

**Original Article****Phenolic, Antioxidant and Acute Toxicological Profiles of Phenolic-rich *Terminalia catappa* as Anti-aflatoxin B1 Agent in Poultry Feeds*****¹Yusuf R. S., ²Abubakar, A., ¹Ado, M. A. and ¹Garba, S.****¹Department of Biochemistry, Bauchi State University, Gadau, Bauchi, Nigeria****²Department of Biological Sciences, Bauchi State University, Gadau, Bauchi, Nigeria**

Submitted: July 2026; Accepted: August 2026; Published: September 2026

ABSTRACT

Aflatoxin B1 which is the most toxic among the aflatoxins when ingested by birds has become a serious threat to the poultry farmers in developing world. This causes great loss to the poultry farmers due to mortality resulting from toxicity and oxidative stress induced by aflatoxin b-1 in the respective birds. As such, natural antioxidants are required to formulate the poultry feeds to serve as anti-aflatoxin b1 agent. Therefore, this study aims at determining the antioxidant, acute toxicity and phenolic profile of phenolic-rich extract of *Terminalia catappa* leaves as anti-aflatoxin b1 agent. determining the antioxidant, acute toxicity and phenolic profile phenolic-rich extract of *Terminalia catappa* leaves. Phenolics analysis, toxicological profile and antioxidant analysis of the extract were carried out using standard methods. The phenolics profile reveals the presence of flavonoids (31.52 ± 3.50 mg/g), total phenols (105.90 ± 5.20 mg/g) and tannins (85.50 ± 4.40 mg/g). The extract shows appreciable 2, 2-diphenyl picryl hydrazyl radical scavenging activities (DPPH), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (ILP) at concentrations of 125, 250 and 500 μ g/mL which compared well with Gallic acid at the same concentration. Furthermore, the LD₅₀ of the extract in Wistar rats is greater than 5000 mg/kg bw (LD₅₀ > 5000 mg/kg). Hence, the extract could be classified as practically non-toxic or relatively harmless. Therefore, the exhibition of phenolic-rich extract of *Terminalia catappa* leaves excellent antioxidants and acute toxicity profiles qualify the extract to be used as an anti-aflatoxin b1 agent in poultry feeds.

Keywords: Phenolic, *Terminalia catappa*, Acute toxicity, Antioxidant, Anti-aflatoxin B1***Corresponding author's email:** ruqayyahsyusuf@sazu.edu.ng, +2347042021335)**INTRODUCTION**

Poultry and mycotoxins have been closely linked for several decades, as they are one of the most sensitive animal species to the toxic effects of mycotoxins (1). In the

poultry industry, the ingestion of aflatoxin B1 (AFB1) lead to the impairment of all the essential productive parameters and decreased resistance to common infectious diseases due to the depression of the humoral and cellular immune

responses, resulting in substantial annual economic losses (2). Furthermore, there is public concern for the possible contamination of toxic residues into poultry products, such as meat and eggs, resulting in a potential hazard to human health, since the AFB1 residues may pass to these products at deficient levels (1). The toxicity of AFB1 occurred as a result of its bioactivation into a highly reactive AFB1- 8, 9-epoxide. The epoxidation of the terminal furan ring of AFB1 was responsible for the production of epoxide radicals that attacked the cellular macromolecules such as DNA, RNA and other protein constituents (3). There is also accumulated evidence to show that generation of oxidative stress as a result free radical attacked several organs in birds (4).

Terminalia catappa Linn. (Combretaceae) (Plate 1) commonly refers to as tropical almond is a large tree that grows in tropical countries. It is a native to Australia, Cambodia, India, Japan, Laos, Malaysia, Thailand and widely distributed all over the globe (5). *T. catappa* is very rich in phenols which could arrest oxidative stress in the system and now being used for various folkloric medicine with scientific reports of anti-inflammatory, antimicrobial, antioxidant, and antidiabetic activities among others (5).

The aim of this study was to determine the phenolic, antioxidant and acute toxicological profiles of phenolic-rich *T. catappa* as anti-afalatoxin-B1 agent in poultry feeds



Plate 1: *Terminalia catappa*

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh *T. catappa* leaves were obtained within the premises of Federal University of Technology, Minna, Niger State, Nigeria. The plant was authenticated at the Botany Department, Sa'ad Zukur University, Gadau, Bauch State Nigeria with voucher number. The specimen was deposited in the herbarium of the department for reference.

Chemicals and Reagents

Gallic acid, Tannic acid, Quercetin, 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH), Picric acid, Ferric chloride, Sodium carbonate, garlic acid, Tannic acid, Quercetin, Thiobarbituric acid, Acetone, Methanol are products of Sigma Chemicals Co, St Louis, USA.

