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THE PHYTOCHEMICAL CONTENTS OF *Telferia occidentalis* (FLUTED PUMPKIN) AND THE EFFECT OF ITS AQUEOUS EXTRACT ON BLOOD GLUCOSE AND ENZYMATIC ANTIOXIDANT OF STRESS INDUCED RATS

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ABSTRACT
Background: Oxidative stress plays a major role in the development of chronic and degenerative diseases. The human body counteracts oxidative stress by producing antioxidants in situ or acquiring them through food.
Objective: To determine the phytochemical content of *T. occidentalis* and the effects of its aqueous extract on some parameters in stress induced Wistar rats.
Methods: The phytochemical content of the leaves was analysed using standard methods. Twenty adult male Wistar rats were divided into 4 groups of 5 rats each (3 test groups and 1 control group) and acclimatized for 3 days. Persistent stress was induced using water immersion induced stress from the 4th to the 17th day. The stressed control group received animal feed and water only, while other stressed groups received animal feed, water and graded dosages of *T.occidentalis* for 14 days. Blood samples were collected before and after for biochemical parameters. The data obtained from the study were analysed using IBM SPSS Statistics version 21.0. Two way Analysis of Variance was performed and means were separated using Duncan's new multiple range test. Significance level was set at $p<0.05$.
Results: Flavonoid content of *T.occidentalis* was 32.60±0.20mg/100g, while alkaloids and carotenoids recorded 496.49±1.37mg/100g and 28.79±0.38mg/100g, respectively. The group treated with *T.occidentalis* showed a significant ($p<0.05$) decrease in blood glucose when compared to the stress control. The group treated with the extract showed an increase ($p<0.05$) in catalase activity but a decrease ($p<0.05$) in malondialdehyde among the treatment groups of rats compared to the stress control group of rats after 14 day treatment period.
Conclusion: This study has proven that *Telferia occidentalis* is safe for consumption and is an effective tool in stress management.

Keywords: *Telferia occidentalis, Phytochemicals, Blood glucose, Stress, Antioxidants*

INTRODUCTION
Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, diabetes, cardiovascular and neurodegenerative diseases (1). Oxidative stress generates free radicals in the body that leads to the production of reactive oxygen species (2). They have been implicated to play crucial roles in mutagenesis, carcinogenesis and aging (3). Reactive oxygen species are produced naturally in cell following stress or respiration, they are also produced by radiation, bacterial and viral toxin, smoking, alcohol and psychological or emotional stress. Antioxidant acts as a defence mechanism that protect against deleterious effect of oxidative reaction produced by reactive oxygen species (ROS) in a biological system (4).
Several activities of man generate free radicals endogenously and exogenously (5), which sadly result in debilitating pathological diseases (6). These pathological states are caused by the imbalance between the free radicals produced and the capacity of antioxidants to neutralize the free radicals (3). Of interest is the fact that the biological system has been packaged to nullify the destructive effect of these radicals generated through the inherent enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione reductase (7). However, there are also synthetic antioxidants that are taken into the body to enhance the activities of the enzymatic ones but regrettably, have been indicted to possess health-related risk (7,8,9). This has led to the resurgence of the search for antioxidants of plant based origin that will be compatible with the biological system as well as boost the capacity of enzymatic ones with the aim of averting the health-related problems accredited to synthetics (10).
Phagocytes release free radicals to destroy invading pathogenic microbes as part of the body's defence mechanism against disease (11, 12). Reactive oxygen specie is also beneficial in their physiological roles in the function of a number of cellular signaling systems (13,14), therefore at low or moderate levels free radicals are vital to human health. Nevertheless, when produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) (15). In other words, oxidative stress results from an imbalance between formation and neutralization of free radicals.

In Nigeria, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals, antioxidant and essential amino acids (16). Phytochemicals found in vegetables are strong antioxidants and they reduce the risk of chronic disease by protecting against free-radical damage, detoxifying carcinogens, and influencing processes that alter the course of tumour cells (17, 18).

