannic acid) are water soluble astringent, bitter polyphenolic compounds of high molecular weight found occurring naturally in the vacuoles or surface wax of quite a number of parts of many edible and non-edible plants. For instance, tannins have been found in plant parts such as barks, flowers, fruits, leaves, stems, spices, nuts etc. (Mole, 1993; Reed, 1995; Puupponen-Pimiä et al., 2001). Also, tannins are found in plant-based products such as chocolate, wines, nuts-almonds, acorns, hazelnuts, walnuts, areca nut, and pecans, peanut butter, chickpeas, fruit juices, ciders, beer, cheeses, some fermented foods, ice creams, condiments, cigarette smoke, caffeine-containing foods and beverages, smoked foods, sour cream, spices, herbs and herbal preparations, animal feed products etc) (Navia, 2002; Wolke & Parrish, 2005; Vattem et al., 2005). It is also found in medicinal nutgalls built by some insects on *Quercus infectoria* Olivier and several other *Quercus* spp. The

presence of tannins in plants and plant products is specifically for the purposes of defense against predation and growth regulation (Salunkhe et al., 1990; Ferrell & Thorington Jr., 2006; Jacobs, 2011). It has been shown that about 180 families of dicotyledons and 44 families of monocotyledons, widely distributed in different geographical regions, contain tannins with varying complexities and composition profiles (Fleck & Layne, 1990; Mole, 1993; Harold, 2004; Makkar et al., 2006). The main families of dicotyledons, which contain tannins include Aceraceae, Actinidiaceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae, Dipterocarpaceae, Ericaceae, Grossulariaceae and Myricaceae while those of monocotyledons include Najadaceae and Typhaceae.

Originally, the term "tannin" was used to describe substances in vegetable extracts used for converting animal skins into stable leather (also the term referred to the use of wood tannins from oak in tanning animal hides into leather; hence the words "tan" and "tanning" for the treatment of leather) (Schofield et al., 2001).

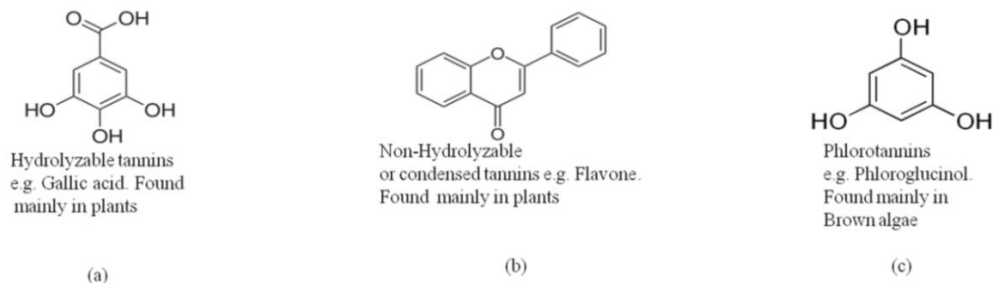


Figure 1. Structures and classes of tannins as described from Wikipedia, the free encyclopedia website. Permission is granted to copy and use under the terms of free license of the Wikimedia Commons (usually CC-BY, CC-BY-SA, GFDL or GPL/LGPL) or in the public domain. (http://commons.wikimedia.org/wiki/Commons:Reusing_content_outside_Wikimedia) Under the courtesy of Bryan Derksen, 17th February, 2007 (a), Neur Otiker, 8th April, 2008 (b), and Edgar 181, 30th August, 2007 (c), respectively.

These substances were later identified as polyphenolic compounds with varying molecular weights and complexities and having sufficient hydroxyls and other suitable groups like carboxyls for enhancement of formation of strong complexes with various macromolecules such as polysaccharides.

Tannins are yellowish to light brown amorphous masses that may be powdery, flaky, or spongy in nature. Chemical structures analysis has shown that tannins are majorly divided into hydrolysable and proanthocyanidins (condensed tannins) (Freudenberg, 1920; Muller, et al., 1992). Hydrolysable tannins have a central hydroxyl groups esterified to phenolic carboxylic acids with a carbohydrate at the center. If the esters contain gallic acid and ellagic acid, it is referred to as gallotannins while the combination with hexahydroxydiphenic acid is referred to as ellagitannins. Hydrolysable tannins react with acid, alkalis and enzymes to yield glucose, phenolic acid or polyhydroxyalcohols (Salunkhe, 1990). Condensed tannins are also referred to as flavolans or procyanidins and are mainly polymerized products of flavan-3-ol (catechin) and flavan-3,4-diol or a mixture of these (Figure 1) and mainly found in legume plants (such as dry bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum*), chickpea (*Cicer arietinum* L.), faba bean (syn. broad bean, field bean; *Vicia faba* L.), cowpea (*Vigna unguiculata* L.) and lentils (*Lens culinaris* L.)) (Strumeyer & Malin, 1975). Cereals containing polyphenolic compounds are barley and millet. Millet contains carbohydrate C-C linked to a flavonoid nucleus, which is resistant to hydrolysis (Reichert et al., 1980). The third category of tannins is the phlorotannins, which are also called phloroglucinols that are mainly found in the Brown algae (Figure 1).

The use of tannins is depended on their chemical structure and dosage (Ferrell and Thorington Jr., 2006; Harold, 2004). The property of tannins inhibiting predation is not clear albeit many plants employing tannins to deter animals exemplified by the fact that animals consuming excessive amounts of plants rich in tannins either fall ill or die (Ferrell & Thorington Jr., 2006). Similarly, humans usually find the bitter taste of foods containing high amounts of tannins, unpalatable (Harold, 2004). Although methods have been designed to

leach tannins from such plants in order to make them palatable, condensed tannins from plant tissue, which become complexed to a carbohydrate or protein matrix and become quite insoluble due to a high degree of polymerization (Deshpande, 1986), are difficult to extract for analysis.

