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Comparative Study on the Antioxidant Activities of Ethyl acetate and Methanolic Leaf Extracts of *Celosia argentea*

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Abstract: The present study was carried out to compare the secondary metabolites, *in vitro* and *in vivo* antioxidant activities as well as the safety of ethyl acetate and methanolic extracts of *Celosia argentea* leaves in cadmium-induced oxidative stress in rats. The secondary metabolite screening was done by standard methods while the *in vitro* antioxidant activity of the extract was evaluated using ammonium thiocyanate, reducing power and diphenyl picryl hydrazyl (DPPH) radical scavenging models. In the *in-vivo* antioxidant and toxicological studies, thirty rats (*Rattus novergicus*) weighing 137.05 ± 5.84g were completely randomized into six groups (A-F) of five animals each. Animals in group A received orally 0.5ml of distilled water for 7 days while those in groups B, C, D, E and F received same volume corresponding to 8 mg/kg body weight (bw) of cadmium, in addition to simultaneous administration of distilled water, 100 mg/kg b.w of ascorbic acid, 100, 200 and 400 mg/kg b.w. of the extract respectively. Biochemical indices of *in vivo* antioxidant activities and toxicity were evaluated in the animals after the treatment period. The ethyl acetate extract of *C. argentea* contained saponins (1.67%), tannins (0.65%), cardenolide and dienolides (1.20%) and phenolics (0.42%) whereas the methanolic extract contained saponins (3.20%), tannins (0.65%), cardenolide and dienolides (0.006%) and phenolics (5.72%). Reducing sugar, steroids, and glycosides were only detected in the ethylacetate extract. The ethyl acetate extract and ascorbic acid, at 50 mg/ml, inhibited linoleic acid oxidation by 51.00 and 24.2% respectively whereas the methanolic extract produced 51.01% inhibition. Ethylacetate extract at 10, 50 and 100 mg/ml produced reducing power of 0.116, 0.092 and 0.127 nm whereas the methanolic extract produced 0.131, 0.185 and 0.183nm when compared with ascorbic acid that gave 0.092, 0.089 and 0.107 nm. The 100 µg/ml of both the ethyl acetate and methanolic extracts scavenged 82% and 30% respectively of the DPPH radical as against 65% in ascorbic acid. Both the extracts attenuated the cadmium chloride treatment related reduction in the activities of superoxide dismutase, catalase, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate transaminase as well as the levels of uric acid, albumin, total and conjugated bilirubin, total protein and the Cd elevated levels of malondialdehyde in the serum and tissues of the animals in a manner similar to that of the ascorbic acid treated animals and the non-Cd treated animals administered distilled water; with the ethyl acetate producing a better result. The totality of the results conferred antioxidant activity on the ethyl acetate extract and methanolic extract by the phenolic components of the extracts via induction of the antioxidant enzymes and scavenging of free radical. The extracts also reversed cadmium induced changes in the biomarkers of liver damage.

KEYWORDS: *Celosia argentea*, Amaranthaceae, Ethyl acetate extract, methanolic extract, Antioxidant, Lipid peroxidation, Cadmium, Oxidative stress

1.0 Introduction

Food provides not only essential nutrients needed for life, but also serve as sources of bioactive compounds needed for disease prevention and health promotion. Previous epidemiologic studies have consistently shown that diet plays a crucial role in the prevention of chronic diseases (Willet, 1994; Temple, 2000). Consumption of fruits, vegetables and grains have equally been associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataract and age related functional decline (Willet, 1994 and 1995; Temple, 2000). The enhancement of good health, and delay in the onset, or even cure of

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these diseases by fruits, vegetables and grains is largely attributed to their antioxidant property which they do by terminating free radical chain reactions, scavenging free radical intermediates, and inhibiting other oxidation reactions arising from reactive oxygen and nitrogen species (ROS and RNS).

Celosia argentea belongs to the family Amaranthaceae and it is locally known as *Sokoyokoto*, *Erimonu* and *Fara-alayatu* respectively in Yoruba, Igbo and Hausa speaking areas of Nigeria respectively. It is an herbaceous vegetable plant grown in Nigeria. The red "soko" has red pigments on the leaves which differentiate it from the green variety, green "soko". The stem of the red soko is about 60 cm tall, typically simple and erect. The leaves which may be about 8 cm long and 4 cm broad are alternate, glabrous and petiolate. The chaffy, terminal inflorescence is about 12 cm long in mature plants. The flowers which may be variously coloured are typically red in some instances (Gill, 1992). *Celosia argentea* has been reported to be useful in hepatitis, mouth sores, excessive menstruation and leucorrhoea and as an aphrodisiac (Dalimartha, 2007; Koh *et al.*, 2009. The whole plant can be used to treat dysentery and dysuria while a poultice can be applied over broken bones; the seed has also been shown to have antipyretic, anti-inflammatory and hypotensive properties (Dalimartha, 2007) and the flowers are hemostatic, diuretic and tonic (Imaoka *et al*., 1994). A previous study showed that the aqueous extract of *Celosia argentea* leaf attenuated cadmium-induced oxidative stress in rats (Malomo *et al*., 2011). In this present study, the comparison between the antioxidant activities of ethyl acetate extract and methanolic extract as well as their safety in rats was carried out to complement already existing information in the open scientific literature.

2.0 Materials and Methods

2.1 Plant material and authentication

The vegetable was harvested from a farmland at Edidi, Kwara State, Nigeria in May, 2010. It was authenticated at the Forestry Research Institute of Nigeria, Ibadan, Nigeria and a visual specimen was deposited at the Herbarium Unit of the Institute under FHI 108372.

