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## A Comparative Study of the Antioxidant Potential of *Foeniculum vulgare*, *Ocimum basilicum*, *Syzygium aromaticum*, *Lagenaria breviflora*, and *Luffa cylindrical*

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Oxidative stress is implicated in various diseases, and medicinal plants are valuable sources of natural antioxidants. This study evaluates the antioxidant activity of five medicinal plants—*Foeniculum vulgare*, *Ocimum basilicum*, *Syzygium aromaticum*, *Lagenaria breviflora*, and *Luffa cylindrica*—using multiple in vitro assays. Antioxidant activity was assessed using nitric oxide (NO) scavenging, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assays. Results were compared with ascorbic acid as a standard antioxidant. Among the five plants, *L. breviflora* exhibited the highest hydroxyl radical scavenging activity ( $83.54 \pm 1.85\%$ ,  $p > 0.05$  vs. ascorbic acid). *F. vulgare* showed the strongest NO and DPPH scavenging activity, while *O. basilicum* demonstrated the highest ferric reducing antioxidant power. *S. aromaticum* had the highest total antioxidant capacity. The tested medicinal plants exhibit significant antioxidant properties, with *L. breviflora*, *F. vulgare*, and *O. basilicum* showing the strongest activity in different assays. These findings support their potential application in mitigating oxidative stress-related diseases, but further studies are needed to evaluate their phytochemical composition and therapeutic relevance.

**Keywords:** Medicinal plants, antioxidants, oxidative stress, radical scavenging activity, bioactive compounds.

## 1. Introduction

Antioxidants are molecules that neutralize reactive oxygen species (ROS) and mitigate oxidative damage to cellular components. An excessive accumulation of ROS leads to oxidative stress, a condition associated with the pathogenesis of various diseases, including cancer, metabolic syndrome, atherosclerosis, and neurodegenerative disorders (Kiran *et al.*, 2023). Oxidative stress arises from an imbalance between ROS production and the body's antioxidant defenses, resulting in lipid peroxidation, protein dysfunction, DNA damage, and cell death (Pizzino *et al.*, 2017; Juan *et al.*, 2021).

Plants possess a complex antioxidative defense system comprising phenolic compounds, glutathione, tocopherols, and carotenoids that help neutralize ROS and maintain cellular homeostasis (Dumanović *et al.*, 2021). For centuries, medicinal plants have played a crucial role in traditional medicine, with their bioactive compounds serving as the basis for drug synthesis and disease management (Marrelli, 2021). The therapeutic

potential of medicinal plants lies in their rich reservoir of natural antioxidants, which contribute to their pharmacological benefits (Igbashio *et al.*, 2023).

Medicinal plants have been used in developing and developed countries for thousands of years because they are natural and cause comparatively fewer complications (Jamshidi-Kia *et al.*, 2018). *Foeniculum vulgare* (fennel) contains flavonoids, glycosides, and phenolic compounds such as quercetin and trans-anethole, which contribute to its antioxidant activity (Manzoor *et al.*, 2016; Noreen *et al.*, 2023). *Basilicum* (sweet basil) exhibits a broad spectrum of pharmacological activities, including antioxidant, antimicrobial, and anti-inflammatory properties, attributed to its essential oils and phenolic compounds (Dhama *et al.*, 2023).

*Syzygium aromaticum* (clove) is rich in eugenol, a potent antioxidant that has demonstrated protective effects against oxidative damage and microbial

infections (Batiha *et al.*, 2020). *Lagenaria breviflora* and *Luffa cylindrica* (sponge gourd) have been traditionally used in herbal medicine, with reported antioxidant and antibacterial properties due to their bioactive constituents (Adeyemi *et al.*, 2018; Anastopoulos and Pashalidis, 2020).

Despite the vast diversity of medicinal plants, many remain understudied in terms of their antioxidant potential (Singh, 2015). This study aims to evaluate the antioxidant activity of *Foeniculum vulgare*, *Ocimum basilicum*, *Syzygium aromaticum*, *Lagenaria breviflora*, and *Luffa cylindrica* to identify their potential applications in oxidative stress-related disease management.

