

# 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 HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> 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

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

## 1 Introduction

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

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

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

## 4 A) SEEDS COLLECTION AND PLANTING

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45 prevent the production of free radical and to scavenge already produced ones, lower lipid peroxidation status and  
46 elevates catalase and superoxide dismutase (antioxidant enzymes) both in vivo and in vitro. The vegetable has  
47 been found to protect and ameliorate oxidative brain and liver damage induced by malnutrition in rats (Kayode,  
48 2010). Hepatoprotective property of polyphenol extracts of leaves on acetaminophen-induced liver damage has  
49 been reported (Nwanna and Oboh, 2007). Ethanol and aqueous extracts of fluted pumpkin also protect the liver  
50 cells against garlic-induced oxidative damage with aqueous extract being more effective than ethanol extract.  
51 The utilization of *T. occidentalis* leaves in folk medicine in the treatment of certain diseases in Nigeria in which  
52 reactive oxygen species involvement could be attributed to antioxidant and free radical scavenging ability.

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

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

## 86 2 II.

## 87 3 Materials and Methods

### 88 4 a) Seeds collection and planting

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

105 which has been reported to cause pulmonary edema (Ajibesin et al., 2002). The vegetable also possesses anti-  
106 inflammatory activity, potential to cause pollution such as sulfur dioxide (SO<sub>2</sub>), oxides of nitrogen (NO<sub>x</sub>),  
107 carbon dioxide (CO<sub>2</sub>) and III. and uniformity of sunlight and water supply.

## 108 **5 Results**

## 109 **6 Phytochemicals**

### 110 **7 b) Preparation and application of simulated acid rain**

111 Simulated nitric and sulphuric acid rain (SNAR and SSAR) concentrations of pH 2.0, 3.0, 4.0 were separately  
112 prepared and a controlled pH of 6.0. Each using different quantity of acid. For pH 2.0 concentration, 30 ml of  
113 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.  
114 The distilled water of pH 6.0 was used as control (Mofunanya and Egah, 2017). A total of thirty-five poly bags  
115 were used; fifteen for simulated nitric acid rain, fifteen for simulated sulphuric acid rain and five for the control  
116 that is, five for each pH concentration replicated three times. Before SNAR and SSAR application, the poly bags  
117 were arranged in a completely randomized block design. Application began with 50 ml of simulated acid rain at  
118 the initial growth period. The amount varied with the increase in plant growth. Simulated acid rain of various  
119 concentrations was applied using a domestic hand-spraying unit on the plants as well as soil. Application was  
120 carried out at an interval of two days for thirteen weeks.

### 121 **8 c) Sample preparation**

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

### 128 **9 d) Sample Analysis**

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

### 138 **10 e) Data analysis**

139 Data obtained in this study were subjected to analysis of variance (ANOVA) using the Statistical Package for  
140 Social Science, (SPSS), Version 15.0 ??SPSS, 2003). Results were also expressed as percentage difference and  
141 differences between mean values were determined at 5% probability. a) Simulated nitric and sulphuric acid  
142 rain impacts on qualitative phytochemicals of *Telfairia occidentalis* leaf, stem, and root *Telfairia occidentalis*  
143 leaf, stem, and roots (plant parts) were screened for the presence of alkaloids, glycosides, saponins, tannins,  
144 flavonoids, reducing compounds, polyphenols, anthraquinones, hydroxymethyl anthraquinones, steroids and  
145 terpenoids (Table 1). Results revealed the presence of alkaloids, saponins, flavonoids, reducing compounds,  
146 polyphenols in aqueous and ethanol extracts of all plant and control plant. Tannins were absent in root samples  
147 of both extracts. Steroids and terpenoids were present in the leaf and root of *T. occidentalis* but absent in  
148 stem samples. Glycosides were present in the stem and root samples but absent in the leaf. Phlobatanins,  
149 anthraquinones, and hydroxymethyl anthraquinones were absent in both aqueous and ethanol extracts of leaf,  
150 stem and root extracts of acid treated and control samples. Steroids were absent in aqueous extract of leaf treated  
151 with both simulated acid rain and in the control. Simulated acid rain (SNAR and SSAR) impacts resulted in  
152 significant (P=0.05) decrease and increase in phytochemical contents of *T. occidentalis* with pH 2.0 depicting the  
153 highest decrease and pH 4.0 the lowest decrease. The quality of phytochemicals varied according to concentrations  
154 of simulated acid rain and plant parts. The leaf had the highest amount of phytochemicals, followed by the stem  
155 and root. Alkaloids and saponins were higher in the root than in the leaf and stem. Impacts of SNAR and SSAR  
156 caused a significant increase on glycosides content of root, leaf saponins, root saponins in SSAR treated and  
157 a decrease in root saponins content of SNAR treated plant part. SSAR impact was more on reducing compounds,  
158 steroids, and terpenoids with higher reductions in these phytochemicals than with SNAR. Mean value decrease  
159 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  
160 ± 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