Plant Materials and Authentication

Fresh *T. catappa* leaves were obtained within the premises of Federal University of Technology, Minna, Niger State, Nigeria. The plant authenticated at the Botany Department, Sa'ad Zukur University, Gadau, Bauch State Nigeria with voucher number SAZU-002.

Sample preparation

The leaves of *T. catappa* leaves were thoroughly washed and dried under room temperature. The dried leaves were milled

using electric blender (Binatone BLG 450, United Kingdom). The powdered sample was kept in an airtight plastic container at room temperature (28 ± 2) °C until further use

Extraction of phenolics from *T. catappa* Leaves

Method described by Busari *et al.* (6) was adapted for the extraction of phenolic-rich extract. Fifty grams (50 g) of the powdered sample was placed in a round bottom flask and reflux with 400 mL of 40 % acetone and 60 % methanol mixture at 50°C for 4 hours. Thereafter, the extract was filtered with muslin cloth after which the methanol/acetone solvent evaporated at low pressure in a rotary vacuum evaporator. The phenolic-rich filtrate of *T. catappa* leaves obtained was lyophilized to obtain phenolics rich extract which was stored at -4°C.

Quantification of Phenolic Compounds

Quantification of total phenol

Total phenol content of the extract was quantified as described by Busari *et al.* (7). Briefly, exact 0.5 mg/mL of either extract was dissolved in methanol and each of the dissolved sample was mixed with exact 2.9 mL of 2 % Na₂CO₃. The mixture was incubated in a laminar flow at room temperature for two minutes followed by addition of 0.1 mL of 10% Folin-Ciocalteu reagent. The mixture was then incubated in a laminar flow for another 30 min at 37 °C. The absorbance of the reaction mixture was taken at 750 nm with a Shimadzu UV-Spectrophotometer UV-1800 model (Japan). Exactly 0.5 mg/mL of gallic acid in methanol was used as standard. The phenolics content of the extract was expressed as milligram gallic acid

equivalents (mg gallic acid/g extract). All samples were analyzed in triplicates.

Quantification of flavonoids

The flavonoids content was quantified as described by Busari *et al.* (7) using spectrophotometric techniques. About 0.5 mg/mL of the extract and quercetin were prepared in absolute methanol and 250 µL of each of mixture was mixed with deionized water (1.6 mL) and 100 µL of 5% Na₂CO₃. The samples were left for 6 min after which 150 µL of 10 % AlCl₃ solution was pipetted into the mixture followed by 5 min incubation at 37 °C. About 500 µL of 1 M NaOH was added to halt the reaction followed by 15 min incubation at 37 °C. The absorbance of the resulted mixture was read at 510 nm using a Shimadzu UV-Spectrophotometer against the blank (contained 250 µL of absolute methanol instead of the extract or quercetin). The 0.5 mg/mL of quercetin in methanol was used as standard. The flavonoids content was quantified as milligram quercetin equivalents (mg quercetin/g extract).

Quantification of tannins

The procedure used in determining the tannins contents was described by Busari *et al.* (6). Exactly 0.2 g of the extract was weighed into a 50 mL beaker followed by addition of 20 mL of 50 % methanol. The beakers were covered with aluminum foil and placed in a water bath with shaker at 80 °C for 1 hour. The extract was allowed to cool, filtered with double layered Whatman No.41 filter paper into a 100 mL volumetric flask with addition of 20 mL of water followed by the addition of Folin-Denis reagent (2.5 mL) and 17 % Na₂CO₃ (10mL). Water was added to the mixture to 100 mL volume and allowed to stand for

20 mi for colour development after proper mixing; the absorbance was read against blank with spectrophotometer at 760 nm. The same procedure was followed for tannic acid standard. The tannins content was quantified as milligram quercetin equivalents (mg tannic acid/g extract).

Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay of the phenolic-rich extract was determined by spectrometric method as described by Yunusa *et al.* (8). Briefly, different concentrations of the plant extracts and Gallic acid (250 – 1000 µ/mL) in 1mL of distilled water. The prepared sample were mixed with phosphate buffer (3.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixtures were incubated at 50 °C for 20 minutes. Then, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, then centrifuged for 10 minutes at 12,000 x g. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%). The absorbance was read at 700 nm against a blank with spectrophotometer.

