Free radical scavenging activity of *Telfairia occidentalis* extracts and their effects on iron-sulphate induced lipid per oxidation

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Abstract: Non-enzymic natural antioxidants of plant origin are known to play a key role in inflammation linked with free radicals and oxidative stress. This study investigates the free radical scavenging activity of various *Telfairia occidentalis* leaves extracts in relation to their inhibitory effects on lipid peroxidation. The extracts markedly exhibited antioxidants activity especially the ethyl acetate (EE) and chloroform (CE) extract which scavenged 1,1-diphenyl-2-picryhydraxyl (DPPH) radicals significantly ($p<0.05$) by 76.34 and 76.62% respectively at 600µg/ml with minimum effects (53.34%) obtained with the methanol (ME) extract. Similarly the extracts scavenged iron/EDTA/H_2O_2 induced hydroxyl radical formation in a dose- dependent manner as the ME, EE and CE extracts scavenged the radicals at 300µg/ml by 40.55, 24.23 and 19.64% respectively. Interestingly, ME, EE and CE extracts inhibited iron-induced lipid per oxidation significantly ($P<0.05$) by 93.56, 76.24 and 70.54% respectively at 300µg/ml. Phytochemical analysis showed the presence of phenols and flavonoids at 2.58, 1.98, 3.24 and 0.840, 0.300, 0.400mg/g garlic acid and quercetin equivalence at 300 µg/ml concentrations respectively. Bioactive constituents of the leaves extracts shows antioxidants potentials, radical scavenging and inhibitory effects on lipid per oxidation which were highly expressed in methanol extract and may be a template for drug discovery.

Keywords: Antioxidant potential, extracts, Lipid per oxidation, radical scavenging, *Telfairia occidentalis.*

INTRODUCTION

Oxidative stress have been implicated in the pathogenesis of several inflammatory disorders as reactive oxygen species such as super oxides, hydroxyl radicals and other oxidants are unstable molecules that bring about catalytic chain reaction of lipid per oxidation and oxidation of protein, DNA and other macromolecules of membrane structures (Gulcin *et al.,* 2002b). Although the presence of oxygen is vital to cellular metabolism, however, increased levels of oxygen can lead to the production of deleterious reactive oxygen species (ROS) which are able to cause damage to the cell when produced uncontrolled. Free radicals are generally unstable and simply reactive some of which are ROS such as, super oxide ($0_2^{\cdot-}$), hydroxyl ($^{\cdot}OH$), peroxyl ($R0_2^{\cdot}$), alkoxyl ($RO^{\cdot}$) and hydroperoxyl ($H0_2^{\cdot}$), while nitrogen free radicals are nitric oxide ($NO^{\cdot}$) and nitrogen dioxide ($^{\cdot}NO_2$) (Freidovich, 1997; Iovine *et al.,*2008). They can be generated in a wide variety of chemical and biological systems including the formation of plastic, the ageing of paint, the combustion of fuel and in the human body (Halliwell and Gutteridge, 1999; Gulcin, 2006).

Although free radicals play some roles in certain cellular functions, their uncontrolled productions have been linked to cardiovascular diseases, arthritis, diabetes, chronic inflammatory disease, neurodegenerative disease, cancer, aging and several other chronic diseases because of their ability to introduce oxidative damage to bio molecules like lipids, deoxyribonucleic acid (DNA) and proteins leading to oxidative stress (Kourounakis *et al.,* 1999).

Oxidative stress is referred to the situation of serious imbalance between production of reactive species and antioxidant defense. It can results from diminished antioxidants like mutation affecting antioxidant defense enzyme such as copper and zinc super oxide dismutase enzyme (Cu, ZnSOD), manganese super oxide dismutase enzyme (MnSOD) and glutathione per oxidases (GSH PX). For example, many xenobiotics are metabolized by conjugation with GSH; high doses can deplete GSH and cause oxidative stress. Depletion of dietary antioxidants and other essential dietary constituents increased production of Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) can also leads to oxidative stress (Sies, 1997).

Lipid per oxidation which is also called the oxidative deterioration of lipids contributes to the development or generation and progression of many diseases such as inflammation, ischemia/reperfusion, artherosclerosis, Alzheimer's disease, aging, cancer etc (Lai *et al.,* 2001).

