GAS Journal of Clinical Medicine and Medical Research (GASJCMMR)

OPEN ACCESS

Volume 2, Issue 7, 2025 Journal Homepage: https://gaspublishers.com/gasjcmmr/ ISSN: 3049-1568
Email: gaspublishers@gmail.com

Antioxidant Activities of Isolated Endophytic Fungi from Vitex Doniana Using DPPH and Frap Techniques

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Received: *10.07.2025* | ***Accepted:*** *27.07.2025* | ***Published:*** *12.08.2025*

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DOI: *10.5281/zenodo.16814341*

Abstract **Case Report**

Endophytic fungi are recognized for producing bioactive secondary metabolites, including those with antioxidant properties. This research assessed the antioxidant capacity of six fungal isolates: Cryptococcus sp.,Trichophyton sp., Trichophyton sp., Acremonium sp., and two strains of Aspergillus flavus, through IC_{50} and Ferric Reducing Antioxidant Power (FRAP) assays. The IC_{50} values ranged notably from 42.521 µg/mL to 9.303×10^{13} µg/mL, while ascorbic acid (used as a standard) had an IC_{50} of 25.000 µg/mL. Trichophyton sp. and Aspergillus flavus (B) exhibited significant antioxidant activity with low IC_{50} values and high FRAP ratings, indicating a strong ability to reduce oxidative stress. Conversely, Trichophyton sp. and Acremonium sp. showed minimal activity. These results highlight certain endophytic fungi as potential natural sources of antioxidants, applicable in pharmaceuticals, cosmetics, or food industries. Further investigation into metabolite characterization and compound isolation is advised for a deeper understanding of their bioactive profiles.

Keywords: Endophytic Fungi, Antioxidant Activity, FRAP Assay, IC_{50} Values, Ascorbic Acid.

Citation: Baiwa, F. I., Mudi, S. Y., Hausa, S. S. K., & Gumel, S. A. (2025). Antioxidant activities of isolated endophytic fungi from Vitex doniana using DPPH and FRAP techniques. *GAS Journal of Clinical Medicine and Medical Research, 2*(7), 9-13, ISSN: 3049-1568.

INTRODUCTION

Endophytic fungi are organisms that inhabit living plant tissues without inflicting harm, recognized for producing an array of secondary metabolites with diverse biological effects (Rajamanikyam *et al*., 2017). These metabolites include antibiotics, anticancer agents, and antioxidants. Given the increasing incidence of health problems associated with oxidative stress—such as cancer, diabetes, and neurodegenerative diseases—the search for new antioxidant sources is essential (Nguyen *et al*., 2020).

Antioxidant compounds are usually evaluated through various in vitro methods such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays. The FRAP assay is favored due to its simplicity and reliability, as it assesses the reducing ability of antioxidants by converting Fe^{3+} to Fe^{2+} (Benzie and Strain, 1996), making it effective for characterizing the antioxidant capacity of fungal extracts at different concentrations.

Prior studies have indicated that several species of endophytic fungi, including Aspergillus, Penicillium, and Trichoderma, possess strong antioxidant characteristics. For instance, Aspergillus niger, extracted from medicinal plants, has shown notable ferric reducing potential and low IC_{50} values, pointing to its therapeutic applicability (Sharma *et al*., 2020). Similarly, specific Trichophyton species have been linked to the production of phenolic compounds contributing to their antioxidant effects (Alvin *et al*., 2014).

Combining the FRAP assay with IC_{50} analysis offers a comprehensive approach to evaluate antioxidant effectiveness. The IC_{50} value indicates the concentration needed to inhibit 50% of oxidative radicals, while FRAP assesses the overall reducing capability of the extracts (Gülçin, 2020). Collectively, these methods provide accurate evaluations of a sample's antioxidant profile, with ascorbic acid often used as a control due to its established radical scavenging properties.

The aim of this research is to investigate the antioxidant attributes of endophytic fungi sourced from Vitex doniana,

utilizing DPPH and FRAP assays, focusing on fungi such as *Cryptococcus sp.,Trichophyton sp., Trichophyton sp., Acremonium sp.,* and two strains of Aspergillus fumigatus.

MATERIALS AND METHODS

Research Location

All laboratory experiments, including antioxidant assessments and spectrophotometric analyses, were conducted at the Department of Chemistry, Bayero University Kano, Nigeria, under controlled conditions. Standard analytical protocols were meticulously followed using calibrated instruments.

