

Impact of Simulated Nitric and Sulphuric Acid

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Abstract

This study evaluated the impact of simulated nitric acid rain (SNAR) and sulphuric acid rain (SSAR) on the nutrient status of *Telfairia occidentalis*. Results of phytochemical screening of *T. occidentalis* revealed the presence of alkaloids, saponins, flavonoids, reducing compounds, polyphenols in all parts with the absence of phlobatanins, anthraquinones and hydroxymethyl anthraquinones in aqueous and ethanol extracts of SNAR and SSAR as well as the control. Tannins, steroids, terpenoids and glycosides were present in some plant parts and absent in others. Impact of simulated HNO₃ and H₂SO₄ acid rain on qualitative phytochemicals resulted in significant ($P=0.05$) increase and decrease in phytochemicals. Ash, protein, fat, fibre and carbohydrate showed decrease owing to SNAR and SSAR impact with increase in root fat content. Protein was significantly reduced at all levels of acidity with leaf percentage reduction of 54.3

Index terms— nutrient status, *telfairia occidentalis*, plant parts, simulated nitric acid rain, sulphuric acid rain.

1 Introduction

The plant *Telfairia occidentalis* (Hooker F.) is a member of the family Cucurbitaceae, tribe; Jolifeae and sub-family Cucurbitoidae. It is a crop of commercial importance grown across the lowland humid tropics in West Africa; Nigeria, Ghana and Sierra Leone being the major producers. *Telfairia occidentalis* is one of the most important vegetables grown in southern Nigeria mainly for its leaves which constitute a significant component in the diet of the people. It is not uncommon to find large quantities of the vegetable being hauled around on the streets and markets in the southern belt of Nigeria (Mofunanya et al., 2008). The nutritional interest in *Telfairia occidentalis* stems from its high contents of essential amino acids, vitamins and mineral nutrients (Fasuyi and Aletor, 2005; Mofunanya et al., 2009) and as a result, hectares of land under cultivation is on the increase to meet the nutritional needs of the ever-increasing population. Locally it is called 'ikong' by the Efiks and Ibibios, 'ugu' by the Igbos, 'egusi iroko' by the Yorubas, 'uwmenkhen' by the Benins in Nigeria. The leaves which are harvested at all stages of growth are used in the preparation of "edikang ikong" a popular delicious soup in Cross River and Akwa Ibom States and "ofe ugu" in the Igbospeaking States.

Telfairia occidentalis is vegetable with high medicinal values. The leaves are rich in protein (25%), fat (18%), ash (14%) fiber (13%) and minerals and vitamins (20%) (Akanbi et al., 2007). The leaves of this vegetable have a high amount of vitamin A adequate to sustain the vitamin A requirement of consumers. It also possesses the hypolipidemic effect and may be used in hypercholesterolemia therapy (Adaramoye et al., 2007). It is also rich in Fe, Mg, K, carotene and vitamin C is remarkable in making the leaves potentially useful food supplements. The essential amino acids contents compared favorably to those of legumes. It is observed that the vegetable provides little dietary energy, making it valuable in energy-limited diets (Aletor and Adeogun, 1995). In traditional medicine, fluted pumpkin is used in reproduction and fertility; it also has the potential to regenerate testicular damage and to increase spermatogenesis (Nwangwa et al., 2007).

Fluted pumpkin has a high amount of antioxidant, free radical scavenging potential and phytochemicals which are of health benefits. Aqueous and ethanol extracts of *T. occidentalis* leaves have the potential to suppress or

prevent the production of free radical and to scavenge already produced ones, lower lipid peroxidation status and elevates catalase and superoxide dismutase (antioxidant enzymes) both in vivo and in vitro. The vegetable has been found to protect and ameliorate oxidative brain and liver damage induced by malnutrition in rats (Kayode, 2010). Hepatoprotective property of polyphenol extracts of leaves on acetaminophen-induced liver damage has been reported (Nwanna and Oboh, 2007). Ethanol and aqueous extracts of fluted pumpkin also protect the liver cells against garlic-induced oxidative damage with aqueous extract being more effective than ethanol extract. The utilization of *T. occidentalis* leaves in folk medicine in the treatment of certain diseases in Nigeria in which reactive oxygen species involvement could be attributed to antioxidant and free radical scavenging ability.

