

Full Length Research Paper

Antioxidant activities of *Solenostemon monostachyus* leaf extract using *in vitro* methods

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***Solenostemon monostachyus* is an important herb that is native to West and Central Africa. The leaves have been traditionally used for various medicinal purposes however the scientific basis for these effects is scarce. The aim of this study was to investigate the *in vitro* antioxidant and free radical scavenging abilities of *S. monostachyus* extract by assessing its hydrogen peroxide scavenging ability, hydroxyl radical scavenging ability and ferric ion reducing ability. Other activities assessed are the ability of the extract to reduce hydrogen peroxide-induced erythrocyte haemolysis and lipid peroxidation. The results showed that the extract possessed significant abilities to scavenge hydrogen peroxide and hydroxyl radicals, and also significant ability to reduce ferric ions. The extract also possessed significant abilities to reduce lipid peroxidation and haemolysis in erythrocytes induced by hydrogen peroxide when compared with the ability of ascorbic acid to do the same. This is ascribed to the possession of antioxidant phytochemicals which acted in synergy, thus the plant could be exploited for pharmacological and nutraceutical purposes.**

Key words: *Solenostemon monostachyus*, antioxidant, free radical, haemolysis, phytochemical.

INTRODUCTION

Diets rich in fruits, cereals and vegetables have been shown to play a crucial role in the prevention of a lot of disorders such as cardiovascular diseases, certain cancer types and even aging (Joshi et al., 2001; Aruoma, 2003; Miller et al., 2000). Thus efforts have been made towards the elucidation of the bioactive potentials of many plants of recent. This has however not translated to the utilization of these plants coupled with the fact that the bioactive potentials of a lot of them are yet to be investigated. *Solenostemon monostachyus* P. Beauv (family Lamiaceae) is an important herb that is

widespread in West and Central Africa. It occurs as an annual weed in anthropogenic habitats and rocky savannahs. It is slightly succulent, aromatic and grows up to 100 cm tall (Mba and Menut, 1994). It has been reported that the plant has been traditionally used in the past for ritual purposes related to pregnancy (Leung et al., 1968). The decoction of the leaves is also taken as a diuretic (Koffi et al., 2009). Research has also shown that the leaves possess antimicrobial activity (Ekundayo and Ezeogu, 2006). The health promoting properties of plants are ascribed to the possession of various phytochemicals especially phenolics and this beneficial activity is related to their antioxidant activity (Heim et al., 2002). Many disorders especially cardiovascular disorders, diabetes, cataract, cancer, ageing are partly caused by reacting

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oxygen species (ROS) and reactive nitrogen species (RNS). Though the cells have intrinsic processes that could deal with these substances; serious pathological consequences may arise when there is excessive formation of free radicals and other ROS and this may overwhelm the activity of the endogenous antioxidant system thus the consumption of fruits, vegetables and beverages as antioxidant supplements is recommended (Knekt et al., 2002). The evaluation of the *in vitro* antioxidant and free radical scavenging activities of *S. monostachyus* leaf extract is one of the aims of the study.

Erythrocytes are prime targets for free radical attack due to the preponderance of polyunsaturated fatty acids (PUFA) in their membranes and the oxygen transport associated with redox active haemoglobin molecules which are promoters of ROS (Sadrazadeh et al., 1984). Thus erythrocytes have been used as model for the study for potential antioxidants since it has been suggested that some phytochemicals from fruits can either protect or increase resistance of erythrocytes to oxidative stress (Youdim et al., 2000; Tedesco et al., 2001). Hydrogen peroxide is an important ROS that could initiate free radical production and lipid peroxidation in erythrocytes by causing the degradation of haem with the release of Fe ions (Puppo and Halliwell, 1988). Thus the other aim of this research is to evaluate whether *S. monostachyus* leaf extract could protect or reduce lipid peroxidation and haemolysis induced by hydrogen peroxide.

