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OXIDATIVE DAMAGE AND DIETARY ANTIOXIDANTS: THE ROLES OF EXTRACT AND FRACTIONS OF *SOLANIUM AETHIOPICUM* LEAVES

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ABSTRACT

Oxidative damage is a factor in human diseases. In this study, effects of extract and fractions of *Solanium aethiopicum* leaves on certain liver function indices, anti oxidative and anti-lipid peroxidation potentials were investigated. Phenols, flavonoids, antioxidative potentials, enzyme assays were determined spectrophotometrically using standardized methods. Results showed rats fed methanol extract (ME) at 50, 100, 150 and 200 mg/kg body weight for 21 days showed significant ($P<0.05$) decreases in liver Malondialdehyde and cholesterol concentrations by 2.530±0.050, 1.780±0.020, 1.670±0.030, 1.480±0.040 mg/ml protein and 69.290±2.660, 46.980±2.500, 39.030±4.820, 34.650±1.660 mg/dl respectively. At these concentrations, ME exhibited significant ($p<0.05$) decreases in serum Gamma glutamyl transferase (GGT) activity by 3.810± 0.010, 2.380 ± 0.010, 2.020 ± 0.010 and 1.190±0.010 IU/L compared with controls (3.830 ± 0.020 IU/L). Phenols and flavonoids of ME, Ethyl acetate fraction (EF) and chloroform fraction (CF) were 3.000±0.003, 2.004±0.001, 1.722±0.005Mg/g Gallic and 6.900±0.065, 7.200±0.001, 8.400±0.075Mg/g Quercetin equivalence respectively. At 300.00µg/ml, extracts and fractions scavenged hydroxyl and DPPH radicals by 46.150±0.008%, 20.190±0.002%, 17.200±0.022% and 83.770±0.001%, 97.110±0.053%, 72.490±0.002 % respectively and inhibited lipid peroxidation by 80.360±0.029%, 84.390±0.003%, and 84.040±0.008%. Results inferred that plant contains bioactive agents with anti-oxidative, anti-lipid peroxidation and hepatoprotective properties

Keywords: *Solanium aethiopicum*, antioxidative, anti-lipidperoxidation, hepatoprotective.

INTRODUCTION

Free radicals have been implicated in the etiology of large number of major known diseases. Oxygen free radicals or more generally, reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. The reactive oxygen species are potentially reactive derivatives of oxygen which are continuously generated inside the human body (Valko *et al*., 2006). They are well recognized for playing a dual role as both deleterious and beneficial species, however the harmful effects of free radicals when overproduced or due to deficiency of enzymatic or non-enzymatic antioxidants causes potential cellular oxidative or biological damage termed oxidative stress and nitrosative stress (Farber, 1994; Valko *et al*., 2006; Kovacic and Jacintho, 2001; Ridnour *et al*., 2005). Oxidative stress is therefore considered to be substantial, if not crucial in the initiation and development of many current conditions and diseases including: inflammation, autoimmune diseases, cataract, cancer, Parkinsons disease, arteriosclerosis and aging (Astley, 2003). Conditions such as heart diseases, neurodegenerative diseases and cancer have been linked with oxidative stress (Astley, 2003). This theory is supported by increasing evidence suggesting/indicating that cellular oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants opposes this and lowers the risk of these diseases (Atoui *et al*., 2005).

Interestingly, the antioxidants both enzymatic and non-enzymatic are known to mediate their effects by directly reacting with reactive oxygen species (ROS), quenching them and or chelating the catalytic metal ions thus offering protection for cell membranes (Robak and Marcinkiewicz, 1995). Recently, there has been a great interest in antioxidants and how they work, especially the

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dietary antioxidants which are major components of plants (Harman, 1956; Halliwell and Gutteridge, 1997). However the use of medicinal plants in developing countries as rich sources of dietary antioxidants is currently under investigation in herbal medicines across African Countries as they have been reported to be safe and without any adverse effects compared with synthetic drugs (Bhatarai *et al*., 2010). Thus a systematic search for useful bioactivities from medicinal plants is now considered to be a national approach in herbal medicine and even drug research since the medicinal values of plants are based on their components phytochemicals which have been found to produce a definite physiological action on the human body (Hill, 1952; Oke and Hamburger, 2002).

