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Exploring the Therapeutic Potential of *Ficus capensis* Stem Extract: Bioactive Compound Analysis and Antioxidant Assessment

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Abstract: The *Ficus capensis* plant has been used for various purposes in traditional medicine, but the therapeutic potential of its stem remains largely unexplored. The ethanol extract of its stem was analyzed for flavonoids, phenol, ascorbic acid, lycopene, and beta-carotene using standard biochemical methods. Antioxidant activities were assessed through lipid peroxidation inhibition, DPPH radical scavenging, and reducing power assays, with BHA as the standard. The analysis revealed significant levels of phenol (146.75 ± 0.93 mgGAE/100g) and flavonoids (122.40 ± 5.62 mgCE/100g), moderate levels of ascorbic acid (4.07 ± 0.04 mg/100g), and trace levels of lycopene (0.45 ± 0.05 mg/100g) and β-carotene (0.23 ± 0.00 mg/100g). The antioxidant assays revealed high activity in a dose-dependent manner, comparable to BHA. At concentrations of 0.625-10.0 µg/mL, the extract showed 4.81-87.36% DPPH radical scavenging activity, 0.193-0.439 nm of reducing power capacity, and 10.96-62.07% inhibition of lipid peroxidation. Notably, the EC_{50} value for DPPH scavenging was 5.03 ± 0.25 µg/mL, compared to 4.08 ± 0.23 µg/mL for BHA. The IC_{50} value for lipid peroxidation inhibition was 7.82 ± 0.40 µg/mL, compared to 4.15 ± 0.38 µg/mL for BHA. Additionally, the reducing power assay yielded an $OD_{0.5}$ value of 12.75 ± 0.78 and 1.81 ± 0.28 for BHA. *Ficus capensis* stem extract contains bioactive compounds with strong antioxidant properties, supporting its potential use against oxidative stress-related diseases.

Keywords: bioactive compound; antioxidant; DPPH; reducing power; lipid peroxidation; *Ficus capensis*; stem.

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1. Introduction

In most cultures worldwide, from ancient times to the present day, medicinal plants are used in folk medicine and serve as the primary treatment for numerous diseases. Out of about 500,000 plant species on earth, less than 10% is used for food by animals and humans [1]. This could imply that more of these plants are used for medicinal purposes. Phytochemicals are naturally occurring bioactive compounds produced by plants for defense. These compounds can be obtained from diverse sources, including whole grains, fruits, vegetables, nuts, and

herbs. To date, researchers have identified over a thousand different phytochemicals. Bioactive compounds in plants possess antioxidant properties, effectively combating oxidative damage arising from free radicals, and are therapeutically beneficial to the body. Free radical damage is a leading element in the development of so many debilitating diseases. Plants' bioactive compounds, such as phenolic compounds, have been scientifically proven to possess medicinal properties [2-5]. These molecules have the potential to improve health outcomes by regulating energy consumption, reducing inflammation and oxidative stress, and managing metabolic dysfunctions [6,7]. A diet rich in flavonoids and carotenoids has been epidemiologically linked to a decrease in various chronic illnesses, such as heart disease, nervous system disorders, and cancer [8,9]. Phytochemicals have been extracted from various plants, which possess biological properties such as antimicrobial [10,11], osteoprotective [12], pesticidal [13], and antibiofouling [14] activities.