MATERIALS AND METHODS

Chemicals and reagents

Thiobarbituric acid, trichloroacetic acid, hydrogen peroxide, ascorbic acid, 1, 10 phenanthroline, anhydrous sodium phosphate (monobasic and dibasic) and sulphuric acid were purchased from Sigma Aldrich Chemical Co. USA. All other reagents and chemicals were obtained commercially and were of the highest grade thus used without further purification. All dilutions were made in double-glass distilled water unless otherwise stated.

Preparation of plant material

Fresh leaves of *S. monostachyus* were obtained from Sampou forest, Bayelsa State, Nigeria. They were sun-dried and later ground to powdery form using a warring blender. Extraction was done in a soxhlet apparatus using absolute methanol as solvent and subsequently concentrated using a rotary evaporator set at 40°C. A residue of 10.4 g was obtained after drying to constant mass. Various concentrations (0.25, 0.5, 1.0 and 2.0 mg/ml) of the extract were prepared and later used for subsequent analysis.

Hydrogen peroxide scavenging activity

This was done for the various extract solutions by replacement

titration according to Zhao et al. (2006) with a slight modification. Briefly, 3 ml of 3% ammonium molybdate was mixed with 1 ml of extract, 1 ml of 0.1 mM hydrogen peroxide, 10 ml of sulphuric acid (2 M) and 7 ml of potassium iodide (1.8 M) in a conical flask. This was titrated with 5.09 M sodium thiosulphate until the yellow colour disappeared. The hydrogen peroxide scavenging activity was expressed as a percentage thus:

$$\% \text{ scavenging activity} = \frac{V_{\text{control}} - V_{\text{sample}}}{V_{\text{control}}} \times 100\%$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the extract was determined as described by Yu et al. (2004) with modifications. This assay is based on the Fenton reaction. Briefly, 1 ml of phosphate buffer (0.2 M, pH 7.2), 0.02 ml of ferrous chloride (0.02 M), 1 ml of extract and 0.5 ml of 1, 10 phenanthroline (0.04 M) were delivered into a test tube. The Fenton 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 was calculated thus:

$$\% \text{ scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Reducing ability

The reducing ability was determined according to the method of Oyaizu (1986) with some modifications. The extract (5 ml) was mixed with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 5 ml of 1% potassium ferricyanide. After incubation for 20 min at 50°C, 0.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. A portion of the upper layer (0.5 ml) was mixed with distilled water (0.5 ml) and 0.1% ferric chloride. After 10 min, the absorbance was measured at 700 nm, the reducing ability was calculated as:

$$\% \text{ scavenging activity} = \frac{A_{\text{sample}} - V_{\text{control}}}{V_{\text{control}}} \times 100\%$$

Erythrocyte lipid peroxidation

Blood (20 to 25 ml) was collected via the vein from a healthy volunteer after obtaining informed consent. It was delivered into a heparinised bottle and centrifuged for 10 min at 4000 rpm at 4°C. It was washed four times with phosphate buffered saline (0.2 M, pH 7.4) and re-suspended to the desired hematocrit level using the same buffer. In order to induce lipid peroxidation, 100 µL of hydrogen peroxide (100 µM) was delivered into 200 µL of the erythrocyte suspension. 200 µL of the extract (as inhibitor) was also delivered into the tube. The entire contents was swirled gently and incubated for 1 h at 37°C. The reaction was halted by the addition of 2 ml of stopping solution (15% w/v TCA, 0.375% TBA, 0.25 M HCl). The entire solution was thereafter incubated in a boiling water bath for 15 min. After cooling, the precipitate was removed by