Determination of beneficial factors in food crops such as tannin antioxidation and subsequent promotion of their nutritional and health values may enhance their market competitiveness, locally and internationally. For instance, in the Kenyan western region, where the studied plants (four vegetables, three grains and cassava roots) are produced for both subsistence and commercial economy, the interpretation of the results of their tannin contents may help improve the profitability at the local market and promote health. In the present study, we report the results obtained from the titrimetric analysis of tannin contents in 8 different food crops (*Crotalaria brevidens* Benth., *Daviesia leptophylla* A. Cunn. ex Don, *Amaranthus hybridus* L., *Solanum nigrum* L., *Arachis hypogaea* L., *Sesamum indicum* L., *Eleusine coracana* Gaertn. and *Manihot esculenta* Crantz.) grown and used in dietary foodstuffs on daily basis in Emuhaya district, western Kenya. The study was conducted to evaluate the composition profiles of tannins in commonly consumed four vegetables, three grains and cassava roots with a view to helping to lay down some groundwork for exploiting partially refined products emanating from these plants such as pea nut butter and a wide range of grades of flour for human consumption and livestock feeds. It is therefore hypothesized that the results from this study may be useful in value-addition and improving the quality and nutritional value of these food crops and thus promotes their production, marketability at local level and subsequent consumption for improved health.

2. Materials and methods

2.1. Study area and collection of study specimen plants

The area under study is Emuhaya district in Vihiga County, western Kenya. It is located at an elevation of 1,551 meters above sea level and covers an area of 173.2 square kilometers.

It is described by the coordinates, 0°1'60" N and 34°37'60" E. The district has reliable annual rainfall ranging from 1800 to 2000 mm. The rainfall is well distributed throughout the year with only two months of relatively dry spells (December through February). The two main rain seasons are the long rains (starting March through August) and the short rains (starting September through November). Temperatures range is between 14°C and 32°C with a mean temperature of 23°C.

The four vegetables (*Crotalaria brevidens* Benth., *Daviesia leptophylla* A. Cunn. ex Don., *Amaranthus hybridus* L. and *Solanum nigrum* L.), three grains (*Arachis hypogaea* L., *Sesamum indicum* L., *Eleusine coracana* Gaertn.) and cassava

roots (*Manihot esculenta* Crantz.) (Figure 2) were randomly collected from several farms and others from market places within Emuhaya district, western Kenya. The average sample consisted of representative amounts of three individual samples from any one given plot and/or shopping centre during market-day.

Under these varying environmental conditions therefore, it was also hypothesized that the four vegetables, three grains and cassava roots grown in Emuhaya district might have varying tannin composition profiles, hence having different medical-based nutritional and physiological impacts on consumers.

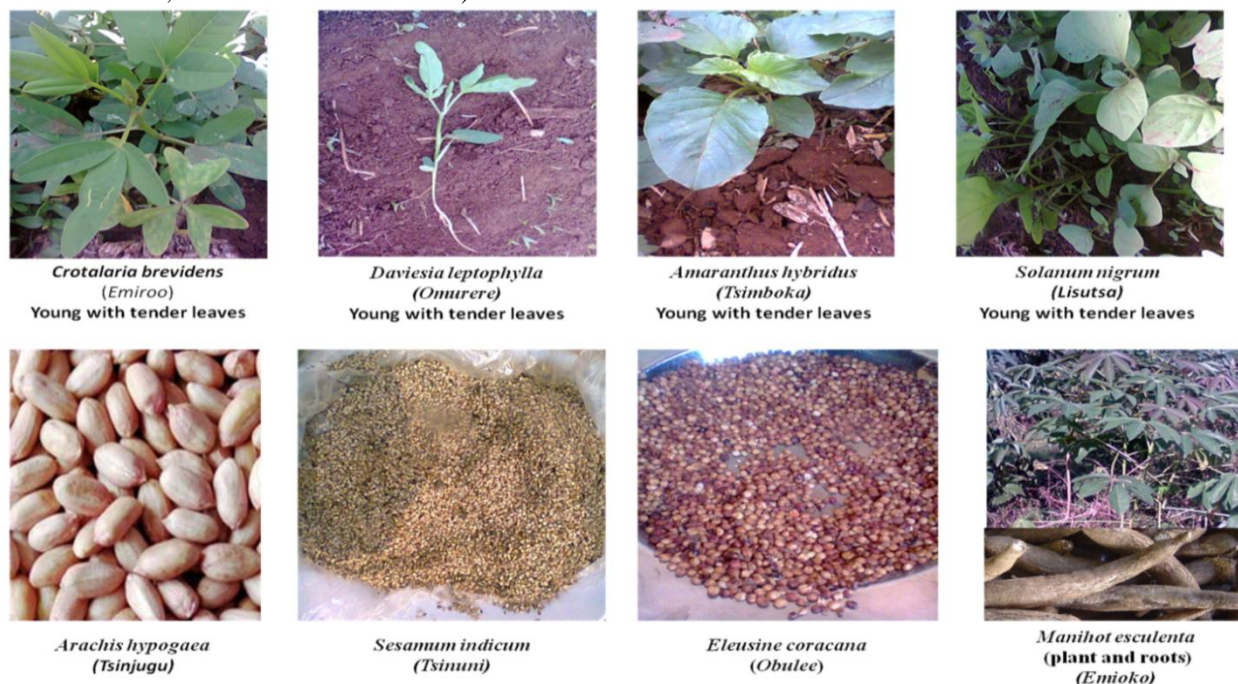


Figure 2. The four vegetables, three grains and cassava roots sampled from Emuhaya district, western Kenya for determination of tannin profiles. These eight crops are traditionally consumed frequently and in large amounts by the local communities, which comprise mainly the Luhya as the majority and Luo and other tribes as the minority. The samples were collected from identified sites during the long rains (starting March through August) when the temperatures are relatively lower than normal. Convincing reports from literature hypothesized that plant specimens harvested during the wet rain season at low temperatures (of below the mean temperature of 23°C in this case) yields high amounts of tannins (Luthar & Kreft, 1999).

