Original Research Article

Assessment of the inherent *in-vitro* antioxidant potential of *Commelina benghalensis* leaf extract

ABSTRACT

Commelina benghalensis is a troublesome but exotic weed native to the African and Asia 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 radicals. 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 product 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 extrant 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 METHOD

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 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 (0.2M, 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

Figure 1 shows the hydrogen peroxide and hydroxyl radical scavenging activities of *Commelina benghalensis* extract. It revealed that the plant extract possessed considerable abilitiy 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 ($p < 0.05$).

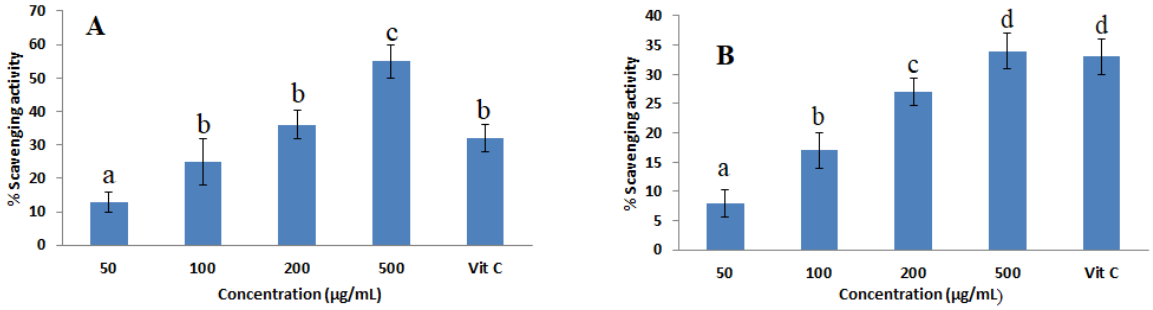

Figure 1. Ability of *Commelina benghalensis* leaf extract to scavenge (A) hydrogen peroxide and (B) hydroxyl radicals. *Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (P < 0.05). Vitamin C (control) was used at 100 µg/ml.*

3.2 Reducing ability

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

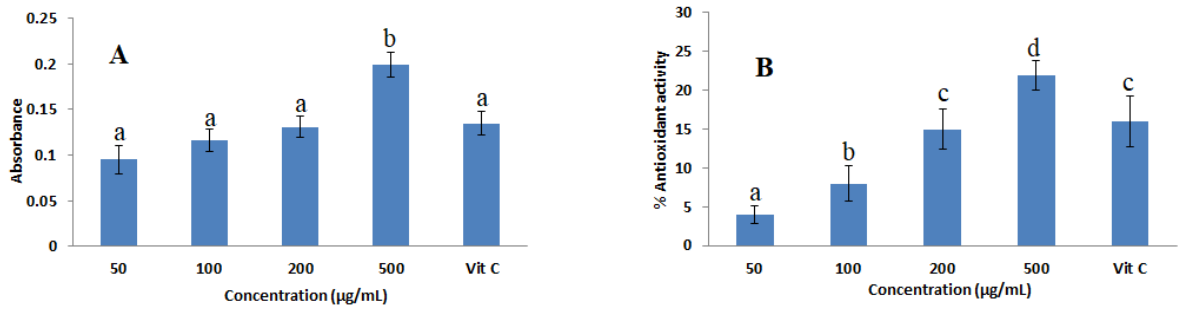

Figure 2. Reducing ability of *Commelina benghalensis* leaf extract. (A) Ferric reducing ability; (B) antioxidant activity via phosphomolybdate reduction. *Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (P < 0.05). Vitamin C (control) was used at 100 µg/ml.*

3.3 Inhibition of erythrocyte damage

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

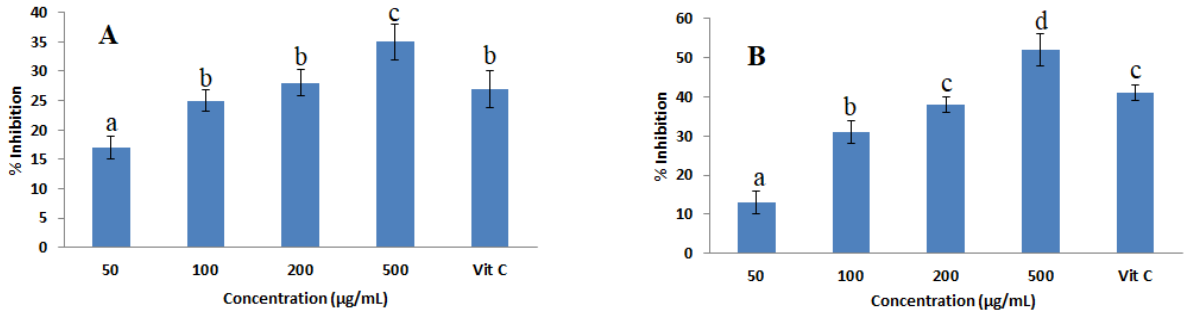

Figure 3. Inhibitory effect of *Commelina benghalensis* leaf extract of hydrogen peroxide induced (A) hemolysis and (B) lipid peroxidation in erythrocytes. *Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (p < 0.05). Vitamin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

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 figure 4. For both tissue homogenates, the response was concentration-dependent ($p < 0.05$).

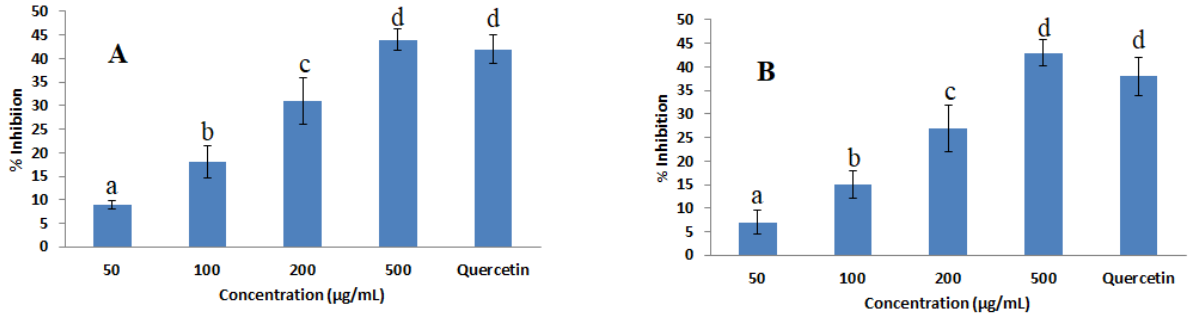

Figure 4. Inhibitory effect of *Commelina benghalensis* leaf extract on ferrous-ascorbate induced lipid peroxidation on (A) liver homogenate (B) Egg yolk homogenate. *Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (P < 0.05). 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 state. 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 (Figure 1). The hydroxyl radical is formed from hydrogen peroxide in a Fenton reaction calalyzed by redox active metal ions. The radical is the most potent among the biologically active free radicals as it

reacts indiscriminate with any molecule it touches hence a principal source of cellular stress [19]. The extract also possess 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 sulphuhydral 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 (figure 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 reactive 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* scavenged hydrogen peroxide, hydroxyl radicals, reduced ferric ions and molybdate, inhibited erythrocyte damage and lipid peroxidation in tissues. Thus the plant is a potent 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.

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