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Evaluation of the Anti-lipid Peroxidative Potential of *Ficus capreifolia* Leaf Extract Using *In-vitro* Models

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Authors' contributions

This work was carried out in collaboration between all authors. Author TO designed the study while author TJG performed the laboratory analysis under the supervision of author NB. Author TO wrote the first draft including management of the literature and statistical analysis. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: To investigate the anti-lipid peroxidation, DPPH radical scavenging and phosphomolybdate reducing abilities of *Ficus capreifolia* leaf extract.
Study Design: The study was designed to determine the anti-lipid peroxidation ability of the extract on erythrocytes and liver homogenate. Other assays are the DPPH scavenging activity and antioxidant evaluation by reducing phosphomolybdate.
Place and Duration of Study: The work was done in the Department of Chemistry, Rivers State University from May 2016 to August 2016.
Methodology: The effect of *Ficus capreifolia* extract on hydrogen peroxide-induced erythrocyte damage and ferrous/ascorbate-induced lipid peroxidation in bovine liver homogenate were evaluated. Other experiments were performed to determine the total antioxidant activity via phosphomolybdate reduction and DPPH radical scavenging activity. Quercetin was used as the positive control for the reduction of lipid peroxidation in liver while vitamin C was used as the positive control for the other experiments.

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Results: The leaf extract significantly ameliorated hydrogen peroxide-induced erythrocyte hemolysis and lipid peroxidation (as indices of erythrocyte damage). The extract also reduced ferrous/ascorbate-induced lipid peroxidation in bovine liver homogenate. The DPPH scavenging activity and total antioxidant activity via the phosphomolybdate method were also remarkable when compared to vitamin C.
Conclusion: The antioxidant effect of of *Ficus capreifolia* leaf extract is attributable to the phytochemicals and provides evidence for the traditional use of the plant. This could be exploited both pharmacologically and for nutraceutical preparations.

Keywords: Ficus capreifolia; erythrocyte; lipid peroxidation; phytochemicals; quercetin.

1. INTRODUCTION

Over the years, there has been significant interest in medicinal plant research which may not be unconnected to the use of plants as first line treatment for various disorders in many regions. Another reason could be the reported frequent side effects associated with conventional chemotherapy compared to the use of plants/plant-derived products. This has led to the proposition that people should be encouraged to consume food rich in botanicals. Plants contain important chemicals that are beneficial to humans since they protect cells and tissues from reactive oxygen species (ROS) mediated attack. These ROS are continuously produced since they are products of functional metabolic processes but the innate antioxidant defenses, which are enzymatic and non-enzymatic, keep them in check. However, overproduction of these ROS could trigger tissue damage as they react with organic molecules such as proteins, nucleic acids, membrane lipids which have been implicated in cancer, inflammation, diabetes, cardiovascular disorders as a result of oxidative stress [1-3].

Ficus capreifolia (Moraceae, also known as the sandpaper fig) is widely distributed in the Neotropical and Afrotropical regions [4]. Various parts of the plant have been used to treat infections and research has it that the plant has antidiabetic potentials [5]. The plant is grossly underutilized and the literature on its potential activity is quite limited thus the aim of this work is to investigate the total antioxidant and anti-lipid peroxidative abilities of the plant using various *in-vitro* models.

2. MATERIALS AND METHODS

2.1 Materials

Quercetin (as quercetin monohydrate), methanol, thiobarbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA) and hydrochloric acid (HCl) were purchased from Sigma Chemicals (USA). All other chemicals were of analytical grade and commercially available. All buffers and solutions were prepared in deionized water and used within 24 hrs.