The percentage FRAP was calculated using the following expression;

$$\% \text{ Inhibition} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Determination of Scavenging Activity Against 1,1-Diphenyl-2-Picryl Hydrazyl Radical

The DPPH Scavenging activity of the phenolics-rich extract and its fractions was determined by spectrometric method as described by Yunusa *et al.* (8). Briefly, 1mL of 125 - 1000µ/mL of phenolics-rich extract and gallic acid were prepared in

separate test tubes using absolute methanol. Afterwards, 2mL of 0.004% DPPH solution in methanol was added. Each of the test samples were properly mixed and incubated at 25°C for 30 minutes. The absorbances of the test mixtures were read against the reagent blank at 517 nm using a spectrophotometer.

The percentage DPPH scavenging activities was calculated using the following expression;

$$\% \text{ Inhibition} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Inhibition of lipid peroxidation

The inhibitory effects of the extracts on lipid peroxidation were determined via thiobarbituric acid reactive substances (TBARS) assay as described by (9) with slight modification. Briefly, 0.5 mL of 10% egg homogenate was added to 0.1 mL of extract or ascorbic acid (control) at various concentrations (50, 100, 200 and 400 µg/mL) and made up to 1 mL with distilled water. Afterward, 0.05 mL of FeSO₄ was added to the mixtures and incubated for 30 minutes. Then, 1.5 mL of acetic acid and thiobarbituric acid in sodium dodecyl sulphate were added, respectively. The resulting reaction mixture was vortexed and incubated at 95 °C for 1 hour. The reaction mixtures were cooled, and 5 mL of butanol was added to each of the reaction mixtures and centrifuged at 1200 x g for 10 minutes and the absorbance of the upper organic layer was read at 532 nm. The percentage inhibition of lipid peroxidation was calculated using the formula below.

$$\% \text{ Inhibition} = A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}} \times 100$$

Acute Toxicity

The acute toxicity was conducted according to Organisation for Economic Co-operation and Development as described by Zarai *et al.* (10). Limit test was used where five females' Wistar rats was administered with a single oral dose of 5000 mg/kg bw after 8 hours fasting. Thereafter, mortality was monitored for 24 hours.

Data analysis

The analysis of variance (ANOVA) followed by *Post hoc* Duncan multiple comparisons test (Statistical Package for

Table 1: Phenolics Contents

Phenolics	mg/g
Total Phenols	105.90 ± 5.20
Flavonoids	31.52 ± 3.50
Tannins	85.50 ± 4.40

Values are Means of triplicates ± SEM.

Ferric reducing antioxidant power of phenolic-rich extract of *T. catappa* leaves

The Ferric reducing antioxidant power of phenolic-rich extract of *T. catappa* leaves is presented in Table 2. Except for the

Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA) at 95% confidence interval was used on the data obtained with *p*-value less than 0.05 was considered significant difference. The data were expressed as mean ± standard error mean of five replicates.

RESULTS

Phenolics Contents

Table 1 showed the result of phenolic compounds in the phenolic-rich extract of *T. catappa* whole plant. The phenolic compounds present are in the following order total phenol > tannins > flavonoids.

lowest concentration (125 µg/mL), there is no significant difference between ferric reducing antioxidant power of phenolic-rich extract of *T. catappa* leaves when compared with that of gallic acid at concentrations 250, 500 and 1000 µg/mL.

Table 2: Ferric reducing antioxidant power of phenolic-rich extract of *T. catappa* leaves

Concentration	125 (µg/mL)	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)
<i>T. Catappa</i>	35.75 ± 0.75 ^a	52.50 ± 1.25 ^a	81.60 ± 0.62 ^a	86.75 ± 1.60 ^a
Gallic acid	37.12 ± 1.05 ^b	50.10 ± 0.75 ^a	82.10 ± 1.60 ^a	88.15 ± 3.80 ^a

Values are expressed as Mean of triplicate ± standard error mean

Values with the same alphabet superscript are not significant difference along the column

The DPPH scavenging activities of phenolic-rich extract of *T. catappa* leaves

The DPPH scavenging activities of phenolic-rich extract of *T. catappa* leaves

is presented in Table 3. Except for 125 (µg/mL) there is significant difference between DPPH scavenging activities between phenolic-rich extract of *T. catappa* leaves when compared with gallic acid at any other concentrations. The

DPPH scavenging activities of phenolic-rich extract of *T. catappa* leaves increased as the concentration increases

Table 3: The DPPH scavenging activities of phenolic-rich extract of *T. catappa* leaves

Concentration	125 (µg/mL)	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)
<i>T. Catappa</i>	32.50 ± 0.80 ^b	49.35 ± 1.30 ^a	71.61 ± 0.36 ^a	80.66 ± 0.40 ^a
Gallic acid	35.70 ± 0.70 ^a	48.15 ± 2.38 ^a	67.24 ± 1.26 ^a	81.40 ± 1.80 ^a

Values are expressed as Mean of triplicate ± standard error mean

Values with the same alphabet superscript are not significant difference along the column

Inhibition of Lipid Peroxidation of phenolic-rich extract of *T. catappa* leaves

The inhibition of lipid peroxidation of phenolic-rich extract of *T. catappa* leaves is presented in Table 4. The inhibition of lipid peroxidation of phenolic-rich extract

of *T. catappa* leaves between compared significantly favorably when compared with gallic acid at all concentrations used. The inhibition of lipid peroxidation of phenolic-rich extract of *T. catappa* leaves also increased as the concentration increases as observed previously.