Solanum aethiopicum is a fruiting plant of the genus *Solanum* mainly found in Asia and Tropical Africa. It is also known as Mock tomato, Garden egg and Ethiopian Nightshade. The leaves are eaten as a leaf vegetable and are actually more nutritious than the fruit. The highly variable fruit of the plant is eaten both raw and cooked and is becoming more popular as a cultivated crop. The fruits and leaves per 100g edible portion have been shown to contain primarily, water, energy, protein, fat, carbohydrates, fibre, calcium, phosphorus, iron, β-carotene and ascorbic acid (Burkill, 2000). Various uses of the plant in indigenous medicine range from weight reduction to treatment of various ailments including asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joints, gastro-esophageal reflux disease, constipation and dyspepsia (Bello *et al*., 2005). Although, few studies has proven and support the folkloric use of the plants in local foods and medicinal preparations, however this study was aimed at investigating the possible therapeutic values of the leaves extract and fractions in relation to oxidative damage and its possible medicinal value as food and drug supplements.

MATERIALS AND METHODS

Materials

Some of the laboratory materials used in this study include weighing balance, PH meter, micropipette, spectrophotometer and glass wares. Reagents includes: methanol, follinciocalteu, gallicacid, quercetin, sodium carbonate, thiobarbituric acid, trichloroacetic acid, 2-deoxyribose, 1,1-dipheny-2-picrylhydrazyl radical (DPPH). All other reagents were of the best pure analytical grade available and were obtained from Sigma Chemical Company USA.

Experimental Animals

Thirty Wistar strain male rats weighing between 140-160 g were obtained from breeders at Ilorin, Nigeria and were allowed free access to food (commercial laboratory rodent diet or feed pellet) and water ad -libitum throughout the experimental period. My institute and national guide for the care and use of laboratory animals were followed in handling these animals throughout the experimental process. For the experimental treatment, the animals were randomly divided into five groups (A,B,C,D and E) containing six rats in each group. The control group A, were fed with normal pellets while group B, C, D and E received the plant methanol extract at 50, 100, 150 and 200 mg/kg body weight respectively, for a period of 21 days alongside the normal feed pellet and water.

Plant materials

Solanium aethiopicum leaves were purchased from a local main market in Oshogbo, Nigeria and were identified and authenticated with voucher herbarium number LHO 284 issued by Dr. Ogunkunle, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso Nigeria.

Methods

Fresh healthy leaves of the plant were air-dried at room temperature and powdered. 10 grams of the powdered leaves was extracted in 100ml of methanol in the cold for 72 hours. The solvent was concentrated at temperature below 40^0C and the resulting methanol extract was subjected to liquid-liquid chromatographic separation technique to obtain the corresponding chloroform and ethyl acetate fractions of the extract (Ogundipe *et al*., 2000). Total Flavonoids content was determined using Aluminium chloride Colorimetric method (Chang et al., 2002). The calibration curve was made by preparing quercetin solutions at different concentrations in methanol. Total phenol was determined by Folin ciocalteu reagent (McDonald *et al*., 2001). The phenol values were expressed in terms of Gallic acid equivalent. Free radical scavenging activities were determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Mensor *et al*., 2001), while the hydroxyl radicals scavenging effect and the inhibition of lipid peroxidation were determined in-vitro using deoxyribose assay method (Halliwell , 1987) and the reactions of peroxides with Thiobarbituric acid (Ruberto *et al*., 2000) . In the in-vivo experiment, after 21 days of treatment, the animals were sacrificed by cervical dislocation, the blood was collected directly from the heart into plain and well- labelled sample bottles and were centrifuged at 4000 rpm for 5 minutes to obtain the serum. The liver was excised and its homogenate was prepared using 50Mm Tris-Hcl, 1.15Kcl phosphate buffer. Serum gamma glutamyl transferase (GGT) activity was determined by the method of Szasz (1969). Tissue total protein, Malondi aldehyde (MDA) and cholesterol concentrations were determined by the Biuret method (Burtis, 1999), reaction of MDA with Thiobarbituric acid (De-Zwart, 1999) and the quantitation of the cholesterol released from the enzyme hydrolysis and oxidation of cholesteryl esters (Richmond, 1988).

STATISTICAL ANALYSIS

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

Interest in medicinal plants have been revived in recent times because of their efficacy in providing cost effective therapy to several diseases without any adverse side effect especially when compared with synthetic drugs (Borek , 1999) . They possess secondary metabolites known as phyto chemicals which have been found to be responsible for the antioxidant properties of many green plants (Xianquan *et al*., 2005).

RESULTS AND DISCUSSION

Table 1. Malondialdehyde (MDA) concentrations in the liver of various treated groups with methanol extract of *Solanum aethiopicum* leaves for 21days.