Lipid per oxidation is caused by the reaction between molecular oxygen and lipids with the subsequent

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formation of lipid hydroperoxides. When lipids are oxidized they are degraded especially in cell membranes where free radicals steals electrons from the lipids usually by several reactive oxygen species (Hydroxyl radicals, Hydrogen peroxides, etc.,) (Halliwell and Gutteridge, 1999; DeZwart *et al.,* 1999). It most often affects polyunsaturated fatty acids proceeding via a free radical chain reaction mechanism (Halliwell and Gutteridge, 1990). However to counteract these effects the human body synthesizes defense mechanisms such as the synthesis of enzymatic antioxidants which are capable of preventing oxidative damage while lately non-enzymatic anti oxidants of natural origin especially in plants are presently been advocated to possess great antioxidants potentials to checkmate the deleterious effects of free radicals in the body.

Medicinal plants have been known to be herbs, vegetables, trees or flowers of plants with biologically active chemical substances and other chemical compounds (Sofowora, 1996) which have curative properties. They serve as therapeutic alternatives, safer choices or effective treatment and it has been shown that the pharmacological activities of many plant secondary metabolites have been used as antioxidants, anticancer, anti malarial, antimicrobial, immuno modulatory etc (Halliwell, 2007; Palash, 2009).

Telfairia occidentalis also called fluted pumpkin from the family of C*ucurbitaceae* is an important leaf and seed vegetable nutritive than exotic vegetables and it is indigenous to southern Nigeria and grown in the forest zone of West and Central Africa (Nigeria, Ghana and Sierra Leone). It probably originated in South-East Nigeria and distributed by Igbos who has been cultivating this crop since time immemorial (Horsefall and Spiff, 2005). It is a popular medicinal plant known locally for its anti diabetic, anti plasmodial, hypo glyceamic, hypo lipidemic and anti bacterial properties with most of these been reported in various parts and different preparations of the plant in the laboratories (Aderibigbe *et al.,* 1999; Nwozo *et al.,* 2004; Okokon *et al.,* 2007; Adaramoye *et al.,* 2007). The leaf is of high nutritional medicinal and industrial values rich in protein (29%), fat (18%) and minerals and vitamins (20%). The nutritional value of pumpkin seeds is higher than that of the leaves while the seed also contains oil which is used for cooking (Akoroda, 1990; Horsefall and Spiff, 2005; Kayode *et al.,* 2009; Kayode *et al*., 2010). Although, the usage of Telfairia occidentalis leaves in folk medicine in Nigeria for treatment of some diseases have been suggested to be as a result of the antioxidant ability of its bioactive constituents. This study was therefore undertaken to evaluate the anti oxidant potential of the plant leaves extracts through the investigation of its free radical scavenging activity and their effects on Iron-Sulphate induced lipid per oxidation.

MATERIALS AND METHODS

Reagents

Reagents used in this study includes; methanol, follinciocalteu, gallic acid, quercetin, sodium carbonate, thiobarbituric acid, trichloroacetic acid, hydrogen peroxide, ethylacetate, chloroform, N-hexane, 2-deoxyribose and 1,1-dipheny-2-picrylhydrazyl radical (DPPH), all of which were of analytical grade and products of Sigma chemical company USA.

Plants material and extract preparation

The plants were purchased from a farmland at Gbongan and authenticated by the botany unit of the department of pure and applied biology of my institution. Fresh healthy leaves of the plant were air-dried at room temperature inside the Biochemistry laboratory for two weeks and powdered while 10 grams of the powdered leaves was extracted in 100ml of methanol in the cold for 72 hours. 30g of the methanol extract was further subjected to fractionation by liquid-liquid chromatography using, N-hexane, chloroform and ethyl acetate by the method of (Ogundipe *et al*., 2000) to obtain the chloroform and ethyl acetate extract.

Phytochemical study

Phenols and Flavonoids were measured spectrophotometrically based on the reduction of Folin Ciocalteu by the phenolic compounds as described by (Mc Donald *et al*., 2001), and the ability of Aluminium chloride to form complexes with flavonoids (Chang *et al*., 2002) using gallic acid and quercetin as standards with absorbance read at 760nm and 415nm respectively.