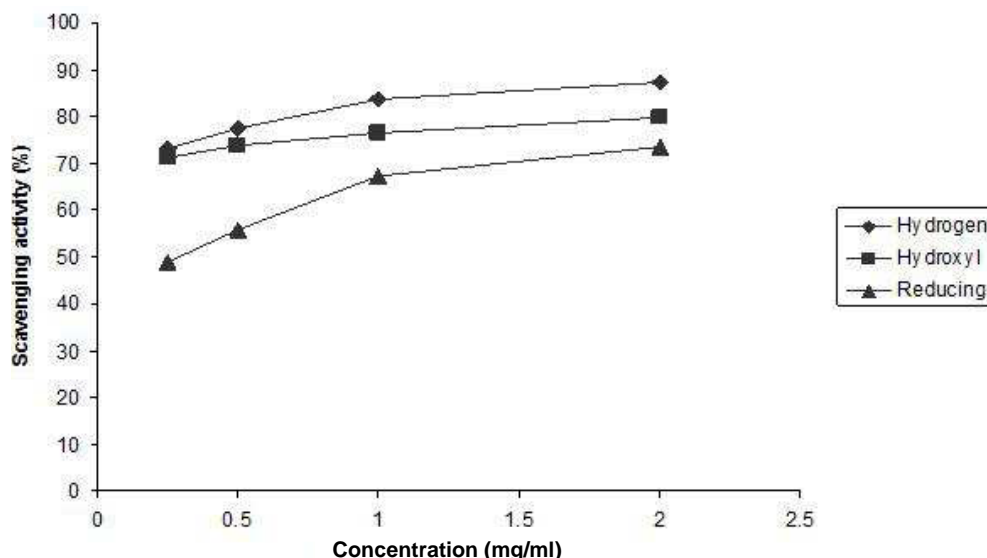


Figure 1. Scavenging activities of *Solenostemon monostachyus* leaf extract. 'Hydrogen' represents hydrogen peroxide scavenging activity; 'hydroxyl' represents hydroxyl radical scavenging activity while 'reducing' represents reducing abilities.

centrifugation at 3000 rpm for 5 min and the absorbance of the resulting supernatant measured at 532 nm. Increase in absorbance indicates greater lipid peroxidation.

Erythrocyte haemolysis

In order to induce haemolysis, 100 μ L of 100 μ M hydrogen peroxide was added to 200 μ L of erythrocytes (as prepared earlier). 200 μ L of the extract was also added. Contents were gently swirled and incubated for 3 h at 37°C. Thereafter, 8 ml of phosphate buffered saline (0.2 M, pH 7.4) was added and centrifuged at 3000 rpm for 10 min. Absorbance of the supernatant was read at 540 nm. Increase in absorbance indicates greater haemolysis.

Statistical analysis

In all cases, ascorbic acid (2.0 mg/ml) was used as a reference antioxidant. For the control experiment, neither extract nor ascorbic acid was added. For experiments involving erythrocytes, treatment without extract or ascorbic acid was taken as either 100% lipid peroxidation or 100% haemolysis. All experiments were done four times. Representative values were expressed as mean \pm SEM. Where appropriate, data were subjected to analysis of variance (ANOVA) or two-tailed students *t*-test. Confidence level exhibited at $P < 0.05$ was considered statistically significant. The concentration of the extract and ascorbic acid to reduce lipid peroxidation and haemolysis by 50% (that is IC_{50}) was graphically estimated using a non-linear regression algorithm.

RESULTS

The hydrogen peroxide scavenging activities, hydroxyl

radical scavenging activities and the reducing abilities of the extract are shown in Figure 1. All the various concentrations of the extract possessed significant antioxidant activities when compared with ascorbic acid.

In all cases, the antioxidant activities increased with increase in the concentration of the extract. The hydrogen peroxide scavenging activity of ascorbic acid (2.0 mg/ml) was lower than the activities recorded for 2.0 mg/ml of the extract; however, the hydroxyl radical scavenging activity and the reducing ability of ascorbic acid (2.0 mg/ml) were significantly higher than the extract ($P < 0.05$) (Table 1). In all the antioxidant models, significant differences were exhibited among the various concentrations ($P < 0.05$). Table 2 shows the inhibitory activities of the extract on hydrogen peroxide induced erythrocyte lipid peroxidation and haemolysis. It was observed that there was a concentration dependent increase in the abilities of the extract to inhibit lipid peroxidation and haemolysis induced by hydrogen peroxide ($P < 0.05$). However, ascorbic acid was better at inhibiting both lipid peroxidation and haemolysis (IC_{50} values of extract and ascorbic acid are shown in Table 3).

