

Original Research Article

Phytochemical analysis and antibacterial activity of acetone extract of *Terminalia catappa* Linn. leaves

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Abstract

Purpose: Traditional medicine has used the leaves of *Terminalia catappa* for various health benefits, with established antibacterial, antidiabetic, anti-inflammatory, antioxidant, antiviral, and hepatoprotective characteristics. This study aims to perform a phytochemical analysis and antibacterial assay of the acetone extract from *T. catappa* leaves.

Methods: The dried leaves were subjected to extraction using 50 % acetone, followed by preliminary phytochemical screening, Gas chromatography-mass spectroscopy (GC-MS) analysis, and antibacterial testing of the extract.

Results: Preliminary phytochemical screening identified the presence of carbohydrates, flavonoids, saponins, alkaloids, glycosides, tannins, phenols, terpenoids, and steroids/triterpenoids. GC-MS analysis showed the presence of epiglobulol and thirteen additional phytochemicals, which have been reported to possess antibacterial activity or potential, with epiglobulol exhibiting the highest concentration among all the identified phytochemicals. The diameters of the inhibition zones ranged from 0 to 15 mm for *Staphylococcus aureus*, 0 to 12 mm for *Streptococcus pneumoniae*, 0 to 12 mm for *Escherichia coli*, and 0 to 8 mm for *Pseudomonas spp.*, with greater extract concentrations leading to larger inhibition zones—though these were smaller than the inhibition zone diameters observed for gentamicin, which varied between 24.5 and 27 mm across the four tested organisms.

Conclusion: The acetone extract of *T. catappa* leaves underwent phytochemical analysis, revealing numerous constituents known to exhibit antibacterial properties.

Keywords: *Terminalia catappa*, Phytochemical, GC-MS, Antibacterial activity

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INTRODUCTION

Natural products, defined as chemical compounds produced by living organisms, account for over one-third (39.1%) of drugs approved by the Food and Drug Administration (FDA).¹ The existence of

more than 200,000 natural metabolites with various bioactive properties underscores the significance of natural products in discovering new drugs.² The belief that certain plants possessed therapeutic qualities and contained substances now recognized as antimicrobial agents was common long before

germs were understood.³ Since ancient times, humans have turned to plants to treat common infectious diseases, and many traditional remedies continue to be utilized today for a range of ailments.³ While plants such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*), and tea tree (*Melaleuca alternifolia*) oil have shown effective antimicrobial properties against a variety of microorganisms, bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) have been traditionally used for the treatment of urinary tract infections, as noted in several phytotherapy resources.³ *Terminalia catappa* Linn., recognized for its nutritious fruit, also possesses medicinal benefits, with multiple pharmacological studies validating the antimicrobial, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer properties of *T. catappa*, thereby endorsing its traditional applications for treating conditions such as asthma, cardiovascular problems, inflammatory diseases, diarrhea, and allergies.⁴⁻¹³ *T. catappa* is acknowledged for containing medicinally significant phytoconstituents, including phenols, flavonoids, and carotenoids.⁵ Despite the availability of various antibiotics aimed at treating bacterial infections, concerns regarding adverse side effects and drug resistance have prompted researchers to explore herbal treatments as potential alternatives in recent years. The widespread accessibility and affordability of herbal medicines, especially in developing countries, greatly contribute to their exploration and advancement.¹⁴ Even though there exists a wealth of plants historically used for medicinal purposes in traditional remedies, there remains a widespread issue surrounding the validation and documentation of ethnomedicine, particularly in Nigeria.¹⁵ Medicinal plants can serve as valuable sources of antibiotics that may exhibit fewer side effects, relatively high effectiveness, and cost-efficiency. However, for these medicinal plants to gain acceptance as reliable sources, further evaluation is necessary to identify and confirm the bioactive compounds responsible for their effects. Continued research and refinement of these compounds could facilitate the creation of a new generation of antibacterial agents with enhanced efficacy, reduced adverse effects, and a lower propensity for resistance. Limited studies have examined the antibacterial properties of the acetone extract of *T. catappa* leaves.¹⁶⁻¹⁸ Nevertheless, 50 % acetone has been identified as the ideal solvent for extracting phenolic compounds—the key natural compounds associated with the antibacterial activities of herbal

