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Evaluation of Phytochemical Composition, Antioxidant Activity, and Antibacterial Properties of *Terminalia mantaly* H. Perrier Leaf Extract

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Abstract

Terminalia mantaly*, a plant indigenous to Africa, is recognized for its medicinal benefits. This research was conducted to assess the phytochemical composition, antioxidant potential, antibacterial effectiveness, and toxicological properties of the methanolic extract from* Terminalia mantaly *leaves. Phytochemical analysis was performed using standard qualitative methods. The antibacterial activity was evaluated using the agar well diffusion technique. The antioxidant capability of the extract was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins, steroids, triterpenes, and phenols in the extract. The extract demonstrated strong antioxidant activity, with an* IC_{50} *value of 6.837 µg/mL, though this was lower than that of ascorbic acid (0.01 µg/mL). The antibacterial assays showed that the extract was effective against clinical strains of* Staphylococcus aureus*,* Escherichia coli*, and* Salmonella typhi*, with minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) ranging from 7.8125 to 15.625 mg/mL. Fourier-transform infrared (FTIR) spectroscopy identified functional groups such as alcohols, phenols, carboxylic acids, aldehydes, and ketones, which correspond to various secondary metabolites. Additionally, the extract exhibited significant toxicity against brine shrimp larvae, suggesting its potential use as a natural insecticide. Overall, the results indicated that* Terminalia mantaly *leaves contain valuable phytochemicals with notable antioxidant, antibacterial, and toxicological properties, meriting further exploration for pharmaceutical and nutraceutical applications. This study demonstrates the effectiveness of* Terminalia mantaly *against the tested clinical isolates.*

Keywords: Antioxidant, Antibacterial, Fourier-transform infrared spectroscopy, Phytochemical

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INTRODUCTION

Terminalia mantaly H. Perrier (Umbrella tree, Madagascar almond), also known as the brown ivory tree or *Terminalia brownii*, is a plant species indigenous to Africa, particularly prevalent in countries like Tanzania, Zambia, and Zimbabwe. Belonging to the Combretaceae family, this plant has long been used in traditional African medicine for various treatments, including wound healing, gastrointestinal issues, and inflammatory conditions. The pharmacological potential of *Terminalia mantaly* is largely attributed to its rich phytochemical profile. Phytochemicals are bioactive compounds in plants that contribute to their medicinal properties. *Terminalia mantaly* is known to contain several key phytochemicals, such as alkaloids, flavonoids, saponins, tannins, and phenols (Adedapo *et al.,* 2009). These compounds are linked to antioxidant and antibacterial activities, which are crucial for maintaining health and preventing diseases. Antioxidants are vital in protecting the body from oxidative stress, a factor associated with numerous chronic illnesses, including cancer, cardiovascular diseases, and neurodegenerative disorders (Lobo *et al.,* 2010). The antioxidant activity of *Terminalia mantaly* is likely due to its flavonoid and phenolic content, which can neutralize free radicals and mitigate oxidative damage to cells and tissues. Additionally, *Terminalia mantaly* has demonstrated antibacterial properties against various pathogens. These effects may be attributed to the presence of compounds like tannins, saponins, and alkaloids, known for their antimicrobial properties (Jiofack *et al.,* 2010). The antibacterial properties of *Terminalia mantaly* could be beneficial in treating bacterial infections. Given its phytochemical composition, *Terminalia mantaly* leaf extract exhibits significant antioxidant and antibacterial activities, highlighting its potential for therapeutic applications. Further research is necessary to isolate and identify the specific compounds responsible for these effects and to assess their efficacy and safety for medical use.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Terminalia mantaly leaves were gathered from the Botanical Garden of the Department of Plant Biology at Bayero University, Kano, located in Gwale Local Government Area, Kano State, Nigeria. The plant was subsequently identified and authenticated at the herbarium of the Plant Biology Department at Bayero University, Kano. A voucher specimen, labeled BUKHAN031, was deposited for future reference.