Materials and Reagents

All reagents used in the assays were of analytical grade and obtained from reputable suppliers. Key chemicals included: DPPH (2,2-diphenyl-1-picrylhydrazyl), Ascorbic acid (the control antioxidant), Ferric chloride ($FeCl_3 \cdot 6H_2O$), TPTZ (2,4,6-tripyridyl-s-triazine), Acetate buffer (300 mM, pH 3.6), Methanol, Sodium acetate, and Hydrochloric acid (HCl).

Preparation of Fungal Extracts

Crude extracts from fungi were produced using methanol on dried biomass. Specifically, 10 g of powdered fungal material was mixed with 100 mL of methanol and shaken periodically for 72 hours. The mixture was then filtered through Whatman No.1 filter paper and concentrated at 40°C using a rotary evaporator. The resulting extracts were kept at 4°C for later use.

Determination of Antioxidant Activity Using DPPH Assay (IC_{50})

DPPH serves as a stable free radical that generates a deep violet solution. Antioxidants reduce DPPH to a yellow-colored diphenylpicrylhydrazine, and the change in absorbance is measured at 517 nm. A stock solution of DPPH (0.1 mM) was formulated in methanol, with serial dilutions of fungal extracts prepared at concentrations from 1000 µg/mL to 7.8 µg/mL. For each dilution, 1 mL of DPPH solution was added, and the mixtures were kept in the dark for 30 minutes at room temperature before measuring absorbance at 517 nm using a UV-Vis spectrophotometer (model UV-1800, Shimadzu). Ascorbic acid served as a positive control, and all experiments were replicated three times.

% Scavenging Activity Calculation

% Inhibition = ((A_control - A_sample) / A_control) × 100
Where:
A_control is the absorbance of DPPH without extract
A_sample is the absorbance in the presence of extract
The IC_{50} value was derived from the graph plotting % inhibition against concentration using linear regression.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the conversion of ferric tripyridyltriazine (Fe^{3+}-TPTZ) to its ferrous (Fe^{2+}) form by antioxidants in an acidic milieu. The formed Fe^{2+}-TPTZ complex displays a blue color, measurable at 593 nm.
A FRAP reagent was prepared freshly using a mix of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. Various concentrations of fungal extracts (1000 to 7.8 µmol equivalents) were created, and 100 µL of each extract was combined with 2.5 mL of FRAP reagent, mixed, and incubated for 30 minutes at 37°C. Absorbance readings were taken at 593 nm against a blank (methanol + FRAP reagent), using ascorbic acid as a reference antioxidant.

Calibration and Result Expression

A calibration curve was developed with standard ascorbic acid solutions, and the FRAP results for samples were reported as µmol Fe^{2+} equivalents per gram of extract (µmol Fe^{2+}/g).

Statistical Analysis

All experiments were performed in triplicate, with the means ± standard deviation (SD) presented. IC_{50} values were calculated using linear regression in Microsoft Excel. One-way ANOVA was employed to compare the antioxidant activities of different fungal species, with a significance threshold of $p < 0.05$.

RESULTS

The results presented in this study stem from the average values derived from triplicate experimental data.

Table 1: Isolates and their average IC_{50} (µg/mL)

Sample codes	Fungal Species	IC_{50} (µg/mL)	$Log_{10}(IC_{50})$
S1	*Cryptococcus sp.*	587.728	2.77
S2	*Culvularia sp.*	14,438.985	4.16
S3	*Trichophyton sp*.	42.521	1.63
S4	*Acremonium sp.*	9.303×10^{13}	13.97

S5	*Aspergillus f.* (A)	1,053.840	3.02
S6	*Aspergillus f.* (B)	89.781	6.00
AA	Ascorbic Acid (Control)	25.000	1.40

