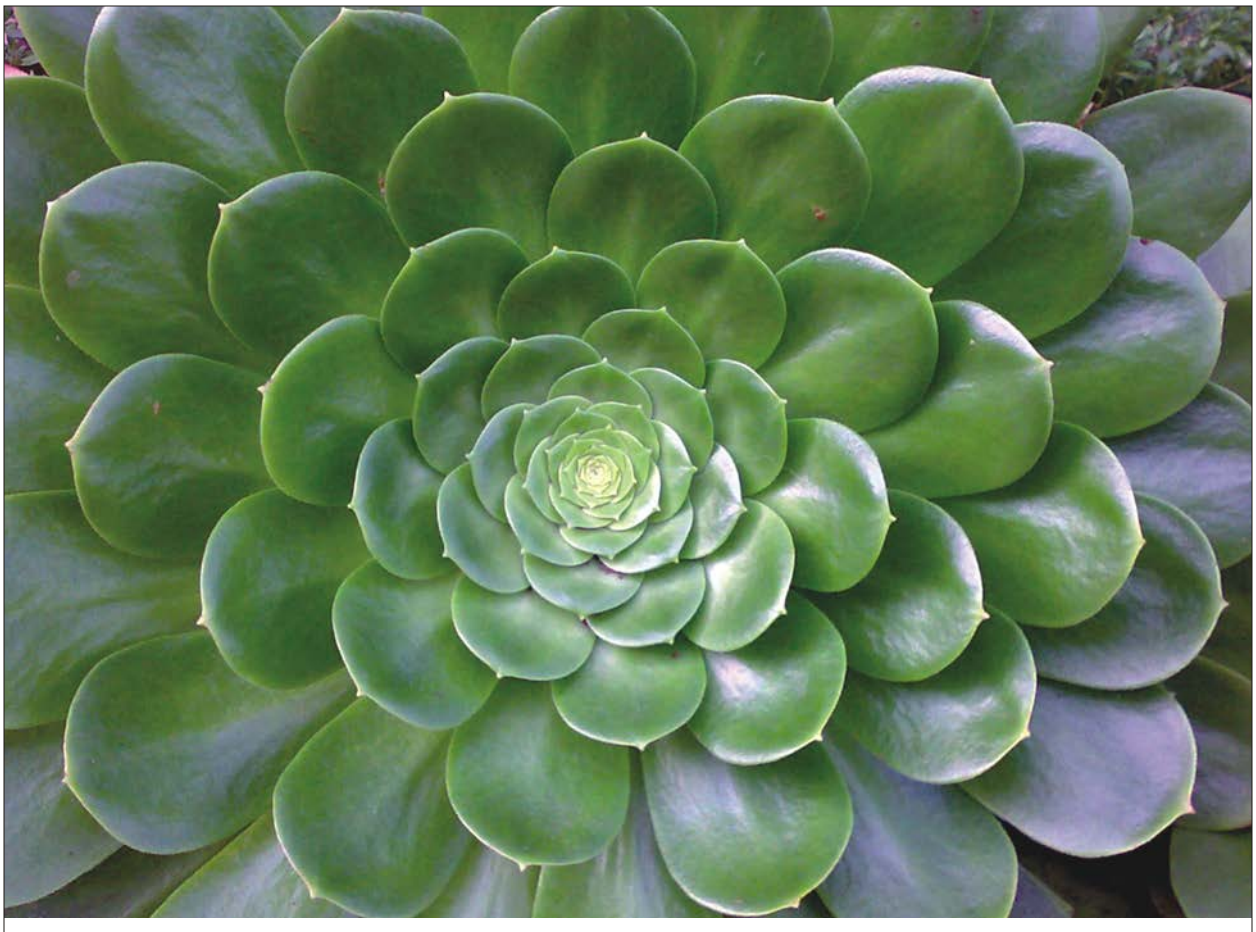

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Tannin Contents of some Economic Plants in Nigeria

