Assessment of the Inherent in-vitro Antioxidant Potential of Commelina benghalensis Leaf Extract

Tebekeme Okoko¹*

¹Department of Biochemistry, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Author’s contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Commelina benghalensis is a troublesome but exotic weed native to the African and Asian subregions used traditionally for the treatment and management of various disorders. The aim of this study was to investigate the potential antioxidant activity of the methanolic leaf extract of Commelina benghalensis using various in vitro models. This was done by investigating the ability of the extract to scavenge hydrogen peroxide and hydroxyl radical. Other activities assessed were the reducing ability, ability to inhibit erythrocyte damage and reduce ferrous-ascorbate induced lipid peroxidation on bovine liver and egg yolk homogenates. The results revealed that the plant extract possessed significant hydrogen peroxide and hydroxyl radical scavenging abilities. The extract also possessed significant ability to reduce ferric ions and molybdate VI. The methanolic extract also significantly inhibited hydrogen peroxide-induced erythrocyte hemolysis and lipid peroxidation. Lipid peroxidation in bovine liver and egg yolk homogenates induced by the ferrous-ascorbate system was also reduced by the extract. In many instances, the effect of the extract was concentration-dependent. (p < 0.05). This antioxidant activity of the extract is ascribed to the phytochemicals which probably acted in
synergy thus the *Commelina benghalensis* leaves could be exploited both nutraceutically and pharmacologically.

**Keywords:** *Commelina benghalensis; hydrogen peroxide; lipid peroxidation; erythrocyte; antioxidant.*

**1. INTRODUCTION**

It is believed that the consumption of plants or diets rich in plants/plant products has been encouraged because of the reported beneficial effects of botanicals. Thus the biological/health promoting effects of a lot of plants have been investigated. However, this seem not to correlate with the reported consumption of such plants. In addition to possessing enormous bioactive potentials, plant/plant products are relatively cheap in addition to the reported less-frequent side effects associated with their use when compared with synthetic drugs. Up till date, many regions of the world rely on plants as first line defense or treatment against a lot of ailments. Many of such plants are weeds thus it is quite interesting that they could have medicinal potentials.

*Commelina benghalensis* is a succulent, astringent troublesome weed native to Africa and Asia [1]. The plant (also called tropical spiderwort) is an exotic, invasive, herbaceous perennial of tropical climate but grows as an annual in temperate regions [2]. The plant which belongs to the family Commelinaceae, possesses 2.5 – 7.5 cm long ovate leaves, erect stem and aerial flowers that are chasmogamous [1,2]. The leaves of *Commelina benghalensis* are used traditionally for the treatment of headaches, constipation, snake bites, skin lump and cancer [3,4]. Based on these reported traditional uses, this current work investigates the potential antioxidant activity of the methanolic extract of *Commelina benghalensis* leaves using various in vitro models. The search for plant based antioxidants has been encouraged because they are assessable and cheap.

**2. MATERIALS AND METHODS**

**2.1 Chemicals**

Ascorbic acid, 1,10 phenanthroline, sulphuric acid, ferrous chloride, hydrogen peroxide, potassium iodide, ferric chloride, potassium ferricyanide, trichloroacetic acid, absolute methanol, n-butanol and thiobarbituric acid were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and commercially available. Where appropriate, buffers and solutions were prepared using double glass distilled water.

**2.2 Preparation of Extract**

*Commelina benghalensis* leaves were harvested from a forest in Obunagha, Yenagoa Local Government area of Bayelsa State. The plant was identified by Prof I Ogidi of the Department of Crop Production, Niger Delta University. The leaves were sundried, pulverized using a blender and soaked in absolute methanol for approximately 72 hrs at room temperature in the dark. The contents were filtered and concentrated using a rotary evaporator set at 40°C. It was further dried to a paste and various concentrations (50 – 500 µg/ml) of the extract were made using distilled water.

**2.3 Hydrogen Peroxide Scavenging Activity**

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al. [5] with some modifications. Briefly 0.5 ml of either extract or vitamin C (as standard) was delivered into a test tube followed by 0.6 ml of 40 mM hydrogen peroxide (prepared in 0.02 M phosphate buffer, pH 7.4). Tube was incubated for 10 min at room temperature. Absorbance was measured at 230 nm against a reagent blank. The hydrogen peroxide scavenging activity was expressed as reported [5].