Telfairia occidentalis has the potential to boost blood level and improves diabetes. In recent time, this vegetable had gained medicinal recognition and subsistence. Fresh leaf is a high-valued health tonic for the treatment of acute anemia. In many tropical countries, anemia constitutes a serious health problem because of the prevalence of malaria and other parasitic infections. In anemic condition, there is a decrease in the level of circulating haemoglobin, less than 13 g dL⁻¹ in males and 12 g dL⁻¹ in females (Okochi et al., 2003). Where, malaria is endemic in the tropics, between 10 to 20% of the populace presents less than 10 g dL⁻¹ hemoglobin (Diallo, 2008). The more vulnerable are the children. *Telfairia occidentalis* leaves are rich in iron which plays a role in the cure of anemia. It has been shown to be blood purifiers (Aletor et al., 2002) and could, therefore, be useful in the maintenance of good health most especially among the poor rural community in developing countries. Vegetables are used to fend off illnesses, help nursing mothers build up their milk and assist rural communities to survive long periods of drought. Furthermore, the fiber content has been reported to have beneficial effects on blood cholesterol and aids in the prevention of bowel diseases, while in diabetic subjects (patients). *T. occidentalis* improves glucose tolerance (Hart, 2005). Diabetes Type 2 associates with the increase in oxidative stress, which probably results either from excess generation of reactive oxygen species or a decrease in antioxidant defenses. But recently, it has come to the knowledge that the most significant factor to increase the free radicals production in diabetes is the hyperglycemic status, which induces damage through overproduction of superoxide radical in the mitochondria (Brownlee, 2001). Superoxide converted to hydroxyls, diffuses through membranes and initiates lipoperoxidation. The oxidation of unsaturated lipids has implications not only for atherosclerosis but also for stability and integrity of the red cell membranes.

The environment is the immanent part of human life, the quality of which plays a critical role in human health. Thus, human health is linked to the quality of the environment. The air quality is of great importance for all living things. The health of plant, animal and human depends on a clean atmosphere. Human activities have continually released into the air elements that have the hydrogen fluoride (HF) producing acid deposition (acid rain) as a result of complex physical and chemical reactions. These reactions are accelerated by sunlight. The transportation of compounds, which convey acid rains through the prevailing wind for thousands of miles raises the pollution to very high rates. Sulphuric acid and nitric acid are the components of acid rain derived largely from fossil fuels combustion (Mofunanya and Soonen, 2017). In Nigeria, increase in population has led to high demand in automobiles, biomass combustion, burning of refuse and traffic emissions have released large quantities of substances into the atmosphere, acidic rain occurs in Nigeria with resultant effect on crop plants. Information on the impact of simulated acid rain on the plant medicinal quality is scares. In-view of the importance of *Telfairia occidentalis* in the diets of the Nigerian people and the antagonizing effect of acid rain, the present study was carried out to evaluate the impact of simulated nitric and sulphuric acid rain on the medicinal potential of *Telfairia occidentalis* (Hooker Fil.).

2 II.

3 Materials and Methods

4 a) Seeds collection and planting

Seeds of *T. occidentalis* were provided by a farmer in Akparabong, Ikom Local Government Area of Cross River State, Nigeria. Polyethylene bags of 16 mm in diameter were bought from the Ministry of Agriculture, Calabar. Nitric and sulphuric acids were purchased from a Scientific Shop all in Calabar, Nigeria. The seeds were sorted for uniformity of size. They were sun-dried for two days to enhance germination and planted in polyethylene bags filled with loamy soil. Two seeds were planted in each bag. On germination, the seedlings were watered with distilled water for a period of two weeks. Simulated nitric and sulphuric acid rain application began after two weeks of germination. The root of *Telfairia occidentalis* possesses antiplasmodial and antimicrobial properties. The blood schizontocidal activity of the root extracts is comparable to that of chloroquine (Okokon et al., 2007). Ethanol and aqueous extracts of *T. occidentalis* root exhibit inhibitory effect on the growth of some Enterobacteriaceae commonly encountered in Nigeria; *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Proteus* sp. Both extracts did not inhibit the growth of some tested fungi; *Aspergillus fumigatus*, *A. flavus*, *Penicillium italicum* and *Geotrichum albidum* (Oboh et al., 2006). Root extracts of *T. occidentalis* also possess antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Klebsiella pneumonia* and *Shigella dysenteriae* (Kayode and Kayode. 2011). Locally, the roots are known as potent human poison and there are reports of their use as fish and human poison. The root extract, though has not been found to be of any practical use in pharmacy, could be used as rodenticide because of high saponin content and cucurbitacin?

which has been reported to cause pulmonary edema (Ajibesin et al., 2002). The vegetable also possesses anti-inflammatory activity. potential to cause pollution such as sulfur dioxide (SO₂), oxides of nitrogen (NO_x), carbon dioxide (CO₂) and III. and uniformity of sunlight and water supply.