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ABSTRACT

The study was carried out to determine the tannin content of five economic plants indigenous to Nigeria. These plants belongs to the families Poaceae (Maize), Fabaceae (Cowpea, Groundnut), Euphorbiaceae (Cassava) and Malvaceae (Okra). The study showed that the percentage tannin content of these plants decreased from week one to week four after planting from 5.20-4.80, 5.40-3.50, 10.20-4.00, 6.90-4.30, 6.30-3.70 for Maize, Cowpea, Groundnut, Cassava and Okra, respectively. There was significant difference in the weekly estimation of tannin content ($F_{3,12}$ = 7.873, p = 0.0036) and between different economic plants examined ($F_{4,12}$ = 3.908, p = 0.0295) at 95% confidence level. The tannin content of the plants altered the nutrient dynamics of the soil; this was observed in the increased mean values recorded for organic matter, organic carbon and sulphate content of the soil within the pH range of 5.95-6.65 from week one to week four after planting.

Key words: Tannin content, economic plants, organic matter and carbon, soil sulphate

INTRODUCTION

Economic plants vary from one region to another and they serve different purposes. Maize (*Zea mays* L.), Cowpea (*Vigna unguiculata* (L.) Walp), Groundnut (*Arachis hypogea* L.), Cassava (*Manihot esculentus* Crantz) and Okra (*Abelmoschus esculentus* L.) are some of the economic plants grown in Nigeria. These plants belongs to different families and genus. They are sources of food for humans, fodder for animals and revenue for the nation. The production of these economic plants has added to the Nation's Gross Domestic Profit (GDP). According to Udoh *et al.* (2005), maize belongs to the family Poaceae and is utilized worldwide for both industrial and food purposes. Cowpea and groundnut are legumes and belongs to the family Fabaceae. Deshpande (1992) reported that legumes play an important role in human nutrition since these are rich sources of protein, calories, certain minerals and vitamins. Cassava belongs to the family Euphorbiaceae. Generally, traditional carbohydrate foods, such as; cassava plays an important role in African diet. However, cassava has been reported to have low protein and anti-nutrients contents (Hahn, 1992; Oboh *et al*., 2002). According to Polson and Spencer (1991), Nigeria alone, produces over 14 m tonnes annually, representing about 25% of sub-saharan Africa's output. Although, it is the third most important food source in the tropical world after rice and maize and provides calories for over 160 m people in Africa. The cyanogenic glucosides present in cassava has reduced its food value to great extent. Okra is widely distributed in Africa and belongs to the family Malvaceae (Doreddula *et al*., 2014). It is commonly used both as food as salad fresh or cooked and for curative purposes, showing low calories, a good source of edible fibre, contains important bioactive compounds (Roy *et al*., 2014). Okra is one of the most consumed vegetable in the countries of Africa

and Asia (Camciuc *et al*., 1998; Avallone *et al*., 2008; Sengkhamparn *et al*., 2010). World production of fruits is estimated at about four million tons with India heading (3.5 million tons) with about 15% of the total vegetable production. The 85% of the production comes from developing countries (Grubben, 1977). In West Africa, Nigeria is the largest producer (1,039.000 t) followed by the Ivory Coast and Ghana.

