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Phytochemical Screening and Evaluation of the Antioxidant Activity of *Ficus capreifolia* Leaf Extract via *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 TJG wrote the first draft while authors TO and NB managed the literature and the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To carry out preliminary phytochemical screening and determine the antioxidant ability of *Ficus capreifolia* leaf extract.

Study Design: The study was designed to evaluate the phytochemical profile of *Ficus capreifolia* leaf and the potential antioxidant activity and comparing to reference antioxidant vitamin C.

Place and Duration of Study: The research was done in the Department of Chemistry, Rivers State University from May to August 2016.

Methodology: The extract was screened for the presence of some phytochemicals. Thereafter, the antioxidant potential was determined by assessing the hydrogen peroxide scavenging ability, hydroxyl radical scavenging activity and ferrous ion reducing ability and comparing them to the corresponding ability of vitamin C.

Results: Phytochemicals detected include tannins, carotenoids, alkaloids, flavonoids, steroids, cardiac glycosides and polyphenols. The extract also scavenged hydrogen peroxide, hydroxyl

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radicals and also reduced ferrous ions significantly when compared to vitamin C.

Conclusion: The leaf extract exhibited significant antioxidant activities which could be ascribed to antioxidant phytochemicals such as flavonoids, polyphenols which were detected. This corroborates the reported traditional use of the plant and can be further exploited pharmacologically.

Keywords: *Ficus capreifolia*; phytochemical; hydrogen peroxide; vitamin C; flavonoids.

1. INTRODUCTION

The utilization of oxygen is key to aerobic metabolism however it is a paradox that reactive oxygen species (ROS) are responsible for the pathogenesis of many disorders [1]. These ROS (both free radicals and other non-radical species) damage membrane lipids, cause DNA mutations and protein damage which could be sporadic. Metabolically, the body has efficient mechanisms to mask, scavenge or dismutate these ROS. The production of these ROS is in fact indispensable for life hence there is a balance between ROS production and antioxidation. Should an imbalance occur (in favour of ROS production), a condition known as oxidative stress occurs and the consequences could be cancer, aging, cardiovascular disease, liver injury etc [2-4]. Plants are rich sources of potential antioxidant compounds hence the intake of diets rich in plants (especially fruits, cereals, vegetables) has been encouraged.

Ficus capreifolia (Moraceae) is a fig shrub that grows in the tropical regions of Africa. The plant (also known as sandpaper fig) grows to about 7 cm in height [5]. The roots and leaves of the plant have been used to treat Schistosomal infections and diabetes [6]. Despite being of potential medicinal value, the plant is grossly underutilized coupled with the limited literature available on studies of the plant. Based on this, this work investigates the antioxidant potential of the *Ficus capreifolia* leaves using some *in vitro* models. The leaves were also screened for the presence of some phytochemicals.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals and reagents were of analytical grade thus used without further purification. All buffers and solutions were prepared using de-ionized water and used within 24 hrs.

2.2 Plant Collection and Identification

The leaves of *Ficus capreifolia* were harvested from its natural environment in Oto-Udu, Udu

Local Government Area, Delta State, Nigeria. The plant was identified and authenticated by Prof. (Mrs.) Green of the Department of Applied and Environmental Biology, Rivers State University, Port Harcourt. A voucher specimen (*F. capreifolia*-2016-05) was deposited at their herbarium.

2.3 Preparation of Extract

Fresh leaves of *Ficus capreifolia* were properly washed with deionized water to remove dirt. The leaves were sundried and pulverized using a warring blender. The extraction was done using Batch Extraction Method with methanol as the extracting solvent. The powdered sample (200 g) was soaked in 1 L of absolute methanol for 72 hours. The supernatant was collected and filtered using Whatman filter paper and concentrated using a rotary evaporator set at 40°C to give a residue. Different concentrations (0.25, 0.50, 1.00 and 2.00 mg/ml) of the extract were prepared and subjected to further analysis.

2.4 Preliminary Phytochemical Analysis

The extract was screened for the presence of alkaloids, flavonoids, polyphenols, saponins, steroids, tannins, cardiac glycosides and carotenoids according to the methods of Bera et al. [4] and Edeoga et al. [7].

