ORIGINAL ARTICLE

In vitro antioxidant effect of aqueous extract of *Solanum macrocarpon* leaves in rat liver and brain

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Lipid peroxidation; Iron(II) sulphate; Reducing power; *Solanum macrocarpon*

Abstract

Objective: Antioxidants are known to offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms. They therefore help in preventing the free radicals induced diseases. *Solanum macrocarpon* is an important medicinal plant with rich source of nutritional and medicinal constituents. The objective of the present study was to investigate the ability of *S.macrocarpon* leaves to protect tissues against iron (Fe^{2+})-induced lipid peroxidation in rat's liver and brain.

Methods: The total phenolic and total flavonoid contents of the aqueous extract of *S.macrocarpon* leaves, as well as its antioxidant potential, free radical scavenging activity, reducing power and protective action on tissues against lipid peroxidation were carried out.

Results: *S.macrocarpon* leaves revealed high phenolic and flavonoid content. Incubating the liver and brain of rats with *S.macrocarpon* leave extract exhibited high percentage inhibition of lipid peroxidation induced by iron(II) sulphate. Moreover, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and reducing power of the extract increased as the concentration increases.

Conclusion: The aqueous extract of *Solanum macrocarpon* leaves possess a powerful antioxidant activity and can offer good protection against oxidative damage to body cells, especially liver and brain.

INTRODUCTION

Solanum macrocarpon is an important medicinal plant with rich source of nutritional and medicinal constituents. The genus *Solanum* comprises over 1000 species worldwide. *S.macrocarpon* has an African ancestry. Spiny wild forms are found throughout the tropical non-arid parts of Africa, their fruits still being gathered occasionally as vegetable, whereas the cultivated forms, called 'Gboma' in West Africa, constitute an important fruit and leaf vegetable, grown for the market and in home gardens [1]. The plant is commonly used for its nutrition and medicinal properties. It is widely cultivated in West Africa where it serves as an important fruit and leaf vegetable. Locally, the leaf is considered very nutritious and is used in preparing soups and stews. The leaves are rich in protein, fat, crude fiber, calcium and zinc [2]. The leaves have a variety of medicinal uses: for example, in Sierra Leone, boiled leaves are chewed to treat throat problems; in Kenya, the crushed leaves are taken to treat stomach problems [1].

Over-production of various forms of activated oxygen species, such as oxygen radicals and non-free radical species is considered to be the main contributor to oxidative stress, which has been linked to several diseases like atherosclerosis, cancer, and tissue damage in rheumatoid arthritis [3]. Fruits and plants are rich sources of phenolic compounds and have been recognized to possess a wide range of properties including antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anticarcinogenic actions. Antioxidant phenolic agents have been implicated in the mechanisms of chemoprevention which refers to the use of chemical substances of natural origin or synthetic to reverse, retard or delay the multistage carcinogenesis process. Numerous crude extracts and pure natural compounds from fruits were reported to have antioxidant and radical-scavenging activities. Phenolics or polyphenols, including flavonoids have received considerable attention because of their physiological functions such as antioxidant, antimutagenic and antitumor activities [4]. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thereby help in preventing the free radical induced diseases [5].

This study was designed to examine the *in vitro* antioxidant potential of *S.macrocarpon* and its protection of tissues against iron(II) (Fe^{2+})-induced lipid peroxidation. Important roles of the total phenolics and total flavonoids as antioxidants cannot be ruled out; therefore, the amounts of total phenolics and total flavonoids in the extract were also determined. The goal of this study therefore was to contribute to the

search for a cheap, safe and readily available remedy against free radical induced diseases. It is hoped that data or information from the present study would raise new possibilities in our quests for an effective plant based remedy against various diseases occasioned by free radicals.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Solanum macrocarpon* were obtained from Bisi market in Ado-Ekiti, Ekiti State, and were authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. A sample of the plant leaf was deposited at the Departmental Herbarium for future reference. The leaves were air-dried and blended. The blended material was then soaked in distilled water for 24 h following which it was filtered using Whatman filter paper no 1. The filtrate was concentrated at 50ºC using the rotary evaporator and dried by means of a freeze dryer.