2.2 Chemicals and assay kits

Ascorbic acid, Para-nitrophenyl phosphate, cadmium sulphate and adrenaline were products of Sigma Aldrich St. Louis, MO, USA, BDH Chemicals Ltd Poole England, MERCK Chemical Co. Germany and LABORATE Chemical Co, India respectively. The assay kits for albumin, bilirubin, uric acid, aspartate transaminase (AST) and alanine transaminase (ALT) were products of Randox Laboratories Limited, UK. All other chemicals used were of analytical grade obtained from Sigma Aldrich, St Louis, USA.

2.3 Animals

A total of forty five albino rats *(Rattus novergicus*) of both sexes weighing 137.05 ± 5.84 g were used in this study. The animals were obtained from Natashco Animal House, Basin. Ilorin, and were acclimatized to housing conditions temperature 22 ± 3◦C, relative humidity 40-45%, and about 12h light/ 12h dark cycle. Water and pellets (Bendel Feed, Ewu, Edo State, Nigeria) were provided for the animals *ad libitum*.

2.4 Preparation of ethyl acetate and methanolic extracts

Celosia argentea leaves were cut from the stem, air-dried at room temperature for 7 days and pulverized using an eluted blender. The powder (1000 g) was extracted each in ethyl acetate and metahnol for 48 hours, filtered and concentrated using rotary evaporator maintained at 40ºC. The ethyl acetate yielded 82 g (corresponding to a % yield of 8.2%) while the methanolic extract produced 60 g (corresponding to a % yield of 6.0%).

2.5 Screening of secondary metabolites in ethyl acetate and methanolic extracts

The screening of ethyl acetate and methanolic extracts of *Celosia argentea* leaves for the presence of alkaloids, steroids, anthraquinones,

cardenolides and dienolides, phenolics, flavonoids, saponins, tannins, triterpenes, reducing sugars and glycosides were according to the methods described by Harborne (1973) and Trease and Evans (1989). The concentrations of the detected secondary metabolites were done as described for saponins (Brunner, 1984), cardenolides, dienolides and phenolics (El-Olemy *et al*., 1994) and tannins (Van-Burden and Robinson, 1981).

2.6 In vitro antioxidant activity of the ethyl acetate and methanolic extracts

The models of ammonium thiocyanate, reducing power and 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging were used to evaluate the extracts of *C. argentea* leaves for *in-vitro* antioxidant activity as described here under:

2.6.1 Ammonium thiocyanate assay

The ammonium thiocyanate assay was carried out on the ethyl acetate and methanolic extracts of *C. argentea* leaves using the method described by Lee *et al* (2002). Briefly, 500 µl of each of the extract, 200 µl of diluted linoleic acid (25 mg/ml in 99% ethanol) and 400 µl of 50 mM phosphate buffer (pH 7.4) were mixed and incubated at 40ºC for 15 minutes. An aliquot (100 µl) from the reaction mixture was added to reaction solution containing 3 ml of 70% ethanol, 100 µl of ammonium thiocyanate (300 mg/ml distilled water) and 100 µl of ferrous chloride (2.45 mg/ml in 3.5% hydrochloric acid). This resulting mixture was then incubated at room temperature for 3 minutes. Absorbance was read at 500 nm. Linoleic acid emulsion without the ethyl acetate and/or methanolic extract served as the control. Ascorbic acid was used as a reference antioxidant. Inhibition of linoleic acid emulsion was calculated using the following expression:

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample} \times 100}{Abs_{control}}$$

2.6.2 Reducing power assays

Reducing power assay of the ethyl acetate and methanolic extracts of *C. argentea* leaves was done as described by Yildrim *et al* (2001). A known volume (0.5 ml) of the separate extract was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated at 50ºC for 30 minutes; thereafter, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The supernatant(s) were collected and added to 2.5ml of distilled water and 0.5 ml of 0.1% ferric chloride. The reference compound used in the model was ascorbic acid. The absorbance of the reaction mixtures was read at 700 nm. An increase in the absorbance of the mixture indicates an enhanced reducing power.

2.6.3 Qualitative and Quantitative Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging Activity

Appropriately diluted stock solutions of the ethyl acetate and methanolic extracts of *C. argentea* leaves were spotted using capillary tubes on pre-coated silica gel thin layer chromatographic (TLC) plates which was activated in an oven at 105ºC for 35 minutes. The plates were developed in solvent systems of different polarities; polar, medium polar and non-polar. Hexane: ethyl acetate (1:1); ethyl acetate: hexane (3:1); methanol (100%, 4ml) and ethyl acetate: methanol (3:2) in order to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the colour changes (yellow on purple background) were noted. The antioxidant activities of the ethyl acetate and methanolic extracts of *C. argentea* were determined using the DPPH free radical scavenging assay by the method of Blois (1958) with minor modifications. The test samples were prepared from stock solution by dilution with 95% methanol. Two drops of DPPH solution was added to each of these test tubes containing *C. argentea* extracts. The positive control was set up using ascorbic acid. The absorbance was read at 517 nm. IC_{50} values (where 50% of the radicals were scavenged by the test sample) was intrapolated from the reference inhibition curve. Percentage scavenging activity of DPPH free

radical for each of the extracts was calculated using the following expression:

% DPPH radical scavenging = [1-As/Ac] x 100

where Ac = absorbance of control and A_S= absorbance of sample

2.6.4 Grouping of the animals

Forty five rats were completely randomized into nine treatment groups (A-I) of five animals each as follows: Group A (control) received distilled water, Group B received 8 mg/kg body weight (b.w.) of cadmium (Cd), Groups C, D, E, F, G, H and I received 8 mg /kg b.w of Cd each and 100 mg/kg b.w. of ascorbic acid, 100, 200 and 400 mg/kg b.w. of the ethyl acetate extract as well as 100, 200 and 400 mg/kg b.w. of methanolic extract of *C. argentea* leaves respectively. The administration of Cd and extract was done orally for seven days.