161 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,  
162 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  
163 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  
164 showed reductions in reducing caused more reductions in reducing compounds than simulated sulphuric acid  
165 rain. At pH 2.0 flavonoids content of stem sample was lower than the control pH 6.0 for SNAR but higher than  
166 the control at pH 3.0 and 4.0. The impact SSAR on stem flavonoids was higher in content than the control. A  
167 similar trend of increase and decrease in phytochemicals due to SNAR and SSAR impacts on alkaloids, glycosides,  
168 tannins, polyphenols, reducing sugars, steroids, and terpenoids were obtained. Glycosides was not detected in  
169 the leaf, tannins was not detected in the root samples of simulated acid rain treated and control plant parts.  
170 Steroids and terpenoids were absent in the stem samples of *T. occidentalis* (Table 2). Impacts of simulated nitric  
171 (SNAR) and sulphuric SSAR) rain acid on the proximate nutrients revealed a significant ( $P=0.05$ ) decrease in ash,  
172 protein, fiber, and carbohydrate content of *T. occidentalis* plant parts. The amounts of these nutrients varied in  
173 plant parts. Proximate nutrients at control (pH 6.0) revealed that ash, protein and fat contents were more in leaf  
174 sample than in the stem and root. Fiber content was more in stem sample than in leaf and root. Fiber content  
175 of leaf and root did not differ significantly. While carbohydrate content in leaf, stem and root did not differ  
176 statistically. The decrease in proximate nutrients varied according to levels of acidity; pH 2.0 depicted highest  
177 reductions in proximate nutrients followed by pH 3.0 and lowest reductions at pH 4.0. Reductions obtained at  
178 pH 4.0 for all proximate nutrients were not statistically significant ( $P=0.05$ ) when compared to the control pH  
179 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  
180 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  
181 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  
182 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  
183 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  
184 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  
185 carbohydrate. Simulated nitric acid rain caused more impact on ash and fiber content of *T. occidentalis* while  
186 impact of SSAR was more on protein and fat (Table 3). All control plant parts had higher proximate nutrient  
187 contents than simulated acid rain treated plant parts. Leaf - - - - - Stem - - - - - Root  
188 - - - - - Anthraquinones Leaf - - - - - Stem - - - - - Root - - - - -  
189 - - - Hydroxymethyl anthraquinones Leaf - - - - - Stem - - - - - Root - - - - -  
190 - - - Steroids Leaf - + - + - + - + - + + Stem - - - - - Root + + + + + + + + + + +  
191 + + - Terpenoids Leaf + + + + + + + + + + + + Stem - - - - - Root + + + + + + + + +  
192 + + + + + + + = Present, + + + =

### 193 11 d) Simulated nitric and sulphuric acid rain impacts on amino 194 acids of *Telfairia occidentalis* leaf, stem, and root

