

Exploring the Free Radical Scavenging Potential of Methanol Stem Bark Extract of *Acacia polyacantha* (Farar Kaya)

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Abstract

Oxidative stress, driven by an imbalance between reactive oxygen species (ROS) and endogenous antioxidants, is a key pathological driver in numerous chronic diseases. While synthetic antioxidants exist, growing concerns over their potential toxicity and side effects have shifted global interest towards natural, plant-derived alternatives. This study investigated the *in vitro* antioxidant potential of the methanol stem bark extract of *Acacia polyacantha* (Farar Kaya). A multi-assay approach was employed to comprehensively evaluate its free radical scavenging capacity, ferric ion reduction capacity, and intrinsic vitamin content. The extract demonstrated a potent and dose-dependent ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, indicating strong electron-donating capabilities, with no statistical significant difference ($p > 0.05$) compared with the standard at the tested concentrations of 25–200 $\mu\text{g/mL}$. The ferric reducing antioxidant power (FRAP) assay also revealed a concentration-dependent efficacy with no statistically significant difference between the extract and the standard. Quantitative analysis revealed remarkably high concentrations of key antioxidant vitamins, including vitamin A ($0.19 \pm 0.11 \text{ mg/g}$), vitamin C ($32.41 \pm 2.12 \text{ mg/g}$), and vitamin E ($0.46 \pm 0.01 \text{ mg/g}$). These findings collectively suggest that *A. polyacantha* stem bark is a rich source of antioxidants, functioning through direct radical neutralization, metal ion reduction, and the provision of essential micronutrients.

Keywords: *Acacia polyacantha*, Antioxidants, FRAP, Free Radical Scavenging, Oxidative stress, and Vitamins

1.0 Introduction

The relentless production of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide is an inevitable consequence of aerobic metabolism [1]. Under physiological conditions, a sophisticated system of enzymatic and non-enzymatic antioxidants maintains redox homeostasis [2]. However, when this balance is disrupted in a state termed oxidative stress, it causes severe damage on cellular macromolecules including lipids, proteins, and DNA. This molecular damage is now recognized as a fundamental etiological factor in the development and progression of a vast array of chronic human diseases, including atherosclerosis, neuro-

degenerative disorders (Alzheimer's and Parkinson's diseases), diabetes, cancer, and aging [3].

While synthetic antioxidants exist, growing concerns over their potential toxicity and side effects have shifted global interest towards natural, plant-derived alternatives [4]. The plant kingdom represents an extensive reservoir of bioactive compounds, particularly phenolic acids, flavonoids, tannins, and vitamins, which exhibit potent antioxidant activities through various mechanisms such as radical scavenging, metal chelation, and singlet oxygen quenching [5].

The genus *Acacia* (Family: Fabaceae) comprises over 1300 species, many of which are deeply rooted

in traditional medicine across Africa, Asia, and Australia [6]. *Acacia polyacantha* (Farar Kaya). in particular, has been employed in folk medicine for the treatment of wounds, inflammation, and various infections [7]. Previous phytochemical investigations of related species have revealed the presence of valuable metabolites like condensed tannins, flavonoids, and terpenoids, which are strongly associated with antioxidant efficacy [7]. However, a comprehensive analysis of the antioxidant mechanisms of *A. polyacantha* stem bark, specifically its reducing power and native vitamin content, remains underexplored[8].

Therefore, this study aims to critically evaluate the *in vitro* antioxidant potential of the methanol stem bark extract of *A. polyacantha* through a battery of tests: (i) assessing its free radical scavenging capacity via the Diphenyl-1-Picrylhydrazyl(DPPH) assay, (ii) determining its ferric reducing antioxidant power (FRAP), and (iii) quantifying its inherent levels of key antioxidant vitamins (A, C, and E). This multi-faceted approach provides a deeper insight into the extract's potential as a source of natural antioxidants for combating oxidative stress.

2.0 Materials and Methods

2.1 Collection and Identification of Plant

The fresh stem bark of *Acacia polyacantha* was collected from Zuru Local Government Area of Kebbi State, Nigeria, August 2024. It was authenticated at the Herbarium unit, Department of Biological Sciences, Federal University Birnin Kebbi, Nigeria. The specimen of the plant was deposited at the herbarium unit of the same department, with a voucher number of FUBKH 0448

2.2 Preparation of stem powder and methanol extract

The stem barks of the plant were rinsed with distilled water, and air dried at room temperature. They were then pulverized to coarse particles, soaked in methanol for 72 hours and filtered using cheese cloth. The filtrates were concentrated using a rotary evaporator at 40°C and further dried in a petri dish at room temperature. Thereafter, the sample was carefully packed in a petri dish and stored in a refrigerator for further use.