2.2 Plant Material and Positive Controls

The leaves of *Ficus capreifolia* were collected from a farmland in Oto-Udu, Udu Local Government Area, Delta State, Nigeria and identified by Prof. (Mrs.) Green of the Department of Applied and Environmental Biology, Rivers State University, Port Harcourt. A voucher specimen has been deposited as F. capreifolia-2016-05 at the herbarium. The leaves were washed, sundried and milled to a fine powder using a waring blender. A weighed sample of the powder was soaked in absolute methanol for approximately 72 hrs. The supernatant was filtered and further concentrated using a rotary evaporator set at 40°C. Different concentrations (0.25, 0.50, 1.00 and 2.00 mg/ml) of the residue (extract) were prepared and used for analysis. Where applicable, either vitamin C or quercetin was used as the positive control after same concentrations (like the extract) were prepared.

2.3 Isolation of Erythrocytes

Whole blood was obtained from a healthy volunteer (after obtaining informed consent) via the vein and delivered into a heparinised canister and centrifuged for 10 min at 2880 x *g* at 4°C. Plasma was removed and cells washed three times using phosphate buffer (0.02 M, pH 7.4) and re-suspended in the same buffer to the desired hematocrit level (5%). The study protocol was performed according to the Helsinki declaration of 1964 and approved by the Institutional Ethical Committee. Erythrocytes were immediately used for further studies.

2.4 Inhibition of Erythrocyte Hemolysis

The inhibition of erythrocyte hemolysis by the extract was performed as reported [6]. Briefly, erythrocytes (200 µL) were incubated with 100 µL of hydrogen peroxide (100 µM) in order to induce hemolysis. The test solution (200 µL extract or vitamin C) was added, gently swirled and incubated for 3 hrs at 37°C. The tube was allowed to cool and 8 mL of phosphate buffered saline (0.02 M, pH 7.4) was added and centrifuged at 2880 x *g* for 10 min. Absorbance of the supernatant was measured 540 nm using a UV-Vis spectrophotometer. The inhibitory activity was expressed as relative activity.

2.5 Inhibition of Erythrocyte Lipid Peroxidation

The method reported by Okoko and Ere [6] was used for the assay. Briefly, 200 µL of erythrocytes were incubated with 100 µL of 100 µM hydrogen peroxide and 200 µL of either extract or positive control (vitamin C). The contents were swirled and incubated for 1 hr at 37°C. The reaction was stopped by adding 2 mL thiobarbituric acid stock reagent (15% w/v TCA, 0.375% TBA, 0.25 M HCl) and incubated in a boiling water bath for 15 min. After cooling at room temperature, the precipitate was removed via centrifugation and the absorbance of the supernatant was measured at 532 nm. The inhibitory activity was expressed as relative activity.

2.6 Inhibition of Liver Lipid Peroxidation

The ability of the extract to inhibit lipid peroxidation in liver was performed according to the method of Yoshiyuki et al. [7]. Briefly, a weighed portion of bovine liver (fresh) was rinsed and homogenized in 0.02 M phosphate buffered saline (pH 7.4) to give a 10% homogenate. This was centrifuged at 2880 x *g* for 10 min at 4°C. The supernatant (0.5 mL) was incubated with 0.1 mL of phosphate buffer (0.02 M, pH 7.4), 0.04 M ferrous chloride (0.05 mL), 0.05 mL of ascorbic acid (0.1 mM) and 0.05 mL of either extract or positive control (quercetin) and left to stand for 1 hr at 37°C. It was incubated in a boiling water bath for 30 min after the addition of 0.9 mL of distilled water and 2 mL of 0.6% thiobarbituric acid. The contents were allowed to cool at room temperature and 5 mL of n-butanol added and shaken vigorously. The absorbance of the n-butanol later (after centrifuging at 2880 x *g* for 30 min) was measured at 532 nm.

2.7 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was determined according to the method reported by Sunil and Ignacimuthu [8]. One millilitre of methanolic DPPH (0.15%) was mixed with 3 mL of extract or vitamin C and incubated in the dark for 30 min. Thereafter, absorbance was measured at 515 nm. The DPPH scavenging activity was calculated as:

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

2.8 Antioxidant Activity via Phospho-molybdate Method

The antioxidant activity via the phospho-molybdate method was determined according to the method of Jayaprakasha et al. [9] as modified [6]. In brief, 0.2 mL of either extract or vitamin C (positive 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. Antioxidant activity was expressed as relative activity.