2.7 Preparation of serum and tissue supernatants

The animals were sacrificed after seven days of treatment using diethyl ether. Blood samples were collected from each animal by cutting the jugular veins. The liver, kidneys and brains of each animal were collected after dissection. The blood samples were centrifuged at 3000 rpm for 10 minutes and the resulting sera were used for biochemical analyses. Weighed organs were homogenized in ice cold 0.25 M sucrose solution (1.5w/v) and centrifuged at 5000 rpm for 15 minutes. The supernatants obtained were stored frozen overnight and used for biochemical analyses.

2.8 Determination of biochemical parameters

Standard methods were employed for the determination of biochemical parameters as described for total protein, total and conjugated bilirubin, albumin, uric acid, catalase, superoxide dismutase, alkaline phosphatase, alanine and aspartate transaminases, gamma glutamyl transferase and lipid peroxidation (Gornall *et al.,* 1949; Jendrassik and Groff, 1938; Doumas *et al*., 1971; Fossati *et al*., 1980; Beers and Sizers, 1952; Misra and Fridovich, 1972; Wright *et al*., 1972; Reitman and Frankel, 1957; Szasz, 1969 and Satoh, 1978).

2.9 Statistical Analysis

Results were expressed as mean of five replicates ± SEM. Data obtained were subjected to one-way Analysis of Variance (ANOVA) using SPSS Software except otherwise stated. Statement of statistical significance was based on $P<0.05$. Bars with alphabets superscripts are different from the control and other test values.

3.0 Results

The screening of secondary metabolites in ethyl acetate extract of *Celosia argentea* var *cristata* leaves (CAVCL) revealed the presence of saponins, reducing sugar, steroids, glycosides, cardenolides and dienolides and phenolics whereas saponins, phenolics, tannins, cardenolides and dienolides were detected in the methanolic extract (Table 1).

The ammonium thiocyanate assay of the ethyl acetate extract revealed a 51% inhibition of linoleic acid oxidation which was higher than the 24.20% of the reference antioxidant, ascorbic acid (Figure 1a). In a similar manner, the methanolic extract produced 51.01% inhibition of the linoleic acid oxidation (Figure 1b). The inhibition of linoleic acid by both the extracts was essentially the same.

The reducing power of both the ethyl acetate extract and methanolic extract of *C. argentea* leaves increased ($P<0.05$) dose dependently and were significantly higher than that of the reference drug, ascorbic acid (Figures 2a and 2b). However, the reducing power of the methanolic extract was higher at all the concentrations than those of the ethyl acetate extract (Figures 2a and 2b).

There were dose dependent increases in the DPPH radical scavenging abilities of both the ethyl acetate and methanolic extracts in a manner similar to that of the reference drug, ascorbic acid (Figures 3a and 3b). However, the increases produced by the ethyl acetate extract was significantly higher than that of the ascorbic

acid whereas the increases by the methanolic extract was significantly lower than that of the ascorbic acid (Figures 3a and 3b). The fifty percent inhibitory concentrations (IC_{50}) for the ethyl acetate extract was 53 µg/ml while that of the methanolic extract was 45.04 µg/ml as against that of ascorbic acid which was 44 µg/ml.

The administration of catalase significantly decreased the activities of both superoxide dismutase and catalase in the liver of the animals (Table 2). These decreases in the activities of these enzymes by cadmium in the present study were significantly reversed by all the doses of the extract and in all case significantly higher than the rats that received distilled water only (Table 2). In all the treatments, the ethyl acetate extract produced more profound reversal of the activities of the enzymes than the methanolic extract (Table 2). The extracts also dose dependently reduced the cadmium related increase in the levels of malondialdehyde (Table 2). Again, the reductions were more pronounced in the cadmium treated animals that were administered the ethyl acetate extract (Table 2).

The reduction in the levels of uric acid, albumin, total protein, total and conjugated bilirubin by cadmium alone were reversed by the solvent extracts in a manner similar to the cadmium treated adnimals that were administered ascorbic acid and the animals that received only distilled water (Table 3). The reversal by the ethyl acetate on all the biomolecules was more profound than thos of the methanolic extract.

Administration of cadmium to the animals significantly decreased the activitties of alkaline phosphatase, alanine and aspartate transferases and gamma glutamyl transferase in the liver of the animals (Tables 4 and 5). These decreases were accompanied with corresponding significant increases in the serum enzymes (Tables 4 and 5). Furthermore, treatment of the animals with ethyl acetate and methanolic extracts signifcantly reversed these cadmium related changes in the activities of the enzymes and in most cases, the activity of the enzymes in the extract treated animals were significantly higher than those of the control animals (Tables 4 and 5).

