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Original Research Article

Evaluation of Chemical Composition, Anti-inflammatory, Antioxidant and Cytotoxic Potential of Leaf and Root Extracts of *Euphorbia graminae*

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ABSTRACT

Reports by several researchers have shown that plants are potent sources of phyto-constituents some of which have been useful in traditional medicine, cosmetics industries and food supplement industries. As part of the ongoing search for therapeutic plants with little or no side effects, this study investigated the nutritive and therapeutic potentials of a common weed *Euphorbia graminae*, so as to provide useful information on the plant.
The leaf and root methanol extracts of *E. graminae* were screened for the presence of phytochemical constituents. The red blood cell membrane stabilization as well as antioxidant assays were also evaluated. The cytotoxic effect of the extracts was also investigated using *Allium cepa* model.
Result revealed that both extracts contained saponins, steroids, cardiac glycosides, flavonoids and triterpenes. The proximate analysis of the leaf revealed the presence of moisture, carbohydrate, protein, ash, fat and crude fibre. The leaf extracts exerted reductive antioxidant potential in dose dependent manner while the root extract showed better reductive potential at lower concentrations. Only the root extract scavenged DPPH- radicals and also exhibited lipid peroxidation inhibitory activity greater than the leaf extract. The leaf extract protected the stressed erythrocytes better at lower concentrations while the root extract protected better at higher concentrations in a dose dependent manner. However, both extracts did not show cytotoxic or genotoxic effect on *A. cepa* chromosome. Therefore *E. graminae* can be considered as plant that could be beneficial in the traditional medicine in the management of oxidative and inflammatory related conditions.

Keywords: *Euphorbia graminae*, nutritional component, phytochemicals, anti-inflammatory, antioxidant, cytotoxic.

Introduction

Plants are known to possess phytochemicals that are effective in the treatment of various illnesses and infections.[1-4] Some are also known to be potent sources of phytoconstituents used in cosmetics and food supplement industries.[5] Although plants have enormous potentials and usefulness to man and other living organisms, some have also proven to be potentially toxic, mutagenic and carcinogenic.[6,7]
Euphorbia species have been reported as being useful in the treatments of ailments such as skin disease, rheumatism, gonorrhea, mucous membrane infections, boil, sores, hypertension and in promoting wound healing.[8-11] Some were found to be useful as purgatives.[12-14] and others as exfoliants.[15]
Euphorbia graminae Jacq. belongs to the family Euphorbiaceae, a morphologically variable herbaceous plant. It is a perennial, 15–30 cm tall, sometimes taller, often branching from the base, and dichotomously branched distally. The leaves are alternate; those toward the base of the stem are ovate or oblong with a few large distantly-spaced teeth, while those toward the stem apices are elliptical to linear and entire.[16,17]
E. graminae, is native to Southern Mexico and has spread as far as Central America to Northern South America and other parts of the world such as Cuba, Lesser Antilles (St. Lucia), Aruba, Bonaire and Nigeria.[16,18]
The nutritional, phytoconstituents, antioxidant, anti-inflammatory as well as the cytotoxic status of *E. graminae* were investigated and reported for the first time in this study. This study is meant to provide information on the possibility of generating an alternative source of novel phytochemicals such as anti-inflammatory and antioxidant compounds from the plant. It is also meant to provide information on the potential nutritive value of this plant as well as give preliminary information on its cytotoxic status thereby helping to salvage public health alongside other possible toxicity tests.

Materials and Methods

Plant Sample
Whole plant samples of *E. graminae* were collected from the wild at the Reforestation Garden, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (07º31": 04º 31" E). The plant material was identified and authenticated at IFE Herbarium (Voucher No.: IFE 16298), Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
Preparation of the Plant Extracts
Fresh leaves and roots excised from *E. graminae* plants during the month of April, 2017 were collected and thoroughly rinsed with water, air-dried for two weeks and grinded separately into powder with manual

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grinding mill. The resulting powders (1 kg) were separately macerated in 3.5 L of methanol for 48 h and were decanted and filtered using Whatman No 1 filter paper. The *E. graminae* methanol leaf and root extracts were concentrated to dryness at 35°C under reduced pressure on Edward High Vacuum Pump rotary evaporator and kept in the desiccator for further analyses.

Proximate Analysis

E. graminae leaves were analyzed for nutritional composition such as carbohydrate, crude fats, protein, moisture content, crude fibre and ash content according to the official method of the Association of Analytical Chemists.[19] The Carbohydrate content was determined by following the method of James and Friday[20] given as: Carbohydrate content = [100 – (protein + fat + moisture + ash + crude fibre)]. The proximate analysis was carried out in duplicates and all values reported in percentage.