products—and is recommended as the optimal solvent for this extraction process.¹⁹ Consequently, this study intends to perform a preliminary phytochemical and GC-MS analysis of the 50 % acetone extract of *T. catappa* leaves, aiming to uncover insights into the various classes and types of natural products found in the plant, in addition to conducting a preliminary antibacterial assessment against common gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Materials

Leaves from *Terminalia catappa*, an autoclave (Genist Technology Private Limited, India), a GC-MS instrument (Gerstel GmbH & Co., Germany), a hot oven (Vilyath Scientific Industries, India), an incubator (Mytemp Mini Digital Heat Incubator, USA), a weighing balance (OHAUS Corporation Pine Brook, USA), acetone, Wagner's reagent, Dragendorff's reagent, Molisch's reagent, acetic anhydride, sulfuric acid, hydrochloric acid, lead acetate solution, ferric chloride solution, 10 % ammonium solution, distilled water, and 0.9 % normal saline.

Methods

Collection and identification of plant materials

The leaves of *T. catappa* were gathered in September 2023 at Kaduna State University (KASU), Kaduna. A taxonomist, U.S. Gallah identified and authenticated the plant at the herbarium within the Department of Biological Sciences at Kaduna State University, Kaduna State.

Drying of plant materials

The fresh leaves of *T. catappa* were dried in the air at room temperature for six weeks, and the dried leaves were ground into a powder using a wooden mortar.

Extraction process from plant materials

The measured powdered sample (216 g) was fully extracted using the maceration method with 2 litres of 50% acetone for three days (72 hours). The solvent in the maceration container was decanted, and the mixture was filtered with No. 1 Whatman filter paper, utilizing a suction pump. A rotary evaporator then concentrated the filtrate, which was subsequently transferred to a hot water bath set at 50 °C and left to dry.

Phytochemicals screening

The acetone extract of *T. catappa* underwent a series of qualitative phytochemical tests following standard methods.²⁰

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was performed with a Shimadzu GCMS-QP2010 system, a gas chromatograph linked to a mass spectrometer (GC-MS) instrument. The Total Ion Count (TIC) method was utilized to identify the compounds. The spectra of the components were compared to a database of known component spectra using the NIST Search software stored in the GC-MS system.

Antibacterial assay

Isolation and characterization of microorganisms.

Clinical isolates of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas spp.*, and *Escherichia coli* were obtained from the Pharmaceutical Microbiology Laboratory at KASU for sub-culturing and confirmation using established protocols.²¹

Serial dilution of the extract

Dried acetone extract of *T. catappa* leaves equivalent to 1.0 g was placed into a test tube containing 1 ml of acetone and dissolved to yield a concentration of 1000 mg/ml. Subsequently, serial dilutions were performed down to 0.1 mg/ml using 0.9 % normal saline.

Susceptibility testing of the test organism with *Terminalia catappa*

Mueller-Hinton agar plates were inoculated with the test organism using a swabbing technique. The susceptibilities of the test organism to the acetone extract of *T. catappa* leaves were assessed using the agar diffusion method. Sterile cork borers (4 mm) were used to create wells on the plates near a Bunsen burner, after which 0.1 ml of different concentrations (100 mg/ml to 0.1 mg/ml) of the acetone extract solution was added to labeled wells. The plates were allowed to sit for one hour to enable proper diffusion before being incubated for 24 hours at 37 °C. After incubation, the diameters of the zones were measured in millimeters using a ruler, with all observations made with the naked eye while viewing the underside of the Petri dish, and the sizes of the zones were documented.

