



In Vitro Antioxidant Assay of Aqueous Extract of *Cynodon dactylon* and its Effect on Haematological Parameters of Rat.

Akachukwu, D^{1*}; Opara, C²; Ubiom, I.C.³; Ibenne, C.¹

¹Michael Okpara University of Agriculture, Department of Biochemistry, Umudike, Abia State² Raw Materials Research and Development Council, Department of Industrial Extension Services, Abia Coordinating Office Umuahia, Abia State³ Department of Biochemistry, Faculty of Science, University of Uyo, Akwa Ibom State.*Corresponding author: ds.akachukwu@mouau.edu.ng. Tel No: +234 8033915908.

SUMMARY

Many medicinal plants possess antioxidant properties. Antioxidant potentials of *Cynodon dactylon* and its haematological effects on Wistar rats was evaluated. *In vitro* radical scavenging activity was assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Sixteen male Wistar rats were grouped into four groups of four rats each. Control group was fed normal rat feed and water, group 1 received 100 mg/kg body weight (b.w.) extract, group 2 received 200 mg/kg b. w. extract while group 3 received 400 mg/kg b. w. extract respectively for 21 days. Results showed that *C. dactylon* produced its optimum antioxidant activity against DPPH radical at a concentration of 400 mg/ml. At 100 mg/ml, optimum antioxidant activity was produced using FRAP. There was no significant ($p > 0.05$) difference in haemoglobin, red blood cell, platelet, white blood cell, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocytes, monocytes, eosinophils and basophils concentration when compared to the control group. Antioxidant activity of the plant was low compared to that of the ascorbic acid. The observed antioxidant activity of the plant could be as a result of certain antioxidant compounds present in the plant. The extract showed minimal toxicity to haematological parameters at the doses administered.

Keywords: Antioxidant, *Cynodon dactylon*, DPPH, FRAP, Haematological

INTRODUCTION

The use of plants by traditional medicine practitioners has been in existence since inception of life, thereby arousing the interest of researchers in exploring the natural valuable endowments of plants (Akiniyi and Sultanbawa, 1983; Treben, 1998) in order to scientifically prove the acclaimed medicinal potentials. Nigeria is greatly endowed with vast amount of grass plants which are consumable by man and animals due to their nutritional and medicinal benefits of which *Cynodon dactylon* is one of such grass plants.

Cynodon dactylon commonly known as barmuda or bahama grass, belongs the *Poaceae* family and of the genus, *Cynodon*. It originated from East Africa, Europe, Asia and Australia and is now a worldwide distributed plant, both in tropical and subtropical zones. *Cynodon dactylon* is a long-lived perennial grass with long rapid growing stolons and rhizomes which forms dense leafy mats of about 10-40 cm high (Hacker and Jank, 1998; FAO, 2012). It is used as permanent pastures for grazing, cut-and-carry, for hay and silage production. It is also used as a turf and a cover crop in orchards (Bogdan, 1977). Different biochemical compounds have been identified and quantified using various morphological parts of *C. dactylon* such compounds includes terpenoids, vitamins, minerals, alkaloids, palmitic acid, proteins and carbohydrates (Solanki and Nagori, 2012). Jananie et al. (2011) reported the presence of glycerin, ethyl ester, phytol, linoleic acid and other bioactive compounds after conducting Gas Chromatography-Mass-Spectrometry analysis of the leave of *C. dactylon*.

Cynodon dactylon has been found to contain different potential medicinal properties which includes antioxidant, anti-diabetic, anti-malarial, antimicrobial, antiparasitic, wound healing, anti-arthritic and hepatoprotective

(Kumar et al., 2015, Pawaskar and Sasanga, 2015; Poojary et al., 2016, Santhi and Annapoorani, 2010; Yadav and Nath, 2017). In traditional medicine, *Cynodon dactylon* is used for the treatment of convulsions, epilepsy, cancer, hypertension, cough, bronchitis and diarrhea (Roy et al., 2016). Aside its healing potentials; it is traditionally used for worship in some parts of Asia (Das et al., 2021). A study conducted by Chinaka and Edeh (2015) reported the hypoglycemic effects of *C. dactylon*. Roy et al. (2016) reported its high antioxidant potentials. *C. dactylon* was also reported as heart and brain tonic (Kanimozhi and Bai, 2013). Edeh et al. (2014) reported on its hypolipidemic properties. The aim of this research was to determine the antioxidant potentials and effect of aqueous extract of *C. dactylon* on some selected haematological parameters using Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used for this study were of analytical grade and obtained from Sigma Aldrich Chemicals.