**2.4 Hydroxyl Radical Scavenging Activity**

The ability of the extract to scavenge hydroxyl radicals was determined based on the Fenton reaction according to the method reported by Yu et al. [6] with modifications. Briefly, 1 ml of phosphate buffer (0.02 M, pH 7.2), 0.02 ml of ferrous chloride (0.02 M), 1 ml of extract or standard and 0.5 ml of 1,10 phenanthroline (0.04 M) were delivered into a test tube. The reaction was initiated by the addition of 0.05 ml of 7 mM hydrogen peroxide. Absorbance was measured at 560 nm.
after 5 min of incubation at room temperature. The relative hydroxyl radical scavenging activity as reported [6].

2.5 Ferric Reducing Ability

The ferric reducing ability was analysed according to the method of Oyaizu [7] as modified [8]. Extract or vitamin C (0.5 ml) was incubated with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (1%) at 50°C. After 20 minutes, 0.5 ml of trichloroacetic acid (10%) was added and centrifuged for 10 minutes at 3000 rpm. A portion of the upper layer (0.5 ml) was mixed with 0.5 ml distilled water and 0.1 ml ferric chloride (0.1%) and incubated for 10 min incubation at room temperature. Absorbance was subsequently measured at 700 nm. Increase in absorbance indicated greater reducing ability.

2.6 Phosphomolybdate Assay

This is based on the reduction of molybdate VI. The antioxidant activity via the phosphomolybdate method was determined according to the method of Jayaprakasha et al. [9] as modified [8]. Briefly, 0.2 mL of either extract or control was mixed with 1 mL of reagent stock (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated in a boiling water bath for 90 min. Absorbance was read at 695 nm after allowing contents to cool.

2.7 Inhibition of Erythrocyte Hemolysis

Erythrocytes were prepared from whole blood as described [8] and re-suspended in phosphate buffered saline (0.02 M, pH 7.4) to desired hematocrit level. In order to induce hemolysis, 100 µL of hydrogen peroxide (100 µM) was incubated with 200 µL of erythrocytes. For the inhibitory assay, 200 µL of either extract or vitamin C was added and contents were incubated for 3 h at 37°C. Thereafter, 8 µL of phosphate buffered saline (0.02 M, pH 7.4) was added to the tube and centrifuged for 2880 x g for 10 min. Absorbance of the supernatant was measured at 540 nm.

2.8 Inhibition of Erythrocyte Lipid Peroxidation

Hydrogen peroxide was used to induce lipid peroxidation in erythrocytes as described [8]. Briefly, 200 µL of hydrogen peroxide (200 µM) was added to 200 µL of erythrocyte. Either extract or vitamin C (200 µL) was added and incubated for 1 h at 37°C. Thereafter, 2 mL of 15% trichloroacetic acid (containing 0.375% thiobarbituric acid and 0.25 M HCl) was added to the contents and incubated in a boiling water bath for 15 min. Tube was cooled and centrifuged at 2880 x g for 10 min. Absorbance of the supernatant was measured at 532 nm.

2.9 Anti-Lipid Peroxidation Activity on Tissue Homogenates

The anti-lipid peroxidation activity of extract on tissue homogenates was performed according to the method of Yoshiyuki et al. [10] with some modifications. Homogenates were prepared from fresh bovine liver and egg yolk as described [11]. Homogenate (0.5 mL) was mixed with 0.1 ml of 0.04 M ferrous chloride, 0.1 ml of 0.1 mM ascorbic acid, 0.2 ml of 0.02 M phosphate buffer (pH 7.4) and 0.5 mL of either extract or control (quercetin monohydrate). Distilled water (1.8 ml) and 2 mL of 2% thiobarbituric acid were also added and contents were incubated in a boiling water bath for 30 min. after cooling at room temperature, 5 mL of n-butanol was added and shaking vigorously. The n-butanol layer was collected via centrifugation and absorbance measured at 532 nm.

2.10 Statistical Analysis

Values are expressed as mean ± SEM (n = 6) and data were analyzed using analysis of variance followed by Duncan’s multiple range test. Significance was set at p < 0.05. All the statistics were carried out using Past3 data analysis package.

3. RESULTS

3.1 Hydrogen Peroxide and Hydroxyl Radical Scavenging Activities

Fig. 1 shows the hydrogen peroxide and hydroxyl radical scavenging activities of Commelina benghalensis extract. It revealed that the plant extract possessed considerable ability to scavenge both hydrogen peroxide and hydroxyl radicals when compared to the standard. For the hydrogen peroxide scavenging activity, the variation was significant between 50 and 100 µg/mL (p < 0.05) but the response exhibited between 100 and 200 µg/mL was not significant (p > 0.05). However, the extract at 500 µg/mL exhibited the highest scavenging activity which
was significant when compared to the other concentrations \( (p < 0.05) \). The hydroxyl radical scavenging activity was concentration-dependent \( (p < 0.05) \).