2.5 Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was performed by replacement titration as reported by Zhao et al. [8] with some modifications. Briefly 1 ml of hydrogen peroxide (0.1 M), 1 ml of the extract, 3 ml of ammonium molybdate (3%), 10 ml of 2 M sulphuric acid and 7 ml of potassium iodide (1.8 M) were introduced into a conical flask. This was then titrated against sodium thiosulphate of (5.09 M). Disappearance of the yellow colour indicated end point of the reaction. The hydrogen peroxide scavenging activity of was expressed as a percentage thus:

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

2.6 Hydroxyl Radicals Scavenging Activity

Hydroxyl radical scavenging activity was investigated using the method described by Bera et al. [4]. Briefly, 1 ml of phosphate buffer (0.2 M, pH 7.2), 1 ml of test solution, 0.02 ml of ferric chloride (0.02 M) and 0.05 ml of phenanthroline (0.04 M) were introduced into a test tube. Reaction was initiated by the addition of 0.05 ml of 7 mM hydrogen peroxide. After 5 min of incubation at room temperature, absorbance was measured at 560 nm using a UV spectrophotometer. Hydroxyl radical scavenging activity was expressed as relative scavenging activity and calculated using the relation:

$$\begin{aligned} & \% \text{ scavenging activity} \\ & = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} = 100\% \end{aligned}$$

2.7 Reducing Ability

The ability of the extract to reduce Fe^{3+} was investigated according to Oyaizu [9] as modified by Okoko and Ere [10]. Test solution (0.5 ml) was mixed with 0.5 ml of phosphate buffer (0.2M pH 6.6) and 0.5 ml of potassium ferricyanide (1%) and incubated at 50°C. After incubation for 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%). After 10 min incubation at room temperature, absorbance was measured at 700 nm. Increase in absorbance indicated greater reducing ability.

2.8 Statistical Analysis

Representative readings were expressed as mean \pm S.E from four replicates. Data were analyzed using either a student's *t*-test (for comparison between two groups) or analysis of variance (ANOVA) for multiple comparisons. Confidence exhibited at $p < 0.05$ was considered statistically significant. Vitamin C was used as the reference control for the antioxidant assessment.

3. RESULTS

Phytochemical analysis of the *Ficus capreifolia* (leaves) revealed the presence of tannins,

carotenoids, alkaloids, steroids, polyphenols, flavonoids and cardiac glycosides. However, saponins were not detected (Table 1).

Table 1. Photochemicals screening test for active components in the *Ficus capreifolia* extract

Active compound	Evidence
Tannis	+
Carotenoids	+
Alkaloids	+
Flavonoids	+
Saponins	-
Steroids	+
Cardiac glycoside	+
Polyphenol	+

+ detected; - not detected

The hydrogen peroxide scavenging ability, hydroxyl radical scavenging ability and the ability of the extract to reduce ferric ion are shown in Figs. 1, 2 and 3. The extract possessed significant ability to scavenge hydrogen peroxide, hydroxyl radical and to reduce ferric ions when compared to the reference compound (vitamin C). Though the responses seem to be concentration-dependent in all cases, the variations were not significant in many cases. The variation of hydrogen peroxide scavenging ability between 0.25 mg/ml and 0.5 mg/ml was significant ($p < 0.05$) while it was not significant ($p > 0.05$) from 0.5 mg/ml to 2.0 mg/ml. For the hydroxyl radical scavenging activity, there was no statistical difference among the various concentrations ($p > 0.05$). For the reducing ability, the difference between 0.25 mg/ml and 0.5 mg/ml was significant ($p < 0.05$), not significant between 0.5 mg/ml and 1.0 mg/ml but significant difference ($p < 0.05$) was exhibited between 1.0 mg/ml and 2.0 mg/ml.