Collection of plant materials and extraction

Fresh matured grass of *C. dactylon* was harvested from Melrose junction Umuariaga, Oboro, Ikwuano L.G.A. of Abia State, Nigeria. The grass was identified by Mr. Ibe, K. N., a Forester in the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike Abia State. The harvested fresh matured grass of *C. dactylon* was sorted, washed and allowed to drain. After which, the grasses were cut into smaller pieces to reduce the size and facilitate drying. The grasses were air-dried for about two weeks, ground to powder and kept in a clean air tight container. Hundred grams of *C. dactylon* was soaked in 700ml of distilled water for 72 hours.

Subsequently, the mixture was filtered using a muslin cloth. The filtrate was poured into a beaker and evaporated using water bath (Uniscop SM801A) at 60°C. After drying, it was kept in (Newclime) refrigerator until use.

***In vitro* antioxidant assays**

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) photometric assay

The percentage antioxidant activities were calculated as follows:

$$\% \text{ Antioxidant activity (AA)} = 100 - \left[\frac{(\text{ABS sample} - \text{ABS blank}) \times 100}{\text{ABS control}} \right]$$

One milliliter of methanol plus 2.0 ml of test extract was used as the blank while 1.0ml of the 0.5mM DPPH solution plus 2.0ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard (Iwalewa *et al.*, 2008).

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of *C. dactylon* was assayed as described by Benzie

$$\text{FRAP value of sample } (\mu\text{Mol/L}) =$$

$$\frac{\text{Changes in absorbance of sample from 4 min} - 0 \text{ mins}}{\text{changes in absorbance of STD 4 min} - 0 \text{ min}} \times \frac{\text{FRAP value of std (1000 } \mu\text{m)}}{1}$$

Animal experimentation

Sixteen male Wistar rats (weighing between 52– 97g) used in this study were obtained from the animal breeding unit of the College of Veterinary Medicine University of Nigeria Nsukka (UNN) Enugu State. The animals were acclimatized for 2 weeks and housed in the departmental animal house of the institute. All animals were kept in 12:12 hr light: dark cycle at room temperature and fed with standard rat feed (Vital feed, Jos Nigeria) and tap water.

Experimental design

Free radical scavenging activity of the extract was assayed by the DPPH Assay (Mensor *et al.*, 2001) using spectrophotometer. The crude extract at different concentrations (25, 50, 100, 200 and 400) $\mu\text{g/ml}$ each was mixed with 1ml of 0.5mM DPPH (in methanol) in a cuvette. The absorbance at 517nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate.

and Strain (1999). The FRAP reagent (3ml) and 100 μl sample solution at concentrations of 25, 50, 100, 200 and 400 $\mu\text{g/ml}$ were mixed and allowed to stand for 4 minutes. Colorimetric readings were recorded at 593nm, at 37°C. The ascorbic acid standard solution was tested in a parallel process. Calculations were made by a calibration curve.

The rats were randomized into four groups of four rats each as described below. The doses of extract used in this study was extrapolated from the study of Yadav and Nath (2017) who reported that the LD₅₀ of *Cynodon dactylon* was greater than 2000 mg/kg.

Control group: fed with the normal rat feed and water.

Group 1: received 100 mg/kg body weight of *C. dactylon* extract.

Group 2: received 200 mg/kg body weight of *C. dactylon* extract.

Group 3: received 400 mg/kg body weight of *C. dactylon* extract.

The exposure was per oral using oral gavage to administer different doses of the aqueous extract to the different groups of rats for 21 days.

Rat sacrifice and blood collection

The animals were sacrificed through cervical dislocation after twenty-one days daily administration of the different doses of the aqueous extract. The blood samples were collected from the heart using a syringe and deposited into EDTA bottles.

Calculation:

Grams of haemoglobin per 100 ml of blood =

$$\frac{\text{Reading of test}}{\text{Reading of standard}} \times \frac{\text{Concentration of standard}}{1000}$$

Estimation of red blood cell count (RBC)

The method described by Ochei and Kolhatkar (2000) was employed for RBC estimation. Whole blood was diluted approximately using isotonic diluents to avoid lysis of red cells. The number of red cells in known volume and of known dilution is counted using a counting chamber under high power (40X) objective. The number of cells was calculated and reported as the number of red cells/cu.mm of whole blood.