5 Results

6 Phytochemicals

7 b) Preparation and application of simulated acid rain

Simulated nitric and sulphuric acid rain (SNAR and SSAR) concentrations of pH 2.0, 3.0, 4.0 were separately prepared and a controlled pH of 6.0. Each using different quantity of acid. For pH 2.0 concentration, 30 ml of each acid was used, 20.1 ml for pH concentration 3.0 and 10.2 ml for pH 4.0 using a pH meter and distilled water. The distilled water of pH 6.0 was used as control (Mofunanya and Egah, 2017). A total of thirty-five poly bags were used; fifteen for simulated nitric acid rain, fifteen for simulated sulphuric acid rain and five for the control that is, five for each pH concentration replicated three times. Before SNAR and SSAR application, the poly bags were arranged in a completely randomized block design. Application began with 50 ml of simulated acid rain at the initial growth period. The amount varied with the increase in plant growth. Simulated acid rain of various concentrations was applied using a domestic hand-spraying unit on the plants as well as soil. Application was carried out at an interval of two days for thirteen weeks.

8 c) Sample preparation

After thirteen weeks of post application of simulated acid rain, the whole plants of *T. occidentalis* grown at various pH concentrations were harvested, and the plant parts (Leaf, Stem and Root) separated. The roots were washed in tape water to remove soil before sun-drying along with other plant parts for one week, and milled separately into powder in an electric mill (National Food Grinder, Model MK 308, Japan). The powdered samples were used to evaluate the impact of simulated nitric and sulphuric acid rain on the medicinal quality (qualitative and quantitative phytochemicals, proximate, amino acids and minerals) contents of *T. occidentalis*.

9 d) Sample Analysis

The presence of phytochemicals in *T. occidentalis* leaf, stem and root were analyzed using standard methods. Alkaloids and glycosides were identified in samples by the method of Sofowora (1993). The presence of tannins, flavonoids, reducing compounds, polyphenols, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were tested for by the method of Trease and Evans (1989). Quantitative determination of flavonoids, alkaloids, saponins in SNAR and SSAR treated samples of the vegetable was carried out by the method of Harbone (1993). Reducing compounds and polyphenols were determined by method described by AOAC (2006). Phytonutrients were analyzed by standard methods. Crude protein was analyzed using the Kjeldahl method. Fat content was determined by the method of AOAC (1995). Ash, fiber, carbohydrate contents of *T. occidentalis* were analyzed by the method of AOAC (2006), minerals (AOAC, 2006) and amino acids (Speckman, 1956; AOAC, 2006).

10 e) Data analysis

Data obtained in this study were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science, (SPSS), Version 15.0 (SPSS, 2003). Results were also expressed as percentage difference and differences between mean values were determined at 5% probability. a) Simulated nitric and sulphuric acid rain impacts on qualitative phytochemicals of *Telfairia occidentalis* leaf, stem, and root *Telfairia occidentalis* leaf, stem, and roots (plant parts) were screened for the presence of alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds, polyphenols, anthraquinones, hydroxymethyl anthraquinones, steroids and terpenoids (Table 1). Results revealed the presence of alkaloids, saponins, flavonoids, reducing compounds, polyphenols in aqueous and ethanol extracts of all plant and control plant. Tannins were absent in root samples of both extracts. Steroids and terpenoids were present in the leaf and root of *T. occidentalis* but absent in stem samples. Glycosides were present in the stem and root samples but absent in the leaf. Phlobatanins, anthraquinones, and hydroxymethyl anthraquinones were absent in both aqueous and ethanol extracts of leaf, stem and root extracts of acid treated and control samples. Steroids were absent in aqueous extract of leaf treated with both simulated acid rain and in the control. Simulated acid rain (SNAR and SSAR) impacts resulted in significant ($P=0.05$) decrease and increase in phytochemical contents of *T. occidentalis* with pH 2.0 depicting the highest decrease and pH 4.0 the lowest decrease. The quality of phytochemicals varied according to concentrations of simulated acid rain and plant parts. The leaf had the highest amount of phytochemicals, followed by the stem and root. Alkaloids and saponins were higher in the root than in the leaf and stem. Impacts of SNAR and SSAR caused asinificant increase on glycosides content of root, leaf saponins, root saponins in SSAR treated and adecrease in root saponins content of SNAR treated plant part. SSAR impact was more on reducing compounds, steroids, and terpenoids with higher reductions in these phytochemicals than with SNAR. Mean value decrease induced on leaf and root flavonoids at pH 2.0 and 4.0 for SNAR impact were 11.40 ± 0.1 , 14.14 ± 0.01 and 8.23 ± 0.02 , 10.60 ± 0.1 mg/100 g. Impact of SSAR had decrease in values of 8.80 ± 0.1 , 10.57 ± 0.01 and 8.29 ± 0.01 ,