Phytochemical Analysis

Methanol leaf and root extracts of *E. graminae* were screened for alkaloids, flavonoids, saponins, tannins, cardiac glycosides, phlobatannins, anthraquinone, xanthoprotein and triterpenes according to standard procedures described by Sofowora[21] and Evans.[22]

Assessment of Anti-inflammatory Activity

Preparation of bovine erythrocytes

Bovine erythrocyte was prepared as described by Oyedapo *et al.*[23] Freshly collected bovine blood sample was poured into clean and dried centrifuge tubes and centrifuged at 3000 rpm for 15 min. The supernatant was carefully removed with the aid of dropping pipettes. The residue (packed cells of erythrocytes) was re-suspended in fresh isosaline, mixed carefully and gently in order to prevent lyses of cells and centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed and the process was repeated until a clear supernatant was obtained. To 2 mL of the residual cell was added 98 mL of isosaline to make 2% (v/v) red blood cells.

Membrane stabilization assay

Membrane stabilizing assay was carried out according to modified procedure of Sadique *et al.*[24,] as reported by Oyedapo *et al.*[23] The assay mixture consisted of hyposaline (0.5 mL), 0.15 M sodium phosphate buffer, pH 7.4 (1.0 mL), varying volumes of isosaline and 2% (v/v) bovine red blood cells (0.5 mL).

The drug control was prepared as test sample without 2% (v/v) bovine red blood cells. The reaction mixture was mixed properly and incubated at 56°C for 30 min. The solution was cooled under running water and then centrifuged at 3000 rpm for 5 min. The supernatant was collected and the absorbance was read at 560 nm against the blank.

The percentage membrane stability activity was estimated from the expression below:

$$100 - \frac{\{(\text{drug test value} - \text{drug control value}) \times 100\}}{\text{Control value}}$$

The control represents 100% lyses. The values represent the average of triplicates ± SEM.

Evaluation of Antioxidant Activity

Estimation of Flavonoids Concentration

The concentration of flavonoids in the methanol leaf and root extracts of *E. graminae* was estimated according to the procedure of Sun *et al.*[25] Leaf (0.1 g) and root (0.1 g) extracts of *E. graminae* were separately dissolved in 20 mL of 70% (v/v) ethanol. To clean dry test tubes (in triplicate) was pipetted 0.5 mL of working solution of sample and diluted with 4.5 mL of distilled water. To each tubes was added 0.3 mL of 5% (w/v) $NaNO_2$, 0.3 mL of 10% (w/v) $AlCl_3$ and 4 mL of 4% (w/v) NaOH. The reaction mixture was incubated at room temperature for 15 min. The absorbance of the products was read at 500 nm against reagent blank. The standard curve for the estimation of total flavonoid concentration were prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of 1 mg/mL rutin standard into clean dry tests tubes. The volumes were diluted into 5.0 mL with distilled water. To each of the test tubes was added 0.3 mL of 5% (w/v) $NaNO_2$, 0.3 mL of 10% (w/v) $AlCl_3$ and 4 mL of 4% (w/v) NaOH. The reaction mixture was incubated at room temperature for 15 min. The absorbance was taken at 500 nm and flavonoid concentration was extrapolated from rutin calibration curve expressed as mg/mL rutin equivalent.

Estimation of Total Phenol

Estimation of total phenol was carried out using Follin Ciocalteu's reagent reaction as reported by Singleton *et al*.[26] The assay involved pipetting 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of garlic acid solution (1.0 mg/mL) in triplicate. The volumes were made up to 1.0 mL with distilled water. To each of the test tubes was added 1.5 mL of Follin Ciocalteu's phenol reagent (1:10). The reaction mixture was incubated at room temperature for 15 min. To the mixture was added 1.5 mL of 10% (w/v) $NaHCO_3$ solution to give a total volume of 4.0 mL. The reaction mixture was further incubated for additional one and half hours.

The estimation of the phenol concentration in the aqueous extract involved pipetting 0.5 mL each of 5 mg/mL *E. graminae* leaf and root extracts into clean dry test tubes separately in triplicate. The volumes were adjusted to 1.0 mL with distilled water. To each of the test tubes was added 1.5 mL of Follin Ciocalteu's phenol reagent (1:10). The reaction mixture was incubated at room temperature for 15 min. To the mixture was added 1.5 mL of 10% (w/v) $NaHCO_3$ solution to give a total volume of 4.0 mL. The reaction mixture was further incubated for additional one and half hours. The absorbance was read at 725 nm against the blank containing all reagents except the standard Gallic acid. The concentration of phenol was extrapolated from garlic acid calibration curve and expressed as mg/mL garlic acid equivalent.

Lipid Peroxidation and Thiobarbituric Acid Reactions

A modified thiobarbituric acid reactive species (TBARS) assay as described by Ohkowa *et al*.[27] was used to measure the lipid peroxide formed using egg yolk homogenate lipid rich media. Egg homogenate [0.5 mL of 10% (v/v)] and 0.1 mL of extract were added to a test tube and made up to 1 mL with distilled water; 0.05 mL of copper sulphate (0.07 M) was added to induce lipid peroxidation and the mixture was incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and then heated at 95°C for 60 min. After cooling, 5.0 mL of butanol was added to each tube and centrifuged for 10 min at 3000 rpm. The absorbance of the organic layer was measured at 523 nm. Percentage inhibition of lipid peroxidation by the extract was calculated using the expression:

$$[(1 - E)/C] \times 100$$

Where C is the absorbance value for fully oxidized control and E is the absorbance in the presence of extract.