The *Ficus* genus, comprising approximately 800 species and 2000 varieties, is of the Moraceae family and is predominantly found in subtropical and tropical forests [15]. Commonly referred to as fig trees, *Ficus* species are widely distributed. Specifically, *Ficus capensis* is a medicinally valuable plant with nearly all its parts utilized for therapeutic purposes. The leaves of *Ficus capensis* are traditionally used to prepare decoctions used to treat infertility in men, respiratory disorders, dysentery, leprosy, and rickets, as documented in studies [16,17]. The root and bark decoctions are used in orthodox medicine to treat cough, stomach ache, edema, infertility, and chest pain [18], while the milky latex from the stems is used to alleviate lung issues and boost milk production in cattle. Despite extensive research on other parts of *Ficus capensis*, the stem's pharmacological properties remain largely unexplored. This study aimed to bridge this knowledge gap by investigating the biologically active compounds present and the antioxidant potential of the ethanol extract of *Ficus capensis* stem. Even though Ficus capensis is widely used in traditional medicine, its stems are systematically discarded as non-medicinal waste during herbal preparation. While leaves, roots, and bark are commonly utilized, the therapeutic potential of the stems remains unexplored. This study addresses this critical knowledge gap by demonstrating that these traditionally overlooked stems contain remarkably high levels of bioactive compounds with potent antioxidant activity comparable to synthetic standards. Our findings challenge conventional practices in ethnopharmacology for selecting plant parts and reveal an untapped therapeutic resource in agricultural byproducts. By validating the medicinal value of stem tissue, this research not only expands the usable biomass of *F. capensis* by 30-40% but also offers a sustainable approach to reduce harvesting pressure on other plant parts while creating value from previously discarded material.

2. Materials and Methods

2.1. Sample collection.

Leaf-bearing stems of *Ficus capensis* were sourced from the botanical garden located within the Department of Applied Biology and Biotechnology at Enugu State University of Science and Technology, Agbani, Nigeria. Identification and authentication of the sample were conducted by Prof. C.S. Eze from the Department.

2.2. Sample preparation/extraction.

The leaves on the stems of *Ficus capensis* were plucked out, and the stems were rinsed with clean water. The stems were chopped into smaller pieces, shade-dried for three weeks, and pulverized into powder.

2.2.1. Sample extraction.

One hundred grams (100g) of powdered sample was extracted with 80 % ethanol (soaked for 72 hours at 25℃). The extract was stored until use in an air-tight container.

2.3. Bioactive compound assay.

2.3.1. Flavonoid analysis.

The colorimetric method developed by Barros *et al*. [19] was modified and used for flavonoid analysis. Briefly, distilled water (2 mL) and $NaNO_2$ solution (5%, 0.5 mL) were added to the sample solution (250 µg/mL, 0.5 mL). Addition of NaOH solution (4%, 2 mL) was made after 6 minutes, and made up to 5 mL with water. The absorbance was measured at 510 nm against a water blank, using catechin as a reference standard. Results were presented as mg catechin equivalents/100g sample (mg CE/100g) of triplicate analysis.

2.4. Total phenol analysis.

The total phenol was evaluated according to the method of Barros *et al*. [19]. One millimeter of Folin-Ciocalteu's phenol reagent was added to the extract solution (1 mL). Then, a saturated Na_2CO_3 solution (1 mL) was added after 3 minutes. This was adjusted with distilled water to 10 mL, followed by 1 hr 30 minutes of incubation. Absorbance was measured at 725 nm. The reference antioxidant was Gallic acid. Results were presented as mg Gallic acid equivalents (GAEs) per 100g extract.

2.4.1. Lycopene and β-carotene contents analysis.

As outlined by Barros *et al*. [20], lycopene and β-carotene contents were analyzed by shaking 100 mg of sample extract with 6 mL mixture of acetone-hexane (4:6) for 60 seconds, filtering through Whatman filter paper, and measuring absorbance at 453, 505, and 663 nm. The lycopene and β-carotene concentrations were calculated thus:

$$\text{Lycopene (mg/100mL)} = 0.0458A603 + 0.372A505 + 0.0806A453 \quad (1)$$

$$\text{ß-carotene (mg/100mL)} = 0.2216A663 + 0.304A505 + 0.452A453 \quad (2)$$

2.4.2. Ascorbic acid content analysis.

As outlined by Klein and Perry [21], metaphosphoric acid (1%, 10 mL) was used to extract a 20 mg sample for 45 minutes at 28°C, passed through filter paper (Whatman No. 4), and then 2,6-dichloro-phenolindophenol sodium salt hydrate (50 µM, 9 mL) was added to the filtrate (1 mL). After 30 minutes, the absorbance was read at 515 nm. A calibration graph of L-ascorbic acid was used to calculate the results, which were reported as mg AE/g (mg ascorbic acid equivalent per gram) of the sample.