195 Analysis of variance revealed that all amino acids of *Telfairia occidentalis* were severely affected by simulated  
196 acid rain. Significant ( $P=0.05$ ) reductions were obtained for both essential (Histidine, isoleucine, leucine, lysine,  
197 methionine, phenylalanine, threonine, tryptophan, and valine) and nonessential (alanine, aspartic acid, arginine,  
198 cysteine, glycine, proline, serine, and tyrosine) amino acids (Table 4). Amino acids reductions varied according  
199 to acidity levels with the highest reductions occurring at pH 2.0, followed by pH 3.0 and the lowest reductions  
200 occurring at pH 4.0 for both simulated nitric and sulphuric acid rain. The quality of amino acids in the various  
201 plant parts also varied with the leaf having the highest amount, followed by the stem and root having the  
202 lowest amount. Amino acids; lysine, threonine, glycine and leucine in leaf, stem and root in control pH 6.0  
203 varied significantly. Histidine, arginine, aspartic acid, serine, glutamic acid, proline, alanine, cysteine, valine,  
204 methionine, isoleucine, and tyrosine did not differ in leaf and stem but varied in root samples. Phenylalanine  
205 did not differ in leaf, stem, and root. Impacts of simulated acid rain resulted in significant ( $P=0.05$ ) increase in  
206 aspartic acid, glutamic acid, proline, and alanine. The results in Table 4 highlight impacts of simulated nitric and  
207 sulphuric acid rain on the mineral nutrient contents of *T. occidentalis*. Analysis of variance revealed significant  
208 ( $P=0.05$ ) reduction in all mineral nutrients posed by simulated acid rain impact compared to the control. Mineral  
209 nutrients reductions varied according to acidity levels and plant parts. The leaf had higher mineral nutrients  
210 than the stem, and the stem had higher mineral nutrients than the roots with the exceptions of K and Na which  
211 were more in the root than in the leaf and stem. However, Ca and Mn were higher in the stem than in the leaf  
212 and root of simulated acid rain treated plants and the control. Highest nutrient reductions occurred at pH 2.0,  
213 followed by pH 3.0, pH 4.0 had the lowest reductions in all mineral nutrients. However, reduction on Ca, Zn, and  
214 P induced by SNAR did not differ statistically at pH 2.0 and pH 3.0. Simulated SNAR impact was higher on K,  
215 Zn, Mn, and Cu with a higher reduction in contents than with SSAR, SSAR impact was higher on Na, Ca, Mg,  
216 and Fe content of the vegetable with significant reductions obtained than with SNAR. Reductions were highest  
217 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  
218 Mg were not statistically different in leaf and stem parts. Reductions posed on Ca in leaf by SNAR at pH 2.0,  
219 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  
220  $\pm$  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,  
221 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

222 respectively compared to value of  $40.51 \pm 0.2$  mg/100 g for the control. Reductions posed by SNAR on root Ca  
223 had values of  $30.50 \pm 0.1$ ,  $33.55 \pm 0.2$  and  $37.79 \pm 0.2$  mg/100 g, SSAR had values of  $30.06 \pm 0.2$ ,  $31.58 \pm 0.2$   
224 and  $34.09 \pm 0.2$  mg/100 g compared to control pH 6.0 value of  $39.01 \pm 0.2$  mg/100 g. Reduction in Iron content  
225 in leaf, stem and root caused by SNAR had mean values of  $19.18 \pm 0.1$ ,  $16.80 \pm 0.2$  and  $10.64 \pm 0.2$  mg/100  
226 g at pH 2.0,  $20.03 \pm 0.1$ ,  $17.78 \pm 0.1$  and  $10.88 \pm 0.2$  mg/100 g at pH 3.0,  $23.15 \pm 0.1$ ,  $18.00 \pm 0.1$  and  $11.99$   
227  $\pm 0.2$  mg/100 g at pH 4.0. Values obtained for SSAR were  $18.80 \pm 0.1$ ,  $16.10 \pm 0.2$  and  $10.05 \pm 0.2$  mg/100  
228 g at pH 2.0,  $19.12 \pm 0.1$ ,  $17.44 \pm 0.1$  and  $10.32 \pm 0.2$  mg/100 g at pH 3.0,  $20.13 \pm 0.2$ ,  $17.91 \pm 0.1$  and  $10.68 \pm$   
229  $0.2$  mg/100 g respectively as against control pH value of  $14.07 \pm 0.1$  mg/100 g. A similar trend of reduction in  
230 mineral nutrient contents according to pH levels and variations in plant parts was also obtained for K, Na, Mg,  
231 Cu, Mn, and P (Table 5).