## 2. Materials and Methods

### 2.1 Plant Collection and Identification

The seeds of *Foeniculum vulgare*, leaves of *Ocimum basilicum*, and dried fruits of *Syzygium aromaticum* were purchased from a local herbal market in Benin City, Edo State, Nigeria. The fresh leaves of *Lagenaria breviflora* were collected from a secondary forest in Oredo Local Government Area, Edo State, while the fruits of *Luffa cylindrica* were obtained from Okpanam town, Delta State.

All plant samples were authenticated by Prof. H.A. Akinnibosun from the Department of Plant Biology and Biotechnology, University of Benin. Herbarium specimens were deposited at the University of Benin Herbarium with the following voucher numbers:

- *Foeniculum vulgare* (UBH-F665)
- *Lagenaria breviflora* (UBH-0461)
- *Ocimum basilicum* (UBH-L283)
- *Syzygium aromaticum* (UBH-S385)
- *Luffa cylindrica* (UBHL-587)

Ethical considerations and permission for plant collection were obtained from the relevant local authorities.

### 2.2 In Vitro Antioxidant Assays

The in vitro antioxidant activities of the plant extracts were evaluated using five standard assays: nitric oxide (NO) radical scavenging assay, DPPH radical scavenging assay, hydroxyl radical scavenging assay, ferric ion reducing antioxidant power (FRAP) assay, and total antioxidant capacity (TAC). All assays were conducted in triplicates, and results were expressed as mean  $\pm$  standard error of the mean (SEM).

#### 2.2.1 Nitric oxide (NO) Radical Scavenging Assay

##### Nitric Oxide (NO) Radical Scavenging Assay

The nitric oxide radical scavenging activity was determined using the Griess Ilosvoy reaction, as described by Marcocci *et al.* (1994), with slight modifications. Briefly, sodium nitroprusside (5 mM)

in phosphate-buffered saline (PBS, pH 7.4) was incubated with varying concentrations (10–500  $\mu$ g/mL) of the plant extracts at 25°C for 150 minutes. The reaction mixture was then treated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 3% phosphoric acid). Absorbance was measured at 540 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan).

The percentage inhibition was calculated as:

$$\text{Radical scavenging activity}(\%) = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100$$

Where  $A_{\text{control}}$  is the absorbance of the control (without extract) and  $A_{\text{test}}$  is the absorbance in the presence of the extract/standard.

#### 2.2.2 DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) Radical Scavenging Assay

The DPPH radical scavenging activity was assessed using a modified method of Jain *et al.* (2008). A 0.1 mM solution of DPPH in methanol was prepared, and 1 mL of this solution was mixed with 1 mL of different concentrations (10–500  $\mu$ g/mL) of the extracts. The reaction mixture was incubated in the dark at room temperature for 30 minutes, after which absorbance was measured at 517 nm. Ascorbic acid served as the standard.

Scavenging activity was calculated using the formula:

$$\text{DPPH radical scavenging activity}(\%) = \left[ \left( \frac{A_0 - A_1}{A_1} \right) \right] \times 100$$

Where  $A_0$  was the absorbance of DPPH radical + methanol;  $A_1$  was the absorbance of DPPH radical + sample extract or standard.

#### 2.2.3 Hydroxyl Free Radical Scavenging Activity

The hydroxyl radical scavenging activity was determined according to the modified method of Chen *et al.* (2013). The reaction mixture contained 1 mM  $\text{FeSO}_4$ , 1 mM EDTA, 1 mM deoxyribose, 20 mM phosphate buffer (pH 7.4), and 10–500  $\mu$ g/mL of the extract. Hydroxyl radicals were generated by the addition of 10 mM  $\text{H}_2\text{O}_2$ . After incubation at 37°C for 1 hour, absorbance was measured at 532 nm. The scavenging effect was calculated using the following formula

$$\text{Scavenging effect}(\%) = \left( \frac{A_2 - A_1}{A_0 - A_1} \right) \times 100$$

Where  $A_2$  and  $A_1$  are the absorbance with or without sample and  $A_0$  is the absorbance without sample and  $\text{H}_2\text{O}_2$ .