2.5. Antioxidant activity assay.

2.5.1. DPPH free radical scavenging assay.

DPPH radical was used to evaluate the free radical scavenging activity of the ethanol extract of *Ficus capensis* stem following Ebrahimzadem *et al.*'s [22] method. The extracts (0-500 µg/mL, 0.3 mL) were mixed with 100 µM methanolic DPPH solution (2.7 mL), shaken, and incubated for 60 minutes. The absorbance was read at 517 nm. BHA was the standard reference. The antioxidant activity was calculated as:

$$\% \text{ Radical Scavenging Activity} = \frac{(ADPPH - As)}{ADPPH} X\ 100 \quad (3)$$

Where: As is the absorbance of the test solution containing the sample, and ADPPH is the absorbance of the DPPH solution.

The EC_{50} value (concentration for 50% radical scavenging activity) was determined from the plot of % RSA vs sample concentration.

2.5.2. Reducing power assay.

This was evaluated with the method outlined by Barros *et al*. [20]. This assay is based on increased absorbance of the mixture. To determine reducing power, potassium ferricyanide (2.5 mL, 1%) and sodium phosphate buffer (0.2M, 2.5 mL, pH 6.6) were added to the sample extract (2.5 mL, concentrations ranging from 0-1000 µg/mL). Next, the mixture was kept in the dark for 20 min at 50°C, followed by the addition of TCA [Trichloroacetic acid (10%, 2.5 mL)]. The mixture was centrifuged (1000 rpm, 8 minutes), and ferric chloride (0.1%, 1 mL) and deionized water (5 mL) were added to the supernatant (5 mL). The absorbance was read at 700 nm, and the results were plotted against extract concentrations. Butylated Hydroxyanisole (BHA) served as the reference standard.

2.5.3. Inhibition of lipid peroxidation assay.

This was assayed as outlined by Barros *et al*. [20]. Goat brain tissue was dissected and blended in a 20 mM ice-cold Tris-HCl buffer of pH 7.4, producing a blend of 50% w/v. It was then centrifuged for 10 minutes at 3000 g, and the supernatant was collected. Next, the supernatant (0.1 mL), ferrosulphate (10 µM, 0.1 mL), ascorbic acid (0.1 nM, 0.1 mL), and the sample extract (0-1000 µg/mL, 0.2 mL) were incubated together at 37°C for 1 h. The reaction was stopped with 28% trichloroacetic acid (0.5 mL) and 2% thiobarbituric acid (0.38 mL), then warmed for 20 minutes at 80°C. Following centrifugation (3000 rpm, 10 min), the absorbance of the MDA-TBA complex was read at 532 nm.

$$IR\ \% = \frac{(A - B)}{A} X\ 100 \quad (4)$$

A is the control absorbance, and B is the compound solution absorbance. The IC_{50} value, representing the concentration of the extract needed to inhibit 50% of lipid peroxidation, was determined from the antioxidant activity curve, with Butylated Hydroxyanisole (BHA) serving as the reference standard.

2.6. Statistical analysis.

Results obtained were presented as Mean ± SD of duplicate analysis. The 50% effective concentration (EC_{50}, IC_{50}, and $OD_{0.5}$-optical density) values were calculated using linear equations deduced from antioxidant activity versus extract concentration plots. Analysis of data was carried out using IBM-SPSS (version 22). Statistical comparisons were made with ANOVA (assuming equal variances), Tukey's post Hoc and Independent t-test. An α level of ≤ 0.05 was considered significant.

3. Results and Discussion

The rising interest in plant-derived remedies has sparked a surge in research aimed at elucidating the chemical composition and pharmacological properties of diverse species of plants, unlocking their potential for nutritional and therapeutic uses. The potential health risks associated with synthetic antioxidants have heightened the search for naturally derived antioxidants, prompting researchers to investigate plant-based options with improved safety profiles [23].