2.9 Statistical Analysis

Representative readings were expressed as mean ± S.E from four replicates. Data were subjected to two tailed student's *t*-test (for comparison between two groups) or analysis of variance (ANOVA) for multiple comparisons. Confidence was set at $p < 0.05$.

3. RESULTS

The ability of the extract to reduce hydrogen peroxide-induced erythrocyte hemolysis and lipid peroxidation is shown in Figs. 1 and 2. When compared to vitamin C, the extract exhibited significant ability at reducing hemolysis which was concentration dependent ($p < 0.05$). Even though the ability of the extract to reduced hydrogen peroxide-induced lipid peroxidation in erythrocytes seemed to be concentration dependent, the variations were not significant ($p > 0.05$) from 0.5 mg/mL to 2 mg/mL.

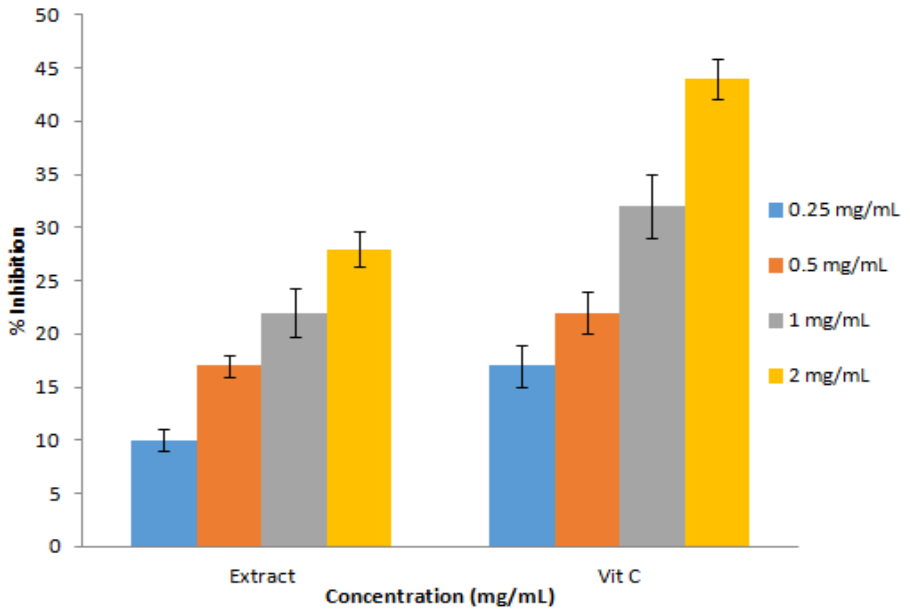

Fig. 1. Inhibitory activity of *Ficus capreifolia* extract on hydrogen peroxide-induced erythrocyte hemolysis