Secondary metabolites are the compounds that do not affect the normal growth and development of a plant but reduce the palatability of the plant tissues in which they are produced (Howe and Jander, 2008). Morant *et al.* (2008) reported that defensive (secondary) metabolites can be either constitutively stored as inactive forms or induced in response to the insect or microbe attack. The former are known as phytoanticipins and the latter as phytoalexins. According to War *et al.* (2012), the secondary metabolites not only defend the plants from different stresses but also increase the fitness of the plants. Tannin, saponin, oxalate, cyanogenic glycosides are examples of plant secondary metabolites. Studies have shown that phenols are found in 100% of a group of medicinal plants in the Caatinga (De Almeida *et al*., 2005; Alencar *et al*., 2009). Active substances that have phenolic groups in their structure have great pharmacological potential (Siqueira *et al*., 2012) with many natural products leading to the development of clinically beneficial drugs (Shu, 1998). Phenols act as a defensive mechanism not only against herbivores but also against microorganisms and competing plants. Tannins are complex polyphenolic compounds with great structural diversity. They have a variable effect in decreasing digestibility of proteins. The diverse effects of tannins on digestion is due to differences in the physiological capabilities of animals to handle them as well as differences in the chemical reactivity of various types of tannins (Aganga *et al*., 1997). According to Atanassova and Christova-Bagdassarian (2009), tannin represent a wide variety of compounds that can be found in fruits, vegetables, dry extract of red wine, dry extract of grape seeds, tea and dry not edible plants. Tannins are also known as proanthocyanidins possessing useful properties such as antioxidant, anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory as well as anti-atherosclerosis and cardiovascular protection. Tannins are the most abundant antioxidants in human diet (Han *et al*., 2007). In addition, tannins also chelate the metal ions, thereby reducing their bioavailability to herbivores. When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. The role of tannins in plant defense against various stresses and their induction in response to insect damage has been studied in many plants (Barbehenn and Constabel, 2011). Constabel (2013) reported that tannins can have ecosystem-level impacts by inhibiting microbial activities and altering nutrient dynamics in soil. Studies have shown that high consumption of tannin is dangerous to health, being a phenolic secondary plant metabolite with one or more hydroxyl substitutes bonded to aromatic ring, it produces anthrocyanides, another toxic product on acid degradation (Getachew *et al*., 2000; Waterman and Mole, 1994). Also, another danger of consumption of high concentration of tannins is that it is not normally extracted either with solvents or detergents thus tannin-protein complexes cannot easily be broken down or digested (Perez-Maldonado and Norton, 1996; Mahato *et al*., 1982).

We deem it necessary to estimate the levels of tannin in five different economic plants, indigenous to Nigeria and some mineral components of the soil under which these plants are grown.

MATERIALS AND METHODS

Seeds of Maize, Cowpea, Groundnut and Okra were procured from School-to-land farm at Iriebe, Rivers State while early maturing Cassava stem cuttings were obtained from National Root

Crop Research Institute Umudike Abia State. Planting bags (40×50 cm) were used, leaving 7 cm at the upper end for irrigation of water. The bags were filled with top soil (0-15 cm) from the University of Port Harcourt Botanical Garden. The planting bags were perforated to avoid water logging. The seeds of the plants were sown on the same day, while the stem cuttings were planted in a slanted manner. The tannin content and number of leaves of the plants were taken weekly. The sulphate content and pH of the soil was monitored weekly while the organic matter and organic carbon content were monitored before planting and four Weeks After Planting (4 WAP).

Determination of tannin (AOAC, 1980): The percentage composition of tannin in the plants was determined using the methods of AOAC (1980) with some modifications. Folin-Denis reagent and saturated sodium carbonate were prepared in accordance with the procedure to analyze the tannin content. Standard solution of tannic acid was freshly prepared by dissolving 10 mg of tannic acid in 100 mL water. A series of tannic; E3 J. Biotechnol. Pharm. Res. 44 acid standards were prepared in the range of 0-2.5 mL, aliquots in 25 mL volumetric flasks, then added to 1.25 mL Folin-Denis reagent and 2.5 mL sodium carbonate solution. The mixture was made up to the volume and the colour was measured after 30 min at 760 nm using a spectrophotometer (Perkin Elmer). The samples were prepared by boiling 1 g of their dried powder in 80 mL of water for 30 min. The samples were cooled, transferred into a 100 mL volumetric flask and diluted to mark. The solution was filtered to get a clear filtrate and analyzed as in the standard. Tannin content was determined by a tannic acid standard curve and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g of dried sample.

$$\text{Soluble tannins (\%)} = \frac{\text{C (mg) extract volume (mL)}}{10 \times \text{aliquot (mL)} \times \text{sample wt (g)}}$$