Table 1: Some secondary metabolites of ethyl acetate extract of *Celosia argentea* leaves

	Ethyl acetate Extract	Methanolic Extract
Secondary Metabolites	**Concentration (%)**	**Concentration (%)**
Saponins	1.67±0.01	3.20±0.10
Tannins	Not detected	0.65±0.01
Alkaloids	Not detected	Not detected
Flavonoids	Not detected	Not detected
Cardenolides and dienolides	1.20±0.05	0.006±0.001
Phenolics	0.40±0.007	5.72±0.01
Steroids	Detected	Not detected
Reducing Sugar	Detected	Not detected
Anthraquinones	Not detected	Not Detected
Triterpenes	Not Detected	Not Detected

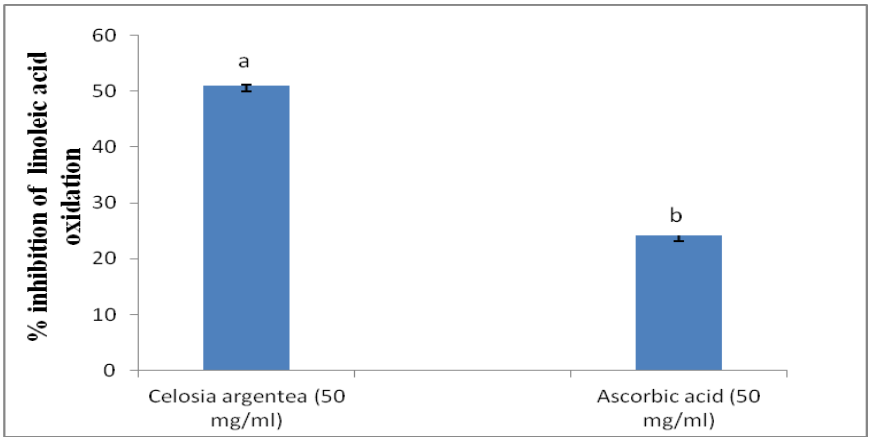

Figure 1a: Inhibition of linoleic acid oxidation by ethyl acetate extract of *Celosia argentea* leaves and ascorbic acid

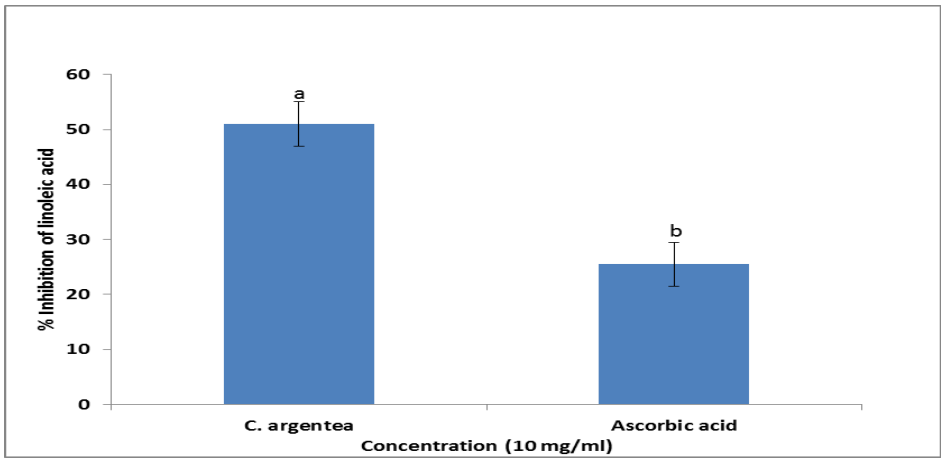

Figure 1b: Inhibition of linoleic acid oxidation by methanolic extract of *Celosia argentea* leaves and ascorbic acid

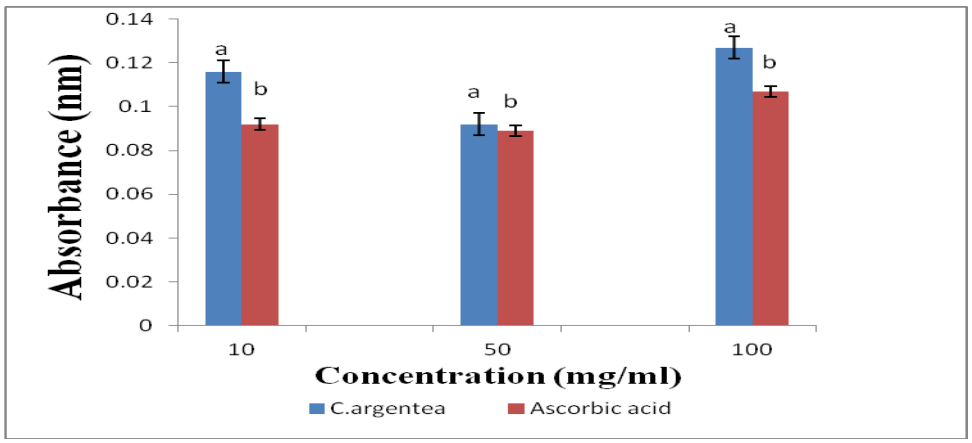

Figure 2a: Reducing power activity of ethyl acetate extract of *Celosia argentea* leaves and ascorbic acid

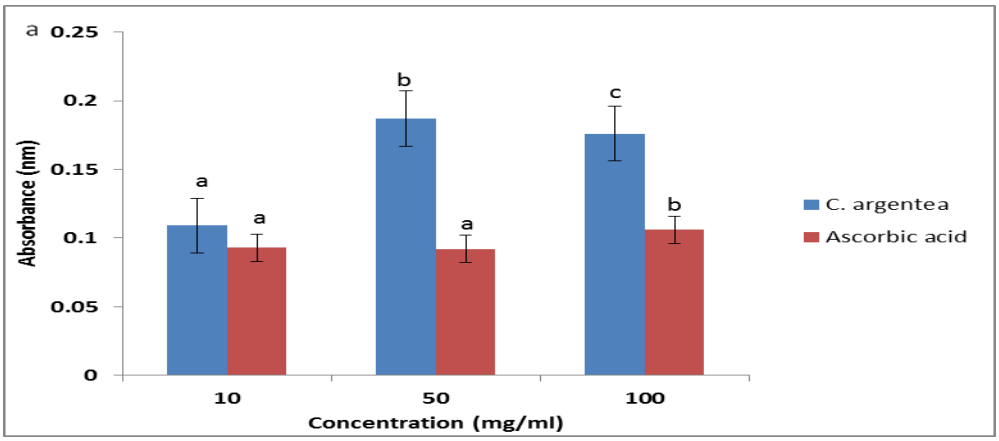

Figure 2b: Reducing power activity of methanolic extract of *Celosia argentea* leaves and ascorbic acid