2.3 Determination of antioxidant activity:

2.3.1 DPPH Radical Scavenging Assay:

The assay assesses hydrogen donation by antioxidants, reducing DPPH from violet to yellow, measured at 517 nm. DPPH scavenging activity was measured using a modified method by [9]. Two milliliters of extract or standard (0.2–1.0 mg/ml) were mixed with 0.5 ml of 0.3 mM DPPH in methanol, stirred, and incubated in the dark for 15 minutes at room temperature. Absorbance was recorded at 517 nm in triplicate, with ascorbic acid as the standard. The percentage of inhibition of DPPH radical or radical scavenging activity (RSA) was calculated using equation 1.

$$\% \text{ DPPH RSA} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs of Control}} \times 100 \text{ -----1}$$

Note: RSA, otherwise known as IC 50 $\times$ 100 or 50 % inhibitory concentration, is the concentration of the antioxidant responsible for scavenging 50 % of DPPH radical by plotting inhibition percentages against concentrations of the sample (sample concentration range from 0 to 1 mg/ mL).

2.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay:

The assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm [10]. Three stock solutions of sodium acetate buffer (300mM, pH=3.6), 10mM of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) in 40mM HCl solution, and 20mM of FeCl_3 (in demineralized water) were prepared separately. The FRAP reagent was prepared freshly by mixing all the three solutions previously mentioned in a ratio of 10:1:1, respectively, and the mixture was warmed in a water bath at 37°C for 6min. 50 μ L of each extract or standard solution was mixed with 1500 μ L of FRAP reagent. The mixture was vortexed and incubated in darkness at room temperature for 5min. Then, the absorbance was measured against control at 594nm. A calibration curve was prepared with ($\text{FeSO}_4 \times 7 \text{H}_2\text{O}$) acid subjected to the above-described procedure. The results were expressed as micrograms of ferrous sulfate equivalents per gram of DW according to the calibration curve of the

standard. A similar procedure was employed on FRAP radicals, at an absorbance of 734 nm.

2.3.3 Quantification of Antioxidant Vitamins

Antioxidant vitamins (A, C, and E) in *Acacia polyacantha* stem bark extract were quantified following the methods described by Omoregie and Osagie [16] with slight modifications.

Vitamin A was extracted from 2 g of the sample using a petroleum ether–ethanol (1:1 v/v) mixture containing 0.01 % BHT. The extract was evaporated under nitrogen, redissolved in methanol, and absorbance was measured at 450 nm against a retinol standard curve.

Vitamin C was determined by extracting 5 g of the sample in 5 % trichloroacetic acid (TCA), followed by titration of the supernatant with standardised 2,6-dichlorophenolindophenol (DCPIP) dye until a persistent pink colour appeared.

Vitamin E (α -tocopherol) was extracted as described for vitamin A, redissolved in methanol,

and quantified by reversed-phase HPLC on a C18 column using methanol–water (98:2 v/v) as mobile phase and UV detection at 292 nm. Quantification was performed using an external α -tocopherol standard curve.

All analyses were carried out in triplicate.

2.4 Statistical Analysis

Experiments were performed in triplicate. Data were expressed as mean $\pm$ SD. Differences were considered significant at $p < 0.05$ using one-way ANOVA followed by Tukey's post-hoc test.

3.0 Results

3.1 DPPH Free Radical Scavenging Activity

The methanol stem bark extract of *Acacia polyacantha* (AP) demonstrated potent dose-dependent DPPH radical scavenging activity, comparable to ascorbic acid (AA) (Fig. 1).