Determination of sulphate by spectrophotometer: Standard solution of sulphate was freshly prepared by dissolving 0.1479 g anhydrous Na_2SO_4 (Sodium Sulphate) in distilled water and diluted to 1000 mL. Buffer solution A was prepared by dissolving 30 g Magnesium Chloride, $MgCl_2.6H_2O$, 5 g Sodium acetate, $CH_3COONa.3H_2O$, 1.0 g Potassium Nitrate KNO_3 and 20 mL Acetic acid CH_3COOH 99% in 500 mL distilled water and made up to 1000 mL while buffer solution B was prepared by dissolving 30 g $MgCl_2.6H_2O$, 5 g $CH_3COONa.3H_2O$, 0.111 g Sodium Sulphate and 20 mL acetic acid in 500 mL distilled water and made up to 1000 mL. Twenty milliliter of the buffer solution was added to 100 mL of sample in a clean 250 mL beaker and the solution was stirred using electromagnetic stirrer. Thereafter, a spoonful of Barium Chloride crystals (20-30 mesh) was added and stirred for 60 sec. The turbidity formed was measured at 420 nm using spectrophotometer. Sulphate standard concentration from 0.01-0.1 mg mL^{-1} were prepared and treated as, unto the samples and the turbidity formed were measured at 420 nm using distilled water reagents as, blank.

$$SO_4^{2-} = \text{Absorbance of sample} \times \text{Gradient of the standard graph}$$

Determination of pH: About 5 g of the air-dry soil was taken in a glass beaker and 10 mL of distilled water was added. The contents were mixed thoroughly with a glass rod and allowed to stand for 30 min. The soil pH was measured using EQUIP-TRONICS Digital pH meter model EQ-610.

Determination of total organic carbon: Two gram of soil was taken in a 500 mL Erlenmeyer flask, 10 mL of 1 M K_2CrO_7 was added and the flask was swirled to mix the contents. Twenty mL of conc. The H_2SO_4 was added to the soil suspension, flask was swirled again for 1 min and allowed to stand for 30 min. After this, 200 mL of water, 10 mL of H_3PO_4 and 1 mL of diphenylamine indicator were added and the contents were titrated against 0.5 M $FeSO_4.7H_2O$ until the colour changed from blue to red. The organic matter was obtained by multiplying total organic carbon values by a conversion factor of 1.27 (AOAC., 1990).

Analysis of data: The data collected were subjected to analysis of variance (ANOVA) and means compared using Least Significant Difference (LSD) at 5% level of probability.

RESULTS

Plant tannin content: The tannin content of the evaluated plants are presented in Table 1. The results showed that the tannin contents decreased from week 1 to week 4 for all the plants evaluated. Groundnut and Maize had the highest tannin content in week one and week four, respectively, while, Maize and Cowpea gave the least tannin content in week one and week four, respectively. There was significant difference in the weekly estimation of tannin content ($F_{3,12}$ = 7.873, p = 0.0036) and between different economic plants examined ($F_{4,12}$ = 3.908, p = 0.0295).

Soil sulphate content: Table 2 shows the sulphate content of the soil under which the different plants thrived. The results showed that the sulphate contents of the soil where Maize, Cowpea, Groundnut and Okra increased from week one to week four respectively, while, that of cassava decreased from week one to week four. However, the soil with Okra had the highest sulphate content four weeks after planting, while, the soil with Maize and Cassava had the least sulphate content. The values of the soil sulphate content obtained weekly were not significantly different ($F_{3,12}$ = 0.167, p = 0.916) and no significant effect was also obtained between different plants examined ($F_{4,12}$ = 1.001, p = 0.444).

Soil organic matter and organic carbon: The results of the organic matter and organic carbon of the soil under, which the different plants thrived for four weeks are shown in Table 3. The

Table 1: Tannin content (%) of plants four weeks after planting

Weeks	Maize	Cowpea	Groundnuts	Okra	Cassava
1	5.200	5.400	10.200	6.900	6.300
2	5.000	3.900	6.300	5.900	5.500
3	4.900	3.700	5.900	5.100	4.500
4	4.800	3.500	4.000	4.300	3.700
SE	0.090	0.430	1.300	0.560	0.570
SD	0.171	0.865	2.601	1.112	1.137

SE: Standard error, SD: Standard deviation

Table 2: Sulphate content of the soil for four weeks

Weeks	Maize	Cowpea	Groundnuts	Cassava	Okra
1	0.0960	0.0810	0.2210	1.7930	0.1430
2	0.1830	0.1230	0.7500	1.3720	0.2320
3	0.1920	0.7130	0.7890	0.2270	1.1230
4	0.1920	0.9310	0.9610	0.1920	1.2320
SE	0.0233	0.2127	0.1598	0.4056	0.2872
SD	0.0467	0.4255	0.3196	0.8112	0.5745