Preparation of Plant extracts

Fresh *Terminalia mantaly* leaves were meticulously cleaned, air-dried, and finely ground into a coarse powder using a grinding machine. The powdered sample was carefully stored in airtight containers to maintain its integrity for further processing. Subsequently, 200 g of the powdered leaves were immersed in 2 liters of methanol in a suitable container. The mixture was left to steep for 72 hours at room temperature (28 ±2^{o}C), with periodic agitation every hour to facilitate efficient extraction. After the steeping period, the extract was meticulously separated from the plant material. Firstly, it was passed through a muslin cloth to remove any coarse debris. Following this, the extract was filtered through a Whatman filter paper (No.1) to ensure clarity and purity of the final solution. The filtered extract was then carefully transferred into a clean evaporating dish. The dish was placed on a water bath set at 50°C to facilitate the evaporation of the solvent. This process continued until all the methanol was completely evaporated, leaving behind the concentrated extract in the dish. Finally, the concentrated extract was carefully collected and stored in suitable containers under appropriate conditions for further analysis and experimentation.

Qualitative Phytochemical Screening of *Terminalia mantaly* Leaf Extract

The *Terminalia mantaly* leaf extract was analyzed for the presence of various phytochemical compounds using established methods similar to those described by Evans (2009).

Test for Carbohydrates

Molish's Test:

To detect carbohydrates, 1 ml of the extract was combined with Molish's reagent in a test tube. Concentrated sulfuric acid was carefully layered down the side of the tube to form a separate lower layer. The appearance of a reddish ring at the interface indicated the presence of carbohydrates.

Test for Saponins

Frothing Test:

In this test, 10 ml of distilled water was added to a portion of the plant extract and vigorously shaken for 30 seconds. The mixture was left undisturbed in a vertical position for 30 minutes. The formation of a stable frothy layer that lasted for 10-15 minutes confirmed the presence of saponins.

Test for Flavonoids

Shinoda Test:

A small portion of the extract was dissolved in 1-2 ml of 50% methanol, and heated magnesium chips were added. A few drops of concentrated hydrochloric acid were introduced to the mixture, resulting in a red color, indicating the presence of flavonoids.

Test for Alkaloids

Wagner's Test:

To test for alkaloids, a few drops of Wagner's reagent were added to a portion of the extract. The formation of a white precipitate indicated the presence of alkaloids.

Test for Steroids and Triterpenes

Liebermann-Burchard's Test:

Equal amounts of acetic anhydride were mixed with a portion of the extract. Concentrated sulfuric acid was then carefully added along the side of the test tube to form a lower layer. The appearance of a red, pink, or purple color indicated the presence of triterpenes, while a blue or green color signaled the presence of steroids.

Test for Cardiac Glycosides

Kella-Killiani's Test:

A portion of the extract was dissolved in glacial acetic acid containing a trace of ferric chloride. The mixture was transferred to a dry test tube, and concentrated sulfuric acid was added to form a lower layer. The appearance of a purple-brown ring at the interface indicated the presence of deoxy sugars, while a pale green color in the upper layer confirmed the presence of cardiac glycosides.

Test for Tannins

Ferric Chloride Test:

To detect tannins, 3-5 drops of ferric chloride solution were added to a portion of the extract. A greenish-black precipitate indicated the presence of condensed tannins, while a blue or brownish-blue precipitate indicated hydrolyzable tannins.

Test for Anthraquinones

Borntrager's Test:

In this test, 5 ml of chloroform was added to a portion of the extract, and the mixture was shaken for 5 minutes. The solution was filtered, and the filtrate was mixed with an equal volume of 10% ammonium solution. A bright pink color in the upper aqueous layer indicated the presence of free anthraquinones.

Antioxidant Activity

The antioxidant potential of *Terminalia mantaly* methanolic leaf extract was assessed by measuring its ability to scavenge free radicals, using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The procedure followed a modified method derived from Sani and Dailami (2015). In brief, 200 µl of a 100 µM methanol solution of DPPH was combined with 100 µl of the leaf extract at different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.8 µg/ml). The mixture was kept in the dark at room temperature for 30 minutes to allow the reaction to occur. The absorbance of each sample, as well as the control and blank, was measured at 517 nm using a spectrophotometer. Each test was repeated three times, and the antioxidant activity was expressed as a percentage of inhibition, calculated using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Microbiological Analysis

Gram staining and Media culturing

The bacterial isolates were subjected to Gram staining to determine their Gram reaction. They were then cultured on selective and differential media to assess their colony color, morphology, and fermentation capabilities (Karzan *et al.,* 2017).