DISCUSSION

Several human disorders have been ascribed to the influence of free radicals and reactive oxygen and nitrogen species which are products of normal cellular processes such as aerobic metabolism. Some of these

Table 1. Comparison of the antioxidant activities (%) of *Solenostemon monostachyus* extract (2.0 mg/ml) and ascorbic acid (2.0 mg/ml).

Variable	Hydrogen (%)	Hydroxyl (%)	Reducing (%)
Extract (2.0 mg/ml)*	87.33 ± 1.15	79.57 ± 0.86	73.33 ± 0.29
Ascorbic acid (2.0 mg/ml)	67.34 ± 1.07	92.76 ± 1.83	87.21 ± 0.93

Values are presented as mean ± SEM. *Denotes significant difference from ascorbic acid ($P < 0.05$). 'Hydrogen' represents hydrogen peroxide scavenging activity; 'hydroxyl' represents hydroxyl radical scavenging activity while 'reducing' represents reducing abilities.

Table 2. Inhibiting activity of *Solenostemon monostachyus* extract on lipid erythrocyte peroxidation and haemolysis.

Concentration	Lipid peroxidation (%)	Haemolysis (%)
0.25	5.07 ± 0.15	2.7 ± 0.62
0.50	14.70 ± 0.30	6.27 ± 0.21
1.00	22.30 ± 0.00	7.37 ± 0.61
2.00	26.67 ± 2.31	18.47 ± 0.56

Values presented as mean ± S.E.M from three independent experiments. LPO (%) represents % inhibition of lipid peroxidation; Haem (%) represents inhibition of haemolysis. Groups are significantly different from one another ($P < 0.05$).

Table 3. IC₅₀ values for extract and ascorbic acid for the reduction of erythrocyte lipid peroxidation and haemolysis.

Variable	Lipid peroxidation (mg/ml)	Haemolysis (mg/ml)
Extract*	3.54	5.64
Ascorbic acid	0.43	1.87

Values were graphically estimated using a non-linear regression algorithm.

highly reactive species can also be induced or enhanced by environmental factors; some of which are inevitable to life, while others are pollutants including genotoxic metals (Shi et al., 2004; Ercal et al., 2001). Though the intrinsic antioxidant system could protect the cells from the damage of these free radicals and reactive species, elevated levels could overwhelm the intrinsic free radical scavenging systems which could lead to a variety of disorders such as mutagenesis, aging, carcinogenesis, diabetes etc (Feig et al., 1994). The use of complementary/alternative medicine especially the consumption of botanicals has been encouraged mostly because of the reported less frequent side effects when compared with modern medicine (Hu et al., 2003). The bioactive property is as a result of the possession of dietary phytochemicals which research has found as effective free radical scavengers, potent metal chelators and decomposition of lipid peroxides (Dufresne and

Fransworth, 2001; Okoko, 2009). The results showed that the extract of *S. monostachyus* possessed significant ability to scavenge hydroxyl radicals, hydrogen peroxide and reducing abilities. It has been demonstrated that Fe ion induce the formation of reactive oxygen species (Avery, 2001; Valko et al., 2005) which causes modification of DNA bases, enhances lipid peroxidation in addition to the alteration of calcium and redox homeostasis (Valko et al., 2004). The reducing ability of the extract could be useful in several ways. Ferric ions are quite redox active and can catalyze reactions to produce radicals including the hydroxyl radical thus the extract could reduce the ferric ions and may lessen its redox active catalysis. The hydroxyl radical attacks proteins, membrane lipids, and other biomolecule it touches (Aruoma, 1999), thus the extract could reduce the hydroxyl radical-mediated oxidation of DNA and membrane lipids. Free radicals can also reduce the