Test animals

All animal procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press, Washington, DC, USA, 2011). All animal experiments were approved by the animal care committee of the Afe Babalola Research Center, Ado-Ekiti. Three rats of Wistar strains (180-220 g) were used for the *in vitro* assay.

Determination of total phenolic content

The total phenolic content of the aqueous leaves extract of *S.marcrocarpon* was determined using Folin-Ciocalteau reagent by the method of Spanos and Wrolstad [6] and modified by Lister and Wilson [7]. Plant extract solution (0.5 ml, 1 mg/l) was added to 2.5 ml of 10% Folin-Ciocalteau reagent and 2 ml of NA_2CO_3 (2% w/v). The resulting mixture was incubated at 45ºC with shaking for 15 min. The absorbance of the sample was measured at 765 nm using UV/visible light. Gallic acid was used as a standard for determining the phenol content and the estimated content was expressed as milligram gallic acid equivalents (GAE) per gram of plant material.

Determination of total flavonoid content

The total flavonoid content of the extract was determined using a colorimeter assay developed by Bao *et al* [8]. The extract (0.2 ml) was added to 0.3 ml of 5% $NaNO_3$ at zero time. After 5 min, 0.6 ml of 10% $AlCl_3$ was added, and after 6 min, 2 ml of NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as milligram quercetin equivalent per gram of plant material.

Assessment of lipid peroxidation

Production of thiobarbituric acid reactive substances (TBARS) was determined using a modified method of Ohkawa *et al* [9], as described by Puntel *et al* [10]. The rats were sacrificed by cervical dislocation method. The liver and brain were removed and placed on ice. One gram of the tissues were homogenized in cold 0.1 M tris buffer at pH 7.4 (1:10 w/v) with about 10 up and down strokes in a Teflon-glass homogenizer. The homogenates were centrifuged at 12,000 rpm for 5 min to yield a supernatant which was used for the assay and pellet that was discarded. The supernatant (100 µl) with or without 50 µl of the freshly prepared pro-oxidant (iron(II) sulphate), different amounts of the plant extract (10-1000 µg) and an approximate volume of distilled water which gives a total volume of 300 µl were incubated at 37ºC for 1 h. The color reaction was carried out by adding 200, 500 and 500 µl of each of 8.1% sodium dodecyl sulphate (SDS), 1.33 M acetic acid (pH 3.4) and 0.6% thiobarbituric acid (TBA), respectively. The reaction mixture was incubated at 97ºC for 1 h and the absorbance was read in a spectrophotometer after cooling the tubes at a wavelength of 532 nm.

DPPH radical scavenging activity

The method described by Liyana-Pathiranan and Shahidi [11] was used to determine the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the plant extract. A solution of 0.135 mM DPPH was prepared in methanol. The solution (1 ml) was mixed with 1 ml of the extract (50-400 mg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. The ability of the plant extract to scavenge DPPH radical was calculated by following equation:

$$(Abs_{control} - Abs_{sample}) / Abs_{control} \times 100\%$$

-$Abs_{control}$; absorbance of DPPH radical + methanol

-Abs_{sample}; the absorbance of DPPH radical + sample extract/standard

Reducing power

The reducing power of extracts was determined using a modified method of Wu *et al* [12] and Yildirim *et al* [13]. Two milliliters of sample was added to 2 ml of 0.2 M phosphate buffer (pH 6.6) and 2 ml of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 25 min, 2 ml of 10% tricholoroacetic acid was added to the reaction mixture and centrifuged for 10 min. Two milliliters of upper layer solution was mixed with 2 ml distilled water and 0.5 ml $FeCl_3$ (0.1%), in the tube. After 10 min the solutions were read at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Distilled water was used as blank, ascorbic acid was the reference standard.

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Statistical analysis

Data are presented as the mean ± standard deviation (SD). The significance of the results was evaluated using one way analysis of variance (ANOVA). Post-hoc testing was performed for comparisons using the Least Significant Difference (LSD) test according to the method described by Zar [14]. In all instances $P < 0.05$ was considered statistically significant.

RESULTS

As to see in Table 1, the aqueous extract of *Solanum macrocarpon* leaves showed high phenolic and flavonoid content.