2.2. Preparation

About 3g of studied vegetable product was extracted while still fresh with distilled deionized water (dd H₂O) into 250 ml volumetric flask for 4 hrs at room temperature and then the samples were filtered for analysis of tannin composition profiles. Dry cereals were grounded using pestle and mortar and extracted in a similar way.

2.3. Tannin assay

Due to the complexity of tannins, several methods have been developed for their quantification and unfortunately, none of them is completely satisfactory (Giner-Chavez, 1996). Nevertheless, major methods for tannin analysis can be classified into three groups: calorimetric methods, protein

binding methods and other methods. Calorimetric methods, which include: - Vanillin assay (Swain & Hillis, 1959), Folin Denis assay (Folin & Denis, 1912), Prussian blue assay (Price & Butler, 1977) and Acid butanol assay (Porter et al., 1986). Protein binding methods is based on the amount of tannins precipitated by a standard protein (Hagerman & Butler, 1989). Proteins such as gelatin, casein, bovine serum albumin, hemoglobin and different enzymes are used and each protein binding assay gives a specific result with tannins from different sources. In a different method, the tannin-protein complex is produced and determined (Martin & Martin, 1982). Other methods include: high-performance liquid chromatography (HPLC), mass spectral analysis, droplet countercurrent chromatography, centrifugal partition chromatography (Okuda et al., 1989) and expensive nuclear magnetic resonance (NMR) techniques.

Analysis was done unlike previously based on the ability of tannins in a candidate sample to precipitate proteins and various other organic compounds including amino acids and alkaloids (Mole, 1993). The analyses of tannins content were performed according to the International Pharmacopoeia (Muller & McAllan, 1992). The 25 ml of infusion were measured into 1 L conical flask, then 25 ml of indigo solution and 750 ml of distilled deionized water (dd H₂O) were added. The 0.1 N KMnO₄ was used for titration until the blue-coloured solution changed to green colour. Then, few drops were added at a time until the solution became golden yellow. Standard solution of indigo carmine was prepared as follows: 6g of indigo carmine was dissolved in 500 ml of dd H₂O by heating. After cooling to room temperature, 50 ml of 95-97% H₂SO₄ was added and diluted to one liter. Finally it was filtered. The blank test was carried by titrating a mixture of 25 ml indigo carmine solution and 750 ml of dd H₂O. All samples were analyzed in triplicate.

2.4. Calculations of tannin contents in samples

The percent tannins content (T %) was calculated using the following formula previously described by Atanassova and Christova-Bagdassarian (2009) :

$$T(\%) = \frac{(V - V_0) \times 0.004157 \times 250 \times 100}{g \times 25}$$

Where V is the volume of 0.1N aqueous solution of KMnO₄ for the titration of the sample in ml

V₀ is the volume of 0.1N aqueous solution of KMnO₄ for the titration of the blank sample in ml,

0.004157 is a constant of the tannin equivalent in 1 ml of 0.1N aqueous solution of KMnO₄,

g is the mass of sample taken for analysis in grams,

250 is a constant of the volume of volumetric flask in ml and

100 is a constant of the percent (%).

2.5. Statistical analysis

The determination of tannin contents were conducted in triplicate and results were expressed as mean ± standard error. Statistical analyses were done by one way ANOVA followed by Student–Newman–Keuls test at α =0.05 as level of significance for popular post hoc multiple comparison test of means.

3. Results and Discussion

The composition profiles of tannins in the four vegetables, three grains and cassava roots obtained from Emuhaya district, western Kenyan are shown in Tables 1 and 2. The plant species contained tannins in the range of 0.42 - 9.49% with the largest quantities of tannins (9.49±0.61%) being recorded in the freshly prepared dark green vegetable of *Crotalaria*