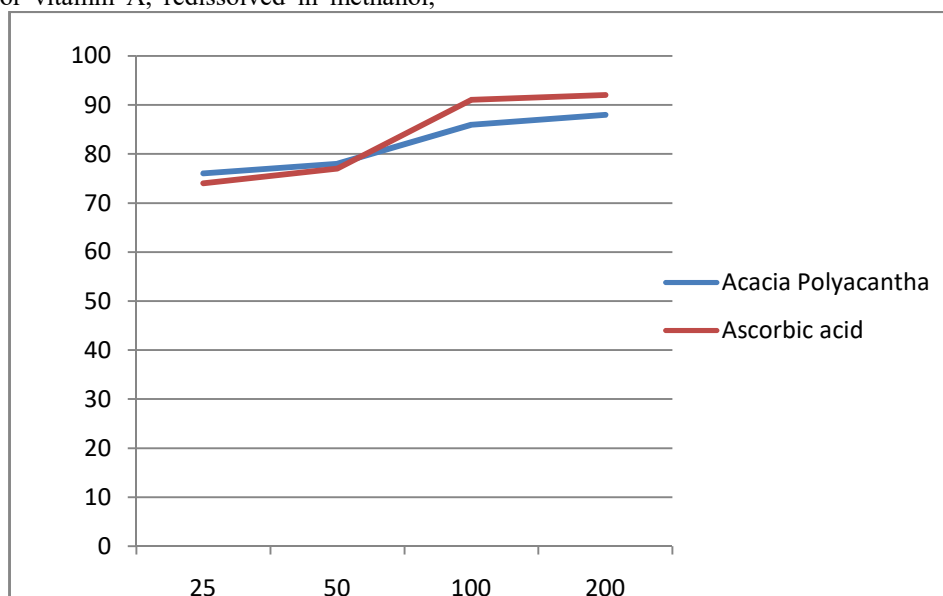


Figure 1: DPPH radical scavenging activity of *A. polyacantha* and ascorbic acid

3.2 Ferric Reducing Antioxidant Power (FRAP)

The FRAC assay demonstrated significant dose-dependent ferric ion reduction by both *Acacia polyacantha* (AP) stem bark extract and ascorbic acid (AA) standard except in 50mg and 200mg

concentrations where the activity of the sample slightly exceeded that of the standard (Figure 2). Absorbance values increased proportionally with concentration for both compounds. Statistical analysis (revealed no significant difference ($p > 0.05$) between AP and AA across tested

concentrations (25–200 mg/mL), suggesting equivalent antioxidant efficacy.

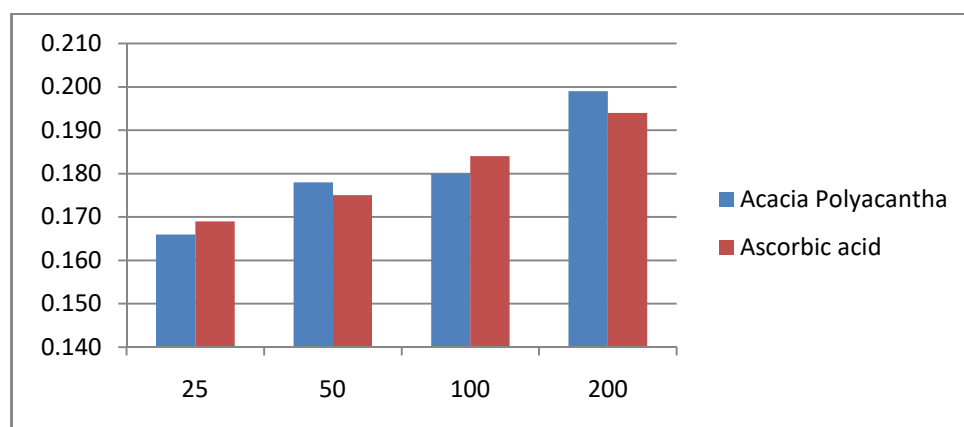


Fig. 2: Comparative FRAC activity of *A. polyacantha* and Ascorbic acid

3.3 Antioxidant Vitamin Content

The antioxidant vitamins content of Vitamin A, C, and E found in *Acacia polyacantha* are presented in (Table 1).

Table 1. Antioxidant Vitamins Content of *Acacia polyacantha*

Vitamin	Concentration(mg/g)
VIT A	0.19±0.11
VIT C	32.41±2.12
VIT E	0.46±0.01

Values are expressed as mean ± standard deviation (n = 3) in mg/g dry weight.

4.0 Discussions

The methanol extract of *A. polyacantha* stem bark exhibited a pronounced and concentration-dependent ability to scavenge DPPH free radicals. The percentage of radical inhibition increased steadily with increasing concentration of the extract, culminating in significant neutralization at the highest doses tested. This linear dose-response relationship is a classic indicator of the presence of potent antioxidant compounds, primarily phenolics and flavonoids, which act by donating hydrogen atoms or electrons to stabilize the free radical [2]. The strong electron-donating capacity observed suggests that the extract's constituents can effectively disrupt the radical chain reactions that propagate oxidative damage within biological systems [11]. These align closely with findings on

other species within the same genus, such as *A. nilotica*, which also demonstrated potent radical scavenging capabilities linked to its phenolic, flavonoid profile, and electron-donating capabilities. This suggests strong antioxidant potential may be a common characteristic among medicinal *Acacia* species [12].