2.2.4 Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed following the method of Benzie and Strain (1996). The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O in a 10:1:1 ratio. The sample extract (10–500 µg/mL) was mixed with FRAP reagent and incubated for 30 minutes at 37°C. Absorbance was recorded at 593 nm. FRAP values were expressed as mg Fe (II) per gram of extract.

2.2.5 Total Antioxidant Capacity (TAC)

TAC was evaluated using the phosphomolybdenum method as described by Prieto et al. (1999). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract, forming a green phosphate/Mo(V) complex. The absorbance was measured at 695 nm. The antioxidant capacity was estimated using the following formula:

$$\text{Total antioxidant capacity(\%)} = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100$$

Where A<sub>control</sub> is the absorbance of the control (without extract) and A<sub>test</sub> is the absorbance in the presence of the extract/standard.

2.3 Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Results were expressed as mean ± SEM of triplicate determinations. One-way ANOVA followed by Duncan's multiple range test was used to determine

statistical significance, with p < 0.05 considered significant.

3. Results and Discussion

3.1 In Vitro Antioxidant Studies

Antioxidants are substances that help to neutralise the damaging effect of free radicals on the cell membrane, DNA, and proteins (Kumar et al., 2024). In this study, the antioxidant potential of the five medicinal plants are shown in Table 1. The results of the antioxidant potential of the five medicinal plants using the hydroxyl assay revealed that *Lagenaria breviflora* hydroxyl radical scavenging potency with an IC<sub>50</sub> value was recorded as 83.54±1.85%, which is the highest percentage of antioxidant activity among all the medicinal plants, and when compared to the standard antioxidant (ascorbic acid), showed no significant difference. The strong free radical-inhibiting potential exhibited by the leaves of *Lagenaria breviflora* may be attributed to a handful of bioactive compounds present in the leaves, including phenols, flavonoids, glycosides, terpenoids, steroids, sesquiterpenes, tannins, and alkaloids, as reported by Banso et al. (2024). These researchers stated that the free radical-inhibiting potentials of *Lagenaria breviflora* leaves likely emanate from phenolics, flavonoids, tannins, and glycosides present within the leaves. Phenolic compounds exhibit redox properties, which are crucial for neutralising free radicals, oxygen singlets, and triplets and decomposing peroxides.

Table 1: Anti-oxidant Potentials of various Medicinal Plants and Ascorbic acid

Medicinal Plants	Hydroxyl (%)	Nitric Oxide (%)	DPPH (%)	TAC (mg/g)	FRAP (mg/g)
<i>F.vulgare</i>	77.01±3.95 <sup>bcd</sup>	77.16±0.96 <sup>b</sup>	90.79±0.58 <sup>a</sup>	218.10±0.33 <sup>a</sup>	1545.67±89.66 <sup>b</sup>
<i>O. basilicum</i>	78.4±2.16 <sup>bc</sup>	75.87±1.21 <sup>b</sup>	63.06±2.41 <sup>c</sup>	179.51±3.81 <sup>c</sup>	1773.33±5.33 <sup>a</sup>
<i>L. breviflora</i>	83.54±1.85 <sup>ab</sup>	75.62±1.06 <sup>b</sup>	79.70±4.47 <sup>b</sup>	195.99±2.08 <sup>b</sup>	1691.67±8.67 <sup>ab</sup>
<i>S.aromaticum</i>	70.34±0.83 <sup>d</sup>	74.75±1.18 <sup>b</sup>	80.82±2.97 <sup>b</sup>	232.05±4.93 <sup>a</sup>	1689.00±5.86 <sup>ab</sup>
<i>L. cylindrical</i>	75.23±2.13 <sup>cd</sup>	74.45±1.38 <sup>b</sup>	22.92±3.06 <sup>c</sup>	142.24±7.11 <sup>d</sup>	1781.67±11.99 <sup>a</sup>
Ascorbic acid	87.01±0.02 <sup>a</sup>	81.35±0.80 <sup>a</sup>	92.62±0.74 <sup>a</sup>	-	912.33±21.70 <sup>c</sup>

Values represent mean ± SEM of five independent determinations. Different superscripts indicate statistically significant differences (p < 0.05) using Duncan's Multiple Range Test.