SE: Standard error, SD: Standard deviation

Table 3: Organic matter and organic carbon content of the soil

Parameters	Maize	Cowpea	Groundnuts	Cassava	Okra
OM					
B4P	6.100±0.153	6.10±0.1530	6.100±0.153	6.100±0.153	6.100±0.153
4WAP	6.900±0.087	7.07±0.0290	7.300±0.029	7.500±0.012	7.900±0.173
OC					
B4P	4.800±0.120	4.80±0.1200	4.800±0.120	4.800±0.120	4.800±0.120
4WAP	5.433±0.068	6.063±0.023	5.748±0.023	5.906±0.009	6.220±0.136

Mean±SE, B4P: Before planting, 4WAP: Four weeks after planting, OM: Organic matter, OC: Organic carbon

analysis of variance showed that the organic matter and organic carbon gave the same trend of result since they are interrelated by a factor. There were significant differences in the organic matter and organic carbon of the soil containing the different economic plants four Weeks After Planting (4 WAP). The analysis of variance showed that there were significant difference in the weeks, different plants and the overall interaction respectively ($F_{1,20} = 296.47$, $p = 1.84\times10^{-13}$, $F_{4,20} = 4.745$, $p = 0.0074$, $F_{4,20} = 4.745$, $p = 0.0074$).

DISCUSSION

Tannin content (6.90-4.30%) in fresh okra leaves obtained was higher than that reported in literature, of 1.2% in fresh okra leaves (Singh *et al*., 2001) and 0.44±0.02% (Caluete *et al*., 2015). The values for cassava tannin content obtained were higher than the range of tannin content for cassava tubers and products which is 0.4±0.0 and 0.1±0.0%, respectively. Moreover, the tannin content values recorded in this study disagree with other reports of cassava flour (Oboh and Elusiyan, 2007), which is 0.4±0.0 for raw cassava flour sample and 0.2±0.0% for fermented cassava flour sample. The tannin content of plants vary from one plant to another. This was supported by the work of Gonzalez-Hernandez *et al*. (2003), which reported that tannin concentrations differ significantly between plant species. The concentration of tannins in plants is not only species-specific but also depends on soil fertility (Keinanen *et al*., 1999) and soil pH (Nicolai, 1988; Northup *et al*., 1995). The amount of variation in number of leaves and tannin content among individuals of plants growing under similar conditions was sufficient to cause significant difference. Coley (1986) reported that variation in tannin content could be due to genetic differences among plants. This was also supported by the work of Verzele *et al*. (1986), which suggested that tannins present in some plants may have different molecular weight. Their tendency to interact with proteins also differs, such that; those with higher molecular weight have more interactions making them less available. Asquith and Butler (1986) earlier reported that protein-tannin interactions are both protein dependent and tannin dependent.

The tannin content of the plants altered the nutrient composition of the soil, this was observed in the increased recorded for organic matter, organic carbon and sulphate content of the soil within the pH range of 5.95-6.65. This result agrees with the work of Halvorson *et al*. (2009). Many studies have shown that high concentration of tannins can be found in plants living in conditions of low soil fertility and low pH (McKey *et al*., 1978; Northup *et al*., 1998). This observation agrees with the report of Constabel (2013), which suggested that tannins can have ecosystem-level impacts by inhibiting microbial activities and altering nutrient dynamics in soil. Also, Aerts and Chapin (1999) reported that plants may influence soil processes through the input to the soil compounds such as tannins. However, reactions between some tannins and soil organic matter might rapidly decrease the solubility of labile soil C and interact with soil N (Halvorson *et al*., 2009).

CONCLUSION

This study shows that different plants contains varying amount of tannins. The tannin content of the plants altered the nutrient dynamics in of the soil, this was observed in the increased recorded for organic matter, organic carbon and sulphate content of the soil within the pH range of 5.95-6.65.

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