3.1. Bioactive compound content.

The concentration of phenol in the extract was ascertained through spectrophotometric analysis of total phenolic content (TPC), a widely accepted methodology for quantifying phenolic compounds in plants. Folin-Ciocalteu reagent was utilized, and the amount of phenol was expressed in terms of gallic acid GAE/100 g of the sample (Table 1). The results revealed that the *Ficus capensis* stem displayed a significantly higher phenol concentration ($p<0.05$) compared to its contents of flavonoids, ascorbic acid, lycopene, and beta-carotene. These findings equally revealed that the ethanol extract of *Ficus capensis* stems possesses significantly higher phenolic content (146.75 mgGAE/100g) compared to previously reported values for other parts of the same species, including fruits (13.97 mgGAE/100g) [24] and leaves (16.47mgGAE) [25]. Phenols exhibit antioxidant activity attributed to their oxidation-reduction properties, enabling them to neutralize free radicals [26-28]. The elevated phenolic content in *Ficus capensis* stems suggests high bioactivity, particularly antioxidant activity. This is consistent with previous reports showing a correlation between phenolic levels and antioxidant capacity. Research by Zhang *et al*. [29] revealed a notable connection between Total Phenolic Content and antioxidant capacity in various plant-based products. Additionally, Saini *et al*. [30] showed that phenolics counteract the formation of reactive oxygen species (ROS), safeguarding plants against molecular damage, pathogens, and pests. Flavonoids, secondary plant metabolites, possess antioxidant activity, with their potency influenced by the number and spatial arrangement of hydroxyl (OH) groups [31]. In this study, the concentration of flavonoid was quantitatively determined using a colorimetric assay with $NaNO_2$ solution. The results were presented as mgCE/100 g of sample. The analysis revealed significantly higher levels of flavonoids than of ascorbic acid, lycopene, and beta-carotene ($p < 0.05$). Flavonoids, a prominent subclass of phenolic compounds, are ubiquitously found in plants, contributing to the flavor, color, and nutritional value of fruits and vegetables [32]. The amount of flavonoids in the stem of Ficus capensis was significantly higher ($p<0.05$) than that reported on the fruits [24].

Table 1. Key bioactive metabolites identified in the ethanol extract of *Ficus capensis* stem.

Parameters	Content
Total Phenol (mgGAE/100g)	146.75 ± 0.93
Flavonoids (mgCE/100g)	122.40 ± 5.62
Beta Carotene (mg/100g)	0.23 ± 0.00
Ascorbic Acid (mg/100g)	4.06 ± 0.05
Lycopene (mg/100g)	0.45 ± 0.05

Values are mean ± SD of duplicate values; mgGAE- milligram gallic acid equivalent; mgCE-milligram catechin equivalent.

3.2. Antioxidant assay.

To evaluate the antioxidant capacity of naturally occurring compounds, relying on one method of analysis is inadequate. This is because various antioxidant assays differ in their underlying principles and experimental conditions. For example, some techniques employ radical scavengers, such as DPPH, while others use metal ions for oxidation, such as in the FRAP assay. Additionally, the time required to generate free radicals through oxidation reactions varies between assays. Therefore, multiple methods are necessary. In the present study, the antioxidant potential of *Ficus capensis* stem was assessed based on its ability to scavenge DPPH radicals, inhibit lipid peroxidation, and reduce $Fe3^{+}$ to Fe^{2+}. The DPPH free radical scavenging assay is a widely used method that employs the stable DPPH radical due to its ease of use and convenience [33]. The assay primarily involves electron transfer reactions, with minimal contribution from hydrogen-atom abstraction [34].