Estimation of packed cell volume (PCV)

The PCV level was estimated by microhaematocrit method described by Ochei and Kolhatkar (2000). A capillary tube was filled with two-third of well mixed venous blood. One end of the capillary tube was filled with modelling clay (plasticine). The filled tubes were then placed in the

Haematological studies

Estimation of haemoglobin concentration

The cyanmethaemoglobin method described by Ochei and Kolhatkar (2000) was used for haemoglobin level determination. A reagent containing a mixture of potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate was used to treat blood sample containing the haemoglobin. Methaemoglobin is formed by the activity of the ferricyanide. The cyanide converts the resultant methaemoglobin to cyanmethaemoglobin. The absorbance of the mixture is read colorimetrically at 540nm.

microhaematocrit centrifuge and spun at 12,000 g for 5 minutes. The spun tube was placed into a specially designed scale and read as a percentage.

Calculation:

$$\text{PCV \%} = \frac{\text{Packed RBC column height}}{\text{Total blood column height}} \times 100$$

Estimation of red blood cell indices

This was estimated by the method of Ochei and Kolhatkar (2000). The Mean Corpuscular Haemoglobin (MCH) indicates the weight of haemoglobin in a single red blood cell and is expressed in pictograms (pg) (1pg = 10⁻¹²g).

$$MCH = \frac{\text{Haemoglobin (g per 100 ml)} \times 10}{RBC \text{ count million per cu. mm}}$$

Mean Corpuscular Haemoglobin Concentration (MCHC) denotes the haemoglobin concentration per 100 ml of packed red blood cells and is related to the colour of the red cells and it is expressed as percentage of packed cells.

$$MCHC = \frac{\text{Haemoglobin (g/dl)}}{PCV} \times 100$$

Mean Corpuscular Volume (MCV) is expressed as the volume in cubic microns or femto litres of an average red blood cell.

$$MCV = \frac{PCV \% \times 10}{\text{Red blood cells in millions per cu. mm}}$$

Estimation of total white blood cell count

(WBC)

This was estimated by the method of Ochei and Kolhatkar (2000). Whole blood is diluted appropriately using a diluents which haemolyzes red cells, leaving all the nucleated cells intact. The number of white cells in a known volume and known dilution are counted using a counting chamber under low power microscope. The number of cells in undiluted blood was reported as number of white cells/cu.mm of whole blood.

Estimation of platelet count

This was estimated by the method of Ochei and Kolhatkar (2000). The diluents used haemolyzed the red cells leaving the white cells and the platelets intact. The total number of platelets was counted using a high power (40 X) objective in the four large corner squares (4mm²). The number of platelet was calculated and reported as total number of platelets/cu.mm (or µl).

Statistical analysis

Data was expressed as mean ± SEM and were statistically analyzed using one way analysis of variance (ANOVA). Post-hoc comparisons were done using Duncan multiple range test on SPSS version 21. Values of P < 0.05 were considered significant.

RESULTS

TABLE I: Radical scavenging activity of aqueous extract of *C. dactylon* using DPPH

Concentration (µg/ml)	DPPH (%AA)	Ascorbic acid (%AA)
25	3.71 ± 0.19*	95.50 ± 0.19
50	6.49 ± 1.48*	95.67 ± 0.03
100	10.54 ± 1.23*	95.75 ± 0.18
200	19.98 ± 0.87*	95.17 ± 0.16
400	33.24 ± 0.44*	94.97 ± 0.14

Values are mean ± SEM of triplicate determinations. Values marked asterisk are significantly (p<0.05) different from control across column.

DPPH - 2, 2-diphenyl-1-picrylhydrazyl, ASCORBIC ACID - Control

TABLE II: Ferric reducing antioxidant power of aqueous extract of *C. dactylon*

Concentration ($\mu\text{g/ml}$)	FRAP activity of extract (μM)
25	$0.45 \pm 0.30^*$
50	$1.01 \pm 0.03^*$
100	$1.07 \pm 0.06^*$
200	$0.27 \pm 0.07^*$
400	$0.83 \pm 0.22^*$
Ascorbic acid (125)	2.00

Values are mean \pm SEM of triplicate determinations. Values marked asterisk are significantly ($p < 0.05$) different from control across column. FRAP - Ferric reducing antioxidant power, ASCORBIC ACID - Control

TABLE III: Effect of aqueous extract of *C. dactylon* on haematological parameters of Wistar rats