The results of the FRAP assay presented a more complex and intriguing profile. The reducing power of the extract did not follow a simple linear progression but showed significant variation across the tested concentrations. Similar patterns have been observed in studies of other plants like *Psidium guajava*, which is often attributed to the complex mixture of compounds, where some may have pro-oxidant effects at lower concentrations or where synergistic/antagonistic interactions occur at specific concentration thresholds[13]. Notably, the extract demonstrated superior ferric ion reduction compared to the standard ascorbic acid at concentrations of 50 µg/mL and 200 µg/mL. However, it was less effective at the 25 µg/mL and 100 µg/mL points. This non-monotonic response is common in plant extracts and may be attributed to the complex interplay between different antioxidant compounds within the extract [14]. At specific concentrations, synergistic effects between various phytochemicals may enhance the overall reducing power, while at others, antagonistic interactions or differential solubility might lead to a less pronounced effect [2]. This finding underscores the importance of identifying an "optimal dose" for maximum efficacy and suggests that the extract contains a mixture of redox-active compounds with varying potencies.

While numerous studies, such as that on *Salvia* species by [15], effectively documented the radical scavenging capabilities of plant extracts, the current study adds a significant additional layer of analysis by quantifying the intrinsic levels of vital antioxidant vitamins (A, C, and E), in a striking finding of an exceptionally high content of these essential antioxidant vitamins in the extract. The quantified levels of vitamin A (0.19 ± 0.11 mg/g), vitamin C (32.41 ± 2.12 mg/g), and vitamin E (0.46 ± 0.01 mg/g), far exceed those typically reported in many common fruits and vegetables. Vitamin C (ascorbic acid) is a premier water-soluble antioxidant, directly scavenging free radicals and regenerating other antioxidants like vitamin E [16]. Vitamin E (α -tocopherol), a lipid-soluble antioxidant, is crucial for protecting cell membranes from lipid peroxidation [16]. The presence of these vitamins in such significant quantities suggests that *A. polyacantha* contributes to antioxidant defense not only through secondary metabolites but also by providing primary antioxidant micronutrients. This dual function enhances its potential nutraceutical value, offering a combined effect of immediate radical quenching and long-term nutritional support for the endogenous antioxidant system[1].

5.0 Conclusion

The findings from this study present a compelling case for the methanol stem bark extract of *Acacia polyacantha* as a potent, multi-mechanistic antioxidant agent. Its efficacy is demonstrated through three distinct pathways: (1) direct free radical scavenging, as evidenced by the DPPH assay; (2) significant ferric ion reduction potential at optimal concentrations; and (3) the provision of remarkably high levels of endogenous vitamins A, C, and E. This triumvirate of activities suggests that the extract could be highly effective in mitigating oxidative stress by targeting different oxidative pathways and cellular compartments.

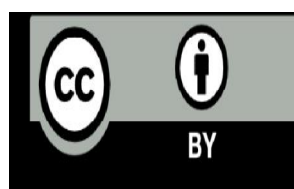
The concentration-dependent effects, particularly the nuanced FRAP results, highlight the complexity of plant extracts and underline the necessity for dose optimization in future applications. The profound vitamin content positions *A. polyacantha* not just as a source of therapeutic phytochemicals but also as a potential functional food ingredient. To translate these promising *in vitro* results into practical applications, subsequent research should focus on bioassay-guided fractionation to identify

the specific compounds responsible for the activity, alongside *in vivo* studies to confirm efficacy and safety in biological models of oxidative stress.