11 D) SIMULATED NITRIC AND SULPHURIC ACID RAIN IMPACTS ON AMINO ACIDS OF TELFAIRIA OCCIDENTALIS LEAF, STEM, AND ROOT

11.30 \pm 0.1 mg/100 g as against control values of 18.30 \pm 0.1 and 13.71 \pm 0.01 mg/100 g respectively. However, impact of SNAR led to increase in stem flavonoids contents with values of 13.10 \pm 0.1, 10.75 \pm 0.01 for SNAR and 14.10 \pm 0.1, 11.36 \pm 0.1 mg/100 g for SSAR compared to control pH 6.0 value of 10.60 \pm 0.1 mg/100 g. Results showed reductions in reducing caused more reductions in reducing compounds than simulated sulphuric acid rain. At pH 2.0 flavonoids content of stem sample was lower than the control pH 6.0 for SNAR but higher than the control at pH 3.0 and 4.0. The impact SSAR on stem flavonoids was higher in content than the control. A similar trend of increase and decrease in phytochemicals due to SNAR and SSAR impacts on alkaloids, glycosides, tannins, polyphenols, reducing sugars, steroids, and terpenoids were obtained. Glycosides was not detected in the leaf, tannins was not detected in the root samples of simulated acid rain treated and control plant parts. Steroids and terpenoids were absent in the stem samples of *T. occidentalis* (Table 2). Impacts of simulated nitric (SNAR) and sulphuric SSAR) rain acid on the proximate nutrients revealed as insignificant ($P=0.05$) decrease in ash, protein, fiber, and carbohydrate content of *T. occidentalis* plant parts. The amounts of these nutrients varied in plant parts. Proximate nutrients at control (pH 6.0) revealed that ash, protein and fat contents were more in leaf sample than in the stem and root. Fiber content was more in stem sample than in leaf and root. Fiber content of leaf and root did not differ significantly. While carbohydrate content in leaf, stem and root did not differ statistically. The decrease in proximate nutrients varied according to levels of acidity; pH 2.0 depicted highest reductions in proximate nutrients followed by pH 3.0 and lowest reductions at pH 4.0. Reductions obtained at pH 4.0 for all proximate nutrients were not statistically significant ($P=0.05$) when compared to the control pH Reduction impacted by SSAR on protein content of stem at pH 2.0 had lower value of 2.37 \pm 0.1 than value at pH 3.0 of 4.51 \pm 0.2; pH 3.0 had lower value than pH 4.0 value of 4.67 \pm 0.1 g/100 g compared to control pH value of 6.10 \pm 0.1 g/100 g. The root mean reduction values were 3.02 \pm 0.1, 3.08 \pm 0.01 and 3.19 \pm 0.1 g/100 g respectively for SNAR. While SSAR had mean reduction values of 2.81 \pm 0.01, 3.25 \pm 0.01 and 3.70 \pm 0.1 g/100 g for pH 2.0, 3.0 and 4.0 respectively compared pH 6.0 value of 3.80 \pm 0.1 g/100 g. A similar trend of lowest decrease at pH 2.0, lower decrease at pH 3.0 and low decrease at pH 6.0 were obtained for ash, fat, fiber and carbohydrate. Simulated nitric acid rain caused more impact on ash and fiber content of *T. occidentalis* while impact of SSAR was more on protein and fat (Table 3). All control plant parts had higher proximate nutrient contents than simulated acid rain treated plant parts. Leaf ----- Stem ----- Root ----- Anthraquinones Leaf ----- Stem ----- Root ----- Hydroxymethyl anthraquinones Leaf ----- Stem ----- Root ----- Steroids Leaf - - - - - Stem - - - - - Root + + + + + Terpenoids Leaf + + + + + Stem - - - - - Root + + + + + + + + + + = Present, +++ =