T. occidentalis leaf is often used as vegetable in the preparation of soups, while the seeds are ground into powder and used as soup thickener (19). According to Oboh (19), *T.occidentalis* has been shown to prevent against garlic –induced oxidative stress. Undoubtedly, past studies have shown that *T. occidentalis* possess antioxidant property (9). Studies have revealed that aqueous extract of *T. occidentalis* has been found to reduce blood glucose levels and also have anti diabetic effects in streptozotocin induced diabetic mice (20).

Therefore, the aim of this study was to determine the phytochemical content of fresh *T. occidentalis* leaves and to assess the effect of aqueous leaf extracts on the blood glucose and enzymatic antioxidant of stress induced Wistar rats.

MATERIALS AND METHODS

Experimental animals

Twenty (20) adult male Wistar rats of comparable sizes and weights ranging from 250-280g were obtained from the Department of Zoology and Environmental Science and housed at the rat cages, Biochemistry Department, both at the University of Nigeria Nsukka. The rats were divided into 4 groups and acclimatized for 3 days in well ventilated cages. During the period of acclimatization, the rats was given animal feed (Finisher pellets; Vital feeds Nigeria) and water *ad libitum*.

Collection of Plant Sample

Fresh leaves of *T.occidentalis* were obtained from Abakpa market at Abakaliki in Ebonyi State. The vegetable was identified at the Department of Food Science and Technology, Ebonyi State University.

Sample preparation

Fresh leaves of *T.occidentalis* were plucked, sorted, washed with clean running tap water, drained in a strainer for 10 minutes, and taken to the laboratory for phytochemical analysis. Two kilogram (2kg) of fresh leaves of *T. occidentalis* were also sorted, washed, drained and ground using a mortar and pestle. Two hundred (200) mls of distilled water was poured into the ground leaves, and the leaves were squeezed to obtain the extract. The aqueous extract was obtained by sieving the mixture using a muslin cloth, and the extract was administered to the rats daily according to their weight through oral gavage. The aqueous extract was stored in airtight containers and refrigerated.

Phytochemical Determination

Flavonoid Determination: The Baham and Kocipia (21) method was used.

Carotenoid Determination: The method described by Onyeka and Nwambaekwe (22).

Alkaloid Determination: Estimated according to the method by Harborne (23).

Induction of stress in rats

The water immersion stress induction method was used for inducing mechanical stress on the rats on a daily basis for 3 hours; this was done from day 4 to day 17. The animals were immobilized in a stress cage and then immersed in water bath at 23± 0.02oC for 3 hours up to the level of the xiphoid process of their sternum (14). All efforts were made to minimize animal sufferings. The animal was housed in groups of 5 animals per cage in a 12-12 hours (8am-8pm) light/dark cycle at 37ºC ± 0.02. They were fed animal feed and water available *ad libitum*. The rats were maintained in their cages except during exposure to stress for 3 hours daily after which they were returned to their cages.

Feeding Trial

The study was conducted for 17 days consisting of 3 days of acclimatization and14 days on stress induction and experimental diets. The groups are summarized below:

Group1- Received feed+ water + 400mg of f *T.occidentalis* leaf extract/ kg BW/day

Group 2 - Received feed + water + 600mg of *T. occidentalis* leaf extract/ kg BW/ day

Group 3 - Received feed + water + 800mg of *T. occidentalis* leaf extract/ kg BW/ day

Group 4 - Received feed + water alone/day

The wistar rats in group 1-3 received *T.occidentalis* leaf extract in graded substitution in addition, received feed and water while rats in group 4 received feed and water alone on a daily basis for 14 days feeding trial. Blood samples were collected for analysis of enzymatic antioxidant and blood glucose on 4th day (before stress induction), 7th day of treatment and 14th day of treatment.

Blood collection

Blood samples were collected from the rat through ocular puncture for biochemical indices determination (enzymatic antioxidant and blood glucose determination). Blood samples were put in non-heparinised sample bottles for biochemical parameters.