### 3.2 Discussion

The antioxidant activity of the medicinal plants varied across the different assays. *Lagenaria breviflora* exhibited the highest hydroxyl radical scavenging potential ( $83.54 \pm 1.85\%$ ), comparable to the standard antioxidant ascorbic acid ( $87.01 \pm 0.02\%$ ), indicating its strong ability to neutralize reactive oxygen species (ROS). The high antioxidant activity of *L. breviflora* is likely attributed to the presence of phenolics, flavonoids, tannins, and glycosides (Banso *et al.*, 2024).

The nitric oxide radical scavenging assay showed no significant differences among the five medicinal plants, although ascorbic acid exhibited the highest activity ( $81.35 \pm 0.80\%$ ). The comparable antioxidant activity among the plants suggests the presence of compounds capable of modulating nitric oxide production and preventing oxidative stress.

In the DPPH radical scavenging assay, *Foeniculum vulgare* demonstrated the highest radical scavenging activity ( $90.79 \pm 0.58\%$ ), nearly equivalent to ascorbic acid ( $92.62 \pm 0.74\%$ ). This suggests that *F. vulgare* possesses potent hydrogen-donating antioxidants such as flavonoids, quercetin, and phenolic compounds (Noreen *et al.*, 2023). In contrast, *Luffa cylindrica* exhibited the lowest DPPH scavenging activity ( $22.92 \pm 3.06\%$ ), indicating a weaker ability to neutralize free radicals.

The Total Antioxidant Capacity (TAC) assay, which assesses the cumulative antioxidant potential of bioactive compounds, showed that *Syzygium aromaticum* had the highest scavenging activity ( $232.05 \pm 4.93$  mg/g), followed closely by *Foeniculum vulgare* ( $218.10 \pm 0.33$  mg/g). Cloves (*S. aromaticum*) are known for their rich polyphenolic content, particularly eugenol, which is a potent antioxidant (Batiha *et al.*, 2020).

The Ferric Reducing Antioxidant Power (FRAP) assay revealed that *Luffa cylindrica* had the highest reducing power ( $1781.67 \pm 11.99$  mg/g), suggesting its strong electron-donating ability. Interestingly, the standard antioxidant ascorbic acid recorded the lowest FRAP value ( $912.33 \pm 21.70$  mg/g), indicating that the tested medicinal plants possess unique phytochemicals contributing to their reducing capacity.

### 4. Conclusion

The study demonstrated that all five medicinal plants exhibited notable antioxidant activity, with varying effectiveness across different assays. *Lagenaria breviflora* displayed the highest hydroxyl radical scavenging activity, while *Foeniculum vulgare* exhibited potent DPPH radical inhibition. *Syzygium aromaticum* demonstrated superior total antioxidant capacity, whereas *Luffa cylindrica* showed the strongest ferric reducing power. These findings highlight the potential of these plants as natural

antioxidants, suggesting their possible applications in nutraceutical and pharmaceutical industries for managing oxidative stress-related diseases. The following recommendations are made from the study:

1. Further in vivo studies should be conducted to assess the bioavailability and metabolism of these antioxidants in biological systems.
2. Isolation and characterization of the active antioxidant compounds should be performed to determine their specific mechanisms of action.
3. Comparative studies with synthetic antioxidants should be carried out to evaluate the efficacy of these medicinal plants in pharmaceutical formulations.
4. Clinical trials should be undertaken to explore the therapeutic potential of these plants in preventing oxidative stress-related diseases.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Contributions

Eluehike, N. conceived the idea and conducted the experiment while Igbashio M.D. prepared the manuscript.

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