11 d) Simulated nitric and sulphuric acid rain impacts on amino acids of Telfairia occidentalis leaf, stem, and root

Analysis of variance revealed that all amino acids of *Telfairia occidentalis* were severely affected by simulated acid rain. Significant ($P=0.05$) reductions were obtained for both essential (Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and nonessential (alanine, aspartic acid, arginine, cysteine, glycine, proline, serine, and tyrosine) amino acids (Table 4). Amino acids reductions varied according to acidity levels with the highest reductions occurring at pH 2.0, followed by pH 3.0 and the lowest reductions occurring at pH 4.0 for both simulated nitric and sulphuric acid rain. The quality of amino acids in the various plant parts also varied with the leaf having the highest amount, followed by the stem and root having the lowest amount. Amino acids; lysine, threonine, glycine and leucine in leaf, stem and root in control pH 6.0 varied significantly. Histidine, arginine, aspartic acid, serine, glutamic acid, proline, alanine, cysteine, valine, methionine, isoleucine, and tyrosine did not differ in leaf and stem but varied in root samples. Phenylalanine did not differ in leaf, stem, and root. Impacts of simulated acid rain resulted in significant ($P=0.05$) increase in aspartic acid, glutamic acid, proline, and alanine. The results in Table 4 highlight impacts of simulated nitric and sulphuric acid rain on the mineral nutrient contents of *T. occidentalis*. Analysis of variance revealed significant ($P=0.05$) reduction in all mineral nutrients posed by simulated acid rain impact compared to the control. Mineral nutrients reductions varied according to acidity levels and plant parts. The leaf had higher mineral nutrients than the stem, and the stem had higher mineral nutrients than the roots with the exceptions of K and Na which were more in the root than in the leaf and stem. However, Ca and Mn were higher in the stem than in the leaf and root of simulated acid rain treated plants and the control. Highest nutrient reductions occurred at pH 2.0, followed by pH 3.0, pH 4.0 had the lowest reductions in all mineral nutrients. However, reduction on Ca, Zn, and P induced by SNAR did not differ statistically at pH 2.0 and pH 3.0. Simulated SNAR impact was higher on K, Zn, Mn, and Cu with a higher reduction in contents than with SSAR, SSAR impact was higher on Na, Ca, Mg, and Fe content of the vegetable with significant reductions obtained than with SNAR. Reductions were highest at pH 2.0 and lowest at pH 4.0. Na, Ca, Fe, Mn, and P varied significantly according to plant parts, while K and Mg were not statistically different in leaf and stem parts. Reductions posed on Ca in leaf by SNAR at pH 2.0, 3.0 and 4.0 were 26.21 \pm 0.2, 27.61 \pm 0.2 and 30.96 \pm 0.2 mg/100 g, SSAR impact caused reductions of 25.12 \pm 0.2, 27.66 \pm 0.1 and 29.59 \pm 0.2 mg/100 g. In the stem, SNAR had mean Ca reduction values of 35.46 \pm 0.1, 37.47 \pm 0.2 and 38.89 \pm 0.2 mg/100 g, SSAR had values of 34.81 \pm 0.1, 35.82 \pm 0.2 and 36.03 \pm 0.2 mg/100 g

respectively compared to value of 40.51 ± 0.2 mg/100 g for the control. Reductions posed by SNAR on root Ca had values of 30.50 ± 0.1 , 33.55 ± 0.2 and 37.79 ± 0.2 mg/100 g, SSAR had values of 30.06 ± 0.2 , 31.58 ± 0.2 and 34.09 ± 0.2 mg/100 g compared to control pH 6.0 value of 39.01 ± 0.2 mg/100 g. Reduction in Iron content in leaf, stem and root caused by SNAR had mean values of 19.18 ± 0.1 , 16.80 ± 0.2 and 10.64 ± 0.2 mg/100 g at pH 2.0, 20.03 ± 0.1 , 17.78 ± 0.1 and 10.88 ± 0.2 mg/100 g at pH 3.0, 23.15 ± 0.1 , 18.00 ± 0.1 and 11.99 ± 0.2 mg/100 g at pH 4.0. Values obtained for SSAR were 18.80 ± 0.1 , 16.10 ± 0.2 and 10.05 ± 0.2 mg/100 g at pH 2.0, 19.12 ± 0.1 , 17.44 ± 0.1 and 10.32 ± 0.2 mg/100 g at pH 3.0, 20.13 ± 0.2 , 17.91 ± 0.1 and 10.68 ± 0.2 mg/100 g respectively as against control pH value of 14.07 ± 0.1 mg/100 g. A similar trend of reduction in mineral nutrient contents according to pH levels and variations in plant parts was also obtained for K, Na, Mg, Cu, Mn, and P (Table 5).