References

- [1] Y. H. Prayogo, W. Syafii, R. K. Sari, I. Batubara, and Danu, "Pharmacological activity and phytochemical profile of acacia heartwood extracts," *Sci. Pharm.*, vol. 89, no. 3, 2021. doi: 10.3390/scipharm89030037.
- [2] G. Moriasi, A. Ireri, and M. P. Ngugi, "In Vitro Antioxidant Activities of the Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* (Schum.)," *J. Evidence-Based Integr. Med.*, vol. 25, pp. 1–9, 2020. doi: 10.1177/2515690X20937988.
- [3] O. S. Ijarotimi, M. R. Fatiregun, and T. D. Oluwajuyitan, "Nutritional , antioxidant and organoleptic properties of therapeutic - complementary - food formulated from locally available food materials for severe acute malnutrition management," *Bull. Natl. Res. Cent.*, 2022. doi: 10.1186/s42269-022-00725-z.
- [4] S. Keser and O. Yilmaz, "Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn," no. May, 2014.
- [5] R. Jame, "Phytochemical and Pharmacological Uses of *Acacia Nilotica* - A Review Phytochemical and Pharmacological Uses of *Acacia Nilotica* - A Review," no. February, 2021, doi: 10.11648/j.ijbc.20180302.11.
- [6] F. A. Ashu *et al.*, "Antistaphylococcal Activity of Extracts, Fractions, and Compounds of *Acacia polyacantha* Wild (Fabaceae)," *Evidence-based Complement. Altern. Med.*, vol. 2020, 2020, doi: 10.1155/2020/2654247.
- [7] V. Issue, A. K. M, A. M. Hamid, and T. E. Hagr, "Gas Chromatography / Mass Spectroscopy Analysis, antibacterial

- Activity of Fixed Oil from *Acacia polyacantha* (Sudanese Kakamout) Seeds,” *Sudan. Online Res. J.*, no. 2, pp. 21–27, 2021, doi: 10.51527/v2i1.4me.
- [8] A. A. A. Abdel-Wareth *et al.*, “Effects of Dietary Bioactive Lipid Compounds of *Acacia nilotica* Bark on Productive Performance, Antioxidant Status, and Antimicrobial Activities of Growing Rabbits under Hot Climatic Conditions,” *Animals*, vol. 13, no. 12, 2023, doi: 10.3390/ani13121933.
- [9] N. M. Thangjam, J. Taijong, and A. Kumar, “Phytochemical and pharmacological activities of methanol extract of *Artemisia vulgaris* L. leaves,” *Clin. Phytoscience*, vol. 6, no. 1, pp. 4–11, 2020, doi: 10.1186/s40816-020-00214-8.
- [10] P. Nanfack, N. Biapa, P. CA, A. M. VJ, B. Moukette, and N. Y. J, “The in vitro antisickling and antioxidant effects of aqueous extracts *Zanthoxylum heitzii* on sickle cell disorder,” *BMC Complement. Altern. Med.*, vol. 13, no. 162, pp. 1–7, 2013.
- [11] R. R. Kotha, F. S. Tareq, E. Yildiz, and D. L. Luthria, “Oxidative Stress and Antioxidants—A Critical Review on In Vitro Antioxidant Assays,” *Antioxidants*, vol. 11, no. 12, 2022, doi: 10.3390/antiox11122388.
- [12] G. A. Engwa *et al.*, “Relationship of oxidative stress and antioxidant response with vaso-occlusive crisis in sickle cell anaemia,” vol. 21, no. 1, pp. 150–158, 2021.
- [13] O. O. Oguntibeju, “A Review of the Relationship between the Immune Response, Inflammation, Oxidative Stress, and the Pathogenesis of Sickle Cell Anaemia,” 2023.
- [14] H. C. Nzelibe, M. M. Abarshi, K. State, and K. State, “ANTIOXIDANT PROPERTIES OF AQUEOUS STEM BARK EXTRACT OF *ANOGEISSUS LEIOCARPUS* AGAINST ETHANOL-INDUCED,” vol. 16, no. 3, pp. 199–205, 2021.
- [15] A. O. OKPANACHI, A. B. ADELAIYE, K. Y. MUSA, A. Mohammed, and Y. Tanko, “Evaluation of the Effect of Aqueous-Methanolic Stem Bark Extract of *Acacia Polyacantha* on Blood Glucose Levels of Normoglycemic Wistar Rats,” *Int. J. Anim. Vet. Adv.*, vol. 4, no. 3, pp. 163–166, 2012, [Online]. Available: <http://www.maxwellsci.com/jp/abstract.php?jid=IJAVA&no=208&abs=01>
- [16] E. S. Omoregie and A. U. Osagie, “Effect of *Jatropha tanjorensis* leaves supplement on the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats,” *J. Food Biochem.*, vol. 35, no. 2, pp. 409–424, 2011, doi: 10.1111/j.1745-4514.2010.00392.x.



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