Reducing Power Assay

The reducing power of the leaf and root extracts of *E. graminae* was determined according to the method of Oyaizu.[28] Different concentration of extract (100 – 1000 µg/mL) in distilled water was mixed with phosphate buffer (0.2 M pH 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min. To the mixture was added 10% (w/v) trichloroacetic acid, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% $FeCl_3$ (0.5 mL) and the absorbance was measured at 700 nm, increased absorbance of the reaction mixture indicated reducing power. Ascorbic acid was used as a standard to compare the reducing power of the extracts.

DPPH Radical Scavenging Assay

The Antioxidant activity of the extracts, on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al*.[11] *E. graminae* extract (0.1 mL) was added to 3 mL of 0.004% MeOH solution of DPPH. Absorbance at 517 nm was taken after 30 min and the percentage inhibition activity was calculated using the expression below;

$$\text{Percentage Inhibition Activity} = \frac{A_0 - A_e}{A_o} \; x \; 100$$

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A_o = Absorbance without extract and
A_e = Absorbance with extract.

Cytotoxicity assay on Allium cepa root meristematic cell
Equal sized onion bulbs (*Allium cepa* L., 2n = 16) of the brown variety was purchased locally at Ife Central market, Ile-Ife, Osun State, Nigeria. The onion bulbs were sun-dried for two weeks and used for the bioassay according to standard procedures.[29,30] The onion bulbs were rinsed thoroughly with distilled water after the careful scrapping and removal of the outer scales and older roots. Thereafter, the bulbs were grown in distilled water for four days to allow new roots to sprout, then transferred into clean vials containing distilled water (negative control) and different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) of extracts for 24 h. The effect of the methanol solvent on the *Allium cepa* root cells was also documented alongside the effect of the various concentrations of *E. graminae* extracts studied.
The roots were excised and fixed in 1:3 acetic acid:ethanol for another 24 h. The fixed roots were hydrolysed in 18% hydrochloric acid for 10 min, squashed and stained with modified Orcein[31] for 20 min. The microscopic examination involved calculation of the mitotic index (MI) per treatment, scoring of aberrant cells at anaphase and telophase and recording of micronuclei in interphase cells. MI was calculated as the number of divided cells/total number of cells. Photomicrographs of selected mitotic cells were documented with an AmScope MT microscope camera version 3.0.0.1 attached to an Olympus light microscope.

Statistical Analysis
Each value represented the mean ± SEM of 3 readings. Data obtained were subjected to Analysis of Variance (ANOVA) at significant level of $p < 0.05$ using the Statistical Analysis Software (SAS) version 9.13 and the means were subjected to Duncan Multiple Range Test.

Results and Discussion

Proximate analysis of E. graminae Leaf
The proximate analysis of *E. graminae* leaf studied revealed appreciable amount of carbohydrate (17.86 ± 0.08%) and protein (65.36 ± 0.04%) components than the other components (Table 1). The presence of high carbohydrate content suggested that they may serve as good sources of energy. Protein is an essential component of human diet needed for the replacement of dead tissues and for the supply of energy and adequate amount of required amino acids.[32] The lower crude fat content observed in *E. graminae* leaf suggests that it can be easily incorporated in weight reducing diet.[33] The low moisture content can prevent susceptibility of the leaves to spoilage by microorganism.[33] The low ash content show that they may have contained little amounts of mineral elements. The results suggests that *E. graminae* leaf is a good source of primary metabolites such as carbohydrate, protein and crude fat and could probably serve more of a dietary function than a therapeutic one.

Phytochemical constituents of methanolic leaf and root extracts of E. graminae
Table 2 shows the result of phytochemical composition of methanol leaf and root extracts of the *E. graminae*. The study revealed the presence of saponins, steroids, cardiac glycosides, triterpenes and flavonoids. Phytochemicals have been reported to possess a wide range of pharmacological properties. Saponins present in plants are known to be cardiotonic in nature.[34,35] Various studies have reported that saponins possess anti-inflammatory, anti-oxidant,[36] antibiotics, anti-microbial and anti-diabetics activities.[37] Flavonoids have been reported to act as antioxidant, anti-cancer, anti-microbial, anti-inflammatory agents.[22,37] Analgesic and antimicrobial activities of many alkaloids have been documented.[38,39] Cardiac glycosides are known to have therapeutic applications useful in the treatment of heart-related problems.[40] Occurrence of all these phytochemicals in leaf and root of *E. graminae* probably contributed to its therapeutic potential.