232 IV.

## 233 12 Discussion a) Impact of SNAR and SSAR on phytochemicals

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

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

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

## 265 13 b) Impact of SNAR and SSAR on proximate nutrients

266 Proximate nutrients of acid rain treated and control plant parts (leaf, stem and root) varied in amount of nutrients.  
267 SNAR and SSAR plant parts had lower proximate nutrients than the control. The leaf samples of *T. occidentalis*  
268 had the highest amount of nutrient, followed by the stem and then the root. However, fiber content was higher  
269 in stem than in the leaf and root. Acid rain impact was evident in the reduction of ash, protein, fat, fiber and  
270 carbohydrate when compared to the control. Reductions in these proximate nutrients due to acid rain stress are  
271 in line with similar reductions in these nutrients in *Amaranthus hybridus* leaf, stem, and root treated with SNAR  
272 and SSAR (Mofunanya and Egah, 2017). Reduction in N which is a component of protein in apple leaves at low  
273 pH has been documented ??Proctor, 1983). Kong et al. (2000) in their research reported that acid rain caused  
274 an increase in free oxygen radicals and a decrease in protein in various organs. The reduction posed by acid  
275 rain impact on these nutrients is disturbing because of their essentiality to health and wellbeing. Proteins are  
276 compounds made up of smaller units of amino acids. When they are broken down during digestion amino acids  
277 are released, which are the building blocks of all protein. Once present in the human body, these amino acids are  
278 used in the synthesis of new proteins including enzymes which are proteins and hormones such as adrenalin ("fight  
279 and flight' hormone). Proteins are also energy source. Proteins are vital in the maintenance of muscle mass and  
280 helpful after strenuous exercise. The needful role of dietary protein in the body is to supply amino acids for the  
281 construction of human proteins. All amino acids are necessary for the synthesis of protein, although cells in the

## 16 CONCLUSION

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282 human body have the potential to synthesize eleven (11) amino acids from raw materials; the remaining nine (283 ??) cannot be synthesized by the body. These nine amino acids are called essential amino acids and must be 284 obtained from plant food (diet). These essential amino acids cannot be stored by the body. Insufficient amount 285 of these essential amino acids prevents the synthesis of necessary proteins resulting in protein deficiency diseases 286 ??Levetin and McMahon, 1999). Deficiency of protein causes wasting and shrinkage of muscle, anemia resulting 287 from the inability to deliver enough oxygen to the cells, caused by lack of dietary iron, edema; a build-up of fluids 288 in the feet and ankles. It causes slow growth in children. Fibers in diet play a role in disease prevention and 289 treatment of colorectal cancer, diabetes, weight loss, high cholesterol, obesity, heart disease and gastrointestinal 290 disorders such as constipation, diarrhea. It promotes Ca absorption. Ash in plant food is very important in that 291 it contains all the mineral nutrient; micro and macronutrients. Carbohydrates are indispensable energy source. 292 Fat in food increases the palatability of food by absorbing and retaining flavors (Antia et al., 2006).

### 293 14 c) Impact of SNAR and SSAR on amino acids

294 Amino acids profile of *T. occidentalis* leaf, stem and root revealed the presence of histidine, lysine, arginine, 295 aspartic acid, threonine, cysteine, glycine, glutamic acid, serine, valine, proline, methionine, leucine, isoleucine, 296 tyrosine, and phenylalanine. These amino acids were present in both SNAR and SSAR and in control plant 297 parts. The present of these amino acids have been reported in *T. occidentalis* (Tindall, 1992; ??asuyi, 2006) and 298 in other cases of biotic stress (Mofunanya et al., 2009;Mofunanya, 2016).

299 Amino acids are the core of orthomolecular medicine. The field of medicine that describes the practice of 300 optimizing bodily functions, in the prevention and treatment of diseases by providing the body with optimal 301 amounts of natural nutrients such as vitamins, dietary minerals, proteins, antioxidants, amino acids and fatty 302 acids. Findings of this research revealed significant reductions in histidine, lysine, arginine, threonine, cysteine, 303 glycine, serine, methionine, leucine, isoleucine, tyrosine, and phenylalanine in plant parts of *T. occidentalis* due 304 to SNAR and SSAR impact. SNAR and SSAR impact however, caused asignificant increase in glutamic acid, 305 aspartic acid, proline, and valine. Accumulation or increase in proline and other amino acids is a usual response 306 of higher plants to biotic and abiotic stress.