3.2.1. DPPH radical scavenging activity.

This study revealed a dose-dependent relationship in the radical scavenging activity of *Ficus capensis* stem and the standard. The ethanol extract of *Ficus capensis* stem reduced DPPH radicals in a concentration-dependent manner comparable with BHA (Figure 1). No statistically significant difference in activity was observed between the extract and BHA at all the concentrations tested. The extract varied significantly($P<0.05$) in DPPH scavenging activity across concentrations, except for the 0.625 and 1.25 µg/mL concentrations, which did not differ significantly. The EC_{50} value of the extract (5.03 ± 0.25 µg/mL) compared with BHA was significantly more potent (EC_{50} value of 4.08 ± 0.23 µg/mL). The activities of the extract and standard compound, BHA, in this study were consistent with previously reported results [35]. The antioxidant capacity of the stem extract, as demonstrated by its DPPH radical scavenging activity (EC_{50} = 5.03 ± 0.25 µg/mL), showed remarkable potency when compared to other Ficus species. Notably, it outperformed F. capensis fruit extract (EC_{50} = 30 µg/mL) [24] and approaches the activity of synthetic antioxidant BHA (EC_{50} = 4.08 µg/mL). These results align with the observed high flavonoid content (122.40 mgCE/100g) in the stems, which exceeds values reported for F. carica latex (34 mgCE/100g) [36]. The basis of the DPPH assay is that antioxidants can concentration-dependently change the purple coloured DPPH solution to the yellow-coloured non-radical DPPH-H form [37]. The extent of this color change can be quantified spectrophotometrically at 518 nm, providing an indicator of a substance's antioxidant ability to scavenge DPPH free radicals [38]. Assessing antioxidant capacity provides valuable insights into the health benefits and functional properties of foods, which can be effectively determined by measuring DPPH radical scavenging capacity [39]. The free radical-scavenging activity observed in the sample may be linked to its high phenol content. Phenols possess free radical scavenging properties through hydrogen or electron donation [40]. This ability confirms their potential as radical scavengers, making them suitable for use as

natural antioxidants. According to Klarić *et al.* [41], phenol content is strongly correlated to radical scavenging activity. The EC_{50} is the extract concentration needed to inhibit half of the DPPH free radicals in a controlled research environment [42]. A smaller EC_{50} value denotes a more potent free radical scavenger and implies a greater antioxidant capacity. Here, the EC_{50} results revealed that the ethanol stem extract required a higher concentration (5.03 ± 0.25 µg/mL) to scavenge 50% of DPPH radicals, whereas the standard antioxidant achieved this at a lower concentration (4.08 ± 0.23 µg/mL). These results are consistent with other studies that reported that phenolic compounds typically exhibit substantial scavenging effects against DPPH free radicals [34, 36, 41, 43].

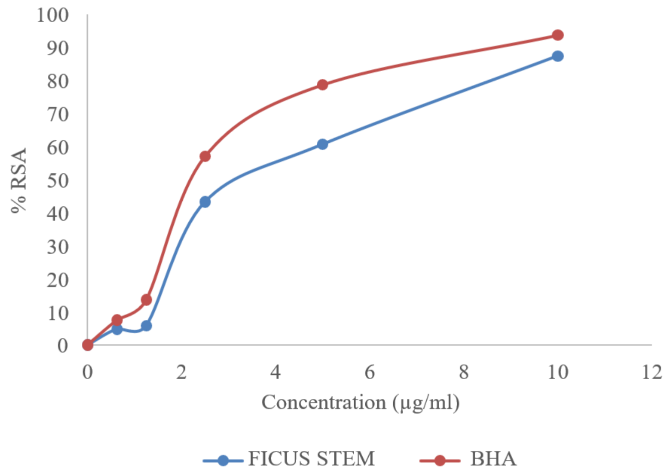

Figure 1. DPPH radical scavenging activity of ethanol extract of *Ficus capensis* compared with BHA.

3.2.2. Inhibition of lipid peroxidation activity.

The inhibitory effect of the ethanol extract of *Ficus capensis* stem on lipid peroxidation was determined using the TBARS assay, utilizing goat brain homogenate as lipid source, and compared with

BHA. The activity of BHA was non-significantly ($p>0.05$) higher than the extract (Figure 2). Inhibitory activity significantly ($P<0.05$) varied among the extract concentrations, with the exception of the 0.625 and 1.25 µg/mL concentrations, which were statistically similar. The inhibitory potency of BHA (IC_{50} =