Antioxidant Assays
Evaluation of phenol and flavonoid concentrations
Polyphenol compounds such as flavonoids possess potent antioxidant activity because of their redox potential, which plays important role in scavenging free radicals, neutralizing the reactive oxygen species or act as an electron donor to peroxide radicals.[41] The role of medicinal plants in the management and control of diseases have been attributed mainly to the antioxidant potentials of their constituents usually a wide variety of polyphenol compounds.[42,43] Analysis of phenols and flavonoids in *E .graminae* leaf and root extracts (Table 3) showed higher phenolic content in the leaf while flavonoid content of the root extract is slightly more than that of the leaf extract. This suggests that polyphenol content of the *E graminae* extracts probably contributed to its various antioxidant activity.

Table 1: Proximate Composition of *Euphorbia graminae* Leaf studied

Nutritional Component	Composition (%)
Protein	15.36 ± 0.04
Moisture	65.36 ± 0.04
Fat	0.32 ± 0.05
Ash	0.83 ± 0.01
Crude fibre	0.27 ± 0.02
Carbohydrate	17.86 ± 0.08

Table 2: Analysis of Phytochemical Compounds Present in *Euphorbia graminae* studied

Phytochemical Compound	*E. graminae* leaf	*E. graminae* root
Saponin	+	+
Steroids	+	+
Cardiac glycosydes	+	+
Phylobatannins	-	-
Xanthoproteins	-	-
Tanins	-	-
Flavonoids	+	+
Triterpenes	+	+
Anthraquinone	-	-
Alkaloids	-	-

Table 3: Concentration of Total Flavonoids and Phenols of Aqueous Leaf and Root Extracts of *E. graminae* studied

	Leaf	Root
Total flavonoid (mg/g RE/g extract)	0.202 ± 0.003	0.207 ± 0.008
Total Phenols (mg/g GAE/g extract)	2.23 ± 0.183	1.08 ± 0.009

Lipid Peroxidation and Thiobarbituric Acid Reactions

Lipid peroxidation inactivates cellular components and plays a key role in oxidative stress in biological systems by cell membrane disruption and cell damage.[44] In the present study, *E graminae* leaf and root extracts demonstrated marked inhibition of lipid peroxidation. The leaf extract showed stronger inhibitory effect in a concentration-dependent manner while the root extract inhibited effectively at lower concentrations (Table 4). The inhibitory activity against lipid peroxidation by *E. graminae* methanol root extract (IC_{50} value 12.22 µg/mL) was greater than that of the leaf extract (IC_{50} value 18.66 µg/mL). This suggests that *E. graminae* extracts probably protected the physicochemical properties of membrane bilayers from free radical-induced cellular dysfunction.

DPPH Radical Scavenging Activity

The DPPH assay is based on the ability of the test compounds to reduce DPPH in the presence of a hydrogen donating antioxidant. A lot of naturally occurring antioxidants have been reported to demonstrate ability to inhibit free radicals within tissues.[45] Figure 1 shows the DPPH radical scavenging activity of the methanol leaf and root extracts of *E. graminae* and trolox (standard). It was observed that only the root extract scavenged DPPH radicals and compare favourably with trolox. Thus *E. graminae* root 'possess hydrogen donating and radical scavenging ability and thereby they can act as primary antioxidant.

Reducing Power

The potentials of the *E. graminae* leaf and root extracts to reduce Fe^{3+}/ferricyanide complex to ferrous form are shown in figure 2. The reducing capacity of a compound indicates its potential antioxidant activity.[41] This is due to the presence of reductants that demonstrate anti-oxidative potential by breaking the free radical chain and donating a hydrogen atom.[46,47] This study showed that *E. graminae* leaf extract exerted reductive antioxidant potential in dose dependent manner while the root extract showed a better reductive potential at lower concentrations. The reductive potential of Ascorbic acid was higher than the extracts of *E. graminae* at all concentrations.

Membrane stabilization assay

Inflammation is a complex biological process, initiated by several factors ranging from chemical injury to environmental pollution, by which body responses to external stimuli such as infections and injuries.[23,38,48] Membrane stabilization has been reported as one of the mechanisms of action through which certain medicinal plant elicits anti-inflammatory activity.[49] Quite a number of extracts have been documented to exert anti-inflammatory activity by stabilizing the red blood cell membrane.[22,23,50,51]

The membrane stabilizing potentials exhibited by *E graminea* (leaf and root) extracts and Diclofenac on red blood cells exposed to heat and hypotonic-induced lyses are as shown in figures 3, 4, 5 and 6 at 0.5, 1.0, 1.5 and 2.0 µg/mL, respectively. It was observed that the leaf extracts protected the stressed red blood cells better at lower concentrations (0.5 and 1.0 µg/mL) and compared favourably with Diclofenac. Furthermore, the root extracts compared favourably with the standard anti-inflammatory drug (Diclofenac) at higher concentrations (1.5 and 2.0 µg/mL) in a dose-dependent manner but stabilizes red blood cells more than the leaf extract.