Antioxidants activity

Antioxidants activities studied in this work includes, free radical scavenging activities which was determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as described by the method of (Mensor *et al*., 2001) using ascorbic acid as standard based on the principle that when DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The change in color from deep violet to golden/light yellow is observed and measured at 518nm.

The hydroxyl radicals scavenging activity was performed using iron/EDTA/H_2O_2 complex following the procedure of (Halliwell *et al*., 1987). Hydroxyl radical generated by reaction of an iron-EDTA complex with H_2O_2 in the presence of ascorbic acid, attack the deoxyribose to form products that upon heating with Thiobarbituric acid at low pH yields a pink chromogen. Added hydroxy radical 'scavengers' compete for the hydroxyl radicals producing diminished chromogen formation. The percentage inhibition of ferrous sulphate- induced lipid per oxidation were determined using a modified thiobarbituric acid reactive substances (TBARS) assay to measure the

lipid peroxide formed using egg yolk as lipid-rich medium as described by (Ruberto *et al,* 2000).

STATISTICAL ANALYSIS

Statistical analysis were based on the Duncan`s experimental analysis with mean standard deviation of sample analysed using the student T test and ($P< 0.05$) i.e., 95% level of significance (Bliss, 1967)

RESULTS

The percentage DPPH radical scavenging activity where highly expressed in the chloroform extract followed by ethyl acetate and methanol extract in the order CE>EE>ME (table 1). All showed good DPPH radical scavenging activities ranging between 53.34-76.34 percent, respectively. Percentage hydroxyl radical scavenging effects between the extracts were in the order ME>EE>CE (table 2). Methanol extract showed better activity than the others and it is dose -dependent with ascorbic acid as standard. Extract also showed good percentage inhibition of lipid peroxidation induced by iron sulphate between 70.59-93.56 percent which convered above 50percent cellular protection (table 3), however the methanol extract showed better activity than the others in a dose –dependent manner. Total Phenolic content were reported as GAE from the standard curve (table 4). The Ethyl acetate extract had higher total phenolic content than others in the order EE>ME>CE, all expressed in a concentration-dependent manner. The total Flavonoids content in Quercetin Equivalent (QE) table 5, were also concentration- dependent expressed in the order ME>CE>EE.

DISCUSSION

Human health is the subject of priority in every life issues, however despite efforts to maintain good health, humans are continuously exposed to different kinds of chemicals such as food additive ,industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil (Stavric, 1994), while reports have shown that most of these chemicals induce a free radical-mediated lipid per oxidation leading to disruption of bio membranes and dysfunction of cells and tissues (Cho *et al*., 2003).

Also, oxidants, free radicals and reactive oxygen species are mostly derived from molecular oxygen known for their catalytic chain reactions initiating lipid per oxidation and cellular oxidative damage. The two most important oxygen-centered free radicals are super oxide and hydroxyl radicals which are derived from molecular oxygen under reducing conditions. Although they have been implicated in certain cell signaling processes however, because of their reactivity free radicals can participate in unwanted side reactions resulting in cell damage, cell injury and death which may contribute to many diseases (Brownlee, 2001; Pacher, 2007; Rajamani *et al*., 2011). Many forms of cancer are thought to be linked with free radical – DNA mutations which affects cell cycle and potentially lead to malignancy (Mukherjee *et al.,* 2004) while some forms of symptoms associated with ageing have been linked with free radical oxidation of cnellular components (Gruber *et al*., 2008).

Interestingly, free-radical have both beneficial and harmful effects, however the body has a number of mechanisms to minimize free-radical induced damage and to repair damage that occurs through enzymatic and non-enzymatic anti-oxidants (Meister and Anderson 1983; Meister, 1988; Rhodes, 2000). Also many researchers have suggested that the best way to neutralize free radical- mediated oxidative stress is to encourage or ingest diet rich in Phytochemical with antioxidants properties or to take dietary supplements of antioxidants (Salawu *et al*., 2006; Badmus *et al*., 2011; Adedosu *et al.,* 2013), which are widely found in nature, especially in plant products as extremely diversified group of chemicals (Materska, 2008).