4. DISCUSSION

The production of ROS and antioxidation are not mutually exclusive as long as life exists. Various physical and chemical processes induce and activate oxidant producing enzymes such as NADPH oxidase, xanthine oxidase etc. These enzymatic reactions produce ROS such as superoxide radical, nitric oxide, hydrogen peroxide, peroxy nitrates [11]. The body has efficient mechanisms (both enzymatic and non-enzymatic) to keep these reactive species in check since they are products of essential processes. However, these ROS could be over produced due to a number of factors and the

consequences are damage to tissues, DNA, proteins which are predisposing to cancer, cardiovascular diseases, diabetes, ageing, neuro degradative disorders, stroke, gastrointestinal diseases, etc [12-15]. Thus antioxidant supplementation is necessary to maintain redox

balance. Plants are the main sources of these antioxidants. Phytonutrients have been recommended to be effective substitutes for most synthetic products since there are reported less-frequent side effects following the consumption of plants or plant-derived products [16].

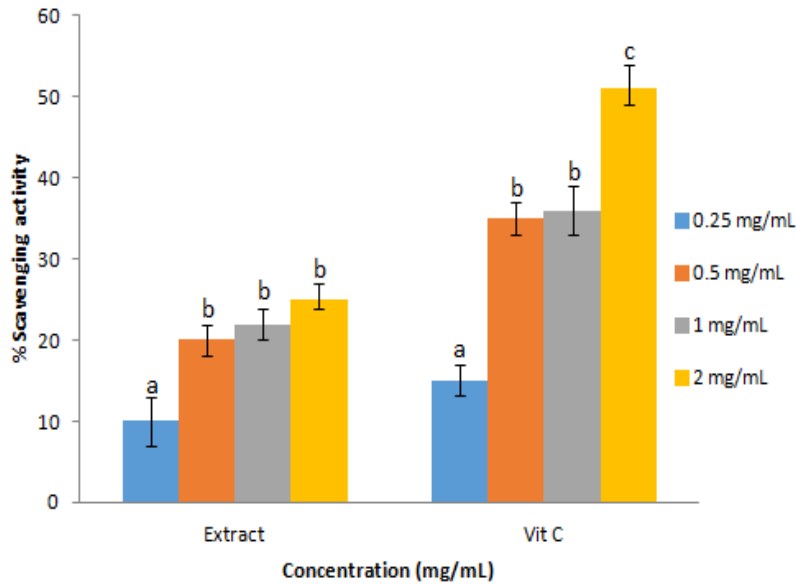


Fig. 1. Hydrogen peroxide scavenging activity of *Ficus capreifolia* leaf and vitamin C
 Each bar represents Mean \pm SEM of four replicates. For each set of bars, values having different superscript letters differ significantly ($p < 0.05$)

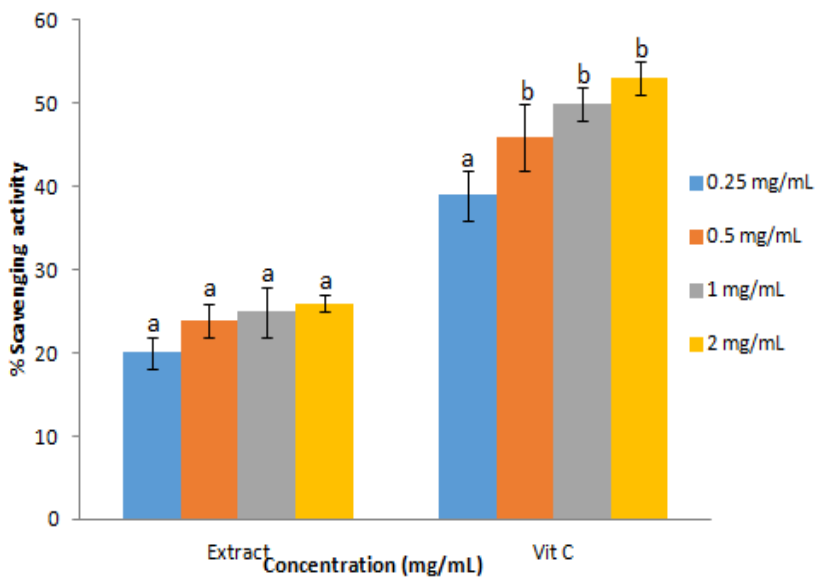


Fig. 2. Hydroxyl radical scavenging ability of *Ficus capreifolia* and vitamin C
 Each bar represents Mean \pm SEM of four replicates. For each set of bars, values having different superscript letters differ significantly ($p < 0.05$)