### 307 15 d) Impact of SNAR and SSAR on mineral nutrients

308 Mineral nutrients reduction induced by simulated acid rain impact is threatening as these are essential to human 309 health. Results of mineral nutrient of leaf, stem, and root revealed a significant decrease in K, Na, Ca, Mg, Mn, 310 Cu, Zn, Fe and P due to SNAR and SSAR impacts when compared to the control. Results of this investigation are 311 in agreement with previous findings of the minerals in *A. hybridus* due to acid rain effect (Mofunanya and Egah, 312 2017). Acid rain of low pH caused a decrease in nitrogen concentration in apple leaves with no effect in levels of 313 K+, Ca2+ and Mg2+. Reductions in these mineral nutrients present problems, when in excess are harmful to 314 the body and when available in desired levels, they contribute immensely to wellbeing. Minerals exercise a role in 315 nearly every human body function ranging from building healthy bones and teeth to energy production, support 316 to the immune system. They are so crucial to health that even slight imbalances of some minerals can produce 317 harmful effects; ranging from low energy levels to severe gastrointestinal problems (<http://younevity.com/index.cfm/my-profile>). Trace minerals are used to neutralize the raw, infected cells in the throat, help the skin to heal 318 faster and reduce scarring, and also for wound cloterization. They heal from sickness. Trace minerals contain Fe 319 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 320 menstrual cycles to proffer fast and better pain relief in women than placebo. The Fe present in trace minerals 321 replaces the Fe lost due to high menstrual flow. Low Fe levels can lead to fatigue (<http://www.elliotthealthcare.com/iron-deficiency.htm>). Trace minerals improve sight. Zinc is essential for the transport of vitamin A from 322 the liver to the eye, also needed to quench free radicals. Trace minerals help to curb cravings.

326 V.

## 327 16 Conclusion

328 Fluted pumpkin leaf, stem, and root showed a wide array of nutritional distinctiveness. This study has revealed 329 that individually simulated nitric acid rain and simulated sulphuric acid rain impacted the medicinal status of 330 *Telfairia occidentalis*. Fluctuation in nutrient contents occasioned by simulated acid rain does not allow the 331 consumers of this vegetable to know the exact amounts of nutrients taken in at any given time. The increase in 332 content of these nutrients creates excess amounts and decrease, insufficient amounts altering their efficacy with 333 attendant health problems.

334 Impact of Simulated Nitric and Sulphuric Acid Rain on the Medicinal Potential of *Telfairia Occidentalis* 335 ??Hooker Fil.) the precursor of collagen called pro-collagen, it also protect the hair follicles from oxidative 336 damage and slows the graying of hair and hair thinning. Lysine simulates collagen to repair hair. Hair follicles 337 also require lysine to function. Hair loss is reduced with lysine. Glycine is not only important for the digestive 338 and central nervous system but also help in collagen production. Proline a non-essential amino acid helps in the 339 production of collagen and cartilage, as well as maintaining the muscle tissues. SNAR and SSAR impact caused

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

[Note: (Hooker Fil.) ]

Figure 1:

**1**

(Hooker Fil.)

Figure 2: Table 1 :

**2**

mg/100 g dry matter

Figure 3: Table 2 :

341

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<sup>1</sup>(Hooker Fil.)

## 16 CONCLUSION

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$\pm$  0.01 and  $6.32 \pm 0.01$ ,  $7.62 \pm 0.1$ ,  $8.71 \pm 0.01$

compared to control value of  $9.80 \pm 0.1$  mg/100 g

respectively. Corresponding values for reducing

compounds of root were  $5.17 \pm 0.01$ ,  $6.18 \pm 0.01$ ,  $7.30$

$\pm 0.1$  for SNAR and  $5.42 \pm 0.02$ ,  $6.51 \pm 0.01$ ,  $7.59 \pm$

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

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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

XVII Protein Fat

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sue

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sion

I

D Stem  $3.10 \pm 0.1$ (39.2)

D

D

D

)

(

Root 5.41  
 $\pm$

Media Fibre Leaf 0.01(12.7)

Re- Stem 12.24

search 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 of

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[Note: Bcompounds of T]

Figure 4:

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**3**

and root

Figure 5: Table 3 :

**4**

(Hooker Fil.)

Figure 6: Table 4 :

**5**

D D D D )  
(

Figure 7: Table 5 :

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

Figure 8:



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346 [Calcium] , Calcium . (Ca) Leaf 26. 21±0.2(20.9) 27. 61±0.2(16.7) 30.96±0.2(6.6) 25.12±02(24.2) 27.66±0.1(16.5)  
347 29.59±0.2(10.7) 33.14±0.20)

348 [ Edition] , Edition

349 [ Edition] , Edition

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

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