brevidens (Figure 2) while the least quantities of tannins (0.42±0.04%) being recorded in the bitter vegetables of *Solanum nigrum*. The dry grains consistently recorded a high level of tannin composition profiles that were not significantly different from one another at α = 0.05 level of significance (Table 2) and this may be due to the presence of condensed tannins, which have been previously recorded in similar dry legume grains (Salunkhe et al., 1990; Atanassova & Christova-Bagdassarian, 2009). While in fresh vegetables, the high level of quantities of tannins may have been probably partly due to environmental conditions of heavy rain season and relatively low temperatures under which the plant samples were harvested from arable farms as previously. These conditions were hypothesized to contribute to high yields of tannin biomolecules (Luthar & Kreft, 1999). The composition profiles of tannins in the plants were comparable to those obtained in other parts of the world such as Sri Lanka (Seresinhe & Pathirana, 2003), Thailand (Kanpukdee & Wanapat, 2007), Pakistan (Gorinstein et al., 2008), Bulgaria (Atanassova & Christova-Bagdassarian, 2009) and in the US (Harris et al., 1970). With regard to ruminant classification levels of safety of tannin consumption rate in feeds adopted by Seresinhe & Pathirana (2003), our composition profiles in the food crops mainly ranged between antinutritional (5–9%) (with four plants: *D. leptophylla*, *S. indicum*, *E. coracana* and *M. esculenta*) to toxic and lethal levels (> 9%) (with two plants: *C. brevidens* and *A. hypogaea*) and with one plant below (*S. nigrum*) and another one within (*A. hybridus*) the beneficial level of tannins (2–4%). Although these plants are mainly human food crops, they are fed to livestock on which human livelihood is directly dependent on their milk, meat, hides, organic fertilizers etc., hence the importance of this comparative analysis. In a study to analyse the levels of tannins in different types of plants in Bulgaria, levels of tannin in edible plants were reported to be significantly lower (ranged from 0.55% to 2.00%) than in non-edible plants (ranged from 8.94% to 93.44%) (Atanassova & Christova-Bagdassarian, 2009). Similarly, in this study, the most preferred plants (*S. nigrum*, *A. hybridus* and *M. esculenta*) by the local community had significantly lower tannin content than those not favoured in the diet. The pattern of tannin content in plants may help explain why a high level of tannin in food products may probably be detrimental to health of the consumer in a number of ways (Chan, 2003; Atoui et al., 2005; Anonymous, 2013) depending on the type of tannin consumed, nutritional and physiological state of the consumer and the source plant of the tannin.

Previous studies have shown varying composition profiles of tannin biomolecules in different plants and plant products worldwide and their corresponding medical-based nutritional values in diets and industrial applications (such as mining and leather industries) (Chung, et al., 1998; Torres et al., 1999; Fraga, 2009; Sakaguchia & Nakajimaa, 1987). Nevertheless, tannins have a lot of conflicts in life albeit having such important socio-economic and health-based values (Ames, 1983; Ascherio et al., 1992). Tannin biomolecules are

holistically very useful, enhancing livelihoods in societies worldwide and fascinating in many different ways in life but sometimes very dangerous molecules.

Table 1. The composition profiles of tannin in four vegetables (*Crotalaria brevidens*, *Daviesia leptophylla*, *Amaranthus hybridus*, *Solanum nigrum*) commonly consumed by local communities in Emuhaya district, western Kenya and the neighbourhood.

Species name of the vegetable consumed [English/common name] (local name(s) by tribe)	Family name [English/common family name]	%Tannin concentration (mean±SE)*
<i>Crotalaria brevidens</i> Benth.var. <i>intermedia</i> (Kotschy) Polhill. [Ethiopian rattlebox] (<i>Kamusuusuu</i> (Kamba), <i>Kipkururiet</i> (Kipsigis), <i>Emiroo</i> (Luhya), <i>Mitoo</i> (Luo), <i>Oleechei</i> (Maa))	Fabaceae [Pea family]	9.49±0.6 ^a
<i>Daviesia leptophylla</i> A.Cunn. ex Don.[Narrow-leaf Bitter-pea] <i>Omurere</i> (Luhya) <i>Anyim</i> (Luo)	Fabaceae [Pea family]	8.38±0.26 ^a
<i>Amaranthus hybridus</i> L. [Smooth amaranth, smooth pigweed, red amaranth, or slim amaranth] (<i>Tsimboka</i> (Luhya), <i>Omboga</i> (Luo))	Amaranthaceae [Amaranth family]	2.49±0.15 ^b
<i>Solanum nigrum</i> L. [Black nightshade] (<i>Isutsa</i> (Luhya), <i>Osuga</i> (Luo))	Solanaceae [Nightshade or Potato family]	0.42±0.04 ^c

*Within a column, means with the same superscript letter(s) are not significantly different at the level of significance, $\alpha = 0.05$ (Student–Newman–Keuls test).

Table 2. The composition profiles of tannin in three grains (*Arachis hypogaea*, *Sesamum indicum* and *Eleusine coracana*) and cassava roots (*Manihot esculenta*) commonly consumed by local communities in Emuhaya district, western Kenya and the neighbourhood.

Species name of the vegetable consumed [English/common name] (local name(s) by tribe)	Family name [English/common family name]	% Tannin concentration (mean±SE)*
<i>Arachis hypogaea</i> Linn. [Peanut, or Groundnut] (<i>Tsinjuku</i>) (Luhya) (<i>Njugu</i>) (Luo)	Fabaceae [Legume or bean family]	9.21±0.39 ^a
<i>Sesamum indicum</i> Linn. [Sesame] <i>Tsinuni</i> (Luhya) <i>Simsim</i> (or “nyim”) (Luo)	Pedaliaceae [Pedalium family or Sesame family]	8.24±0.37 ^a
<i>Eleusine coracana</i> Gaertn. [Finger millet] <i>Obulee</i> (Luhya) <i>Bel</i> (Luo)	Poaceae [Gramineae or true grasses]	8.11±0.20 ^a
<i>Manihot esculenta</i> Crantz. [Cassava] <i>Emioko</i> (Luhya) <i>Omwogo</i> or <i>marebwa magomba</i> or <i>mariep magomba</i> or <i>marewa magomba</i> depending on regional differences in dialects of sub-tribes(Luo)	Euphorbiaceae [Spurge family]	6.99±0.47 ^b

*Within a column, means with the same superscript letter(s) are not significantly different at the level of significance, $\alpha = 0.05$ (Student–Newman–Keuls test).