4.15 ± 0.38 µg/mL) was significantly greater ($P<0.05$) than that of the extract (IC_{50} = 7.82 ± 0.40 µg/mL). As illustrated in Figure 3, the lipid peroxidation inhibitive activity of *Ficus capensis* stem extract increased as the concentration increased, just like BHA, although it was less potent. The standard demonstrated significantly higher ($p<0.05$) inhibition, giving an IC_{50} of 4.15 ± 0.38 µg/mL, whereas the sample had an IC_{50} of 7.82 ± 0.40 µg/mL. Peroxidation of lipids occurs when polyunsaturated fatty acids in the membrane of cells go through oxidative degradation, resulting in the formation of degradative products like malondialdehyde [44]. This process can lead to cell membrane destruction and damage to biological systems. It is linked to various chronic disorders, including liver damage, inflammation, and atherosclerosis [45]. Malonaldehyde is a key indicator of oxidative stress and lipid peroxidation [46]. The reaction between malonaldehyde and thiobarbituric acid is a widely used, sensitive assay method for detecting lipid peroxidation. The *Ficus capensis* stem extract has demonstrated the ability to inhibit lipid peroxidation, likely due to its bioactive compounds. Research suggests that bioactive constituents, and phenols in particular, can repress lipid peroxidation through various mechanisms, such as electron transfer, radical recombination or addition, and free radical quenching [28, 47].

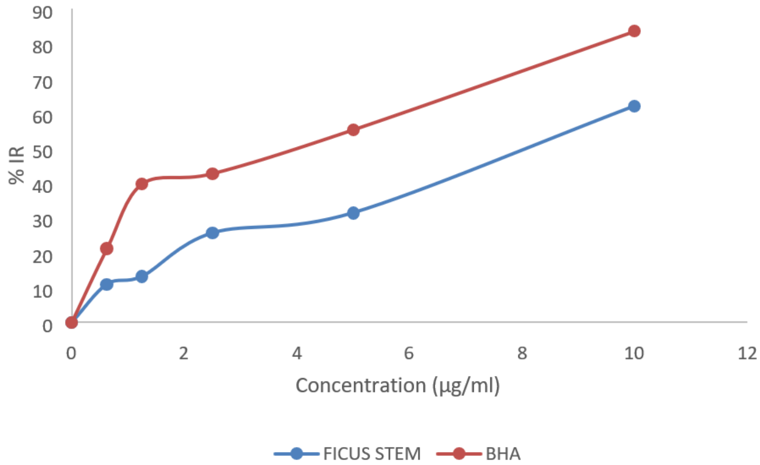

Figure 2. Dose-dependent inhibition of lipid peroxidation by *Ficus capensis* stem ethanol extract compared to the synthetic antioxidant, BHA.

3.2.3. Reducing power activity.

The reducing power assay revealed that the extract successfully reduced the Fe^{3+} to Fe^{2+}. The ethanol extract of *Ficus capensis* stem displayed moderate capacity to reduce Fe^{3+} ions to their ferrous state in comparison with the standard. The activity of BHA was significantly higher ($p<0.05$) than that of the extract (Figure 3). Increasing the concentration of the extracts brought about a rise in the reducing power activity. At the dose of 0.625 to 10 µg/mL, the absorbance of ethanol extract of *Ficus capensis* stem was in the range of 0.193 ± 0.01 – 0.439 ± 0.02, while that of the standard was 0.298 ± 0.01 – 1.091 ± 0.05. The reducing power activity also varied significantly ($P<0.05$) among the extract concentrations, with the exception of between 1.25 and 2.50; 2.50 and 5.0 µg/mL concentrations, which were statistically similar. The dose-response curves for the extract and BHA showed similar patterns but with statistically different $OD_{0.5}$ values of 12.75 ± 0.78 and 1.81 ± 0.28, respectively. Iron (Fe) is essential for various bodily functions, including oxygen transport, cellular respiration, and maintaining proper cellular function, and also serves as a cofactor for numerous enzymes [48]. But, excessive levels of iron can activate Fenton reactions and hydroperoxide decomposition, thereby exacerbating oxidative stress [49]. Consequently, a crucial aspect of an antioxidant compound's efficacy is its ability to reduce Fe^{3+} to Fe^{2+}, thereby mitigating oxidative damage [50]. The reducing power assay evaluates an antioxidant's ability to counteract the oxidative effects of free radicals by measuring its reducing capacity. It relies on the basis that compounds with reduction capability convert Fe^{3+} to Fe^{2+}. This reaction forms a ferric-ferrous complex with a maximal absorption at 700 nm. The assay measures the increase in absorbance, indicating enhanced reductive ability. A higher reducing capacity often correlates with higher antioxidant ability. In the present study, the extract demonstrated increased reducing power, which was dose-dependent and comparable to the standard. This antioxidant activity can be linked to the presence of reductones in the sample, which contribute hydrogen atoms to exert their antioxidant effects [37, 44]. The reductive ability of a substance is a reliable index of its antioxidant properties [51]. As shown in Figure 2, the stem extract demonstrated a higher $OD_{0.5}$ value of 12.75 ± 0.78 µg/mL in comparison with the BHA (1.81 ± 0.28 µg/mL). This suggests that the extract possesses lower reducing power potency than the standard.