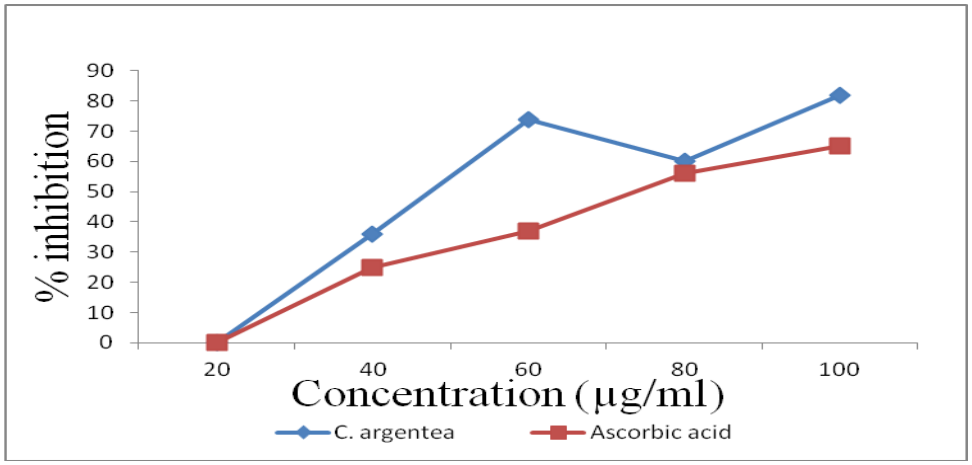

Figure 3a: Diphenyl picryl hydrazyl (DPPH) scavenging activity of ethyl acetate extract of *Celosia argentea* leaves

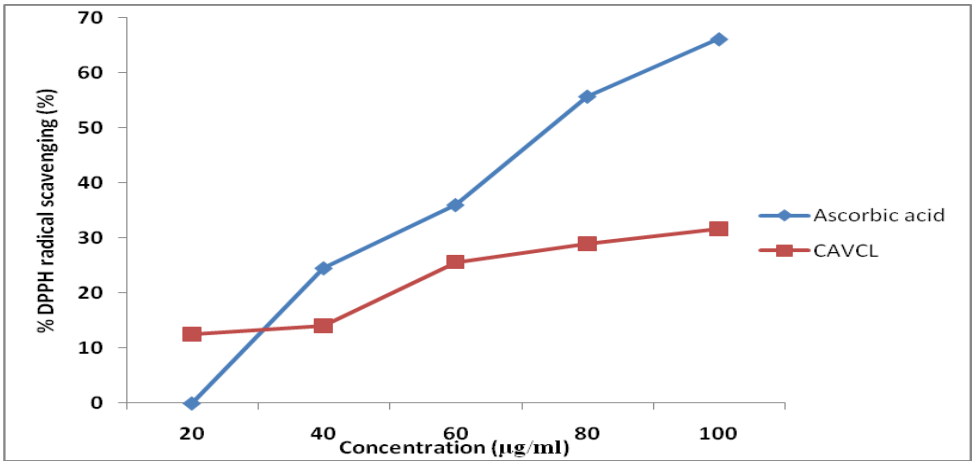

Figure 3b: Diphenyl picryl hydrazyl (DPPH) scavenging activity of methanolic extract of *Celosia argentea* leaves

Table 2: Effects of administration of ethyl acetate and methanolic extracts of *celosia argentea* leaves on the activities of superoxide dismutase, catalase and the levels of lipid peroxidation in the liver of cadmium treated rats

Treatment groups	Liver		
	Superoxide Dismutase (Unit/g)	Catalase (µmol/min/mg)	Lipid peroxidation (nmol/g tissue)
Control	0.62 ± 0.03^{a}	1.78 ± 0.08^{a}	1.45 ± 0.15^{a}
8mg/kg b.w Cd	0.06 ± 0.00^{b}	0.43 ± 0.08^{b}	4.31 ± 0.26^{b}
Cd+100mg/kg b.w. Ascorbic acid	0.59 ± 0.04^{a}	1.44 ± 0.04^{c}	2.02 ± 0.07^{c}
Cd+100mg/kg b.w. of ethyl acetate extract	0.62 ± 0.01^{a}	1.74 ± 0.05^{a}	0.78 ± 0.01^{d}
Cd+200mg/kg b.w. of ethyl acetate extract	4.38 ± 0.14^{c}	1.77 ± 0.02^{a}	1.46 ± 0.14^{a}
Cd+400mg/kg b.w. of ethyl acetate extract	4.98 ± 0.07^{d}	3.96 ± 0.02^{d}	1.44 ± 0.07^{a}
Cd+100mg/kg b.w. of methanolic extract	1.51 ± 0.36^{e}	2.94 ± 0.05^{e}	3.22 ± 0.16^{e}
Cd+200mg/kg b.w. of methanolic extract	1.65 ± 0.08^{e}	1.67 ± 0.15^{f}	2.00 ± 0.08^{c}
Cd+400mg/kg b.w. of methanolic extract	1.42 ± 0.10^{f}	3.35 ± 0.06^{g}	1.46 ± 0.08^{a}