RESULT AND DISCUSSION

Percentage yield of extracts of *Terminalia catappa* leaves

Weight of crude acetone extract = 7.7 g

Weight of plant material = 216 g

The percentage yield was calculated using the formula below:

$$\% \text{ Yield} = \frac{\text{weight of crude acetone extract}}{\text{weight of dried plant material}} \times 100$$

$$\text{Therefore, } \% \text{ Yield} = \frac{7.7 \text{ g}}{216 \text{ g}} \times 100 = 3.6 \%$$

A yield of 3.6 % was obtained for the extraction from the dried *T. catappa* leaves with 50 % acetone. Chemicals that are soluble in water and/or organic solvent may be easier to extract when water and organic solvent are used together.²² However, a higher percentage yield of acetone extract of *T. catappa* leaves of 5.65 % has been reported.²³ The lower percentage yield in this study may be due to the low solvent-to-solid ratio used for the extraction.²⁴

The result of the phytochemical screening of acetone extract of *T. catappa* leaves (table 1), shows the presence of carbohydrates, flavonoids, saponins, alkaloids, glycosides, tannins, phenols, and terpenoids, while anthraquinones were observed to be absent. This is similar to what has been reported of the presence of tannins, saponins, phenols, alkaloids, flavonoids, glycosides and steroids in methanol extracts of *T. catappa* leaves and the absence of anthraquinones in both acetone and methanol extracts.^{4,23} It has been reported that the antimicrobial activities of plant extracts depend on the nature and structure of phenolic compounds, and through their hydroxyl groups and phenolic compounds, they can bind to proteins in bacterial membranes to form complexes.²⁵

Table 1. Phytochemical screening of acetone extract of *Terminalia catappa* leaves

Phytochemical	Test	Inference
Carbohydrates	Fehling's test	+
Flavonoids	Sodium hydroxide test	+
Saponins	Frothing test	+
Alkaloids	Meyer's test	+
Glycosides	Keller-Kiliani test	+
Tanins	Ferric Chloride test	+
Anthraquinones	Bontrager's test	-
Phenols	Liebermann's test	+
Terpenoids	Salkowski test	+

Key: (+) Present (-) Absent

Furthermore, preliminary phytochemical screening of the acetone extract of *T. catappa* detected the presence of tannins; which are potential inhibitors of hydrolytic enzymes used by pathogenic bacteria.²⁶ GC-MS analysis of the acetone extract (table 2) revealed the presence of 26 natural compounds with epiglobulol having the highest concentration (Percentage peak area = 5.4938 %). This correlates with the extracting capability of the

solvent used because an extensive literature review shows that acetone has been used to extract sesquiterpenoids and terpenoids which epiglobulol belongs to. Epiglobulol has been reported to have antibacterial activities and it can strongly inhibit the growth of foodborne microorganisms.²⁷ Epiglobulol and 6,11-Dimethyl-2,6,10-

dodecatrien-1-ol, another detected natural product that has been reported to possess antibacterial activity,²⁸ are also phenolic compounds, and the antimicrobial effects of phenols have long been known and reported.²⁵