Each bar represents Mean ± SE of four replicates

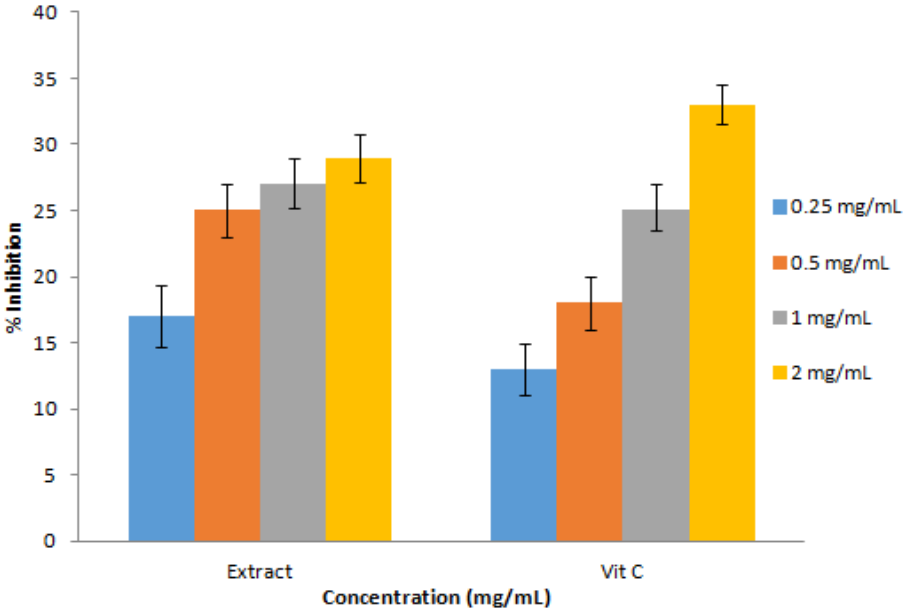

Fig. 2. Inhibitory activity of *Ficus capreifolia* extract on hydrogen peroxide-induced lipid peroxidation in erythrocytes

Each bar represents Mean ± SE of four replicates

The extract also reduced ferrous/ascorbate-induced lipid peroxidation of liver homogenate when compared to quercetin however, the variations were not significant ($p > 0.05$) from 0.25 mg/mL to 1 mg/mL (Fig. 3). In order to further assess the inherent bioactive potential of *Ficus capreifolia* extract, its ability to scavenge DPPH and the total antioxidant ability via phosphomolybdate reduction was measured. For the DPPH radical scavenging ability, the

variations were not significant ($p > 0.05$) from 0.25 mg/mL to 1 mg/mL but difference was significant ($p < 0.05$) between 1 mg/mL and 2 mg/mL (Fig. 4). When compared to vitamin C, the extract possessed significant antioxidant activity via the phosphomolybdate method which was concentration-dependent and the differences were statistically significant among the various concentrations (Fig. 5) ($p < 0.05$).

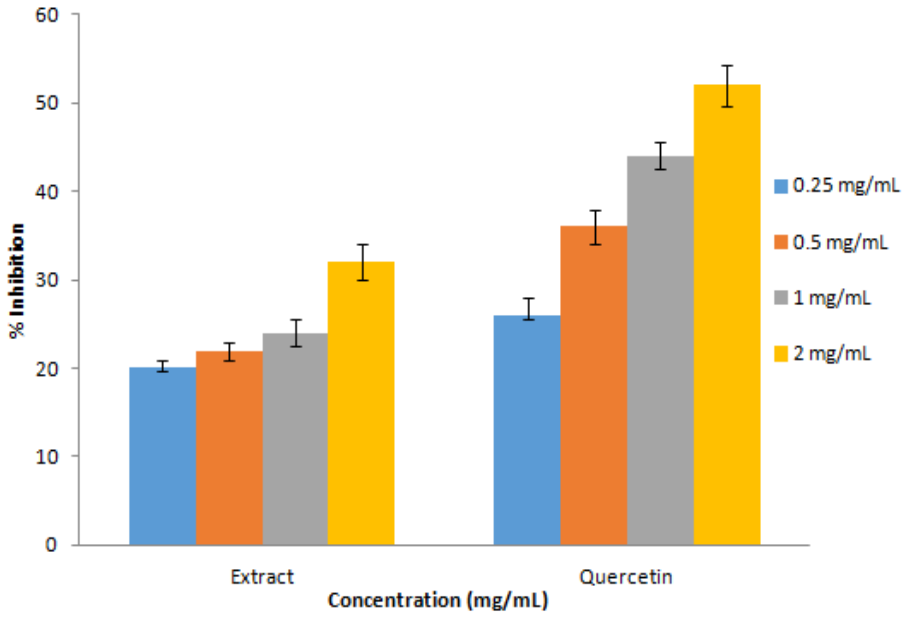

Fig. 3. Inhibitory effect of *Ficus capreifolia* extract on $FeCl_2$-ascorbic acid induced lipid peroxidation in bovine liver homogenate