Values are means ± SEM; Values with superscripts different from the control are significantly different (P<0.05)

Table 3: Effects of administration of ethyl acetate and methanolic extracts of *C. argentea* leaves on liver and serum alkaline phosphate activity of cadmium treated rats

	Alkaline Phosphatase Activity (U/I)	
Group	Liver	Serum
Control	72.44 ± 6.50^{a}	24.15 ± 1.01^{a}
8mg/kg b.w of Cd	32.35 ± 5.57^{b}	54.87 ± 4.20^{b}
Cd+100mg/kg b.w. Ascorbic acid	77.29 ± 7.24^{a}	24.00 ± 1.85^{a}
Cd+100mg/kg b.w. of ethyl acetate extract	71.99 ± 4.48^{a}	24.52 ± 1.57^{a}
Cd+200mg/kg b.w. of ethyl acetate extract	164.45 ± 7.95^{c}	32.86 ± 4.00^{c}
Cd+400mg/kg b.w. of ethyl acetate extract	233.42 ± 2.07^{d}	37.94 ± 1.03^{c}
Cd+100mg/kg b.w. of methanolic extract	50.21 ± 6.00^{e}	35.94 ± 2.05^{c}
Cd+200mg/kg b.w. of methanolic extract	161.88 ± 10.21^{c}	40.89 ± 4.02^{d}
Cd+400mg/kg b.w. of methanolic extract	121.30 ± 7.08^{f}	33.64 ± 3.00^{c}

Values are means ± SEM; Values with superscripts different from the control are significantly different (P<0.05)

Table 4: Effects of administration of ethyl acetate and methanolic extracts of *C. argentea* leaves on the levels of extracellular antioxidants of cadmium treated rats

Treatment groups	Uric acid (µmol/l)	Albumin (g/l)	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)	Total Protein (mg/ml)
Control	7.46±0.39^{a}	8.03±0.47^{a}	6.42±1.11^{a}	4.35±0.35^{a}	178.0±2.27^{a}
8mg/kg b.w Cd	4.98±0.60^{b}	3.61±0.31^{b}	4.01±0.21^{a}	2.25 ±0.55^{a}	161.3±1.44^{b}
Cd+100mg/kg b.w. Ascorbic acid	6.59±0.22^{c}	6.38±0.65^{b}	7.28±0.36^{b}	4.32±0.17^{a}	159.1±1.69 ^{bc}
Cd+100mg/kg b.w. of ethyl acetate extract	6.42±0.16^{bc}	6.43±0.56^{b}	7.10±0.49^{bc}	4.18±0.24^{a}	168.1±5.55^{ab}
Cd+200mg/kg b.w. of ethyl acetate extract	6.91±0.60^{c}	7.51±0.80^{ac}	6.73±0.08^{abc}	4.41±0.43^{a}	176.4±0.98^{a}
Cd+400mg/kg b.w. of ethyl acetate extract	6.64±0.29^{c}	7.09±0.40^{bc}	6.91±0.35^{bc}	4.28±0.19^{a}	180.6±2.48^{a}
Cd+100mg/kg b.w. of methanolic extract	6.95±0.27^{c}	7.15±0.38^{b}	6.21±0.06^{b}	4.10±0.16^{b}	134.28±0.36^{d}
Cd+200mg/kg b.w. of methanolic extract	7.37±0.39^{d}	6.82±0.18^{b}	6.44±0.42^{b}	4.94±0.06^{b}	167.01±0.22^{ab}
Cd+400mg/kg b.w. of methanolic extract	7.11±0.30^{d}	7.55±0.32^{b}	6.27±0.30^{b}	4.80±0.17^{b}	177.21±2.36^{a}

Values are means ± SEM; Values with superscripts different from the control are significantly different (P<0.05)

Table 5: Effects of administration of ethyl acetate and methanolic extracts of *C. argentea* leaves on selected enzymes in the liver and serum of cadmium treated rats

Treatment groups	Alanine transaminase (U/I)		Aspartate transaminase (U/I)		Gamma glutamyl transferase (nm/min/mg protein)	
	Liver	Serum	Liver	Serum	Liver	Serum
Control	3131.82 ± 106.65^{a}	132.48± 17.17^{ab}	1722.26 ± 17.74^{a}	81.92 ± 8.32^{a}	2.60 ± 0.07^{a}	1.80 ± 0.00^{a}
8mg/kg b.w of Cd	1916.02 ± 83.53^{b}	146.88 ± 9.95^{b}	1011.40 ± 19.08^{b}	118.49 ± 10.09^{b}	3.20 ± 0.06^{b}	1.21 ± 0.20^{b}
Cd+100mg/kg b.w. of Ascorbic acid	3126.16 ± 52.05^{a}	62.40 ± 5.88^{c}	1576.98 ± 35.40^{ab}	58.81 ± 3.65^{c}	2.61 ± 0.06^{a}	1.32 ± 0.14^{bc}
Cd+100mg/kg b.w. of ethyl acetate extract	2997.7 ± 147.96^{ab}	316.80 ± 13.15^{d}	1432.76 ± 24.59^{c}	64.10 ± 3.38^{cd}	2.31 ± 0.20^{a}	1.52 ± 0.11^{d}
Cd+200mg/kg b.w. of ethyl acetate extract	3119.5 ± 56.82^{a}	130.60 ± 10.00^{a}	1702.96 ± 20.31^{a}	82.20 ± 6.22^{a}	2.54 ± 0.08^{a}	1.61 ± 0.15^{de}
Cd+400mg/kg b.w. of ethyl acetate extract	3168.74 ± 52.77^{b}	132.80 ± 7.00^{a}	1716.00 ± 14.21^{a}	85.99 ± 4.62^{a}	2.69 ± 0.04^{a}	1.82 ± 0.04^{a}
Cd+100mg/kg b.w. of methanolic extract	2541.21 ± 44.00^{c}	135.94 ± 7.05^{a}	1221.70 ± 10.21^{d}	55.99 ± 3.02^{c}	2.91 ± 0.05^{c}	1.73 ± 0.08^{a}
Cd+200mg/kg b.w. of methanolic extract	266.88 ± 34.21^{d}	140.89 ± 3.02^{a}	1431.04 ± 12.12^{c}	65.21 ± 2.62^{cd}	3.43 ± 0.06^{d}	1.80 ± 0.06^{a}
Cd+400mg/kg b.w. of methanolic extract	2689.30 ± 30.08^{d}	133.64 ± 6.00^{b}	1404.00 ± 11.96^{c}	66.99 ± 4.62^{cd}	3.03 ± 0.02^{cd}	1.82 ± 0.03^{a}