Fasting blood glucose determination

The fasting blood glucose of the rats was determined on days 4, 7 and 14. This was established using the one touch glucometer. The ON button on the glucose monitor was pressed and a code displayed (the displayed code tallied with the code on the container of the reagent strip). A glucose (reagent) strip from the container in the glucose monitoring kit was removed and inserted into the strip slot as far as possible on the glucose monitor. The blood drop symbol blinking indicated that the monitor is ready

for use. Blood sample was dropped on the reagent strip. The blood glucose level of the blood sample displayed in, mg glucose per decilitre of blood. The used strip was discarded after use and the monitor turned off. The blood sugar level of each rat was recorded.

Enzymatic antioxidant

Catalase activity: Determined by the method described by Aebi (24).

Malondialdehydeactivity: Determined according to method described by Paoletti *et al.* (25).

Gluthathione peroxidase activity: Measured according to Ellerby and Bredesen method (26).

Statistical analysis

The data obtained was analysed using IBM SPSS Statistics version 21.0 for mean, standard deviation and Standard error of mean. Mean values and standard deviations were reported, two way Analysis of variance (ANOVA) was performed and the mean separation was done by Duncan multiple range test ($p < 0.05$).

RESULTS

The phytochemical composition of fresh *Telferia occidentalis* leaves is shown in **Table 1**. The flavonoid content of the leaf sample was found to be 32.60 ± 0.20 mg/100g, alkaloid content of 496.49± 1.37 mg/100g and the carotenoid content was 28.79 ± 0.38 mg/100g.

Table 1: Phytochemical content (mg/100g) of *Telferia occidentalis* (Fluted pumpkin) leaves

VARIABLES	*Telferia occidentalis*
Flavonoid	32.60±0.20
Alkaloid	496.49±1.37
Carotenoids	28.79±0.38

Values are expressed as Mean ± SD (n=3)

In **Table 2**, the effect of the aqueous extracts of *Telferia occidentalis* on blood glucose level (mg/dl) in Wistar rats induced with persistent physical stress is shown. Rats fed 400 mg/kg had a decrease ($p>0.05$) in fasting blood glucose on Day 14 of treatment (70.60 ± 7.30 mg/dl) compared to the fasting blood glucose values on Day 7 of treatment (73.00±7.78 mg/dl). Also, the group of rats fed 600 mg/kg showed a significant decrease ($p< 0.05$) in their fasting blood glucose levels on the 14th day of treatment (77.4 ±11.93 mg/dl) compared to the values gotten on the 7th day of treatment (79.20± 11.90 mg/dl), while the stress control rats recorded a significant increase on Day 14 of treatment (145±17.72mg/dl) compared to the fasting blood glucose values gotten on Day 7 of treatment (95.8 ± 5.31 mg/dl).

More so, among the groups, it was observed that the fasting blood glucose in the stress control group (145± 17.72 mg/dl) increased ($p< 0.05$) rapidly when compared to group 1(70.60 ± 7.30 mg/dl), 2(77.4± 11.93 mg/dl), and 3(79.0 ± 8.0 mg/dl) respectively, fed with graded doses of aqueous extract of *Telferia occidentalis*.

Table 2: Effects of aqueous extracts of *Telferia occidentalis* (Fluted pumpkin) leaves on blood glucose level (mg/dl) in Wistar rats

GROUPS	BS	DAY 7 OF TREATMENT	DAY 14 OF TREATMENT	P -VALUE
GROUP 1	$59.20^{a}{}_{a}$±8.41	73.00 $^{a}{}_{a}$ ±7.78	$70.60^{a}{}_{a}$±7.30	0.036
GROUP 2	62.20 $^{a}{}_{a}$± 8.17	79.20 $^{a}{}_{a}$± 11.90	$77.4^{b}{}_{a}$±11.93	0.055
GROUP 3	58.20 $^{a}{}_{a}$± 7.26	$82.6^{b}{}_{a}$± 10.26	$79.0^{b}{}_{a}$ ±8.0	0.002
STRESS CONTROL	59.60 $^{a}{}_{a}$± 10.14	$95.8^{b}{}_{b}$± 5.31	$145^{c}{}_{b}$±17.72	0.000
P-VALUE	0.896	0.009	0.000	

Values are mean ± SD (n=5)

Mean values along the row bearing different alphabetical superscript indicates significant difference ($p<0.05$) within groups.