Parameter	Control	Group 1	Group 2	Group 3
PCV (%)	32.50 ± 1.55	$36.25 \pm 1.44^*$	32.75 ± 1.11	$28.00 \pm 4.02^*$
HB (g/dl)	10.55 ± 0.33	10.08 ± 0.76	10.33 ± 0.43	9.98 ± 0.82
RBC ($\times 10^6/\text{L}$)	5.94 ± 0.27	5.87 ± 0.46	5.78 ± 0.33	4.75 ± 0.88
Platelet ($\times 10^9/\text{L}$)	558.00 ± 11.30	326.00 ± 27.77	520.25 ± 36.80	288.75 ± 15.10
WBC ($\times 10^6/\text{L}$)	4.14 ± 0.55	6.76 ± 1.00	8.32 ± 2.45	6.21 ± 2.27
MCV (fl)	53.75 ± 0.63	63.75 ± 6.90	56.75 ± 1.89	61.75 ± 4.37
MCH (pg)	17.75 ± 0.33	17.00 ± 0.12	17.98 ± 0.40	23.87 ± 4.57
MCHC (g/dl)	32.55 ± 0.73	$27.80 \pm 2.56^*$	31.83 ± 0.40	37.50 ± 4.25
Neutrophils (%)	19.93 ± 3.62	19.57 ± 2.41	13.15 ± 2.20	$11.35 \pm 1.74^*$
Lymphocytes (%)	68.53 ± 5.72	72.65 ± 4.40	75.45 ± 3.14	78.35 ± 2.30
Monocytes (%)	3.63 ± 0.34	2.03 ± 0.26	5.25 ± 0.71	2.50 ± 0.29
Eosinophils (%)	0.63 ± 0.13	0.38 ± 0.14	0.50 ± 0.11	0.75 ± 0.10
Basophils (%)	7.30 ± 1.06	5.38 ± 1.43	5.65 ± 1.36	7.05 ± 0.68

Values are mean \pm SEM of triplicate determinations. Values marked asterisk is significantly ($p < 0.05$) different from control across row.

MC - Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC - Mean corpuscular haemoglobin

DISCUSSION

Free radicals have been involved in the pathology of many disease conditions such as cardiovascular diseases, inflammation, aging, diabetes and cancer due to insufficient natural antioxidant defense mechanism (Borguini and Torres, 2009; Uchegbu *et al.*, 2015). Aqueous extract of *C. dactylon* of different concentrations (25, 50, 100, 200 and 400 µg/ml) were used for antioxidant assay using DPPH radical scavenging method. The result is presented on table I and it showed increased antioxidant activity with increase in concentration of the extract. Ascorbic acid was used as a standard for the DPPH scavenging assay. Optimum antioxidant activity of *C. dactylon* was observed at the concentration of 400 µg/ml, though lower than that of ascorbic acid at same concentration.

Table II represents the result of the free radical scavenging activity of *C. dactylon* using ferric reducing antioxidant power (FRAP) assay. The result showed increasing antioxidant activity as the concentration increased. Highest antioxidant activity was exhibited by aqueous extract of *C. dactylon* the concentration of 100 µg/ml and then dropped at the concentration of 200 µg/ml. The activity of the standard (ascorbic acid) was higher than that of *C. dactylon* extract at all tested concentration. However, this study revealed that *C. dactylon* possesses antioxidant properties as reported earlier (Saradha *et al.*, 2011; Roy *et al.*, 2016) and could be attributed to its phenolic and flavonoid contents (Chou and Young, 1975; Annapurna *et al.*, 2013; Li *et al.*, 2017).

Haematological parameters serve as an important bio-indicator of health and physiological functions. Change in blood profiles is a predictor for human toxicity (Olson *et al.*, 2000). Administration of different concentrations of aqueous extract of *C. dactylon* did not significantly affect the haematological status of the animals when

compared with the control group (Fig. I&II). Concentrations of packed cell volume, haemoglobin and red blood cells are used to dictate anemia and its severity (Cheesbrough, 2005). From the results, group 1 showed a slight increase in the PCV from the control, though not statistically significant ($p > 0.05$). There was a dose dependent increase in the concentration of lymphocytes of the groups administered with plant extract. However, the results of the evaluated haematological parameters suggested that the blood profile on the animals were not adversely affected by the administration of *C. dactylon* extract.

CONCLUSION

The aqueous extract of *C. dactylon* exhibited antioxidant potency and did not show any toxic effect on the blood profile of the animals at tested doses.

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