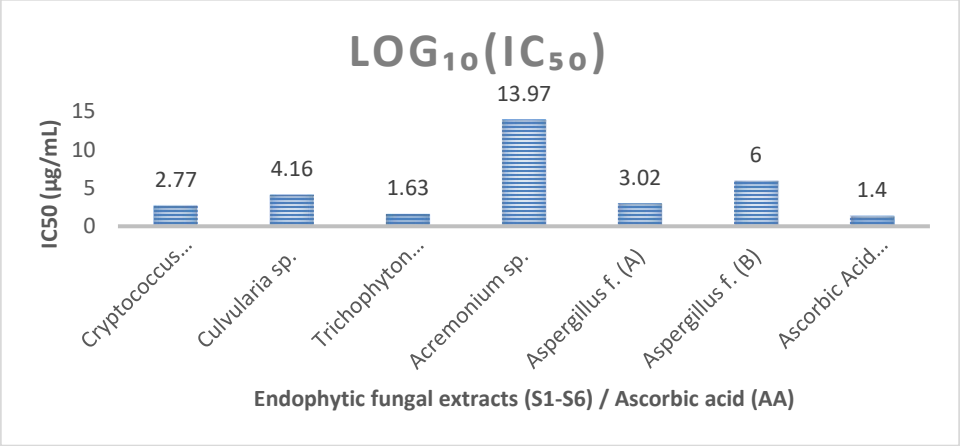

Figure 1. Results of IC50

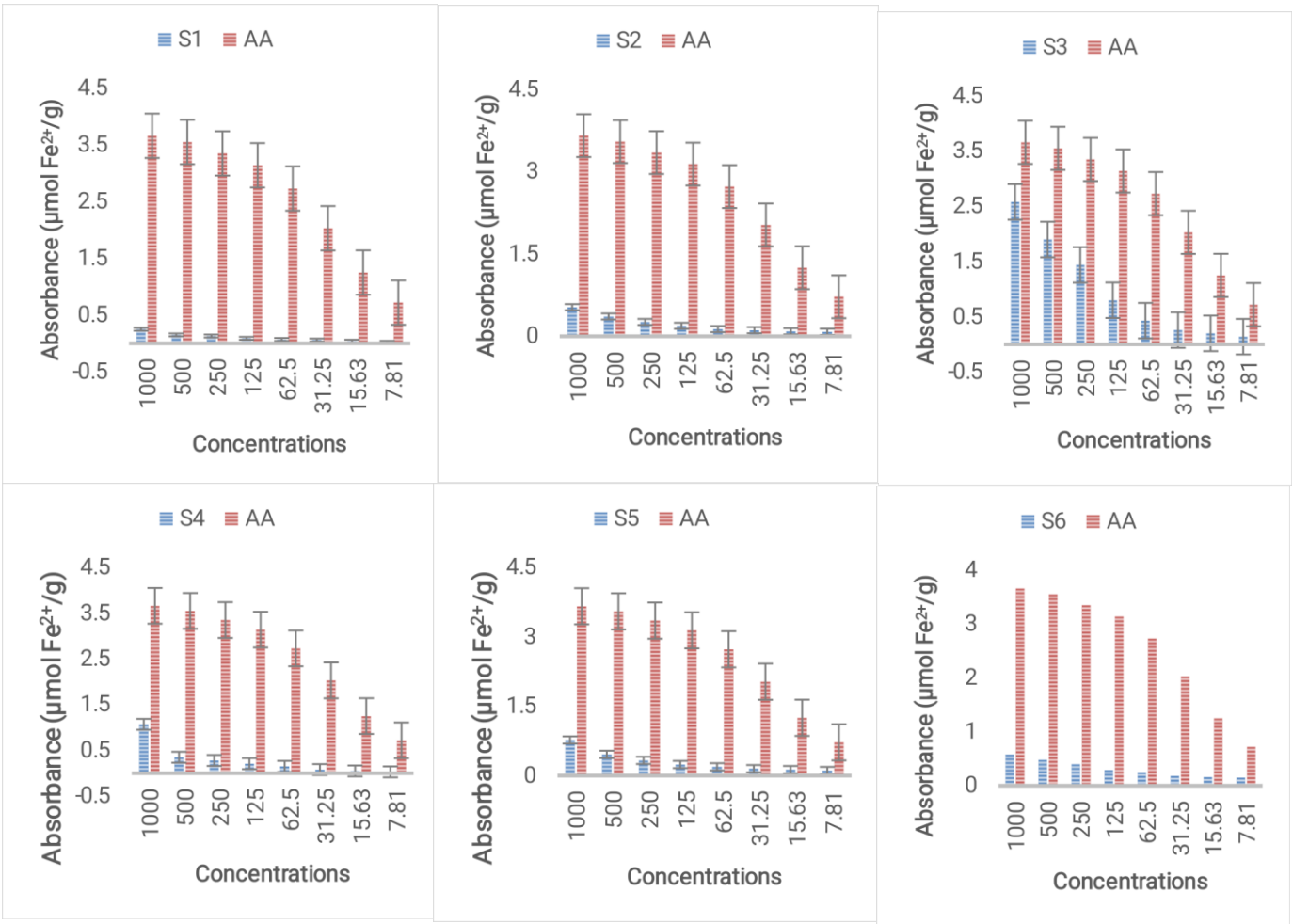

Figure 2. Results of FRAP of the study isolates in µmol Fe^{2+}/g

DISCUSSION

The antioxidant activity of methanolic leaf extracts from Vitex doniana was assessed using IC_{50} values (Figure 1) and FRAP values (Figure 2), highlighting a substantial antioxidant potential. The DPPH assay specifically measures the extract's ability to donate hydrogen atoms to neutralize free radicals, whereas the FRAP assay examines its capacity to convert Fe^{3+} into Fe^{2+}, providing a solid estimate of the antioxidant power present in biological samples (Benzie and Wachtel-Galor, 2018).