Isolation of Bacterial Isolates

Samples were cultured on some selective/differential media such as blood agar, chocolate agar, and MacConkey agar plates at 37°C for 24 hours. Based on colony morphology, selected colonies were sub-cultured onto blood and chocolate agar plates to isolate pure strains. Gram staining was conducted on these isolates, and depending on their Gram reactions, they were inoculated on selective media, including mannitol salt agar, cetrimide agar, and eosin methylene blue agar. Biochemical tests such as catalase, coagulase, and oxidase were performed. The isolated bacteria were stored on nutrient and chocolate agar slants at 4°C for further analysis (Cheesbrough, 2010).

Identification and Characterization

Bacteria identification and characterization were done using the Microgen Identification Kit (XYZ) according to the manufacturer's guidelines (API Biomerieux). Saline suspensions of the bacteria were placed into the test wells, with specific wells (1, 2, 3, and 9) covered in sterile paraffin oil. After incubation for 18-24 hours at 37°C, reagents such as Nitrate A and B, Kovac's, Tryptophan deaminase (TDA), and Voges-Proskauer (VPI and II) were added to certain wells for further testing, and the color changes were recorded. The results were converted into codes and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Antibacterial Susceptibility Test

Preparation of Extract Concentrations

To prepare stock solutions, 0.25 g of each plant extract was dissolved in 1 ml of dimethyl sulfoxide (DMSO). Two-fold serial dilutions were then performed to obtain concentrations of 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml (Srinivasan *et al.,* 2009).

Standardization of Bacterial Inoculum

An inoculum loop was used to transfer overnight cultures into a test tube containing normal saline, adjusting the turbidity to match the 0.5 McFarland standard, following the National Committee for Clinical Laboratory Standard (NCCLS, 2008) guidelines.

Susceptibility Testing of Bacterial Isolates

The antibacterial activity of *Terminalia mantaly* leaf extract against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* was assessed using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions, and wells were filled with various concentrations of the plant extract. The DMSO was used as a negative control, while ciprofloxacin served as a positive control. After incubating at 37°C for 24 hours, the zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the tube dilution method. Plant extracts were diluted in a series, and test tubes with 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml and 15.625 mg/ml were inoculated with bacterial suspensions. After 24 hours of incubation at 37°C, growth was observed. To confirm inhibition, samples were sub-cultured on nutrient agar plates.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by sub-culturing broth from the MIC tubes onto fresh nutrient agar plates. The lowest concentration that showed no bacterial growth after incubation was recorded as the MBC (Adesokan *et al.,* 2008).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The methanol extract of *Terminalia mantaly* was subjected to FTIR analysis to identify functional groups present in the sample. The analysis was conducted at the Instrumental Laboratory, Department of Chemistry, Bayero University, Kano, Nigeria, using a Shimadzu 8400 FT-IR spectrophotometer. A small portion of the extract (0.1 g) was mixed with 0.025 g of dry potassium bromide (KBr) using a mortar and pestle to create a homogenized mixture. This mixture was pressed into a thin KBr film using a hand press, which was then placed into the FT-IR spectrophotometer. Spectra were collected by accumulating 64 scans with a resolution of 4 cm^{-1}, covering a range of 4000 to 400 cm^{-1}. The resulting spectra were plotted as percentage transmittance against wavelength to help identify functional groups based on the observed peaks in the infrared region.

Cytotoxicity Assay

Brine Shrimp Hatching

Brine shrimp (*Artemia salina*) eggs were incubated in seawater, and after 48 hours, the larvae were drawn toward a light source and collected using a pipette. To ensure separation from unhatched eggs, the larvae were aliquoted into small beakers of seawater multiple times (Ibrahim and Abdullahi, 2015).

Brine Shrimp Lethality Assay

The cytotoxicity of the *Terminalia mantaly* extract was assessed using the brine shrimp lethality assay, following a modified protocol adapted from Lilybeth *et al.* (2013). The methanolic extract was dissolved at various concentrations, and the number of surviving larvae was counted after 24 hours of exposure. The median lethal concentration (LC_{50}) was determined using SPSS software version 20.

RESULTS

The *Terminalia mantaly* extract tested positive for flavonoids, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrates, cardiac glycosides, and phenols. However, anthraquinones were not detected (Table 1).