Various studies are presently advocating the use of herbs and other plants products as having beneficial effects in the management, prevention and treatment of diseases associated with free radical -induced oxidative stress, tissue degeneration, inflammation and ageing due to the presence of certain bioactive components of these plants which are suspected to have antioxidant properties and are currently been investigated using various antioxidant indices. In this study, the free radical scavenging activities and the effects of three different extracts of *Telfairia occidentalis* leaves, Methanol extract (ME), Ethyl acetate extract (EE) and Chloroform extract (CE) on lipid per oxidation were investigated as a measure of their antioxidant status.

1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical found in numerous applications as a general antioxidants detector and a screening tool for detecting free radical scavenging activities of anti oxidants in various media (Karki *et al*., 2005; Werber *et al*., 2011). In this study, DPPH application as an index to determined the free radical scavenging effects of *Telfairia occidentallis* extracts as well as its antioxidant status shows that the percentage scavenging activities of DPPH in all the extracts increases significantly ($P\leq0.05$) in a concentration dependent manner (table. 1) while at the maximum concentration (600µg/ml), the methanol (ME), ethyl acetate (EE) and chloroform (CE) extracts scavenged DPPH radical by 53.34%, 76.34% and 76.62% respectively an indication that the extracts might possess hydrogen donating ability to act as an antioxidant by reducing the stable radical DPPH to the yellow colored diphenyl picrylhydrazine.

Table 1: Percentage DPPH radical scavenging activities of various extracts of *Telfairia occidentalis* leaves at different concentrations.

Concentrations (µg/ml)	Methanol extract (ME)	Ethyl acetate extract (EE)	Chloroform extract (CE)
100.00	28.89 %	31.36%	35.36%
200.00	32.74%	42.50%	59.95%
300.00	42.22%	56.26%	62.45%
400.00	43.10%	69.05%	69.05%
500.00	45.65%	71.11%	75.79%
600.00	53.34%	76.34%	76.62%

Table 2: Percentage Hydroxyl radical scavenging activities of various extracts of *Telfairia occidentalis* leaves at different concentrations.

Concentrations (µg/ml)	Methanol extract (ME)	Ethyl acetate extract (EE)	Chloroform extract (CE)
50.00	1.26%	3.17%	1.93%
100.00	17.33%	5.01%	10.09%
150.00	19.12%	9.20%	13.51%
200.00	35.92%	18.92%	15.24%
250.00	37.71%	20.04%	17.60%
300.00	40.55%	24.23%	19.64%

Table 3: Percentage inhibition of lipid peroxidation by various extracts of *Telfairia occidentalis* leaves at different concentrations.

Concentrations (µg/ml)	Methanol extract (ME)	Ethyl acetate extract (EE)	Chloroform extract (CE)
50.00	60.98%	31.52%	34.78%
100.00	72.64%	36.01%	41.36%
150.00	79.33%	39.34%	50.90%
200.00	8 1.96 %	54.32%	62.06%
250.00	91.59%	38.54%	65.60%
300.00	93.56%	76.24%	70.59%

Table 4: Total phenolic content of the various extracts of *Telfairia occidentalis* leaves in mg/g ±SD Gallic acid equivalent (GAE) at different concentrations.

Concentrations (µg/ml)	Methanol extract (ME)Mg/g±SD	Ethyl acetate extract (EE)Mg/g±SD	Chloroform extract (CE)Mg/g±SD
50.00	0.130±0.017	0.110±0.038	0.012±0.014
100.00	0.420±0.086	0.650±0.016	0.370±0.053
150.00	0.730±0.030	1.230±0.026	0.690±0.009
200.00	1.150±0.004	1.860±0.010	1.040±0.002
250.00	1.830±0.079	2.640±0.017	1.500±0.054
300.00	2.580±0.035	3.240±0.044	1.980±0.018

Table 5: Total flavonoids content of the various extracts of *Telfairia occidentalis* leaves in mg/g ± SD (Quercetin equivalent) QE at different concentrations.