In this study, V. doniana exhibited a relatively lower IC_{50} value compared to ascorbic acid, the control, suggesting a high efficacy in radical scavenging. Furthermore, the extract's impressive FRAP value supports its antioxidant capacity. The pronounced reducing ability indicates the presence of phenolic and flavonoid compounds, which are known for their roles in donating electrons and neutralizing oxidative agents (Pang *et al*., 2020).

Prior investigations established Vitex doniana as a source of bioactive constituents like flavonoids, tannins, and phenolics contributing to its antioxidant traits (Okwu and Iroabuchi, 2009). The extract's performance in the DPPH and FRAP assays is consistent with previous findings by Arora *et al*. (2019), who noted that natural plant antioxidants typically exhibit both radical scavenging and ferric reducing activities.

Notably, while the IC_{50} of V. doniana was higher than that of standard ascorbic acid, its FRAP value demonstrated substantial antioxidant strength. This implies that its radical scavenging abilities may be moderate, but it possesses a strong capacity to donate electrons. Such differences might arise from the extract's phytochemical makeup, solvent polarity, or the extraction method utilized (Kusari *et al*., 2021).

Ascorbic acid, functioning as the positive control, presented the lowest IC_{50} and the highest FRAP value, reinforcing its status as a powerful natural antioxidant. It serves as a crucial reference point in antioxidant assays due to its reliable hydrogen-donating and reducing properties (Sharma *et al*., 2020), validating the assessment methodologies used for V. doniana's antioxidant potential.

In conclusion, the findings emphasize the potential of Vitex doniana leaves as a noteworthy source of natural antioxidants. The robust performance of the methanolic extract in both assays suggests the presence of various antioxidant compounds that may act through multiple mechanisms, such as radical scavenging, metal ion chelation, and the inhibition of oxidative enzymes (Kang *et al*., 2021).

CONCLUSION

This study has shown that methanolic extracts of Vitex doniana leaves possess significant antioxidant activity, as confirmed by the DPPH radical scavenging and FRAP assays. The extract demonstrated strong reducing capacity alongside a moderate IC_{50}, indicating its effectiveness against oxidative stress. These findings support the traditional medicinal use of V. doniana and suggest further exploration of its potential as a natural antioxidant in pharmaceutical, nutraceutical, and food preservation fields. Ascorbic acid was the most effective antioxidant in the tests, providing a reliable standard for comparison and confirming the accuracy of the assays utilized. Future phytochemical analyses and in vivo studies are recommended to isolate and identify the specific compounds responsible for the antioxidant effects of V. doniana.

RECOMMENDATIONS

i. Future research should utilize chromatographic and spectrometric methods (e.g., HPLC, LC-MS) to isolate and characterize the antioxidant compounds produced by promising fungi such as Trichophyton sp. and *Aspergillus flavus*.
ii. The antioxidant yield of fungi can vary based on growth conditions; thus, optimizing factors such as pH, substrate type, and temperature may enhance metabolite production.
iii. To ensure safety and practicality, selected fungal extracts should undergo cytotoxicity assessments and in vivo antioxidant evaluations.
iv. Exploring potential correlations between the antioxidant properties of fungi and their host plants may provide insights into metabolic relationships between endophytes and their hosts.
v. Promising isolates should be further examined for their applicability in pharmaceutical formulations, food preservation, and natural skincare products where managing oxidative stress is essential.
vi. Additionally, further isolation of endophytic fungi from diverse plant species and ecosystems is recommended to uncover novel bioactive agents.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to the Department of Science Laboratory Technology at the College of Science and Technology, Jigawa State Polytechnic, Dutse, for providing the necessary laboratory facilities and academic support for the successful completion of this research. Special thanks are extended to principal researcher Fatima Ibrahim Baiwa for her dedication, technical expertise, and leadership throughout the project.

This research was financially supported by the Tertiary Education Trust Fund (TETFund) under the Institutional-Based Research (IBR) grant scheme, and we appreciate the funding that facilitated this work. We also thank the laboratory technologists and research assistants for their crucial contributions during the experimental phases, along with all colleagues and mentors whose feedback enhanced the quality of this research.

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