Studies have established that both saponins and flavonoids possess the abilities to bind to the cellular macromolecules on the surfaces of the membranes thereby preventing the interactions of inflammatory agents from the cell surfaces.[35,52] Also, certain saponins and flavonoids have been reported to exert profound membrane stabilizing effect both *in vivo* and *in vitro*.[22,53] On the basis of these results, it could be inferred that both the leaf and root extracts of *E. graminae* contains principles that protected the stressed red blood cell membrane.

Cytogenetic analysis

The *A. cepa* test has been used in quite a lot of laboratories all over the world[54] in the determination of cytotoxicity and/or genotoxicity effects of various substances.[55] The cytological studies of the leaf and root extracts of *E. graminae* revealed that the effect of the extracts were concentration-dependent as shown in Tables 5 and 6. Cells treated with leaf extract showed lower mitotic index at all tested concentrations than those treated with root extracts. Also, no aberrant chromosome was recorded in the various stages of cell divisions observed during the microscopic examination of the treated mitotic cells of the *Allium cepa* (Figures 7 and 8). This implies that the leaf and root extracts of the *E. graminae* studied did not inhibit or suppress the mitotic divisions of the cells of the *A. cepa* used in the assay and also the extracts did not show any mutagenic, cytotoxic or genotoxic effect that could result in abnormalities in the structure or arrangement of the treated *A. cepa* chromosome.

Table 4: Inhibition of Lipid Peroxidation by the *E. graminae* Extracts studied

Sample	Concentration (µg/mL)	% Inhibition ± SEM (n=3)	Regression equation	IC_{50} (µg/mL)
LEAF	200	31.40 ± 0.03	Y = 30.429x + 14.23	18.66
			(R^2 = 0.6023)	
	400	32.97 ± 0.01		
	600	36.63 ± 0.02		
	800	37.40 ± 0.03		
	1000	38.27 ± 0.02		
ROOT	200	26.63 ± 0.07	Y = 12.553x + 13.789	12.22
			(R^2 = 0.2154)	
	400	26.53 ± 0.07		
	600	23.80 ± 0.03		
	800	23.33 ± 0.06		
	1000	20.10 ± 0.09		

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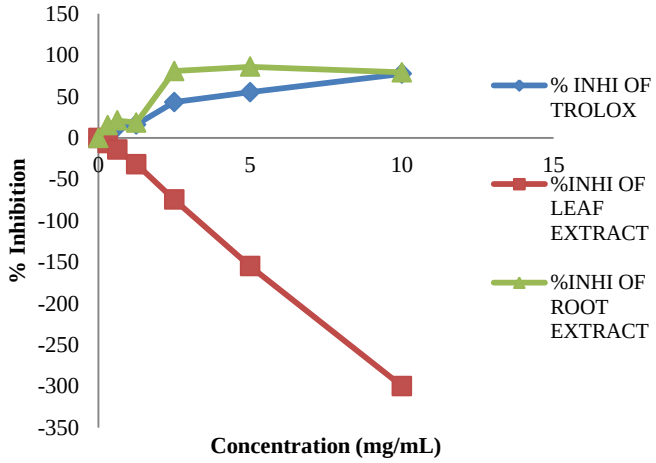

Figure 1: DPPH Radical Scavenging Activity of *E. graminae* Leaf and Root Extracts.

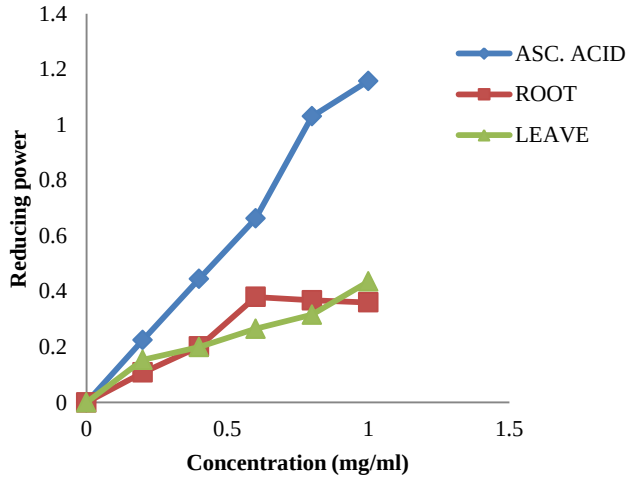

Figure 2: Ferric Reducing Power of *E.graminae* Leaf and Root Extracts.