Values are means ± SEM; Values with superscripts different from the control are significantly different (P<0.05)

4.0 Discussion

Previous epidemiologic studies have consistently shown that diet plays a crucial role in the prevention of chronic diseases (Willet, 1994; Temple, 2000). Secondary metabolites including phenolics, tannins and carotenoids have been implicated to function as small low molecular weight antioxidants (Halliwell and Whiteman, 2004). Secondary metabolites from medicinal plants such as detected in the present study function as antioxidants through direct antiradical chain-breaking of the free radical propagation and interaction with transition metals (Halliwell, 2004). Phenolic compounds function as high level antioxidants because they possess the ability to adsorb and neutralize free radicals as well as quench reactive oxygen species. A strong relationship between total phenolic content and antioxidant activity in fruits, vegetables, grain products and plants of ethno pharmacological application has been reported (Dorman *et al*., 2003). Therefore, the *in vitro* antioxidant effects of both the ethylacetate extract and methanolic extracts obtained from *C. argentea* leaves in this study may be due to the detectable quantities of saponins and phenolic compounds commonly present in the two extracts.

The ammonium thiocyanate assay of the ethyl acetate extract and methanolic extract of *C. argentea* revealed that the ethyl acetate extract and the methanolic extract produced 51.00% and 51.01% inhibition of linoleic acid, which were higher than the 24.20% of the reference antioxidant, ascorbic acid (Figure 1). The percentage of the inhibition of linoleic acid by both the extracts of ethyl acetate and methanol might be attributed to any of and/or the combination of reduction of hydroperoxides, inactivation of free radicals and complexation with metal ions. Earlier studies have attributed antioxidant activity of plant extracts to phenolics and flavonoids (Lolliger, 1991; Omale and Okafor, 2008). Therefore, the antioxidant activity of the extracts may not be unconnected with the presence of phenolics in the extracts.

The reducing ability of a compound generally depends on the presence of reductants (Yokozawa *et al*., 1998), which exhibit antioxidant activity by breaking the free radical chain through donation of a hydrogen atom (Gordon, 1990). The enhanced reducing power observed in the extracts in the present study may be accounted for by the phenolic content. A strong relationship between total phenolic content and reducing activity in fruits and vegetable has been established (Yildrim *et al*., 2001). The result also suggests that polyphenolic components within the ethyl acetate extract and the methanolic extracts of *C. argentea* play an important role in scavenging the free radicals. The results show that both the ethyl acetate extract and the methanolic extract obtained from *C. argentea* leaves in the present study confers electron donor ability on the extracts, and could react with free radicals, convert them to more stable products and terminate radical chain reaction. It is worthy of note that the reducing power of the separate extracts was more than that of the reference drug, ascorbic acid.

DPPH radical is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extracts (Koleva *et al*., 2002; Bhuiyan *et al*., 2009; Tara *et al*., 2012). The assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. Scavenging of antioxidants involve either the transfer an electron or a hydrogen atom to DPPH•, thus neutralizing its free radical character (Pan *et al*., 2008). When a solution of DPPH is mixed with a substance of H donor, it gets reduced into non radical state (Arokiaraj *et al*., 2008). Therefore, the dose dependent increase in the DPPH radical scavenging of both the ethyl acetate extract and methanolic extract in the present study consistently emphasizes the antioxidant activity of the solvent extracts from *C. argentea* leaves. It is worthy of note that the ethyl acetate extract produced the most profound DPPH radical scavenging activity even when compared with the reference drug, ascorbic acid in the present study.

Certain heavy metals including cadmium have been reported to generate reactive oxygen species (Iszard *et al*., 1995). The role of antioxidants is to neutralize the excess of free radicals, to protect the cells from toxic effects and to contribute to disease prevention (Douglas *et al*., 2000).

Catalases are haem containing proteins that protect the cell from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen (Oyedemi and Afolayan, 2011). Depletion of this enzyme may enhance cellular damage caused by accumulation of superoxide and hydrogen peroxides (Pari *et al*., 2007). The reduction in the activity of catalase following the administration of cadmium alone may also suggest oxidative stress. The attenuation of hepatic catalase activities by both the solvent extracts buttresses the antioxidant potential of the ethyl acetate and methanolic extracts of *C. argentea* leaves. It can be deduced from this study that the solvent extracts possesses hepatoprotective activity against cadmium induced liver damage with that of the ethyl acetate displaying the most activity.