Mean values along the column bearing different alphabetical subscript indicates significant difference ($p<0.05$) among groups.

Key: BS- Before Stress

Table 3 shows the changes in Catalase (CAT) antioxidant enzymes in Wistar rats induced with persistent physical stress and fed with graded quantities of aqueous extracts of *Telferia occidentalis* (fluted pumpkin) leaf. It was observed that the CAT activities showed a non significant increase ($p<0.05$) within group 1 and group 2 on the 7th day of treatment (1.21± 7.30), (1.10± 0.03) compared to Day 14 of treatment (1.31 ± 0.06), (1.18± 0.05), respectively. Meanwhile, a significant increase ($p< 0.05$) was observed in the CAT activities in group 2 between Day 7 of treatment (1.15± 0.05) to Day 14 (1.18± 0.05).

It was also observed that the CAT activities on Day 7 of treatment, among group 1 (1.21± 7.30), group 2 (1.15± 0.05) and group 3 (1.10± 0.03) were not significantly different ($p<0.05$). However, the stress control (1.97± 0.13) was significantly different ($p<0.05$) in CAT activity when compared to other groups. Changes in CAT activities among the treatment groups at the end of the treatment period were significantly different ($p<0.05$) from the control group.

Table 3: Effects of aqueous extracts of *Telferia occidentalis* on Catalase (IU/L) parameter in Wister rats

GROUPS	BS	DAY 7 OF TREATMENT	DAY 14 OF TREATMENT	P -VALUE
GROUP 1	$1.22^{a}{}_{a}\pm 0.08$	$1.21^{a}{}_{a}\pm 7.30$	$1.31\ ^{a}{}_{a} \pm 0.06$	0.482
GROUP 2	$1.22\ ^{a}{}_{a}\pm 0.09$	$1.15^{b}{}_{a}\pm 0.05$	$1.24\ ^{a}{}_{a}\pm 0.07$	0.616
GROUP 3	$1.15\ ^{a}{}_{a}\pm 0.07$	$1.10^{a}{}_{a} \pm 0.03$	$1.18^{a}{}_{a}\pm 0.05$	0.571
STRESS CONTROL	$1.18^{a}{}_{a}\pm 0.06$	$1.97^{b}{}_{b}\pm 0.13$	$1.97^{b}{}_{b}\pm 0.13$	0.001
P-VALUE	0.888	0.000	0.000	

Values are mean ± SD (n=5)
Mean values along the row bearing different alphabetical superscript indicates significant difference ($p<0.05$) within groups.
Mean values along the column bearing different alphabetical subscript indicates significant difference ($p<0.05$) among groups.
Key:BS- Before Stress

Table 4 shows the changes in malondialdehyde (MDA) concentrations in Wistar rats induced with persistent physical stress and fed with graded quantities of aqueous extracts of *Telferia occidentalis* (fluted pumpkin) leaf. The result shows that non-significant decrease ($p> 0.05$) in malondialdehyde (MDA) concentrations was observed within each group treated with aqueous extracts of *T. occidentalis* leaves on the 14th day of treatment (end value)when compared to the 7th day of treatment.

More so, among the treatment group of rats, stress control (3.63± 0.42) recorded the highest value ($p<0.05$) of MDA concentration on Day 7 of treatment when compare to other treatment groups of rats that had 1.70 ± 0.28, 1.81± 0.20 and 1.82± 0.19 MDA concentrations, respectively. Also, after the treatment periods (Day 14), groups 1, 2 and 3 with MDA values of 1.41± 0.20, 1.51± 0.26and 1.35 ± 0.26, respectively were significantly lower ($p<0.05$) than the stress control group (3.83±0.67).