Table 4: Inhibition of Lipid Peroxidation of phenolic-rich extract of *T. catappa* leaves

Concentration	125 (µg/mL)	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)
<i>T. Catappa</i>	28.40 ± 2.40 ^a	48.60 ± 0.75 ^a	68.20 ± 2.45 ^a	78.00 ± 0.75 ^a
Gallic acid	28.60 ± 2.60 ^a	49.20 ± 0.60 ^a	70.30 ± 3.20 ^a	82.10 ± 1.30 ^a

Values are Means of triplicate ± SEM.

Values with the same alphabet superscript are not significant difference along the column

Table 5 showed the result for acute toxicity. No mortality observed in any of

the 5 Wistar rats used at the end of 24 hours and beyond

Table 5: Acute Toxicity

Dose	Mortality
5000 mg/kg.bw	No Mortality

DISCUSSION

Phenolic compounds have been reported to have a wide range of medicinal properties (6). The antioxidant activities exhibited by the extracts via DPPH scavenging capacities could be due to their hydrogen atom or electron donation ability. This hydrogen-donating ability could be as a result of the presence of phenolic compounds in the extracts as these secondary metabolites have been found to possess antioxidant activities. The electron donating ability of

antioxidants in plant extracts is usually reflected by the ability of such antioxidants to reduce iron (Fe) in the oxidation state of Fe³⁺ to Fe²⁺. Thus, the higher the activity of the antioxidants denotes the higher electron donating ability or reducing ability (8). Therefore, the significant activities of the extracts imply that they were able to reduce Fe³⁺ to Fe²⁺ revealing their electron- donating ability, which in turn implies the possibility of using the extracts in preventing oxidation of biomolecules in cells. The exhibition of excellent

antioxidant properties by the extract as seen from percentage DPPH scavenging activities, ferric reducing antioxidant power and inhibition of lipids peroxidation might be as a result of phenolic compounds such as flavonoids, phenol and tannins found in phenolic-rich extract of *T. catappa* leaves content. This result agrees with the report of Busari *et al.* (6) who reported higher phenolics content (phenols, flavonoids and tannins) of methanol/acetone extract of *N. latifolia* fruit than its methanol extract. Furthermore, the displaying of better antioxidant effects is in tandem with the previous study reported on antioxidant effects of methanol extract of *T. catappa* (9) which might be as a result of phenol contents (11)

Therefore, the excellent antioxidant activities (*in vitro*) of the in phenolic-rich extract of *T. catappa* leaves could make it a better candidate for oxidative stress related toxicity such as metal toxicity and aflatoxin-induced toxicity as reported by (12).

In addition, the non-mortality of the five animals after the toxicological study showed that the LD₅₀ of the of phenolic-rich extract of *Terminalia catappa* is greater than 5000mg/kg.bw in Wistar rats (LD₅₀ > 5000mg/kg.bw).

CONCLUSION

The extract is classified as practically non-toxic or relatively harmless. Therefore, the exhibition of phenolic-rich *Terminalia catappa* excellent antioxidant and absolutely acute toxicity profiles qualify the extract to be used as an anti-aflatoxin-b1 agent in poultry feeds.

Authors' contributions

Authors YRS designed and conceptualized the study; AA Identified the plant; YRS, AMA and GS participated in the extraction, data collections and data analysis; YRS, AMA and GS performed the laboratory analysis; YRS and AA interpreted the data; YRS prepared the first draft of the manuscript, reviewed by AA and AMA. All authors contributed to the development of the final manuscript and approved its submission.

Conflict of interest

None

Acknowledgements

The authors are sincerely grateful to both Technical and Non-technical staff of Department of Biochemistry Sa'ad Zukur University Gadau, Bauchi State, Nigeria for their contributions towards the success of this research. We also acknowledge the Tertiary Education Trust Fund (TETFUND), Federal University of Technology Minna Nigeria, who funded this research work with grant number: TETF/DR&D/CE/UNI/GADAU/IBR/2025/VOL1.

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