Table 2: GCMS analysis of acetone extract of *Terminalia catappa* leaves

Phytochemical compound detected	Retention time, Rt (mins)	Percentage peak area (%)
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	9.6517	3.1562
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	9.6941	2.9144
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	9.7554	4.8473
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	9.8953	0.9738
6,11-Dimethyl-2,6,10-dodecatrien-1-ol	19.5876	0.2731
N-(5-Amino-1,3-benzothiazol-2-yl)acetamide	23.4103	0.1632
Vitamin E	23.4504	0.1016
Epiglobulol	25.6306	5.4938
trans-Traumatic acid	25.6625	0.6164
Cyclopentaneundecanoic acid	25.6832	0.7451
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2 α ,4 α .,8 β)]-	25.7065	0.4791
(E,Z)- α -Farnesene	25.7252	1.3545
Lupeol	25.7791	0.9138
Sesquirosefuran	26.0452	0.4398
3,7,11-Trimethyl-dodeca-2,4,6,10-tetraenal	26.1363	2.1205
Tricyclo[6.3.3.0]tetradec-4-ene,10,13-dioxo-	26.2179	1.2028
Farnesol formate	26.2872	0.5046
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	28.7358	0.3751
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	28.7744	0.3098
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	30.4650	0.3110
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	30.505	0.2642
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	30.5817	0.4079
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	30.6704	0.4017
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	30.7698	0.4944
1H-Indole, 5-methyl-2-phenyl-	30.8227	0.4504
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	30.8869	0.6000
Benzo[h]quinoline, 2,4-dimethyl-	30.9280	0.4104
1-Nitro-.beta.-d-arabinofuranose, tetraacetate	30.9770	0.4081
1H-Indole, 5-methyl-2-phenyl-	31.0228	0.4428
2-quinoxalinamine, 3-chloro-N-ethyl-	31.0506	0.2816
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	31.0699	0.2881
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.1173	0.7333
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.1480	0.2922
Trimethylsilyl-di(trimethylsiloxy)-silane	31.2576	1.7200
2-Hydroxyphenethyl alcohol, 2TMS derivative	31.2833	1.2308
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.3936	1.3070
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	31.4150	0.8195
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.4605	0.5429
1H-Indole, 5-methyl-2-phenyl-	31.5847	3.1348
Oleic Acid	31.6227	0.9858
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.6568	1.1678
Cyclohexane, 1,1'-(2-propyl-1,3-propanediyl)bis-	31.7114	1.6367

2-Ethylacridine	31.7424	0.9829
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	31.7801	1.6011
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.8254	2.1242
Tetrasiloxane, decamethyl-	31.8743	1.5094
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.8962	0.7803
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	31.9334	1.8982
1H-Indole, 5-methyl-2-phenyl-	31.9632	0.8839
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	31.9950	1.4913
Trimethylsilyl-di(trimethylsiloxy)-silane	32.0320	2.6105
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	32.0882	1.9718
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	32.1288	2.6016
Maltose	32.2034	3.8058
7,11-Hexadecadienal	32.2340	1.1652
Docosanoic acid	32.2597	1.9659
3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	32.3217	3.0849
2-Myristynoyl-glycinamide	32.3615	2.6205
2-(Cyclopropylsulfanyl)-1,3-benzothiazole	32.4335	3.6399
2-Hydroxyphenethyl alcohol, 2TMS derivative	32.4773	1.4577
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	32.4978	1.5359
1H-Indole, 5-methyl-2-phenyl-	32.5464	2.5535
Trimethylsilyl-di(trimethylsiloxy)-silane	32.5809	1.7090
2-Hydroxyphenethyl alcohol, 2TMS derivative	32.6345	2.3435
Indolizine, 2-(4-methylphenyl)-	32.7148	1.0020
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	32.8567	2.7737
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	32.9097	1.3279
1H-Indole, 5-methyl-2-phenyl-	33.0408	2.5965
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	33.1850	0.9742
Adamantane-1-(3,3-dichloropropyn-1-yl)	33.2329	0.8244
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	33.3742	0.5237
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	33.4178	0.3244

GC-MS analysis also detected the presence of other natural compounds that have been reported to possess antibacterial activities, such as 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester,²⁹ 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester,³⁰ N-(5-amino-1,3-benzothiazol-2-yl)acetamide,³¹ cyclopentaneundecanoic acid,³² farnesol formate,³³ octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl³⁴ and oleic acid.³⁵ Other natural products detected which have potential antibacterial activity are indolizine, 2-(4-methylphenyl)-, an indolizine derivative,³⁶ 2-(cyclopropylsulfanyl)-1,3-benzothiazole, a benzothiazole derivative,³⁷ 1H-Indole, 5-methyl-2-phenyl, an indole derivative,³⁸ benzo[h]quinoline, 2,4-dimethyl,³⁹ and naphthalene, Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2 α ,4 α .,8 α β)],⁴⁰ Several studies on the GC-MS analysis of aqueous and ethanolic extracts of *T. catappa* leaves, reported a diverse group of natural compounds.⁴¹⁻⁴³ However, none of the phenols or terpenoids detected in this study was reported. This can be attributed to the use of 50 % acetone as the extracting solvent,

which has a relatively different extraction capability when compared to more polar solvents.²²