Within a cell, the superoxide dismutase (SODs) constitutes the first line of defence against ROS (Alscher *et al*., 2002). Superoxide dismutase is one of the chief cellular defence enzymes that dismutase superoxide radical to water and oxygen. Superoxide dismutase (SOD) is an antioxidant enzyme which mops up free radicals. The decreased activity of the antioxidant SOD in the hepatic tissue of Cd treated rats suggested a failure of the antioxidant defense-system to overcome the influx of free radicals generated by cadmium. It may also be a consequence of a direct inhibitory effect on SOD activity via Cd-enzyme interaction (Patra *et al*., 1999; Casalino *et al*., 2002). In contrast however, the significant increase in SOD activity obtained in this study following the administration of the solvent extracts to the Cd treated animals may be that the extract stimulated the *De novo* synthesis of these antioxidant that eventually mop up the free radicals generated by cadmium. The presence of phenolics in the extracts may be implicated in the mechanism of enhanced synthesis of SOD. Antioxidant phenolic substances can interact with transition metals, inhibits ROS-generating enzymes such as xanthine oxidase or induce nitric oxide synthase and improve the endogenous cellular antioxidant mechanisms (Halliwell and Whiteman, 2004). This finding in the present study is similar to that reported by Pari *et al* (2007).

The extracellular fluid of the body contains certain preventive low molecular weight antioxidants such as transferrin and albumin that bind extracellular iron and copper (metals involved in free radical generation) in non-reactive state and thereby diminish their ability to accelerate lipid peroxidation (Gutteridge, 1995).

The presence of uric acid and albumin also scavenges free radicals in the extracellular body fluid. This antioxidant ensures limited survival of reactive oxygen species such as superoxide and hydrogen peroxide in the extracellular fluid. Loss of these antioxidants (albumin and uric acid) in the extracellular fluid of only cadmium treated animals in the present study may be an indicator of ongoing biological oxidative stress (Gutteridge, 1995). The attenuation of the loss of these molecules by the solvent extracts suggests antioxidant activity which may be attributed to the presence of the secondary metabolites detected in the solvent extracts in the present study. The findings in this study are consistent with the previous report by Malomo *et al.* (2011) where aqueous extract of *Celosia argentea* var *cristata* leaves attenuated cadmium-induced oxidative stress in rats. The findings with respect to the total protein and bilirubin (total and conjugated) in the present study are also similar to that obtained for the uric acid and albumin in the serum of the animals. The ability of the solvent extracts to reverse the cadmium treatment related reductions in these extracellular antioxidant biomolecules consistently emphasis the ability to alleviate the free radical mediated oxidative stress in the animals.

Lipid peroxidation is the biological damage caused by free radicals, which are formed during oxidative stress. It is also used to investigate the oxidative damage of proteins and lipid peroxidation of the membrane and lipoproteins as a possible pathogenic mechanism for liver injury (Kocic *et al*., 1998). The increase in the level of lipid peroxidation in the serum of the animals treated with cadmium alone further suggest excessive formation of free radicals and activation of lipid peroxidation system. The reduction in the level of lipid peroxidation in the serum of the animals by the ethyl acetate and methanolic extracts of *C. argentea* leaves

suggest an anti-lipid peroxidation effect of the plant. The antioxidant effect of natural sources is due to the active compounds present in the plants in which some were also detected in the solvent extracts in the present study. The findings with respect to lipid peroxidation are similar to the previous report by Zeynep *et al.* (2004) and Malomo *et al.* (2011).

In toxicity studies, the determination of the activities of various enzymes in the tissues and body fluids can be used as indicators of damage to the organ or compromise of integrity of such organs which will adversely affect the normal functioning of such organ(s). Alkaline phosphatase is an enzyme that is associated with the plasma membrane and the endoplasmic reticulum (Wright, and Plummer, 1974). ALP is required in certain amounts for proper functioning of organs. Furthermore, other enzymes such as the transaminases occupy a central position in the metabolism of amino acids whereas the gamma glutamyl transferase, a membrane – localized enzyme that plays a major role in glutathione metabolism and resorption of amino acids from the glomerular filterate and from the intestinal lumen (Kaplan, and Pesce, 1996), is the most sensitive enzymatic indicator of hepatobiliary disease (Mayne, 1998). Alterations in the levels of these enzymes in the tissues with attendant corresponding changes in the serum are indications of cellular toxicity on the tissues (Afolayan and Yakubu, 2009). The loss of AST and ALT following the administration of cadmium alone is quite understandable since any damage to the plasma membrane by way of oxidative stress leading to membrane permeability will consequently result in the leakage of cellular components including the cytosolic enzymes, and in this instance, the transferases. The antioxidant properties of the solvent extracts (ethyl acetate and methanol) became apparent again as they reverted the trend of the activities of the enzymes towards the control values. In an attempt to reverse these trend produced by the administration of cadmium alone on the activities of these enzymes, the activities of some of these enzymes were increased to levels higher than the controls. This is suggestive of some of the components of the extract which might have influenced the mechanism for the synthesis of the enzyme probably as a consequence of adapting to the effect of the chemical compound, cadmium chloride, in this instance.

In conclusion, the present study supports that the ethyl acetate extract and methanolic extract of *Celosia argentea* leaves possess *in vitro* and *in vivo* antioxidant activities as well as attenuate the effects of cadmium related toxicity in rats. Ethyl acetate extract of *C. argentea* provided a better potent source of natural antioxidant than the methanolic extract.

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