### 3.2 Reducing Ability

The reducing abilities of the extract is shown in Fig. 2. It revealed that the variations in the ferric ion reducing abilities of the extract was not significant from 50 to 200 \( \mu g/mL \) However, the response exhibited by the extract at 500 \( \mu g/mL \) was significantly higher than the other concentrations \( (p < 0.05) \). The reducing ability via the phosphomolybdate method was concentration-dependent \( (p < 0.05) \).

### 3.3 Inhibition of Erythrocyte Damage

The ability of the extract to reduce hydrogen peroxide-induced erythrocyte hemolysis and lipid peroxidation is shown in Fig. 3. The ability of the extract to reduce hemolysis was significant between 50 and 100 \( \mu g/mL \) but there was no significant difference exhibited by the extract 100 and 200 \( \mu g/mL \) \( (p > 0.05) \). The extract at 500 \( \mu g/mL \) exhibited the highest ability to reduce hemolysis. The ability of the extract to inhibit erythrocyte lipid peroxidation was concentration-dependent \( (p < 0.05) \).

### 3.4 Anti-Lipid Peroxidative Activity on Tissue Homogenate

The ability of the extract to inhibit ferrous-ascorbate induced lipid peroxidation on liver and egg homogenates is shown in Fig. 4. For both tissue homogenates, the response was concentration-dependent \( (p < 0.05) \).

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**Fig. 1. Ability of Commelina benghalensis leaf extract to scavenge (A) Hydrogen peroxide and (B) Hydroxyl radicals**

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly \( (p < 0.05) \). Vitamin C (control) was used at 100 \( \mu g/ml \).

**Fig. 2. Reducing ability of Commelina benghalensis leaf extract. (A) Ferric reducing ability and (B) Antioxidant activity via phosphomolybdate reduction**

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly \( (p < 0.05) \). Vitamin C (control) was used at 100 \( \mu g/ml \).
Fig. 3. Inhibitory effect of *Commelina benghalensis* leaf extract of hydrogen peroxide induced (A) Hemolysis and (B) Lipid peroxidation in erythrocytes

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (*p* < 0.05). Vitamin C (control) was used at 100 µg/ml

Fig. 4. Inhibitory effect of *Commelina benghalensis* leaf extract on ferrous-ascorbate induced lipid peroxidation on (A) Liver homogenate and (B) Egg yolk homogenate

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (*P* < 0.05). Quercetin (control) was used at 100 µg/ml

4. DISCUSSION

The production of free radicals and other reactive oxygen/nitrogen species is inevitable in the biological system under both pathological and non-pathological states. These highly reactive species attack important biological molecules in cells and tissues however their activities are kept in check by robust antioxidant systems which are either enzymatic or non-enzymatic. Should an imbalance occur to the detriment of the antioxidants, a condition called oxidative stress sets in which has been implicated in disorders such as diabetes, cardiovascular disorders, cancer, ageing, stroke, arthritis, neurological disorders etc. [12-14].

Hydrogen peroxide is a stable reactive oxygen species (ROS) but highly diffusible because of its lipophilicity. It is produced from superoxide via dismutation (i.e. reactions catalyzed by superoxide dismutase), reactions with electron donors and chain reactions [15]. Hydrogen peroxide is involved in many physiological processes such as hypoxic signal transduction, cell differentiation, proliferation and mediating immune response [14,16]. Hydrogen peroxide is pivotal among the ROS since it is generated from almost all oxygen radicals [17]. Excess hydrogen peroxide is reduced to water by catalase however stressors could elevate its level above tolerable limit where it attacks important biological molecules such as lipids, proteins and DNA to cause cell and tissue damage [17,18]. Thus the scavenging of hydrogen peroxide is key to cellular protection. In the current study, *Commelina benghalensis* extract scavenged hydrogen peroxide and hydroxyl radicals when compared to the antioxidant standard vitamin C (Fig. 1). The hydroxyl radical is formed from hydrogen peroxide in a Fenton reaction catalyzed by redox active metal ions. The radical is the most potent among the biologically active free radicals as it reacts indiscriminately with any molecule it touches hence a principal source of cellular stress [19]. The extract also possesses
significant ability to reduce ferric ions and molybdate VI. The reducing ability is important as it could slow or stop the Fenton reactions thus the production of powerful oxidants could be reduced.