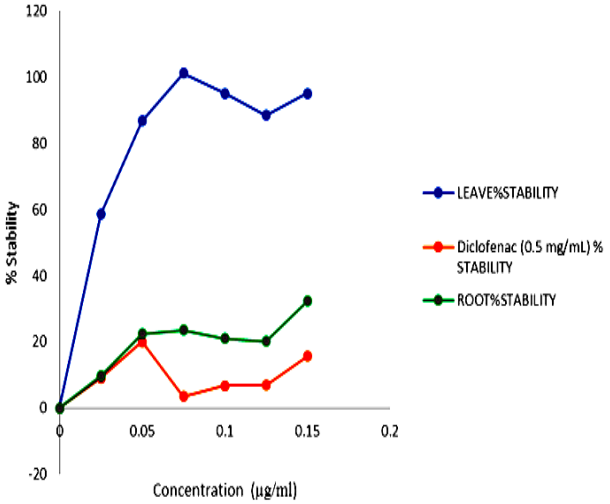

Figure 3: The Graph of Percentage Membrane Stability against Concentration of Leaf and Root Extracts of *E.graminae* and diclofenac at 0.5 mg/mL.

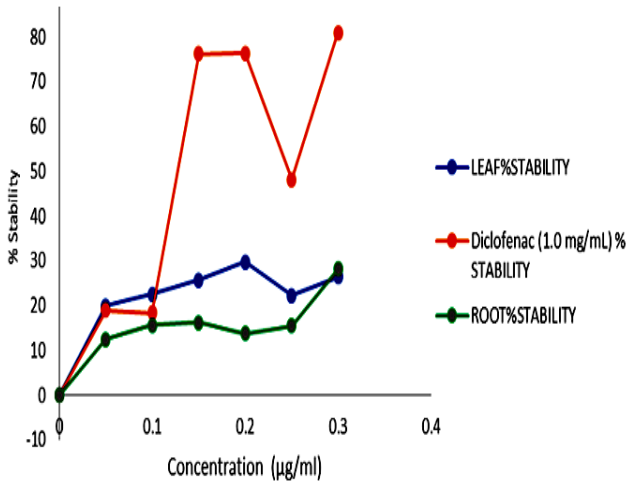

Figure 4: The Graph of Percentage Membrane Stability against Concentration of Leaf and Root Extracts of *E.graminae* and diclofenac at 1.0 mg/mL.

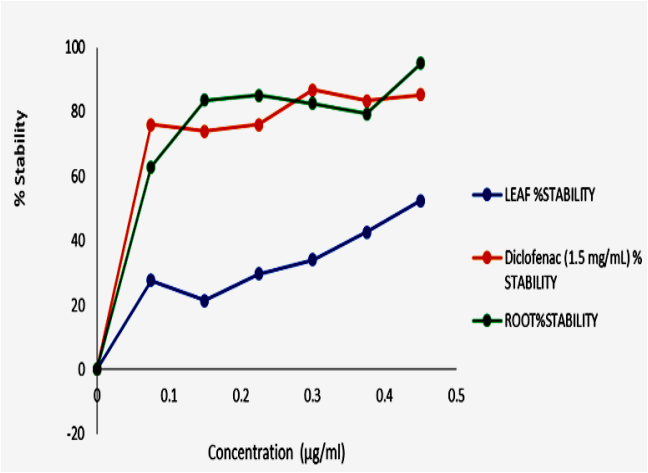

Figure 5: The Graph of Percentage Membrane Stability against Concentration of Leaf and Root Extracts of *E.graminae* and diclofenac at 1.5 mg/mL.

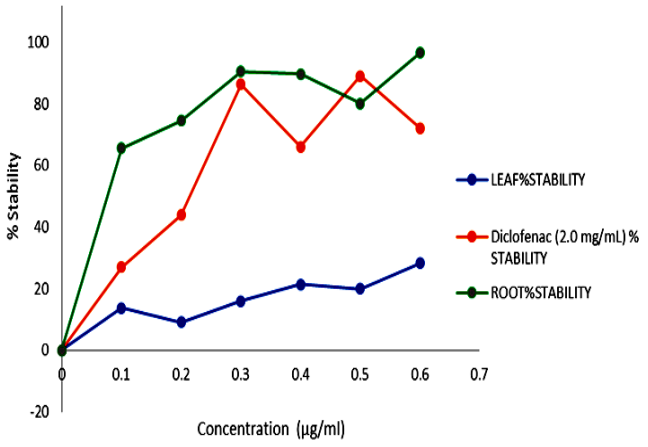

Figure 6: The Graph of Percentage Membrane Stability against Concentration of Leaf and Root Extracts of *E.graminae* and diclofenac at 2.0 mg/mL.

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Table 5: Cytological Effect of *Euphorbia graminae* Leaf Extracts on *Allium cepa* Root Tips.