Historically used as a universal antidote in combination with activated charcoal and magnesium oxide, nowadays, tannin is used to treat cold sores and fever blisters, diaper rash and prickly heat, HFE hereditary hemochromatosis, poison ivy, ingrown toenails, sore throat, sore tonsils, spongy or receding gums and skin rashes, to stop bleeding and to protect against 6-hydroxydopamine-induced toxicity

(Chung et al., 1998; Nobre-Junior et al., 2008; Anonymous, 2013). Orally, tannin is administered for bleeding cases, chronic diarrhoea, dysentery, bloody urine, painful joints and persistent coughs (Anonymous, 2013). Tannins are also used as a douche for white or yellowish vaginal discharge (leukorrhoea) and commercially used in foods and beverages as a flavouring agent and in the manufacture of ointments

and suppositories, which are applied to treat hemorrhoids, tan hides, manufacture ink, resins, wood adhesives and anti-corrosive primers and kill dust mites on furniture (Torres et al., 1999; Anonymous, 2013). Besides, tannins have been known to cause acceleration of blood clotting process, reduction of blood pressure (cardiovascular disease and hypertension), decrease in the serum lipid level, modulation of immunoresponses etc. and being antioxidant and antimicrobial (against many fungi, yeasts, bacteria, protozoal parasites and viruses, hence important in food processing industry) and antimutagenic and anticarcinogenic (due to antioxidative property of tannins) (Morton, 1970; Chung et al., 1998; Akiyama et al., 2001; Lü et al., 2004; Atoui et al., 2005; Kolodziej et al., 2005; Souza et al., 2006; Nobre-Junior et al., 2008; Anonymous, 2013). Tannins too, detoxify reactive oxygen species (ROS) and prevent them from causing damage to cellular macromolecules and organelles through multi-mechanisms (Krinsky, 1989; Pool-Zobel et al., 1997; Han et al., 2007; Verma et al., 2011). Extracts from condensed tannins of some indigenous foods in Kenya have shown antidiabetic effects (Kunyanga, et al., 2011).

In view of the fact that about 80% of the world's population depends on traditional medicines, which are rich in tannins, for their healthcare needs (World Health Organization [WHO], 1996), it has been however shown that if care is not taken and the medicines are consumed in large amounts, they may cause liver damage, heart failure, kidney damage, skin damage and/or cause nose or throat cancer (Chan, 2003; Atoui et al., 2005; Anonymous, 2013). Tannins have also been identified with adverse nutritional and physiological effects such as carcinogen (causing esophageal cancer due to betel nuts and herbal teas), gastroenteritis, inhibition of gut bacteria, which contribute to digestion in the body and foods rich in tannins have been known to have low nutritional value among others (Chung et al., 1998). Polyphenols have been demonstrated to cause unique congenital malformations (Ross, 1998; Ross, 2000; Strick et al., 2000; Paolini et al., 2003; Spector et al., 2005; Barjesteh van Waalwijk van Doorn-Khosrovani et al., 2007; Zielinsky et al., 2012) and anemia (Matuschek & Svanberg, 2002) in humans.

It is therefore apparent that with these conflicting ideas regarding the use and application of tannins, thorough understanding of the composition profiles of tannins is required as they naturally occur in different plants, particularly those plants, which are frequently consumed in large amounts just like the four vegetables, three grains and cassava roots (*C. brevidens*, *D. leptophylla*, *A. hybridus*, *S. nigrum*, *A. hypogaea*, *S. indicum*, *E. coracana* and *M. esculenta*) under the current study from Emuhaya district, western Kenya. This understanding is essential so that the composition profiles are known and maintained in the dietary applications, particularly as is required in the diet of mostly plant-based products (chocolate, wines, nuts-almonds, acorns, hazelnuts, walnuts, areca nut, and pecans, peanut butter,

chickpeas, fruit juices, ciders, beer, cheeses, some fermented foods, ice creams, condiments, cigarette smoke, caffeine-containing foods and beverages, smoked foods, sour cream, spices, herbs and herbal preparations, animal feed products etc) or else tannin composition profiles may exceed the required limit and cause undesired adverse nutritional and physiological effects (Chung et al., 1998; Chan, 2003; Atoui et al., 2005; Anonymous, 2013). In the synthesized food products, the tannin concentration will therefore be modeled alongside that one(s) naturally found in plants for provision of optimal health in humans since the amount and type of tannins synthesized by plants varies considerably depending on plant species, cultivars, tissues, geographical location, stage of development and environmental conditions (Giner-Chavez, 1996). For instance, *A. hybridus* tannin composition profile found in our study in Kenya was $2.49 \pm 0.15\%$ compared to that of $0.087 \pm 0.04\%$ obtained from Pakistan (Gorinstein et al., 2008). While cassava hay (*M. esculenta*) in Thailand showed a tannin composition of 2.2% (Kanpukdee, 2007) compared with that evaluated in Kenya, $6.99 \pm 0.47\%$, probably the difference being the use of different cultivars and/or varieties. Determining percent composition of tannins in food products is important as moderate tannin levels of $< 4\%$ in forage legumes were found to cause relatively high growth rate and milk production from cows whereas those of $> 6\%$ affected growth rate and milk production (Giner-Chavez, 1996). In poultry industry, tannin levels of 3 – 7% caused death while those of 0.5 – 2% caused depression on growth rate and egg production from layers while tannin levels of $> 5\%$ were lethal in swine diet (Giner-Chavez, 1996). Such comparative analysis and quantification of tannins present in dietary foodstuffs will enhance a productive evaluation of their nutritional effects on consumers and thus help provide guidance on using these plants and their corresponding plant products in maintaining optimal health. This is particularly important as tannins have well-known health-promoting effects, which have been demonstrated in *in vitro* studies to include: improvements of the mental functions such as cognition, memory, intelligence, motivation, attention and concentration (Papandreou et al., 2009). However, how these effects holistically apply to the entire organism and their clinical outcome in human diseases in particular, remains a controversially discussed topic in nutrition science and disease prevention (Halliwell, 2007), particularly in the light of conflicting uses and applications of tannins. In a similar circumstance of controversy, a convincing hypothesis has been advanced to explain that a diet with a high concentration of flavonoids may play a medically useful role in the control and management of cardiovascular diseases (Habauzit & Morand, 2012). In addition to many unconfirmed health-related risks associated with the dietary tannins, there is need to conduct a worldwide research to evaluate composition profiles of all plants and plant products used as human food and animal feeds based on their plant species, cultivars, tissues, geographical location, stage of development and environmental conditions so that a health-based informed