Each bar represents mean ± SE of four replicates

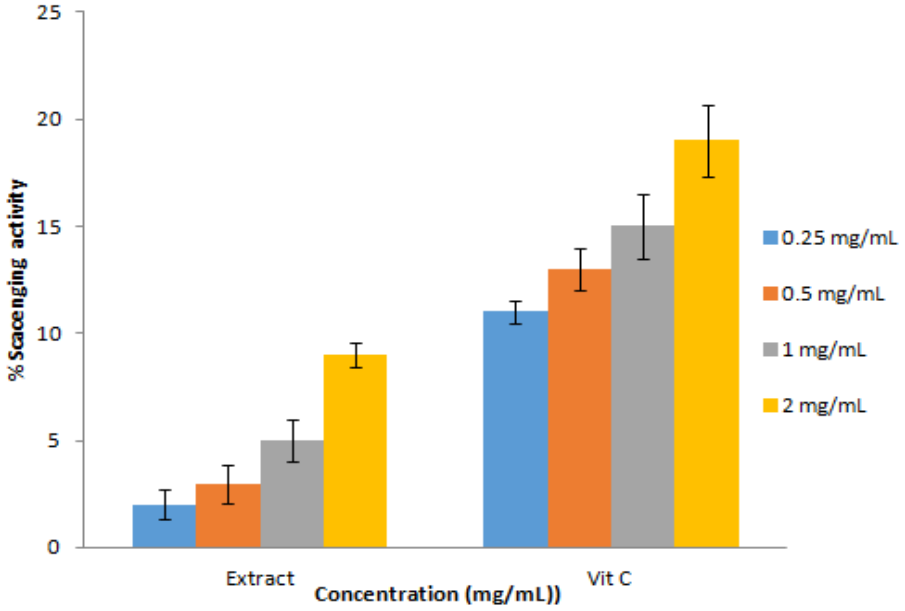

Fig. 4. DPPH radical scavenging ability of *Ficus capreifolia* extract

Each bar represents mean ± SE of four replicates

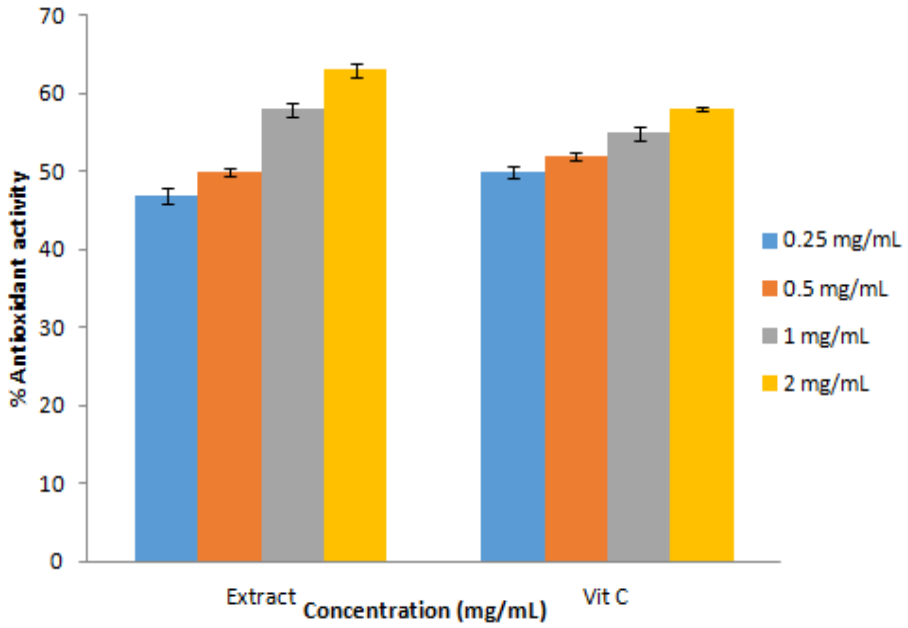

Fig. 5. Total antioxidant activity of *Ficus capreifolia* extract via the phosphomolybdate method
Each bar represents mean ± SE from four replicates