Table 1. Qualitative Phytochemical screening of methanol leaves extract of *Terminalia mantaly*

Metabolites	Inferences
Saponins	+
Flavonoid	+
Alkaloid	+
Tannins	+
Cardiac glycoside	+
Carbohydrate	+
Triterpenes	+
Phenol	+
Anthraquinones	-
Steroid	+

The antioxidant capacity of the methanolic extract of *Terminalia mantaly* leaves was assessed using the DPPH radical scavenging method, with ascorbic acid as the positive control. The extract demonstrated significant radical scavenging activity, achieving 100%, 96.46%, and 93.30% at concentrations of 1000 µg/mL, 500 µg/mL, and 250 µg/mL, respectively, surpassing the standard, which exhibited 92.0% activity (Table 2).

Table 2. Antioxidant activities of Methanolic leaf extract of *Terminalia mantaly*

Analyte	Concentration (µg/mL) / % Inhibition							
	1000	500	250	125	62.5	31.25	15.6	7.8
Terminalia mantaly	100	96.46	93.30	84.57	77.37	71.33	65.58	52.38
Ascorbic acid	92.0	93.3	93.0	93.4	93.2	92.1	92.3	90.8

The inhibition of DPPH radicals by the *Terminalia mantaly* extract increased in a concentration-dependent manner, as indicated by the IC_{50} values (the concentration required to achieve 50% inhibition). When comparing the DPPH radical scavenging ability, the methanolic extract was less effective than ascorbic acid. The IC_{50} value of the methanolic extract of *Terminalia mantaly* leaves was determined to be 6.837 µg/mL.

Table 3: Antioxidant Activities of the Methanolic Leaf extract of *Terminalia mantaly*

Sample	IC_{50} (µg/mL)
Terminalia mantaly	6.837
Ascorbic acid	0.01

The methanol extract of *Terminalia mantaly* leaves demonstrated antibacterial activity against all tested clinical isolates, including *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*, at concentrations of 250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL (Table 4). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the extract were found to be 7.8125 mg/mL and 15.625 mg/mL, respectively, for *Salmonella typhi*, while *Escherichia coli* and *Staphylococcus aureus* showed similar values.

Table 4: Antibacterial susceptibility of methanol leaf extract of *Terminalia mantaly*

Clinical isolates	Concentration (mg/ml)/Diameter zone of inhibition (mm)						MIC	MBC
	250	125	62.5	31.25	CPR	DMSO		
S. aureus	18	16	12	10	38	06	7.8125	15.625
E. coli	22	17	15	11	37	06	7.8125	15.625
Salmonella typhi	24	20	18	13	40	06	7.8125	15.625

Table 5. Peak Positions and Associated Functional Groups of Isolated Compounds from *Terminalia mantaly* Methanolic Leaf Extract by FTIR

IR-value (cm^{-1})	Functional group	Secondary metabolite
3204.64	O-H Stretching vibration of alcohol and phenols	Steroid, terpenoids, saponins, phenols, carbohydrates
2921.62	C-H (Stretching), C=O Stretching of Carboxylic acid and ketone	Flavonoids, terpenoids, steroid, saponins
1607.60	C=C Stretching, N-H bending of primary amines	Alkaloid, steroid, terpenoids, saponins, fatty acid
1451.60	C-N stretching of aromatic amines, C-H bending	Alkaloids
1015.29	C-H stretching of aromatic hydrocarbon, C-O Stretching	Terpenoid, steroid , saponins, carbohydrate

The brine shrimp lethality assay results for the methanol extract of *Terminalia mantaly* leaves reveal a concentration-dependent toxic effect on the larvae. Higher concentrations of the extract, specifically 100 µg/mL and 1000 µg/mL, resulted in increased mortality rates, with complete mortality (100%) occurring at 1000 µg/mL. The LC_{50} value of 39.483 µg/mL indicates the concentration at which 50% of the brine shrimp larvae are expected to die, providing a measure of the extract's toxicity.

Table 6: Brine Shrimp Lethality Assay (BSLA) for *Terminalia mantaly* Methanol Extract

	Conc. (µg/mL)	Number of Surviving Nuplii After 24 hrs			Total Number of Death	% Mortality	LC_{50} (µg/mL)
		T1	**T2**	**T3**			
Vernonia amygdalina extract	10	9	8	9	4	13.3	39.483er
	100	2	3	2	23	76.6	
	1000	0	0	0	30	100	