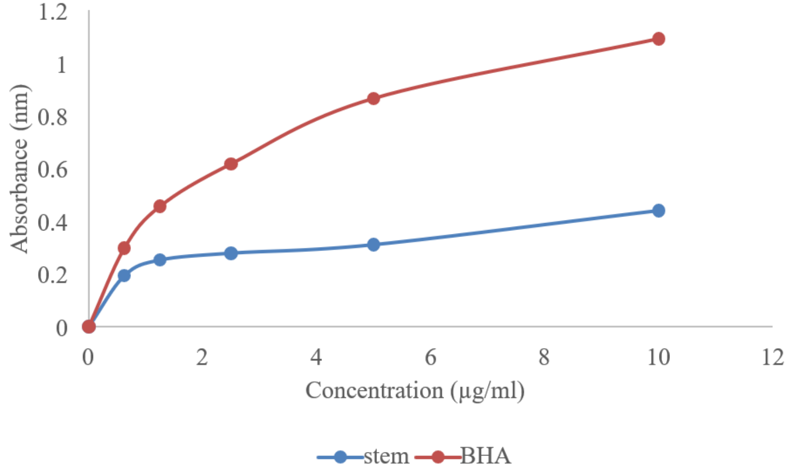

Figure 3. Reducing power activity of ethanol extract of *Ficus capensis* stem compared with BHA.

4. Conclusions

The study concludes that the stem of the *Ficus capensis* plant has significant potential due to its rich bioactive compound content (flavonoids, phenols, ascorbic acid, lycopene, and beta-carotene). The ethanol extract of its stem demonstrated robust antioxidant activity, as evidenced by its high reducing power, DPPH radical-scavenging activity, and lipid peroxidation inhibition. These mechanisms contribute to the extract's antioxidant properties, highlighting its potential to mitigate oxidative stress and related disorders. Challenges of the study include limited mechanistic insights, the need for *in vivo* validation, and extraction variability. However, this study suggests that *Ficus capensis* stem extract holds promise for combating oxidative stress-related diseases, comparable to the standard antioxidant BHA. The findings support the therapeutic potential of *Ficus capensis* stem extract and underscore its usefulness in traditional medicine. Prospects involve clinical trials for oxidative stress-related diseases, synergistic formulations with other antioxidants, and industrial applications in nutraceuticals. Advanced techniques (HPLC-MS, omics) could uncover novel compounds. Despite promising *in vitro* results, further research is needed to standardize protocols, assess bioavailability, and translate findings into therapeutics. Bridging these gaps could position the *Ficus capensis* stem as a sustainable, natural antioxidant for health and industrial use.

Author Contributions

Conceptualization, O.N.A.; methodology, O.N.A and C.E.A; software, I.I.U.; validation, O.N.A.,C.E.A., E.I.A., and C.E.O.; formal analysis, O.N.A.; investigation, O.N.A. and I.I.I.; resources, E.I.A.; data curation, C.E.O.; writing—original draft preparation, O.N.A.; writing—review and editing, O.N.A. and I.I.U; visualization, E.I.A.; supervision, C.E.A.; project administration, O.N.A. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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