Treatment group	Malondialdehyde (MDA) concentration (Mg/ml protein±SD)
A Control	2.790 ± 0.040
B (50 Mg/kg body weight)	2.530 ± 0.050
C (100Mg/kg body weight)	1.780 ± 0.020
D (150 Mg /kg body weight)	1.670 ± 0.030
E (200 Mg/kg body weight)	1.480 ± 0.040

·Values are given as mean and standard deviation of six determinations.

Table 2. Cholesterol concentrations in the liver of various treated groups with methanol extract of *Solanum aethiopicum* leaves for 21days.

Treatment group	Cholesterol concentrations (Mg/dl±SD)
A Control	91.780 ± 6.740
B (50Mg/kg body weight)	69.290 ±2.660
C (100Mg/kg body weight)	46.980 ± 2.500
D (150 Mg/kg body weight)	39.030 ± 4.820
E (2OOMg/kg body weight)	34.65 ± 1.660

·Values are given as mean and standard deviation of six determinations.

Table 3. Serum Gamma Glutamyl Transferase activity of various treated groups with methanol extract of *Solanum aethiopicum* leaves for 21days.

Treatment group	Gamma GlutamylTransferase activity (IU/L ± SD)
A Control	3.830 ± 0.020
B (50 Mg/kg body weight)	3.810 ± 0.010
C (100 Mg/kg body weight)	2.380 ± 0.010
D (150 Mg/kg body weight)	2.020 ± 0.010
E (200Mg/kg body weight)	1.190 ± 0.010

·Values are given as mean and standard deviation of six determinations.

Table 4. Total protein concentration in the liver of various treated groups with methanol extract of *Solanum aethiopicum* leaves for 21days.

Treatment group	Total protein concentration (g/dl ±SD)
A Control	2.250 ± 0.175
B (50 Mg/kg body weight)	2.770 ± 0.660
C (100 Mg/kg body weight)	3.510 ± 0.660
D (150 Mg/kg body weight)	3.570 ± 0.640
E (200 Mg/kg body weight)	3.960 ± 0.366

·Values are given as mean and standard deviation of six determinations.

Table 5. Total phenolic content of the methanol extract (ME), ethyl acetate fraction (EF) and chloroform fraction (CF) of *Solanum aethiopicum* leaves (mg/g. Gallic Acid Equivalent GAE).

Concentration (mg/ml)	Total phenolic content (mg/g ±SD) Gallic acid Equivalent (ME)	Total phenolic content (mg/g ±SD) Gallic acid Equivalent (EF)	Total phenolic content (mg/g ± SD)Gallic acid Equivalent (CF)
0.025	1.222± 0.040	0.777 ± 0.007	0.622 ± 0.001
0.050	1.811±0.002	1.166 ± 0.002	0.922 ± 0.001
0.075	2.311 ±0.001	1.466 ± 0.008	1.000 ± 0.002
0.100	2.577 ± 0.001	1.722 ±0.001	1.422 ± 0.006
0.125	2.711 ±0.002	1.866 ±0.007	1.555 ± 0.001
0.150	3.000 ±0.003	2.044 ±0.001	1.722 ± 0.005

Mean Value ± Standard deviation of three replicates.

Table 6. Total flavonoids content of the methanol extract (ME), ethyl acetate fraction (EF) and chloroform fraction (CF) of *Solanum aethiopicum* leaves (mg/g. Quercetin Equivalent QE).

Concentration (mg/ml)	Total flavonoids content (mg/g ±SD) Quercetin Equivalent (ME)	Total flavonoids content (mg/g ±SD) Quercetin Equivalent (EF)	Total flavonoids content (mg/g ±SD) Quercetin Equivalent (CF)
0.025	1.400 ±0.001	1.000 ±0.001	1.200 ±0.001
0.050	3.200 ±0.004	1.640± 0.001	3.000 ± 0.003
0.075	4.000 ±0.002	1.980 ±0.002	5.600 ±0.004
0.100	4.100 ±0.002	3.040 ± 0.001	6.800 ± 0.001
0.125	6.600 ±0.003	4.010 ±0.004	7.610 ±0.001
0.150	6.900 ±0.065	7.200±0.001	8.400 ±0.075

Mean Value ± Standard deviation of three replicates.

Table 7. Percentage DPPH radical scavenging activity of the methanol extract (ME), ethyl acetate fraction (EF) and chloroform fraction (CF) of *Solanum aethiopicum* leaves.