decision can be made. But the ultimate effect of tannin in the consumer will largely depend on the type of tannin under consideration, nutritional and physiological state of the consumer, composition profile of tannin in the consumed product and source plant.

Although research on tannins has a long history, considerable additional research must be carried out before details of tannin chemistry are elucidated and the conflicting nutritional effects of tannins are made clear. Limited information is available on the chemical nature of polyphenols referred to as tannins in different types of foods and feeds commonly used throughout the world (Freudenberg & Weinges, 1962). Advanced techniques such as HPLC and nuclear magnetic resonance should provide better and new information on the biosynthesis and structure of tannins in plant materials. In general, research dealing with the relationship between the chemical nature of the tannins within and between different grains, vegetables and other plant species and their nutritional and physiological effects should be emphasized in human dietary habits.

4. Conclusions

The tannin content significantly varied amongst the four vegetables, three grains and cassava roots sampled from Emuhaya district, western Kenya. The study showed that the composition profile of tannin was relatively high in *C. brevidens*, *A. hypogaea*, *D. leptophylla*, *S. indicum*, *E. coracana* and *M. esculenta* and existed in moderate amounts in *A. hybridus* but in the lowest amounts in *S. nigrum*. The results however, provide scientific rationale for dietary exploitation of these plants by the local communities and may lay down some groundwork for exploiting partially refined products such as pea nut butter and a wide range of grades of flour for human consumption and livestock feeds. However, consumption of tannins in the diet may sometimes be necessary for optimal health but a lot of caution needs to be taken for their conflicting medically-based nutritional and physiological effects.

Acknowledgments

Authors wish to express their gratitude to the Department of Chemistry, Maseno University for administrative assistance, in particular to the Chief Technologist, Mr. Richard Chepkui. Sincere thanks also go to Mr. Daniel Buyela of the Department of Botany, Maseno University for confirming the identity of the plant specimens.

References

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*, *Journal of Antimicrobial Chemotherapy*, 48 (4), 487–491.

Ames, B. M. (1983). Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*, 221, 1256 – 1263.

Anonymous, (2013). Find a Vitamin or Supplement. Tannic Acid (Acide Tannique, Ácido Tánico). WebMD. Natural Medicines Comprehensive Database Consumer Version. <http://www.webmd.com/vitamins-supplements/ingredientmono-217-TANNIC%20ACID.aspx?activeIngredientId=217&activeIngredientName=TANNIC%20ACID>, as retrieved on Friday, August 16th, 2013, 12:57PM East Africa time.

Ascherio, A., Rimm, E. B., Giovanucci, E. L. Colditz, G. A., Rosner, B., & Willett, W. C. (1992). A prospective study of nutritional factors and hypertension among US men. *Circulation*, 86, 1475-1484.

Atanassova, M., & Christova-Bagdassarian, V. (2009). Determination of tannins content by titrimetric method for comparison of different plant species. *Journal of the University of Chemical Technology and Metallurgy*, 44 (4), 413-415.

Atoui, K., Mansouri, A., Bosku, G., & Kefalas, P. (2005). Tea and herbal infusions: their antioxidant activity and phenol profile. *Food Chemistry*, 89, 27 – 36.

Barjesteh van Waalwijk van Doorn-Khosrovani, S., Janssen, J., Maas, L. M., Godschalk, R. W., Nijhuis, J. G., & van Schooten, F. J. (2007, August). Dietary flavonoids induce MLL translocations in primary human CD34⁺ cells. *Carcinogenesis*, 28 (8), 1703–1709. doi:10.1093/carcin/bgm102.

Chan, K. (2003). Some aspects of toxic contaminants in herbal medicines. *Journal of Chemosphere*, 52, 1361–1371.

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: a review. *CRC Critical Reviews in Food Science and Nutrition*, 38 (6), 421-464.

Deshpande, S., Cheryan, M., & Salunkhe, D. K. (1986). Tannin analysis of food products. *CRC Critical Reviews in Food Science and Nutrition*, 24 (4), 401-449.

Ferrell, K. E., & Thorington Jr., W. R. (2006). *Squirrels: the animal answer guide*. Baltimore, MD: Johns Hopkins University Press. ISBN 0-8018-8402-0, pp. 91.

Fleck, D. C., & Layne, J. N. (1990, October). Variation in tannin activity of acorns of seven species of central florida oaks. *Journal of Chemical Ecology*, 16 (10), 2925-2934.

Folin, O., & Denis, W. (1912). On phosphotungstic-phosphomolybdic compounds as color reagents. *Journal of Biological Chemistry*, 12, 239-245.

Fraga, C. G. (editor), (2009). *Plant Phenolics and Human Health: Biochemistry, Nutrition and Pharmacology*. ISBN 9780470287217, (ISBN-13: 978-0470287217), The Wiley-IUBMB Series on Biochemistry and Molecular Biology (Book 1), pp. 593.