Table 4: Effects of aqueous extracts of *Telferiaoccidentalis*on Malondialdehyde (mg/dl) parameter in Wister albino rats

GROUPS	BS	DAY 7 OF TREATMENT	DAY 14 OF TREATMENT	P VALUE
GROUP 1	$1.45^{a}{}_{a}\pm 0.22$	$1.70\ ^{a}{}_{a} \pm 0.28$	$1.41^{a}{}_{a}\pm 0.20$	0.652
GROUP 2	$1.84\ ^{a}{}_{a}\pm 0.31$	$1.81\ ^{a}{}_{a}\pm 0.20$	$1.51^{a}{}_{a}\pm 0.26$	0.619
GROUP 3	$1.71\ ^{a}{}_{a}\pm 0.33$	$1.82^{a}{}_{a}\pm 0.19$	$1.35^{a}{}_{a} \pm 0.26$	0.449
STRESS CONTROL	$1.70^{a}{}_{a}\pm 0.37$	$3.63^{b}{}_{b}\pm 0.42$	$3.83^{b}{}_{b}\pm 0.67$	0.022
P-VALUE	0.849	0.000	0.000	

Values are mean ± SD (n=5)
Mean values along the row bearing different alphabetical superscript indicates significant difference ($p<0.05$) within groups.
Mean values along the column bearing different alphabetical subscript indicates significant difference ($p<0.05$) among groups.
Key: BS- Before Stress

The changes in the glutathione peroxidise enzymes (GPX) in Wistar rats induced with persistent physical stress and fed graded levels of *Telferia occidentalis* aqueous leaf extract is shown in **Table 5**. It was observed that group 1(400 mg/kg), group 2(600 mg/kg) and group 3(800mg/kg) had a non significant ($p>0.05$) decrease in the end values (Day 14 of treatment) when compared to the GPX level obtained on Day 7 of treatment. After the 17th day of treatment, it was observed that the GPX activities among the treatment groups showed that group 1 (10.45± 0.43 mg/dl) had the highest GPX level, followed closely by Group 2 (10.28± 0.28 mg/dl) and Group 3 (10.11± 0.32 mg/dl). However the GPX level were similar ($p>0.05$) among the treatment groups after the 14 days treatment period, but were significantly different ($p<0.05$) when compared to the stress control group

Table 5: Effects of aqueous extracts of *Telferia occidentalis* on Gluthathione Peroxidase (mg/dl) parameter in Wistar rats

GROUPS	BS	DAY 7 OF TREATMENT	DAY 14 OF TREATMENT	P VALUE
GROUP 1	$10.89^{a}{}_{a}\pm 0.51$	$11.02\ ^{a}{}_{a} \pm 0.43$	$10.45^{a}{}_{a}\pm 0.43$	0.670
GROUP 2	$10.71\ ^{a}{}_{a}\pm 0.52$	$10.79\ ^{a}{}_{a}\pm 0.34$	$10.28^{a}{}_{a}\pm 0.28$	0.626
GROUP 3	$10.72\ ^{a}{}_{a}\pm 0.38$	$10.45^{a}{}_{a}\pm 0.34$	$10.11^{a}{}_{a} \pm 0.32$	0.482
STRESS CONTROL	$10.51^{a}{}_{a}\pm 0.32$	$17.57^{b}{}_{b}\pm 0.48$	$16.82^{b}{}_{b}\pm 0.59$	0.000
P-VALUE	0.945	0.000	0.000	

Values are mean ± SD (n=5)
Mean values along the row bearing different alphabetical superscript indicates significant difference ($p<0.05$) within groups.
Mean values along the column bearing different alphabetical subscript indicates significant difference ($p<0.05$) among groups.
Key:BS- Before Stress