IV.

12 Discussion a) Impact of SNAR and SSAR on phytochemicals

Quantitative determination of phytochemicals in plant parts of *T. occidentalis* treated with SNAR and SSAR and the untreated control revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, reducing sugars, steroids, and terpenoids in both aqueous and ethanol extracts while phlobatanins, anthraquinones, and hydroxymethyl anthraquinones were absent in both extracts. The impact of simulated nitric and sulphuric acid rain on *T. occidentalis* provoked significant ($P=0.05$) reductions in some of the investigated phytochemicals; alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols, reducing sugars, steroids, and terpenoids. Phytochemicals are of great importance to the health needs of the people. The reduction in these phytochemicals orchestrated by SNAR and SSAR impact has positive and negative effects. *Telfairia occidentalis* is a rich source of phytochemicals like other vegetables. These plant chemicals have both therapeutic and protective potentials essential in disease prevention and maintenance of the state of well being, by stimulating catalysts (enzymes) in the liver that neutralize some carcinogens and helping the body stimulate others.

Phytochemicals present in this vegetable are responsible for these health benefits. Tannins are used in the treatment of ulcerated or inflamed tissues and cancer prevention. Thus, tannins contained in this vegetable may serve as a potential source of bioactive compound in the prevention and treatment of cancer. Plants rich in tannins have been used in Ayurvedic medicine for the treatment of diarrhea, leucorrhoea, and rhinorrhoea (Douglas et al., 2009). Tannins are polyphenols that are astringent, making them useful in drawing tissues together thus, limiting blood flow. They help to maintain healthy circulation and strengthens capillary (Ejike and Ajileye, 2007). Flavonoids are polyphenolic compounds widely distributed in many plant varieties. Flavonoids are antiviral, antiinflammatory, antitumor and anti-platelets agents. They are potent antioxidants that are soluble in water, they are free radical scavengers, which prevent oxidative cell damage and possess anticancer activity. Hydroxyl flavonoids are responsible for the free radical scavenging effects of most plants (Usunobun and Egharebva, 2014). Flavonoids aid in the alleviation of cholesterol levels in patients with cardiovascular complication reduces high blood pressure. They also reduce the chances of heart disease. Isoflavones help to reduce osteoporosis and menstrual pains in women. The phytochemical; proanthocyanidins possess the ability to improve dental health and also to reduce urinary tract diseases. They fight atherosclerosis. Phytochemicals help to boost the immune system, reduce chronic inflammation which is of immense benefit to obese individuals with inflammatory markers (<http://benefits-of-phytochemicals/>).

Saponins; triterpenoid saponins are useful for skin care that found in a herb licorice root (*Glycyrrhiza glabra*). This saponin promotes nutrients absorption. The major ingredient in many medicinal plants is saponins. Triterpene, sponins, and their aglycones are used as analgesic, antioedema and antimicrobial, anti-inflammatory, antipyretic, antiulcerogenic, and fibrinolytic agents (Mofunanya and Nta, 2016).