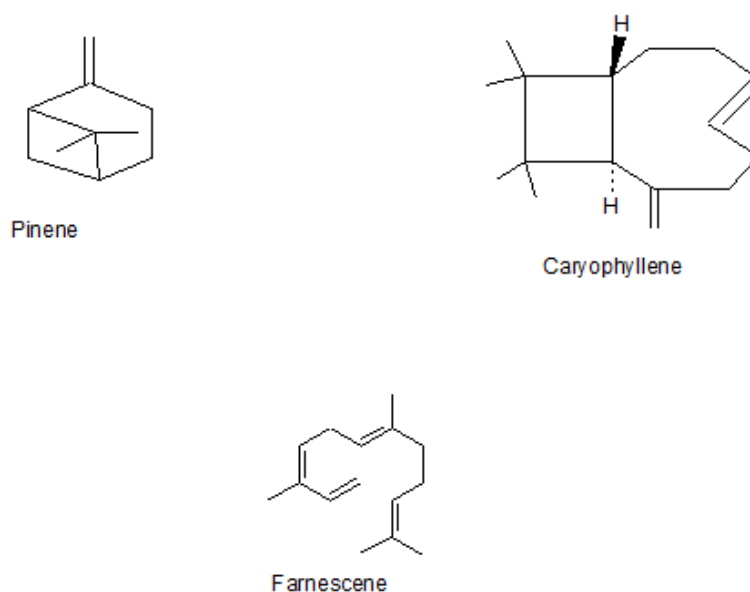


Figure 2. Some compounds isolated from *Solenostemon monostachyus*.

safety and nutritional value of food especially oily and dairy foods via the peroxidation of the lipids in cells and tissues. Hydrogen peroxide is an important reactive oxygen species that is formed by the reaction catalyzed by superoxide dismutase as part of the antioxidant cascade, xanthine oxidase and phagocytes (Fridovich, 1974; Zweier et al., 1994; Carter et al., 1994). It has the capacity to damage cells and macromolecules such as proteins and DNA. It also generates hydroxyl radicals via the Fenton reaction with Fe. Though hydrogen peroxide can be reduced to water via catalase activity, excessive production could cause severe circumstances hence the need for other scavengers which could even be as supplements.

S. monostachyus could serve as a good supplement because of its hydrogen peroxide scavenging potential. Erythrocytes are the most abundant cells in vertebrates. Their unique morphology and physiological nature are exploited in drug delivery and targeting (Hamidi and Tajerzadeh, 2003). Because of the preponderance of polyunsaturated fatty acids in the erythrocyte membranes, they are highly susceptible to oxidative damage whose consequences are lipid peroxidation and haemolysis. This has been a proposed mechanism for erythrocyte cell injury and death (Miki et al., 1987). Erythrocyte haemolysis can be caused by some haemoglobinopathies, oxidative drugs and redox active metals (Ko et al., 1997). Oxidative stress on erythrocytes induced by hydrogen peroxide is manifested as alteration of membrane proteins, changes in cell shape and distinct

echinocyte formation (Ajila and Prasada, 2008). Changes in erythrocyte membrane ion permeability, formation of disulphide bonds and activation of proteolysis are also consequence of oxidative challenge on erythrocytes (Tavazzi et al., 2000; Davies and Goldberg, 2001). Thus the inhibition or the reduction of hydrogen peroxide induced erythrocyte damage could reverse or reduce the aforementioned morphological and physiological changes in the erythrocytes and could restore the function of the oxygen-carrying cells. *S. monostachyus* is a prime candidate that could reverse the oxidative damages. Some compounds have been isolated from the leaves of *S. monostachyus*. Examples are the terpenoids β -pinene, oct-1-en-3-ol, β -caryophyllene, octan-3-ol, and *E, E*- α -farnesene (Figure 2) (Eyele et al., 2006). Dietary polyphenols, carotenoids and terpenes have exhibited significant medicinal potentials (Wang et al., 2008; Zhou et al., 2006). The observed effects of *S. monostachyus* could be ascribed to these antioxidant phytochemicals which acted in synergy.

The occurrence of *S. monostachyus* is part of nature's generosity to mankind thus should be exploited for pharmacological and nutraceutical purposes. However, the extract should be fractionated for characterization and further research.

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