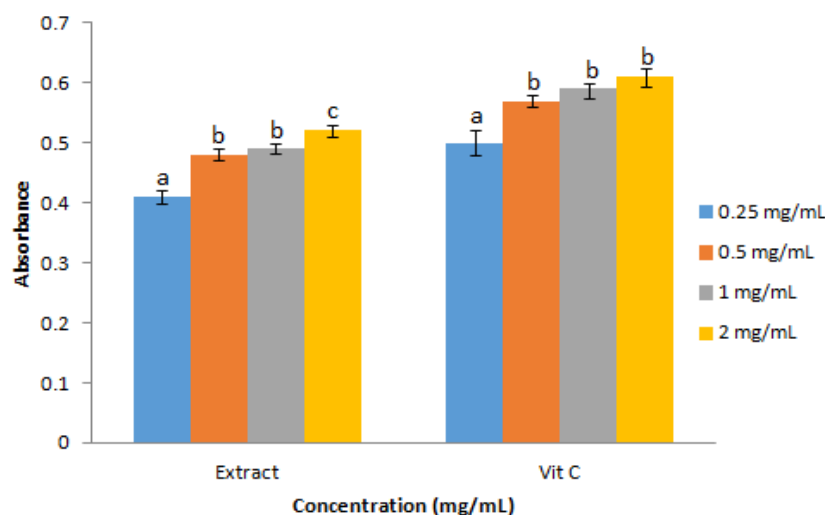


Fig. 3. Reducing ability of *Ficus capreifolia* and vitamin C

Each bar represents Mean \pm SEM of four replicates. For each set of bars, values having different superscript letters differ significantly ($p < 0.05$)

Hydrogen peroxide is a potent oxidant produced via phagocytosis and in reactions catalyzed by xanthine oxidase, superoxide dismutase [17]. Though converted to water by catalase or glutathione peroxidase, overproduction damages various cellular targets due to its diffusible nature [17-19].

The study revealed that *Ficus capreifolia* leaf scavenged hydrogen peroxide which was significant when compared to vitamin C (Fig. 1).

The hydroxyl radical is the most harmful in the biosystem because of its non-selective reactivity [20-21]. In the presence of transition metal ions (such as Fe^{3+} and Cu^{2+}), hydrogen peroxide could generate hydroxyl radical via the Fenton reaction [22]. Thus the reduction of these redox active transition metal ions and the scavenging of the hydroxyl radical are indispensable at reducing free radical induced damage. *Ficus capreifolia* extract significantly scavenged hydroxyl radical and also reduced ferric ions. This ability could be due to important phytochemicals inherent in the plant. Preliminary phytochemical screening revealed the presence of tannins, carotenoids, alkaloids, steroids, polyphenols, flavonoids and cardiac glycosides (Table 1). Flavonoids and other polyphenols have been reported to possess immense antioxidant potential by scavenging hydroxyl radicals, hydrogen peroxide and also possess metal reducing abilities [23-26]. Thus the intake of diet rich in flavonoids (fruits and vegetables) has been inversely associated with the development of coronary heart disease,

cancer, cerebrovascular disease etc [27]. In addition to polyphenols, the antioxidant activities of alkaloids, tannins and carotenoids have also been reported [28-32]. Thus the observed *in-vitro* antioxidant activity could be due to synergy exhibited by the various phytochemicals. However, this is yet to be investigated.

5. CONCLUSION

Ficus capreifolia extract exhibited high *in-vitro* antioxidant and free radical scavenging potentials when compared to vitamin C so could be exploited both pharmacologically and neutraceutically. The various *in-vitro* models indicate that *Ficus capreifolia* extract is a significant source of natural antioxidants, which could be helpful in preventing the progress of various diseases due to oxidative stress. Follow-up studies using various *in-vivo* models are recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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