DISCUSSION

Phytochemicals, which are bioactive compounds found in plants, significantly contribute to their medicinal properties. Through qualitative phytochemical screening of *Terminalia mantaly* leaf extract, a diverse array of compounds was identified including alkaloids, flavonoids, saponins, cardiac glycosides, tannins, steroids, triterpenes, and phenols. Alkaloids are known for their analgesic, antimicrobial, and anti-inflammatory effects (Atanasov *et al.,* 2021). Flavonoids possess potent antioxidant capabilities and are recognized for their anti-inflammatory, antiviral, and anticancer properties (Oliveira *et al.,* 2020). Saponins are associated with antimicrobial and anti-inflammatory activities (Sparg *et al.,* 2004) while cardiac glycosides are utilized in treating heart conditions (Ahmad, 2013). Tannins offer antioxidant and anti-inflammatory benefits (Haslam, 2007) and steroids and triterpenes contribute to anti-inflammatory and antimicrobial effects (Barnes *et al.,* 2005; Huang *et al.,* 2019). Phenols, noted for their antioxidant properties, play a crucial role in plant defense (Mouradov and Spangenberg, 2014). The absence of anthraquinones and carbohydrates suggested they are either minimally present or not present in the extract. Anthraquinones are typically known for their laxative and antimicrobial effects (Tiwari and Rana, 2020), while carbohydrates are vital for plant metabolism (Sarwar and Saleem, 2019).

The methanolic extract of *Terminalia mantaly* leaves exhibited significant antioxidant activity with an IC_{50} value of 6.837 µg/mL. Although this is lower than ascorbic acid's IC_{50} of 0.01 µg/mL, which is a more potent antioxidant, the presence of compounds such as phenolics, flavonoids, and tannins in the extract likely contributes to its antioxidant properties (Mothana *et al.,* 2011; Gbenou *et al.,* 2013). These compounds help neutralize free radicals, which are implicated in various diseases including cancer and cardiovascular conditions.

In terms of antibacterial activity, the methanol extract of *Terminalia mantaly* leaves effectively inhibited the growth of tested clinical isolates. The extract's antibacterial efficacy, which increased with concentration, was confirmed by MIC and MBC values, demonstrating its ability to both inhibit and kill bacteria at relatively low concentrations. The observed antimicrobial activity is likely due to the presence of tannins, flavonoids, and alkaloids, all known for their antimicrobial properties (Akinpelu, 2009; Dzoyem *et al.,* 2017).

The FTIR analysis identified several key functional groups in the extract. Peaks at 3204.64 cm^{-1} corresponded to O-H stretching vibrations typical of alcohols and phenols, found in compounds such as steroids and terpenoids (Sun *et al.,* 2019). The peak at 2921.62 cm^{-1} indicated C-H stretching and C=O stretching associated with carboxylic acids and ketones, present in flavonoids and saponins (Yang et al., 2020). The 1607.60 cm^{-1} peak was linked to C=C stretching and N-H bending, characteristic of alkaloids and other compounds (Pan et al., 2019). Peaks at 1451.60 cm^{-1} and 1015.29 cm^{-1} were associated with C-N stretching and C-H stretching, relevant to alkaloids, terpenoids, and carbohydrates (Tang *et al.,* 2021).

The brine shrimp lethality assay demonstrated that the methanol extract of *Terminalia mantaly* leaves has significant toxic effects, as indicated by high mortality rates at elevated concentrations. This aligns with prior research showing the toxicity of *Terminalia* species against various organisms (Aly *et al.,* 2016; Prasad *et al.,* 2017). The observed toxicity may be attributed to the bioactive compounds present, such as tannins, flavonoids, and alkaloids. These results suggest that *Terminalia mantaly* could potentially serve as a source of natural insecticides or pharmaceutical agents. Further studies are needed to isolate and identify the specific bioactive compounds responsible for the observed toxicity and to evaluate the plant's potential in broader toxicological and therapeutic contexts.

CONCLUSION

The phytochemical analysis of the methanol extract from *Terminalia mantaly* leaves has identified a range of bioactive compounds, underscoring its potential as a source of natural therapeutic agents. The FTIR analysis of the extract revealed the presence of functional groups associated with various bioactive compounds, including alcohols, phenols, carboxylic acids, aldehydes, ketones, and amines. These findings indicate that the methanol extract of *Terminalia mantaly* leaves exhibits notable antioxidant and antibacterial properties. To fully understand the therapeutic potential of these effects, additional research is needed to isolate and characterize the specific compounds responsible for these activities and to explore their potential applications in medicine.

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