Table 3 presents the sizes of inhibition zones resulting from the antibacterial properties of the acetone extract of *T. catappa* leaves against chosen gram-positive and gram-negative bacterial species. Utilizing the disc plate method for microbiological testing,⁴⁴ the antibacterial efficacy of plant extracts can be classified according to their inhibition zones as follows: < 6 mm - weak activity, 6-10 mm - moderate activity, 11-20 mm - strong activity, and >21 mm - very strong activity. Consequently, the acetone extract of *T. catappa* leaves demonstrated moderate to strong antibacterial effectiveness against the tested gram-negative and gram-positive bacteria in this study.

The antibacterial strength of the extract was observed to rise with increased concentrations, and these effects were significantly different (P< 0.05) among the gram-positive bacteria assessed in this study. The enhancement in antibacterial potency with higher concentrations may be linked to the increased presence of bioactive compounds.⁴⁵

Table 3. Diameters of the inhibition zones obtained by the effect of acetone extract of *Terminalia catappa* leaves against selected Gram (+) and Gram (-ve) bacterial species

Concentration (mg/ml)	Test organisms			
	Gram (+ve)		Gram (-ve)	
	<i>S. aureus</i>	<i>S. pneumonia</i>	<i>E. coli</i>	<i>Pseudomonas spp</i>
100	15 ± 0.25 ^b	12 ± 0.21 ^b	12 ± 0.28 ^b	8 ± 0.25 ^b
10	8.5 ± 0.17 ^c	7 ± 0.16 ^c	5.5 ± 0.20 ^c	5 ± 0.33 ^b
1	0.00	0.00	0.00	0.00
0.1	0.00	0.00	0.00	0.00
Gentamicin (0.01)	27 ± 0.33 ^a	25 ± 0.35 ^a	26 ± 0.33 ^a	24.5 ± 0.31 ^a

Note: 0.00- no zone of inhibition. Values are expressed as: means ± SEM of three independent experiments. One One-way analysis of variance (ANOVA) followed by Dunnett's Post Hoc Test, different superscripts along the column indicate significant differences between treatment groups at P < 0.05.

Similar antibacterial effects of *T. catappa* extracts have been reported when both aqueous and methanolic extracts were evaluated against *Escherichia coli* and *Staphylococcus aureus*.⁴⁶ No significant differences (P > 0.05) were noted in the antibacterial activities observed at various concentrations tested on *Pseudomonas spp* in this study, as both the 10 mg/ml and 100 mg/ml concentrations yielded comparable antibacterial effects (P > 0.05). Gram-negative bacteria, especially *Pseudomonas spp*, exhibit lower sensitivity to variations in concentration and types of antibacterial substances due to their unique cell structure and notable intrinsic resistance levels.^{47,48}

CONCLUSION

The acetone extract of *Terminalia catappa* demonstrated a rich array of phytochemical compounds, including epiglobulol, a well-known natural compound known for its potent antibacterial properties, found in the highest concentration. This extract exhibited promising antibacterial activities against the studied gram-positive and gram-negative bacteria, reinforcing the traditional use of this plant in treating various infectious diseases. Future investigations centered on activity-guided fractionation and the isolation of bioactive compounds responsible for these antibacterial effects may unveil new compounds that could serve as a basis for developing more effective antibacterial medications from *Terminalia catappa*.

Conflict of interest

The authors declare no conflict of interest.

Authors Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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