Concentration	Total Number of Cells	Mitotic Index (mean ± S.E.M)	Interphase	Prophase	Metaphase	Anaphase	Telophase
Water	4316	$0.56^{C} \pm 0.09$	$654.33^{A} \pm 186.02$	$668.00^{BA} \pm 36.59$	$32.67^{A} \pm 12.44$	$31.00^{A} \pm 7.81$	$52.67^{A} \pm 31.28$
0.2 mg/mL	3293	$0.65^{BC} \pm 0.07$	$390.00^{BA} \pm 14\ 5.72$	$646.00^{B} \pm 87.08$	$27.67^{A} \pm 23.67$	$22.33^{A} \pm 16.37$	$11.67^{B} \pm 6.69$
0.4 mg/mL	3019	$0.92^{A} \pm 0.05$	$84.33^{C} \pm 61.064$	$890.67^{BA} \pm 67.49$	$15.33^{A} \pm 6.49$	$10.67^{A} \pm 4.33$	$5.33^{B} \pm 2.60$
0.6 mg/mL	3068	$0.98^{A} \pm 0.03$	$6.33^{C} \pm 4.485$	$946.33^{A} \pm 186.11$	$33.33^{A} \pm 14.53$	$14.67^{A} \pm 2.60$	$10.33^{B} \pm 4.84$
0.8 mg/mL	2626	$0.99^{A} \pm 0.003$	$0.00^{C} \pm 0.00$	$836.00^{BA} \pm 58.05$	$8.00^{A} \pm 3.61$	$9.00^{A} \pm 4.16$	$15.67^{B} \pm 8.09$
1 mg/mL	3097	$0.82^{BA} \pm 0.05$	$133.67^{BC} \pm 133.67$	$779.33^{BA} \pm 52.59$	$15.67^{A} \pm 4.702$	$10.0^{A}0 \pm 2.65$	$7.00^{B} \pm 1.53$

*Means with same letters along the column are not significantly different at $p \leq 0.05$.

Table 6: Cytological Effect of *Euphorbia graminae* Root Extracts on *Allium cepa* Root Tips.

Concentration	Total Number of Cells	Mitotic Index (mean ± S.E.M)	Interphase	Prophase	Metaphase	Anaphase	Telophase
Water	4316	$0.560^{B} \pm 0.09$	$654.33^{A} \pm 186.02$	$684.67^{BA} \pm 46.37$	$32.67^{BA} \pm 12.441$	$31.00^{A} \pm 7.81$	$19.33^{BA} \pm 3.38$
0.2 mg/mL	3069	$0.588^{B} \pm 0.13$	$387.67^{BA} \pm 131.79$	$580.67^{B} \pm 110.34$	$51.00^{A} \pm 14.572$	$21.00^{BA} \pm 9.16$	$27.33^{A} \pm 11.69$
0.4 mg/mL	2995	$0.863^{A} \pm 0.06$	$130.00^{BC} \pm 65.57$	$820.67^{A} \pm 30.84$	$13.67^{B} \pm 5.7831$	$7.33^{B} \pm 4.33$	$5.00^{B} \pm 2.89$
0.6 mg/mL	2868	$0.969^{A} \pm 0.01$	$6.33^{C} \pm 3.18$	$878.00^{A} \pm 71.50$	$6.67^{B} \pm 4.1767$	$6.67^{B} \pm 4.25$	$7.00^{B} \pm 4.04$
0.8 mg/mL	2853	$0.997^{A} \pm 0.01$	$1.67^{C} \pm 1.67$	$860.00^{A} \pm 65.06$	$10.67^{B} \pm 5.4874$	$11.00^{BA} \pm 4.35$	$5.00^{B} \pm 1.73$
1 mg/mL	2923	$0.945^{A} \pm 0.10$	$5.33^{C} \pm 5.33$	$918.00^{A} \pm 96.42$	$11.33^{B} \pm 1.333$	$12.33^{BA} \pm 3.67$	$7.00^{B} \pm 1.00$