DISCUSSION

Fresh vegetables are important sources of nourishment and a vital ingredient in healthy and balanced diets. Phytochemical compositions of *Telferia occidentalis* leaves revealed very high presence of alkaloid. Alkaloids have medicinal usefulness as agents possessing anti-inflammatory, anti-carcinogenic, and bacterial activities (27); therefore the high level of alkaloid in this leaf sample, could suggest that the vegetable could be useful in these areas. The result further showed the presence of flavonoids and carotenoids in the fresh leaf sample. The presence of flavonoid and caroteniods in the leaf sample in Table 1 suggest that consumption of *Telferia occidentalis* is essential and has a free radical scavenging activity that protects the cells against oxidative stress.

Free radical formation and increased lipid peroxidation leads to damage of cells and also increased insulin resistance due to oxidative stress (28). There was significant ($p<0.05$) increase in the fasting blood glucose of stress control groups of rats observed in this study. This is as a result of increased insulin resistant arising from generation of excessive free radicals. The result is in agreement with what was stated by Brownlee (29), who found that oxidative stress plays a pivoted role in the development of diabetes and diabetic complications. However, there was a non significant ($p>0.05$) decrease in the fasting blood glucose observed among rats in group 1 and group 2. This shows that the *Telferiaoccidentalis* had the ability to hinder the effect of free radicals; this may be due to the presence of phytochemicals in the sample that had antioxidant properties.

Catalase as an antioxidant plays a central role in the decomposition of hydrogen peroxide to water and oxygen to prevent cells from oxidative damage (30). The result revealed a non significant ($p<0.05$) increase in catalase activity among the treatment groups of rats, after treatment period. However, the stress control had a significant decrease in catalase activities compared to the other treatment groups. Increased activity of catalase was achieved on the administration of *Telferia occidentalis*. This supports the finding of Mansour, and Mossa(31), who reported that supplementation of diet with *Telferia occidentalis* enhanced the activity of catalase. Also, agreed with the work done by (32) who stated that aqueous extract of *Telferia occidentalis* enhanced catalase activity in stress induced rats.

Measurement of lipid peroxidation is a gold marker of oxidative damage caused by reactive oxygen species, and the assessment of MDA is a reliable method to gain such determination (30). Highly reactive oxygen metabolites especially hydrogen peroxide acts on unsaturated fatty acids of phospholipid components of membranes to produce MDA, a lipid peroxidation product when in excess leads to cell death (31). Among the groups after the treatment period, the stress control group recorded a significant increase ($p<0.05$) in serum MDA level compared to other groups that had a decrease in serum MDA. The decrease in MDA may be attributed to decreased generation of reactive oxygen specie (ROS) caused by scavenging action of the antioxidant present in the leaf sample. The decreased lipid peroxidation in physically stressed rats treated with aqueous extract of *Telferia occidentalis* is in consonance with the fact that the vegetable is efficient in the management of oxidative stress.

The antioxidant enzyme, glutathione peroxidase (GPX) work together with catalase, to eliminate active oxygen species and prohibit the harmful effect of oxidant molecules on tissues and cells. Deviation in physiological concentrations of these enzymes may result in defect of body defence system and vulnerability to oxidative damage (33). Therefore the decreased activities of GPx may be due to overproduction of ROS, especially hydrogen peroxide. The increased MDA levels in this study supported this hypothesis since it is a biomarker of oxidative stress.

CONCLUSION

The study revealed some of the biologically active phytochemicals present in *T.occidentalis.* From the results of this present study, it was concluded that *Telferia occidentalis* reduced serum Malondialdehyde MDA level and increased some non enzymatic antioxidants (catalase) activity in rats. These may be due to the antioxidants effect of the phytochemicals present in the leaf sample which reduced the effect of reactive oxygen species on the cells and tissues. The study therefore suggests that *Telferia occidentalis* (fluted pumpkin) can improve the antioxidant status when consumed

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