4. DISCUSSION

The balance between the production of ROS and antioxidation ensures redox balance hence is of utmost importance to cells and tissues. However disruption of this balance could be caused by various stressors which overproduce these reactive oxidants and one of the implications is lipid peroxidation. Oxygen-derived free radicals and other non-radical reactive species attack polyunsaturated fatty acids (PUFAs) in membranes. This disrupts membrane organization which could lead to functional loss of the cell [10]. Lipid peroxidation is also considered as the main molecular mechanism involved in oxidative damage to cells and tissues [11]. One of the most important ROS is hydrogen peroxide (H_2O_2), that is produced in reactions catalyzed by xanthine oxidase, NAD(P)H oxidase etc. At physiological levels, H_2O_2 regulates cell shape changes, recruitment of immune cells and other functions but at supraphysiological levels, it damages cells and tissues coupled with its high diffusibility [12]. Several disorders due to damage to proteins, nucleic acids and membranes have been attributed to H_2O_2-mediated oxidation [13]. In this study, H_2O_2 was used to initiate erythrocyte damage which was determined by assessing lipid peroxidation and hemolysis. These are implicated in some hemoglobinopathies [14-15]. Because of the presence of redox active metals (iron and iron-protoporphyrin IX), hydrogen peroxide can produce the hydroxyl radical which is considered as the most potent among the biologically active free radicals [16]. Erythrocytes have high PUFA content in their membrane thus is highly susceptible to oxidative attack hence used as model to study oxidative damage [17-18]. Oxidative damage (which could be manifested as hemolysis) contributes to the senescence of normal red cells and shorter life span for pathogenic red blood cells [18]. The results reveal that the extract reduced H_2O_2-induced erythrocyte damage significantly when compared to vitamin C. Furthermore, it reduces the morphologically changes associated with erythrocyte peroxidation thus could maintain the integrity of the red blood cells.

The extract also reduced lipid peroxidation in liver homogenate induced by the ferrous/ascorbate system. The liver homogenate is an oxidizable molecular target in this model for assessing lipid peroxidation [19]. Antioxidants reduce lipid peroxidation induced by the ferrous/ascorbate system by quenching free radicals [20].

Phytochemicals have been proposed to reduce the risk of certain diseases, preventing lipid peroxidation. They do so by neutralizing,

quenching or decomposing peroxides thus the antioxidant potential of any substance is linked to the inhibition of lipid peroxidation [21].

The extract also possessed significant ability to scavenge DPPH radical and to reduce phosphomolybdate when compared to vitamin C. This reveals that the extract could transfer and/or donate electrons to neutralize reactive species.

Phytochemical analysis of the plant revealed the presence of tannins, carotenoids, alkaloids, steroids, polyphenols, flavonoids and cardiac glycosides [22]. Flavonoids and other polyphenols exert their antioxidant effects by direct radical scavenging and augmentation of cellular antioxidant defenses thus play important roles in the protection of DNA, protein and tissues against damage due to intrinsic and extrinsic factors [23-25]. Alkaloids have also been reported to possess significant antioxidant potentials. It has been suggested that they form adducts with ROS to reduce the oxidation process [26-27]. Hence the observed bioactivites of the plant could be due to the combined action of various antioxidant phytochemicals.

5. CONCLUSION

Oxidation reactions contribute to some disorders hence it is believed that antioxidants could reduce the incidences of such oxidative stress-mediated disorders such as stroke, aging, diabetes, cardiovascular diseases etc. The current work reveals that *Ficus capreifolia* has a significant ability to reduce H_2O_2-induced erythrocyte damage and lipid peroxidation in bovine liver homogenate when compared to antioxidant standards. Thus the plant could be exploited pharmacologically and probably nutraceutically for the prevention of oxidative stress-mediated disorders. However, follow-up studies using *in vitro* and *in vivo* investigations are programmed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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