Freudenberg, K. (1920). *Die Chemie der Natürlichen Gerbstoffe*. Berlin: Springer Verlag.

Freudenberg, K., & Weinges, K. (1962). *Catechins and flavonoid tannins*. In: *The Chemistry of Flavonoid Compounds*, pp. 197-216, Geissman, T. A., editor, Oxford, Pergamon Press.

Giner-Chavez, B. I. (1996). *Condensed tannins in tropical forages*. PhD Thesis, Cornell University, Ithaca, NY, USA.

Gorinstein, S., Lojek, A., Ciz, M., Pawelzik, E., Delgado-Licon, E., Medina, O. J., Moreno, M., Salas, I. A., & Goshev, I. (2008). Comparison of composition and antioxidant capacity of some cereals and pseudocereals. *International Journal Food Science and Technology*, 43, 629–637.

Habauzit, V., & Morand, C. (2012). Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: an update for clinicians. *Therapeutic Advances in Chronic Disease*, 3 (2), 87-106.

Hagerman, A. E., & Butler, L. G. (1989). Choosing appropriate methods and standards for assaying tannin. *Journal of Chemical Ecology*, 15, 1795-1810. doi:10.1007/BF01012267.

Halliwel, B. (2007). Dietary polyphenols: Good, bad, or indifferent for your health? *Cardiovascular Research*, 73 (2), 341–347.

Han, X., Shen, T., & Lou, H. (2007). Dietary Polyphenols and their biological significance. *International Journal of Molecular Science*, 8, 950 – 988.

Harold, M. (2004). *On food and cooking: the science and lore of the kitchen*. New York: Scribner. ISBN 0-684-80001-2, pp. 714.

Harris, H. B., Cummins, D. G., & Burns, R. E. (1970). Tannin content and digestibility of sorghum grain as influenced by bagging. *Agronomy Journal*, 62 (5), 633-635. doi:10.2134/agronj1970.00021962006200050025x.

Jacobs, J. (2011). What Foods Have Tannic Acid? <http://www.livestrong.com/article/433531-what-foods-have-tannic-acid>