13 b) Impact of SNAR and SSAR on proximate nutrients

Proximate nutrients of acid rain treated and control plant parts (leaf, stem and root) varied in amount of nutrients. SNAR and SSAR plant parts had lower proximate nutrients than the control. The leaf samples of *T. occidentalis* had the highest amount of nutrient, followed by the stem and then the root. However, fiber content was higher in stem than in the leaf and root. Acid rain impact was evident in the reduction of ash, protein, fat, fiber and carbohydrate when compared to the control. Reductions in these proximate nutrients due to acid rain stress are in line with similar reductions in these nutrients in *Amaranthus hybridus* leaf, stem, and root treated with SNAR and SSAR (Mofunanya and Egah, 2017). Reduction in N which is a component of protein in apple leaves at low pH has been documented (Proctor, 1983). Kong et al. (2000) in their research reported that acid rain caused an increase in free oxygen radicals and a decrease in protein in various organs. The reduction posed by acid rain impact on these nutrients is disturbing because of their essentiality to health and wellbeing. Proteins are compounds made up of smaller units of amino acids. When they are broken down during digestion amino acids are released, which are the building blocks of all protein. Once present in the human body, these amino acids are used in the synthesis of new proteins including enzymes which are proteins and hormones such as adrenalin ("fight and flight" hormone). Proteins are also energy source. Proteins are vital in the maintenance of muscle mass and helpful after strenuous exercise. The needful role of dietary protein in the body is to supply amino acids for the construction of human proteins. All amino acids are necessary for the synthesis of protein, although cells in the

human body have the potential to synthesize eleven (11) amino acids from raw materials; the remaining nine (??) cannot be synthesized by the body. These nine amino acids are called essential amino acids and must be obtained from plant food (diet). These essential amino acids cannot be stored by the body. Insufficient amount of these essential amino acids prevents the synthesis of necessary proteins resulting in protein deficiency diseases (Levetin and McMahon, 1999). Deficiency of protein causes wasting and shrinkage of muscle, anemia resulting from the inability to deliver enough oxygen to the cells, caused by lack of dietary iron, edema; a build-up of fluids in the feet and ankles. It causes slow growth in children. Fibers in diet play a role in disease prevention and treatment of colorectal cancer, diabetes, weight loss, high cholesterol, obesity, heart disease and gastrointestinal disorders such as constipation, diarrhea. It promotes Ca absorption. Ash in plant food is very important in that it contains all the mineral nutrient; micro and macronutrients. Carbohydrates are indispensable energy source. Fat in food increases the palatability of food by absorbing and retaining flavors (Antia et al., 2006).

14 c) Impact of SNAR and SSAR on amino acids

Amino acids profile of *T. occidentalis* leaf, stem and root revealed the presence of histidine, lysine, arginine, aspartic acid, threonine, cysteine, glycine, glutamic acid, serine, valine, proline, methionine, leucine, isoleucine, tyrosine, and phenylalanine. These amino acids were present in both SNAR and SSAR and in control plant parts. The presence of these amino acids have been reported in *T. occidentalis* (Tindall, 1992; Asuyi, 2006) and in other cases of biotic stress (Mofunanya et al., 2009; Mofunanya, 2016).

Amino acids are the core of orthomolecular medicine. The field of medicine that describes the practice of optimizing bodily functions, in the prevention and treatment of diseases by providing the body with optimal amounts of natural nutrients such as vitamins, dietary minerals, proteins, antioxidants, amino acids and fatty acids. Findings of this research revealed significant reductions in histidine, lysine, arginine, threonine, cysteine, glycine, serine, methionine, leucine, isoleucine, tyrosine, and phenylalanine in plant parts of *T. occidentalis* due to SNAR and SSAR impact. SNAR and SSAR impact however, caused a significant increase in glutamic acid, aspartic acid, proline, and valine. Accumulation or increase in proline and other amino acids is a usual response of higher plants to biotic and abiotic stress.

15 d) Impact of SNAR and SSAR on mineral nutrients

Mineral nutrients reduction induced by simulated acid rain impact is threatening as these are essential to human health. Results of mineral nutrient of leaf, stem, and root revealed a significant decrease in K, Na, Ca, Mg, Mn, Cu, Zn, Fe and P due to SNAR and SSAR impacts when compared to the control. Results of this investigation are in agreement with previous findings of the minerals in *A. hybridus* due to acid rain effect (Mofunanya and Egah, 2017). Acid rain of low pH caused a decrease in nitrogen concentration in apple leaves with no effect in levels of K⁺, Ca²⁺ and Mg²⁺. Reductions in these mineral nutrients present problems, when in excess are harmful to the body and when available in desired levels, they contribute immensely to wellbeing. Minerals exercise a role in nearly every human body function ranging from building healthy bones and teeth to energy production, support to the immune system. They are so crucial to health that even slight imbalances of some minerals can produce harmful effects; ranging from low energy levels to severe gastrointestinal problems (<http://youneivity.com/index.cfm/my-profile>). Trace minerals are used to neutralize the raw, infected cells in the throat, help the skin to heal faster and reduce scarring, and also for wound clotterization. They heal from sickness. Trace minerals contain Fe which help in the formation of hemoglobin in the blood, and in turn transports oxygen to the cells (<http://www.oohoi.com/healthy-living/vitamin-info/benefits-of-iron.htm>). They also contain Mg and are used in painful menstrual cycles to proffer fast and better pain relief in women than placebo. The Fe present in trace minerals replaces the Fe lost due to high menstrual flow. Low Fe levels can lead to fatigue (<http://www.elliottthehealthcare.com/iron-deficiency.htm>). Trace minerals improve sight. Zinc is essential for the transport of vitamin A from the liver to the eye, also needed to quench free radicals. Trace minerals help to curb cravings.