*Commelina benghalensis* leaf extract also reduced hydrogen peroxide-induced hemolysis and lipid peroxidation in erythrocytes. Erythrocytes are highly vulnerable to lipid peroxidation due to constant exposure to high oxygen tension coupled with their high amount of polyunsaturated fatty acids and presence of transition metals that are redox active [20]. Thus coupled with their ease of isolation, the erythrocyte is an excellent model for the study of oxidative damage [21]. The erythrocyte also has robust antioxidant systems but increased oxidative stress could occur in pathological states such as β-thalassemia, sickle cell anaemia, glucose-6-phosphate dehydrogenase deficiency [20]. This could manifest as disruption in membrane permeability, increase in lipid peroxidation, hemolysis, oxidation of sulphhydral groups and proteolysis [22]. Hydrogen peroxide is neutral and liposoluble thus easily diffuses across the erythrocyte membrane to cause damage if in excess [23].

The extract also reduced chemically-induced lipid peroxidation in liver and egg homogenate (Fig. 4). In the current experiment, lipid peroxidation was induced in the homogenates using the ferrous-ascorbate system. This system (at low concentrations) has been reported to initiate radical formation from lipid hydroperoxides [24].

Phytochemicals are non-nutritive, naturally occurring compounds that are often products of secondary metabolism. They are antioxidant in nature because they act as reducing agents, donate hydrogen to quench free radicals and chelate and/or reduce redox active metals [25]. Hence intake could be beneficial against ROS-mediated disorders. Phytochemicals are also strongly bioactive because they modulate signal transduction pathways [26,27]. For instance, the antioxidant and anti-inflammatory potentials of flavonoids, polyphenols, carotenoids, terpenoids and alkaloids have been reported [28-30]. These phytochemicals have been detected in the plant in various studies [1,31].

Hence the bioactive potential of the plant extract could be attributed to the various phytochemicals which might have acted in synergy. Further work is aimed at characterizing compounds in the plant with bioactive potentials.

5. CONCLUSION

This current work reveals that the methanolic extract of *Commelina benghalensis* leaves scavenged hydrogen peroxide, hydroxyl radicals, reduced ferric ions and molybdate, inhibited erythrocyte damage and lipid peroxidation in tissues. Thus the plant could be an invaluable source of antioxidants hence could serve as a cost effective food/feed additive. Further isolation and characterization of bioactive compounds is required and already in progress. The assessment of the bioactive potential in cell line based systems is also recommended.