- d/., as retrieved on Saturday, August 10th 2013, 5:16PM East Africa time.
- Kanpukdee, S., & Wanapat, M. (2007). *Effects of mangosteen (Garcinia mangostana) peel and sunflower and coconut oil supplementation on rumen fermentation, milk yield and milk composition in lactating dairy cows*. MSc Thesis, MEKARN-SLU <http://www.mekarn.org/MSC2005-07/thesis07/such1.htm>, as retrieved on Friday, August 16th 2013, 2:26 PM East Africa time.
- Kolodziej, H., & Kiderlen, A. F. (2005). Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. *Phytochemistry*, 66 (17), 2056–2071.
- Krinsky, N. I. (1989). Antioxidant functions of carotenoids. *Free Radical Biology and Medicine*, 7, 617–635.
- Kunyanga, C. N., Imungi, J. K., Okoth, M., Momanyi, C., Biesalski, H. K., & Vadivel, V. (2011). Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. *Journal of Food Science*, 76 (4), C560-7.
- Lü, L., Liu, S. W., Jiang, S. B., & Wu, S. G. (2004). Tannin inhibits HIV-1 entry by targeting gp41. *Acta pharmacologica Sinica*, 25 (2), 213–218.
- Luthar, Z., & Krefit, I. (1999). Influence of temperature on tannin content in different ripening phases of buckwheat (*Fagopyrum esculentum* Moench.) seeds. *Fagopyrum*, 16, 61–65.
- Makkar, H. P. S., Dawra, R. K., & Singh, B. (2006, September, 17). Tannin levels in leaves of some oak species at different stages of maturity. *Journal of the Science of Food and Agriculture*, 54 (4), 513–519. doi:10.1002/jsfa.2740540403.
- Martin, J. S., & Martin, M. M. (1982). Tannin assays in ecological studies: lack of correlation between phenolics, proanthocyanidins and protein-precipitating constituents on mature foliage of six oak leaves. *Oecologia*, 54, 205–211.
- Matuschek, E., & Svanberg, U. (2002). Oxidation of polyphenols and the effect on *in vitro* Iron accessibility in a Model Food System. *Journal of Food Science*, 67, 420–424.
- Mole, S. (1993). The Systematic Distribution of Tannins in the Leaves of Angiosperms: A Tool for Ecological Studies. *Biochemical Systematics and Ecology*, 21 (8), 833–846.
- Morton, J. F. (1970). Tentative correlations of plant usage and esophageal cancer zones. *Economic Botany*, 24, 217–226.
- Muller, H., & McAllan, A. B. (1992). Tannins: Their biochemistry and nutritional properties. *Advances in plant cell biochemistry and biotechnology*, 1, 151–217.
- Navia, J. (2002). Could Tannins Explain Classic Migraine Triggers? [Online], <http://www.widomaker.com/~jnavia/tannins/tannexpl.htm>, as retrieved on Friday, August 16th 2013, 3:36 PM East Africa time.
- Nobre-Junior, H. V., Maia, F. D. de Oliveira, R. A., Bandeira, M. A. M. Pessoa, C. do Ó., Moraes, M. O. Cunha, G. M. A., & Viana, G. S. B. (2008). Neuroprotective actions of Tannins from *Myracrodruon urundeuva* on 6-Hydroxydopamine-induced Neuronal Cell Death. *Journal of Herbs Spices and Medicinal Plants*, 13 (2), 41.
- Okuda, T., Yoshida, T., & Hatano, T. (1989). New methods of analyzing tannins. *Journal of Natural Products*, 52, 1–31.
- Paolini, M., Sapone, A., & Valgimigli, L. (2003). Avoidance of bioflavonoid supplements during pregnancy: a pathway to infant leukemia? *Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis*, 527 (1–2), 99–101.
- Papandreou, M. A., Dimakopoulou, A., Linardaki, Z. I., Cordopatis, P., Klimis-Zacas, D., Margariti, M., & Lamari, F. N. (2009). Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. *Behavioural Brain Research*, 198 (2), 352–358.
- Pool-Zobel, B. L., Bub, A., Liegbei, U. M., Treptow-Van, L. S., & Rechkemmer, G. (1997). Mechanisms by which vegetables consumption reduces genetic damage in humans. *Cancer Epidemiology Biomarkers and Prevention*, 7, 891–899.
- Porter, L. J., Hrstich, L. N., & Chan, B. G. (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25, 223–230.
- Price, M. L., & Butler, L. G. (1977). Rapid visual estimation and spectrophotometric determination of tannin content in sorghum grain. *Journal of Agricultural and Food Chemistry*, 25, 1268–1273.
- Puupponen-Pimiä R., Nohynek, L., Meier, C., Kähkönen, M., Heinonen, M., Hopia, A., & Oksman-Caldentey, K. M. (2001, May). Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology*, 90 (4), 494–507. doi:10.1046/j.1365-2672.2001.01271.x
- Reed, J. D. (1995, May). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science*, 73 (5), 1516–1528.
- Reichert, R. D., Fleming, S. E., & Schwab, D. J. (1980). Tannin deactivation and nutritional improvement of sorghum by anaerobic storage of H₂O, HCl, or NaOH- treated grain. *Journal of Agricultural and Food Chemistry*, 28, 824–829.
- Ross, J. A. (1998). Maternal diet and infant leukemia: a role for DNA topoisomerase II inhibitors? *International Journal of Cancer – Supplement*, 11, 26–28.
- Ross, J. A. (2000). Dietary flavonoids and the MLL gene: A pathway to infant leukemia? *Proceedings of the National Academy of Sciences USA*, 97 (9), 4411–4413.
- Sakaguchia, T., & Nakajima, A. (1987). Recovery of Uranium from seawater by immobilized Tannin. *Separation Science and Technology*, 22 (6), 1609–1623.
- Salunkhe, D. K., Chavan, K. S., & Kadam, S. (1990). *Dietary Tannins: Consequences and Remedies*. Boca Raton, FL: CRC Press.
- Schofield, P., Mbugua, D. M., & Pell, A. N. (2001). Analysis of condensed tannins: a review. *Animal Feed Science and Technology*, 91, 21–40.
- Seresinhe, T., & Pathirana, K. K. (2003). Forage tannins in ruminant nutrition. *Tropical Agricultural Research and Extension*, 6, 29–43.
- Souza, S. M. C., Aquino, L. C., Milach Jr, A. C., Bandeira, M. A., Nobre, M. E., & Viana, G. S. (2006). Antiinflammatory and antiulcer properties of tannins from *Myracrodruon urundeuva* Allemão (Anacardiaceae) in rodents. *Phytotherapy Research*, 21 (3), 220–225.
- Spector, L. G., Xie, Y., Robison, L. L., Heerema N. A., Hilden, J. M., Lange, B., Felix, C. A., & Davies, S. M. (2005). Maternal diet and infant Leukemia: The DNA Topoisomerase II inhibitor Hypothesis: A report from the children's Oncology Group. *Cancer Epidemiology Biomarkers and Prevention*, 14 (3), 651–655.
- Strick, R., Strissel, P. L., Borgers, S., Smith, S. L., & Rowley, J. D. (2000). From the Cover: Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. *Proceedings of the National Academy of Sciences USA* 97 (9), 4790–4795.
- Strumeyer, D. H., & Malin, M. J. (1975, January). Condensed tannins in grain sorghum: isolation, fractionation and characterization. *Journal of Agricultural and Food Chemistry*, 23 (5), 909–914. doi: 10.1021/jf60201a019.
- Swain, T. & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. 1. The quantitative analysis of phenolic constituents. *Journal of Agricultural and Food Chemistry*, 10, 63–68.
- Torres, J., Olivares, S., De La Rosa, D., Lima, L., Martínez, F., Munita, C. S., & Favaro, D. I. T. (1999). Removal of mercury (II) and methyl mercury from solution by tannin adsorbents. *Journal of Radioanalytical and Nuclear Chemistry*, 240 (1), 361–365.
- Vattem, D. A., Ghaedian, R., & Shetty, K. (2005). Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. *Asia Pacific Journal of Clinical Nutrition*, 14 (2), 120–130.
- Verma, P. K., Raina, R., Singh, S. P. & Sultana, M. (2011). Oxidative stress: Pharmacology of Vitamin E. *Journal of Veterinary Pharmacology and Toxicology*, 10, 1–7.
- WHO, (1996). World Health Organization, *The World Health Report, 1996: Fighting Diseases, Fostering Development*, Geneva.
- Wolke, R. L. & Parrish, M. (2005). *What Einstein told his cook 2: the sequel: further adventures in kitchen science*. Vol. 2. W. W. Norton & Company. ISBN978-0-393-05869-7, pp. 384.
- Zielinsky, P., Piccoli, A. L., Manica, J. L. L., Nicoloso, L. H., Vian, I., Bender, L., Pizzato, P., Pizzato, M., Swarowsky, F., Barbisan, C., Mello, A., & Garcia, S. C. (2012). Reversal of fetal ductal constriction after maternal restriction of polyphenol-rich foods: an open clinical trial. *Journal of Perinatology*, 32, 574–579.