Concentration (µg/ml)	(%)DPPH radical Scavenging activity (ME)	(%)DPPH radical Scavenging activity (EF)	(%)DPPH radical Scavenging activity (CF)
50.00	50.890 ± 0.023	72.760 ± 0.060	46.490 ± 0.675
100.00	52.540 ± 0.075	82.120 ± 0.032	47.730 ± 0.028
150.00	65.060 ± 0.004	89.680 ± 0.024	50.340 ± 0.010
200.00	74.000 ± 0.060	93.370 ± 0.027	62.450 ± 0.011
250.00	81.570 ± 0.011	95.460 ± 0.065	67.400 ± 0.029
300.00	83.770 ± 0.001	97.110 ± 0.055	72.490 ± 0.002

Mean Value ± Standard deviation of three replicates.

Table 8. Percentage Hydroxyl radical (OH) scavenging activity of the methanol extract (ME), ethyl acetate fraction (EF) and chloroform fraction (CF) of *Solanum aethiopicum* leaves.

Concentration (µg/ml)	(%) Hydroxylradical Scavenging activity (ME)	(%) Hydroxylradical Scavenging activity (EF)	(%) Hydroxylradical Scavenging activity (CF)
50.00	11.880 ± 0.005	1.680 ± 0.001	8.060 ± 0.001
100.00	19.800 ± 0.022	6.490 ± 0.004	10.540 ± 0.004
150.00	35.870 ± 0.009	11.290 ± 0.005	11.940 ± 0.003
200.00	43.040 ± 0.007	14.900 ± 0.002	13.010 ± 0.001
250.00	43.580 ± 0.008	15.630 ± 0.085	13.660 ± 0.007
300.00	46.150 ± 0.008	20.190 ± 0.002	17.200 ± 0.022

Mean Value ± Standard deviation of three replicates.

Table 9. Percentage Inhibition of lipid peroxidation by the methanol extract (ME), ethyl acetate fraction (EF) and chloroform fraction (CF) of *Solanum aethiopicum* leaves.

Concentration µg/ml)	(%)Inhibition of lipid peroxidation (ME)	(%)Inhibition of lipid peroxidation (EF)	(%)Inhibition of lipid peroxidation (CF)
50.00	32.690 ± 0.016	53.170 ± 0.033	53.90 0 ± 0.048
100.00	55.280 ± 0.055	58.450 ± 0.010	60.400 ± 0.026
150.00	64.870 ± 0.055	62.320 ± 0.013	71.980 ± 0.009
200.00	72.530 ± 0.031	70.540 ± 0.014	73.640 ± 0.001
250.00	77.980 ± 0.050	80.520 ± 0.025	80.610 ± 0.075
300.00	80.360 ± 0.029	84.390 ± 0.003	84.040 ± 0.008

Mean Value ± Standard deviation of three replicates.

These medicinal plants provide a template for the development of safer natural antioxidants from their extracts and fractions which can be used in place of synthetic antioxidants (Pulido, 2000). Interestingly, indigenous vegetables play important roles in human diets although consumed innocently in most African Countries without any information on their dietary and health benefits as they were merely reported to be more nutritive, healing or relieve and less expensive compared with exotic drugs, hence it is pertinent to explore the bioactive components of these herbs as commonly consumed as food or as medicinal herbs or concoctions and in various preparations in some west African natives.

Solanium aethiopicum, commonly called "Igbagba" in the South-Western Nigeria is a household medicinal plant with edible leaves and fruits. However,with the higher burden of free radicals causing imbalance in homeostatic phenomena between oxidants and antioxidants in the body (Tiwari, 2000). This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases such as atheroslerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and parkinsonism (Noguchi and Niki, 1999). These free radicals as products of various chemicals and environmental toxicants causes several cellular damages in different organs of the body through their metabolic activation and also initiate the peroxidation of membrane poly-unsaturated fatty acids which results in the generation of reactive oxygen species (ROS) and finally cell necrosis (Recknagel, 1989).

In the present study, it was observed that the methanol extract of *Solanium aethiopicum* leaves administered to rats for twenty one days at 50, 100, 150 and 200 mg/kg body weight respectively lowered the liver malondialdehyde concentrations significantly ($P<0.05$) in a concentration dependent manner compared with the controls (Table 1), the corresponding values are 2.790±0.040, 2.530±0.050, 1.780±0.020, 1.670±0.030 and 1.480±0.040 mg/ml protein respectively. Evidence of lipid peroxidation by increased levels of malondialdehyde is one of the primary means by which researcher have associated oxidative processes with an overall of decrease of cellular functions. The increased MDA level is also an index used to identify free radicals-induced injuries (Janero, 1990).