ETHICAL APPROVAL

The study protocol was performed according to the Helsinki declaration of 1964 and approved by the Institutional Ethical Committee.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Ibrahim J, Ajaegbu VC, Egharevba HO. Pharmacognostic and phytochemical analysis of *Commelina benghalensis* L. Ethnobotanical Leaflets. 2010;14:610-615.
2. Culpepper AS, Flanders JT, York AC, Webster TM. Tropical spiderwort (*Commelina benghalensis*) control in Glyphosate-resistant cotton1. Weed Tech. 2004;18(2):432-436.
3. Kabir MS, Hasanat A, Chowdhury TA, Rashid MM, Hussain MM, Ahmed S. Study of antidiarrheal and anthelmintic activity methanol extract of *Commelina benghalensis* leaves. Afr J Pharm Pharmacol. 2016;10(32):657-664.
4. Mbazima VG, Mokgotho MP, February F, Rees DJ, Mampuru LJ. Alteration of Bax-to-Bcl-2 ratio modulates the anticancer activity of methanolic extract of *Commelina benghalensis* (Commelinaceae) in Jurkat T cells. Afr J Biotechnol. 2008;7(20):3569-3576.
5. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by...
antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10(6):1003-1008.
6. Yu W, Zhao Y, Shu B. The radical scavenging activities of radix puerariae isoflavonoids: A chemiluminescence study. Food Chem. 2004;86(4):525-529.
7. Oyaizu M. Studies on products of browning reaction: The Jap J Nutr Diet. 1986; 44(6):307-315.
8. Okoko T, Ere D. Reduction of hydrogen peroxide-induced erythrocyte damage by carica papaya leaf extract. Asian Pacific J Trop Biomed. 2012;2(6):449-453.
9. Jayaparakasha GK, Jena BS, Negi PS, Sakariah KK. Evaluation of antioxidant activities and antimutagenicity of turmeric oil: A byproduct from curcumin production. Zeitschrift Für Naturforschung C. 2002; 57(9-10):828-835.
10. Yoshiyuki K, Michinori K, Tatado T, Shigeru A, Hiromichi O. Studies on scutellariae radix. IV. Effects on lipid peroxidation in rat liver. Chem Pharm Bull. 1981;29:2610-2617.
11. Ndoni S, Ere D, Okoko T. Assessment of the in vitro anti-lipid peroxidative activity of costus afer stem extract. Oxidants Antioxid Med Sci. 2017;6(2):30-34.
12. Graves DB. The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. J Physics D: Appl Physics. 2012;45(26):263001.
13. Roberts RA, Smith RA, Safe S, Szabo C, Tjalkens RB, Robertson FM. Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. Toxicol. 2010;276(2):85-94.
14. Figueira TR, Barros MH, Camargo AA, Castilho RF, Ferreira JC, Kowaltowski AJ, Sluse FE, Souza-Pinto NC, Vercesi AE. Mitochondria as a source of reactive oxygen and nitrogen species: From molecular mechanisms to human health. Antioxid Redox Signal. 2013;18(16):2029-74.
15. Buettner GR, Ng CF, Wang M, Rodgers VG, Schafer FQ. A new paradigm: Manganese superoxide dismutase influences the production of H2O2 in cells and thereby their biological state. Free Rad Biol Med. 2006;41(8):1338-1350.
16. Lennicke C, Rahn J, Lichtenfels R, Wessjohann LA, Seliger B. Hydrogen peroxide–production, fate and role in redox signaling of tumor cells. Cell Comm Signal. 2015;13(1):1-9.
17. Jian Z, Li K, Liu L, Zhang Y, Zhou Z, Li C, Gao T. Heme oxygenase-1 protects human melanocytes from H2O2-induced oxidative stress via the Nrf2-ARE pathway. J Invest Dermatol. 2011;131(7):1420-1427.
18. Kaneko S, Kawakami S, Harayama Y, Wakamori M, Itoh E, Minami T, Takada Y, Kume T, Katsuki H, Mori Y, Akaike A. A critical role of TRPM2 in neuronal cell death by hydrogen peroxide. J Pharmacol Sci. 2006;101(1):66-76.
19. Aruoma Ol. Free radicals and international nutrition. Asia Pacific J Clin Nutr. 1999; 8:53-63.
20. Zhu QY, Holt RR, Lazarus SA, Orozco TJ. Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. Exp Biol Med. 2002;227(5):321-329.
21. Kolanjappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clinica Chimica Acta. 2002;326(1-2):143-149.
22. Tavazzi B, Di Pierro D, Amorini AM, Fazzina G, Tuttobene M, Giardina B, Lazzarino G. Energy metabolism and lipid peroxidation of human erythrocytes as a function of increased oxidative stress. Eur J Biochem. 2000;267(3):684-689.
23. Herken H, Uz E, Özüyurt H, Söğüt S, Virit O, Akyol Ö. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. Mol Psychiatry. 2001;6(1):67-73.
24. Schafer FQ, Qian SY, Buettner GR. Iron and free radical oxidations in cell membranes. Cell Mol Biol (Noisy-le-Grand, France). 2000;46(3):657-662.
25. Embusodó ME. Spices and herbs: Natural sources of antioxidants–a mini review. J Func Foods. 2015;18:811-819.
26. Neergheen VS, Bahorun T, Taylor EW, Jen LS, Aruoma OI. Targeting specific cell signaling transduction pathways by dietary and medicinal phytochemicals in cancer chemoprevention. Toxicol. 2010; 278(2):229-241.
27. Bosch R, Philips N, Suárez-Pérez JA, Juarranz A, Devmuran A, Chalensouk-Khaosaat J, González S. Mechanisms of photoaging and...
cutaneous photocarcinogenesis and photoprotective strategies with phytochemicals. Antioxidants. 2015;4(2): 248-268.
28. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014;6(2): 466-488.
29. Cör D, Knez Ž, Knez Hrnčič M. Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of ganoderma lucidum terpenoids and polysaccharides: A review. Molecules. 2018;23(3):649.
30. Rehman S, Khan H. Advances in antioxidant potential of natural alkaloids. Curr Bioac Comp. 2017;13(2): 101-108.
31. Anusuya NA, Gomathi RA, Manian SE, Sivaram VE, Menon AN. Evaluation of basella rubra L. Rumex nepalensis spreng and Commelina benghalensis L. For antioxidant activity. Int J Pharmaceut Sci. 2012;4:714-720.

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