Lipid peroxidation inhibition

The inhibitory effect of *S.macrocarpon* leaves aqueous extract with Fe^{2+}-induced lipid peroxidation in isolated rat liver and brain homogenates is presented in Tables 2&3. *S.macrocarpon* leaves caused a significant decrease in the liver and brain TBARS levels.

DPPH radical scavenging activities

The scavenging activities of DPPH exerted by the extract were summarized in Fig.1. The scavenging effect of extracts in the range of 50-250 mg/ml increased in a concentration-dependent manner.

Reducing power

Fig.2 shows that the reducing power of the extract has also a dose-dependent effect. The reducing ability of both the extract and the standard drug (ascorbic acid) increased with increasing concentrations.

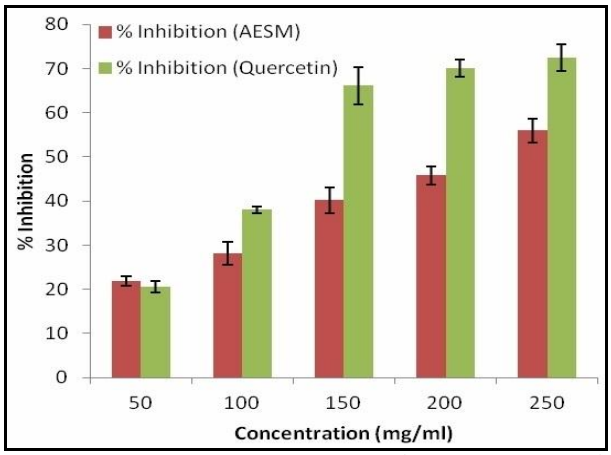

Figure 1. DPPH radical scavenging activity of the aqueous extract of *Solanum macrocarpon* (AESM) and quercetin as standard. Each value represents mean ± SD (n = 3).

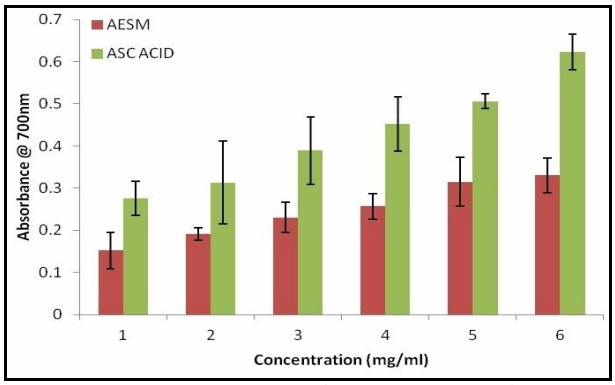

Figure 2. The reducing power of aqueous extract of *Solanum macrocarpon* (AESM) and ascorbic acid standard. Each value represents mean ± SD (n = 3).

Table 1. Total phenolic and total flavonoid contents of *Solanum macrocarpon* leaves aqueous extract as well as IC_{50} values for DPPH radical scavenging ability of the extract and quercetin (mean ± SD, n = 3)

Total phenols	Total flavonoids	DPPH radical scavenging ability of the extract (IC_{50})	DPPH radical scavenging ability of quercetin (IC_{50})
104 ± 0.03 mg GAE/g	35.85 ± 0.1 mg quercetidn/g	4.35 mg/ml	2.74 mg/ml

Table 2. Inhibitory effect of *Solanum macrocarpon* leaves extract on iron(II) sulphate-induced lipid peroxidation in a rat liver

Concentration (mg/ml)	µMol TBARS/mg tissue liver	% Inhibition	Logarithm Equation (r2)	IC_{50}
Basal	1.574 ± 0.32*****	91.86 ± 1.32	Y = -30.47 ln (X) + 90.685, R^2 = 0.8026	3.8
Control	19.283 ± 0.77	-		
3.33	3.298 ± 0.21*****	82.86 ± 1.78		
6.67	3.935 ± 0.48*****	79.62 ± 1.66		
13.33	6.222 ± 0.48*****	67.76 ± 1.19		
20.00	11.675 ± 0.34	39.44 ± 0.63		
26.67	11.975 ± 0.24	37.88 ± 1.24		

The results are expressed as means of three experiments in duplicate ± standard deviation. **Basal** = liver homogenate without extract or Fe^{2+}; **control** = homogenate containing Fe^{2+} only. *****$P < 0.05$ compared with control.