However, the ability of the extract to reduce MDA level in a concentration-dependent manner in rats (Table 1) may be an indication of its ability to protect membranes against the deleterious effect of oxidants and free radicals thus maintaining membrane integrity via its reduction and probably as an antioxidant.

Hypercholesterolemia and hyper triglyceridemia are risk factor for predicting coronary heart disease (Rosamond *et al*., 2007). In a similar manner, the methanol extract showed a decreased in the level of cholesterol concentration in the liver in a dose-dependent manner (Table 2). The observed decreased in cholesterol level may result from the increase in HDL level or from reduced liver fatty acid synthesis. The behaviour exhibited by the extract may be attributed to its ability to alter cholesterol metabolism (Villacorta *et al*., 2007), suggestive of an antioxidant property capable of protecting very low-density lipoprotein from oxidation which facilitates uptake by the liver and hence its removal from the plasma.

It has been observed that certain serum enzyme levels could be indicative of liver function and that the elevation of the levels of these enzymes could be suggestive of an early liver damage (Wooton, 1964; Varley, 1969). The results obtained in Table 3, shows that the extract exhibited a significant decrease in the serum gama glutamyl tranferase (GGT) activity in a dose-dependent manner. This then suggests the positive modulatory role of the extracts for possible hepatoprotective ability (Dajas *et al*., 2005). Results of the liver total protein concentration (Table 4) show that the extracts elicit a general increases in the treated groups, indicative of the possible ability to increase or alter protein synthesis.

Furthermore, the *in-vitro antioxidant* analysis of the methanol extract (ME), ethyl acetate fraction (EF) and

chloroform fraction (CF) of the leaves showed that the total phenolic content (Gallic acid equivalent (GAE)) and the total flavonoids contents (Quercetin equivalent (QE)) of these extracts were expressed maximally in a concentration-dependent manner (Table 5 and 6). A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Rice-evans *et al*., 1995; Marja, 1999; Sugihara *et al*., 1999). Flavonoids are phenolic compounds present in plants and conserved in plant derived foods while the concepts of its ability to provide positive health effects is currently gaining acceptance as dietary antioxidant (Earlejman *et al*., 2004). Thus, the results of the *in-vivo* studies are further correlated with the presence of flavonoids and phenolic compounds in the plant extracts and its fractions from the in-vitro study.

Hydroxyl radicals and other reactive oxygen species (ROS) are constantly generated in every cell during its normal life under aerobic conditions and it is well recognized that several physiological and pathological processes are linked to oxidative stress (Halliwell and Gutteridge, 1997). In this regard, our investigation shows that the methanol extract (ME), ethyl acetate fraction (EF) and the chloroform fraction (CF) of *Solanium aethiopicum* leaves, scavenged 1-1-di phenyl-2picrylhydrazyl radical (DPPH) and Hydroxyl (OH) radicals maximally by 83.770, 97.110, 72.490 and 46.150, 20.190, 17.200 %, respectively (Table 7 and 8). Property exhibited by the extracts and fractions are linked to their possible bioactive contents mostly phenolic compounds with potent antioxidant property.

Lipid peroxidation is generally thought to be the major mechanism of bio membrane injury promoted by ROS, free radicals and other oxidants which are strongly affected by transition metals those catalyses many of the reactions involved in this process (Halliwell and Gutteridge, 1997). It is also known that superoxide and derived forms of activated oxygen besides mediating lipid peroxidation are capable of oxidizing protein thiol groups, causing enzyme deactivation, initiating generation of more reactive and distinctive species (Halliwell and Gutteridge, 1984) oxidizing nucleic acids.

The present study revealed that the extracts and fractions conferred 50% protection at maximum concentrations on lipid peroxidation induced by $FeSO_4$ as they inhibited lipid peroxidation by 80.360, 84.390 and 84.040 % respectively (Table 9).

Interestingly, it has been proposed that accumulation of oxidants and oxidative damage and mutations in mitochondria DNA play a pivotal role in most diseases especially the degenerative diseases and in the aging processes. However properties exhibited by the extract and fractions of *Solanium aethiopicum* leaves are anti oxidative, anti lipid peroxidation, anti cholesterolemic and hepato protectives which are suggestive of maintaining cellular and membrane integrity from invading oxidants and radicals.

CONCLUSION

In conclusion, properties exhibited by *Solanium aethiopicum* extracts are indicative of its positive roles as dietary antioxidants locally with certain bioactive agents which may be exploited as chemotherapeutic agent in the management, control and or prevention of numerous pathological effects or indices associated with oxidants, oxidative damage and aging and as potential food supplement.

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