V.

16 Conclusion

Fluted pumpkin leaf, stem, and root showed a wide array of nutritional distinctiveness. This study has revealed that individually simulated nitric acid rain and simulated sulphuric acid rain impacted the medicinal status of *Telfairia occidentalis*. Fluctuation in nutrient contents occasioned by simulated acid rain does not allow the consumers of this vegetable to know the exact amounts of nutrients taken in at any given time. The increase in content of these nutrients creates excess amounts and decrease, insufficient amounts altering their efficacy with attendant health problems.

Impact of Simulated Nitric and Sulphuric Acid Rain on the Medicinal Potential of *Telfairia Occidentalis* (Hooker Fil.) the precursor of collagen called pro-collagen, it also protect the hair follicles from oxidative damage and slows the graying of hair and hair thinning. Lysine stimulates collagen to repair hair. Hair follicles also require lysine to function. Hair loss is reduced with lysine. Glycine is not only important for the digestive and central nervous system but also help in collagen production. Proline a non-essential amino acid helps in the production of collagen and cartilage, as well as maintaining the muscle tissues. SNAR and SSAR impact caused

340 a significant increase in amino acids aspartic acid, glutamic acid, proline, and alanine. Plant disturbances with other stressors have been reported to ¹

[Note: (Hooker Fil.)]

Figure 1:

1
(Hooker Fil.)

Figure 2: Table 1 :

2
mg/100 g dry matter

Figure 3: Table 2 :

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¹(Hooker Fil.)

± 0.01 and 6.32 ± 0.01 , 7.62 ± 0.1 , 8.71 ± 0.01
 compared to control value of 9.80 ± 0.1 mg/100 g
 respectively. Corresponding values for reducing
 compounds of root were 5.17 ± 0.01 , 6.18 ± 0.01 , 7.30
 ± 0.1 for SNAR and 5.42 ± 0.02 , 6.51 ± 0.01 , $7.59 \pm$

Year 0.01 for SSAR when compared to value of 8.73 ± 0.02 mg/100 g for the control. Simulated nitric acid
 2018

54

Volume Proximate Nutrients Ash Plant part Leaf Stem $4.20 \pm 0.1(20.9)$ pH 2.0 $3.20 \pm 0.01(50.8)$ Root 2.81
 XVIII Protein Fat

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I

D Stem $3.10 \pm 0.1(39.2)$

D

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(

Root 5.41
 \pm

Medicine

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Leaf $0.01(12.7)$

Stem 12.24

Root \pm

$0.01(12.9)$

14.92

\pm

$0.1(11.2)$

12.40

\pm

$0.1(13.0)$

Global Carbohydrate Leaf Stem $84.60 \pm 0.1(6.5)$ $85.65 \pm 0.02(5.4)$ Root $83.24 \pm 0.1(3.3)$? Results are mean

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[Note: Bcompounds of T]

Figure 4:

3

and root

Figure 5: Table 3 :

4

(Hooker Fil.)

Figure 6: Table 4 :

5

D D D D)
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Figure 7: Table 5 :

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D D D D)
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[Note: Bproline in their tissues (]

Figure 8:

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[Calcium] , Calcium . (Ca) Leaf 26. 21±0.2(20.9) 27. 61±0.2(16.7) 30.96±0.2(6.6) 25.12±0.2(24.2) 27.66±0.1(16.5) 29.59±0.2(10.7) 33.14±0.20)

[Edition] , *Edition*

[Edition] , *Edition*

[? Results are mean of three replicates on a dry weight basis ± standard deviation; P=0.05 ? Simulated nitric acid rain (SNAR)]
 ? Results are mean of three replicates on a dry weight basis ± standard deviation; P=0.05 ? Simulated nitric acid rain (SNAR), (Simulated sulphuric acid rain (SSAR)

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