Table 3. Inhibitory effect of *Solanum macrocarpon* leaves extract on iron(II) sulphate-induced lipid peroxidation in a rat brain

Concentration (mg/ml)	µMol TBARS/mg brain	% Inhibition	Logarithm Equation (r2)	IC_{50}
Basal	1.995 ± 0.14*****	90.15 ± 1.35	Y = -28.37 ln (X) + 98.283, R^2 = 0.9006	5.5
Control	21.223 ± 0.16	-		
3.33	1.433 ± 0.22*****	92.91 ± 1.56		
6.67	2.401 ± 0.22*****	88.14 ± 1.89		
13.33	6.797 ± 0.36*****	66.46 ± 4.05		
20.00	8.094 ± 0.27*****	60.18 ± 1.39		
26.67	10.418 ± 0.88	47.93 ± 2.08		

The results are expressed as means of three experiments in duplicate ± standard deviation. **Basal** = liver homogenate without extract or Fe^{2+}; **control** = homogenate containing Fe^{2+} only. *****$P < 0.05$ compared with control.

DISCUSSION

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many of the phenolics have been shown to contain high levels of antioxidant activities [15]. The mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals [16]. The significance of high content of phenolics and flavonoids in plant extracts has been recognized to exhibit various biological activities such as anti-inflammatory, anti cancerogenic, antiviral, vasodialatory and antimicrobial effects. In the present study, by comparing the IC_{50} value for the free radical scavenging activity of the *Solanum macrocarpon* extract and that of the reference (quercetin), it can be deduced that leaves of *S.macrocarpon* could offer protection against oxidative damage to body cells.

Lipid peroxidation is one of the main manifestations of oxidative damage and play important role in the toxicity of many xenobiotics [17]. Iron overload results in the formation of lipid peroxidation products, which have been demonstrated in several tissues including the liver, brain and kidneys [18, 19]. The increased lipid peroxidation in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reaction that generate ROS, such as the hydroxyl (•OH) radical, formed from hydrogen peroxide (H_2O_2) through Fenton reaction. Free iron in the cytosol and mitochondria can cause considerable oxidative stress damage by increasing superoxide radical ($O_2•^-$) production, which can react with Fe^{3+} to regenerate $FeSO_4$, thus neutralizing its free radical character either by transferring an electron or hydrogen atom to Fe^{2+}. The observed decrease of the present study in the Fe^{2+}-induced lipid peroxidation in rat liver and brain homogenates in the presence of *S.macrocarpon* leaves extracts could be attributed to different antioxidant compounds like flavonoids, phenols, vitamin C, *etc* [20], as well as the ability of phytochemicals to chelate Fe^{2+} and scavenge free radicals produced by the Fe^{2+} catalyzed product of ROS [21].

The study revealed that, for means of inhibition of lipid peroxidation, the extract is most effective at the lowest dose tested (3.33 mg/kg) in both the liver and the brain. Thus, it can be suggested to be an effective protective agent against free radical induced tissue damage.

With regard to DPPH radical scavenging activity, it was demonstrated that the extract showed a concentration dependent activity By comparing the IC_{50} value for the DPPH scavenging activity of the extract and that of the reference (quercetin), it can be deduced that the leaves of *S.macrocarpon* could offer protection against oxidative damage to body cells. Its nutritional and medicinal uses are therefore very relevant. In reducing power determination, the antioxidant activity of sample was measured by its ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products.

Antioxidants capacity is a widely used parameter for assessing medicinal values of plant in the treatment of various diseases and this capacity was exhibited by *S.macrocarpon* leaves. The protection offered by this antioxidant compounds demonstrated high electron donating capacity which initiates chain termination in the lipid peroxidation mechanism and transforming reactive free-radical species into more stable non-reactive products. Therefore, it can be concluded that aqueous extract of *Solanum macrocarpon* leaves possess powerful antioxidant activity and can offer good protection against oxidative damage to body cells, particularly liver and brain.

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COMPETING INTERESTS

The authors declare no conflict of interest and that the research was conducted in the absence of any commercial or financial relationships.

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