*Means with same letters along the column are not significantly different

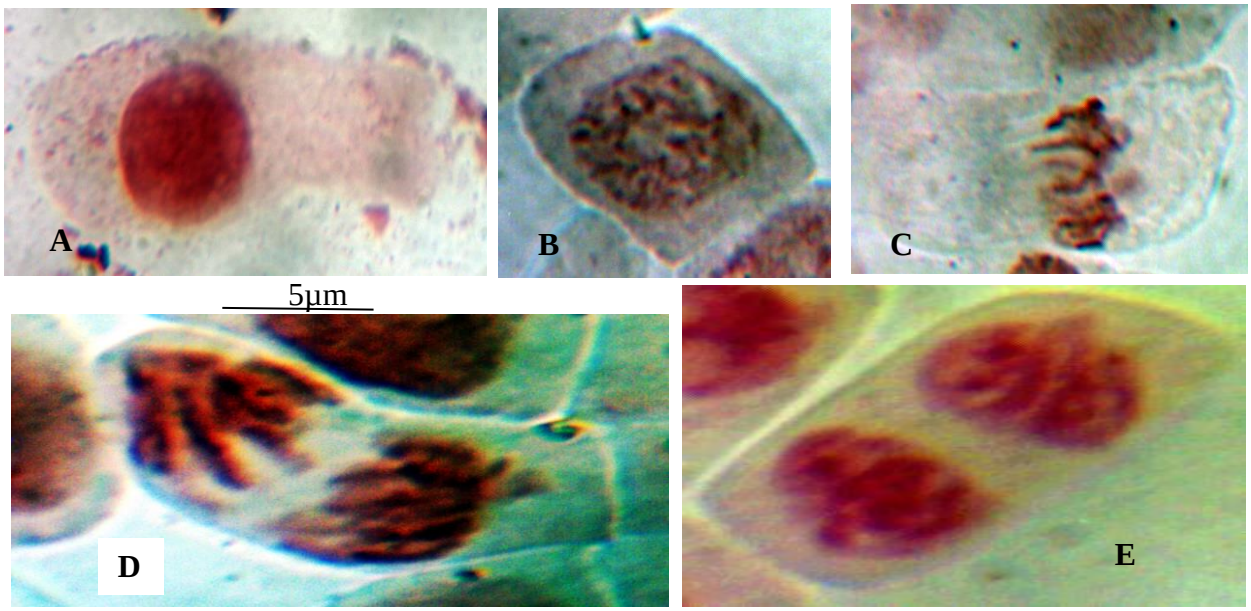

Figure 7: Mitotic cells of *Allium cepa* studied After Treatment with E. graminae leaf extract. A: Interphase; B: Prophase; C: Metaphase; D: Anaphase; E: Telophase.

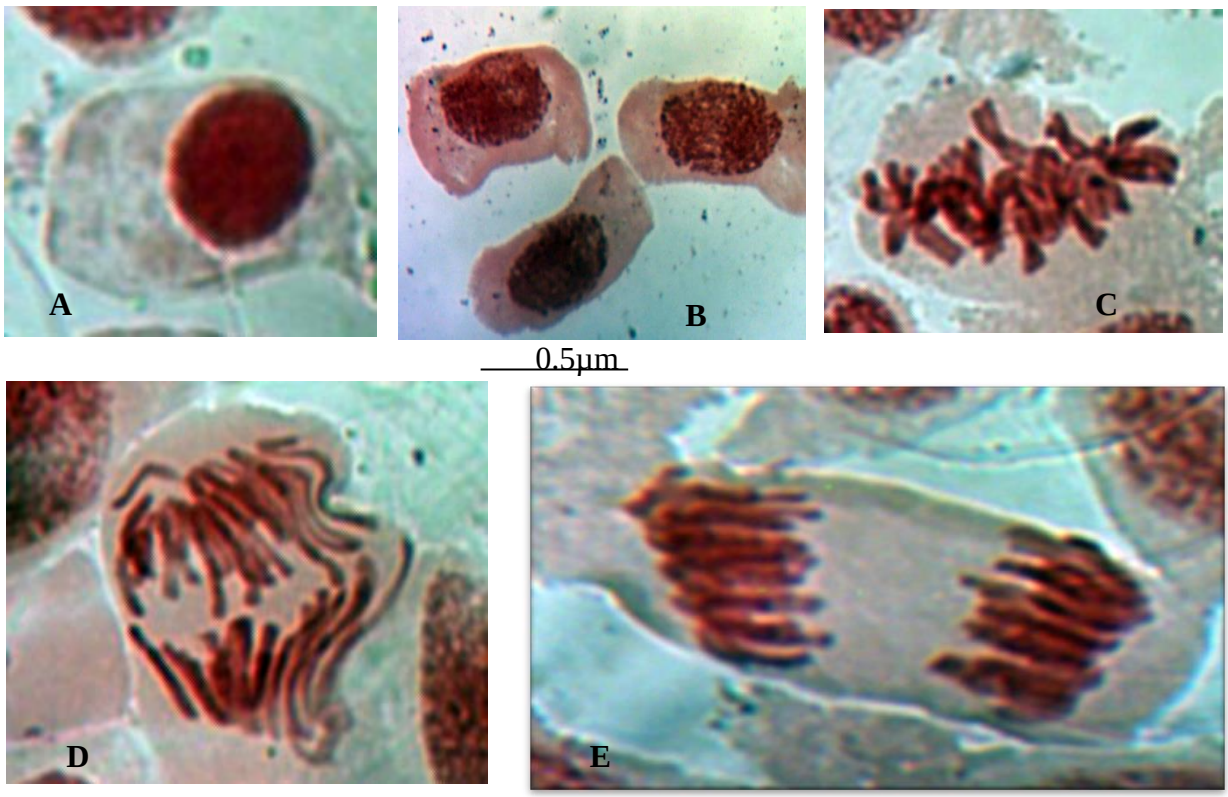

Figure 8: Mitotic cells of *Allium cepa* studied after Treatment with *E. graminae* Root extract A: Interphase; B: Prophase; C: Metaphase; D: Anaphase; E: Telophase.

Conclusion

This study revealed that the plant *Euphorbia graminae* contains some vital nutritional and bioactive components that could be beneficial as food and useful in the management of oxidative and inflammatory related conditions in traditional medicine. However, further toxicity studies needs to be carried out on this plant to ensure its safety for human consumption. Also, more study on the pharmaceutical exploration of the plant *Euphorbia graminae* is recommended.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this work will be borne by them.

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