Concentrations (µg/ml)	Methanol extract (ME) Mg/g ±SD	Ethyl acetate extract (EE) Mg/g ±SD	Chloroform extract (CE)Mg/g±SD
50.00	0.060±0.001	0.040±0.001	0.120±0.002
100.00	0.170±0.003	0.090±0.002	0.140±0.001
150.00	0.300±0.001	0.090±0.001	0.160±0.003
200.00	0.480±0.002	0.120±0.003	0.250±0.004
250.00	0.620±0.005	0.200±0.001	0.370±0.001
300.00	0.840±0.002	0.300±0.001	0.400±0.003

Mean Value ± Standard deviation of three replicates.
Gallic acid used as control and value with significant level ($p \leq 0.05$) .

The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge, 1999). Its attacks on cellular components results in pathological effects .Further examination of the ability of the extracts to act as hydroxyl radical scavenging agent using ferric EDTA with hydrogen peroxide and ascorbic acid shows that the extract exhibited strong scavenging effect of hydroxyl radical as they inhibited the degradation of 2-deoxy-2-ribose into fragments by hydroxyl radicals generated from the reacting medium. Highest scavenging activity obtained were 40.55%, 24.23% and 19.64% at 300µg/ml concentrations for the ME, EE and CE extract respectively (table.2). Thus this scavenging ability could inhibit lipid damage in systems susceptible to toxic effects of oxidants, radicals and reactive oxygen species (ROS).

Since the presence of uncontrolled amount of various oxidants, free radicals and ROS readily attacks biological membranes initiating a self-propagating chain reaction leading to destruction of membrane lipids. The effects of such lipid peroxidation reactions as well as their products have been found to be dangerous to cells and tissues viability (Mylonas, 1999). From this study, the lipid-rich media(egg yolk) undergoes rapid lipid peroxidation producing peroxides when incubated with ferrous sulphate, while the extracts showed inhibition of peroxidation effect in all concentration (table 3). The extracts, ME, EE and CE inhibited lipid peroxidation by 93.56%, 76.24% and 70.59% respectively at 300µg/ml concentrations. The highest anti-lipid peroxidation activity was obtained in the methanol extract. This property exhibited by the extracts may be considered important as the plant is consumed by some local tribes as edible vegetables.

Phenolic compounds may produce their beneficial effect by scavenging free radicals (Halliwell, 2008) as studies have shown that consumption of food with high phenolic compounds decreases cardiovascular diseases, have high antioxidants and free radical scavenging potentials (Marja *et al*., 1999; Gibson *et al*., 2008). The expression of phenolic compounds in the three extracts varied and at 300µg/ml concentration, 2.580, 3.240 and 1.980 mg/g gallic acid equivalence (GAE) respectively of total phenols were obtained (table 4). The presence of phenols in the extracts are pointer to their possible antioxidants activities.

Also, flavonoids are the most common group of poly phenolic compounds in the human diet and are found ubiquitously in plants. Flavonols, the original bio flavonoids such as quercetin, are also found ubiquitously but in lesser quantities. The widespread availability and relatively low toxicity compared to other secondary metabolites from plant origin means that a good number of animals including humans ingest a significant amount of these compounds in their diet (Donald and Cristobal 2012). In this study, considerable amount of flavonoids were obtained in these extract ranging between 0.300-0.840 mg/g Quercetin Equivalence (QE) respectively (table 5). Flavonoids possess anti oxidant activity and have become popular because of their health benefits as various functions such as anti-allergic, anti-cancer, anti oxidants, anti- inflammatory, anti-atherosclerosis and anti –viral have been linked to flavonoids (Barjesteh van Waalwijk *et al*., 2007; Donald and Cristobal 2012).

CONCLUSION

The bioactive constituents of the leaves extracts mainly polyphenols as shown from this study correlated with the free radicals scavenging and their inhibitory effects on lipid peroxidation especially the methanol extract from the results of this study, hence *Telfairia occidentalis* may be considered of great importance nutritionally and in medicinal/therapeutic uses as expressed by the plants antioxidant status which is one of the defense mechanisms against free radical-induced oxidative stress .The plant may therefore be a source of chemo preventive agent for prevention of diseases linked with free radicals, oxidants and oxidative stress.

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