

PHYTOCHEMICAL CHARACTERIZATION AND IN VITRO α -AMYLASE INHIBITORY ACTIVITY OF TERMINALIA CATAPPA LINN. ROOT EXTRACTS

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ABSTRACT

Background: Terminalia catappa Linn. is a medicinally important member of the family Combretaceae. Although leaves, bark and fruits have received substantial attention, the root remains comparatively less characterized. **Objective:** To characterize ethanolic and aqueous root extracts of T. catappa and evaluate their in vitro antidiabetic potential by α -amylase inhibition. **Methods:** Root extracts were assessed for extraction yield, qualitative phytochemical profile and selected heavy metals. The ethanolic extract was further characterized by HPTLC at 254 and 366 nm, LC-MS and GC-MS. α -Amylase inhibition by ethanolic (EETC) and aqueous (AETC) extracts was evaluated at 12.5, 25, 50 and 100 μ g/mL using acarbose as reference. **Results:** EETC and AETC yielded 14.35% and 9.64% w/w, respectively. Both extracts tested positive for carbohydrates, tannins, phenols, flavonoids and saponins. Mercury, arsenic, cadmium and lead were not detected at a method detection limit of 0.05 ppm. HPTLC showed multiple reproducible peaks at both wavelengths. LC-MS tentatively indicated gallic acid, ellagic acid, catechin and β -sitosterol, while GC-MS fragmentation suggested γ -sitosterol. α -Amylase inhibition increased with concentration. At 100 μ g/mL, EETC, AETC and acarbose produced $77.50 \pm 1.25\%$, $74.52 \pm 1.02\%$ and $86.00 \pm 1.12\%$ inhibition, respectively. **Conclusion:** T. catappa root extracts possess a chemically diverse phytochemical profile and appreciable in vitro α -amylase inhibitory activity. The ethanolic extract showed consistently higher activity than the aqueous extract, supporting further chemical standardization and enzyme-focused investigation.

Keywords: Terminalia catappa; root; phytochemical screening; HPTLC; LC-MS; GC-MS; α -amylase; acarbose; in vitro antidiabetic activity

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INTRODUCTION

Medicinal plants remain a major source of structurally diverse natural products and have contributed directly or indirectly to modern drug discovery. Plant-derived preparations are particularly relevant in traditional health systems, where complex mixtures of phenolics, flavonoids, tannins, terpenoids and other secondary metabolites may contribute to biological activity.¹

Terminalia catappa Linn. (Combretaceae), commonly known as tropical almond or Indian almond, is widely distributed in tropical and subtropical regions. Different parts of the plant have been investigated for antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anticancer and antidiabetic activities. Reviews of the species consistently identify phenolic and flavonoid constituents as important components of its pharmacological profile.^{1,2}

The chemistry of *Terminalia* species is notable for hydrolysable tannins and related phenolic markers. Gallic acid and ellagic acid are among compounds frequently examined in *Terminalia* extracts, and validated chromatographic procedures have been reported for their analysis in members of this genus.⁴

Diabetes mellitus is characterized by persistent disturbances in glucose homeostasis and is associated with both metabolic and oxidative abnormalities. One pharmacological approach to postprandial glucose control is inhibition of digestive carbohydrases. α -Amylase participates in the hydrolysis of dietary starch; therefore, inhibition of this enzyme can slow the generation of absorbable carbohydrates after a meal.^{2,7}

Natural-product screening against α -amylase is widely used to identify extracts and compounds with potential antidiabetic activity. Acarbose is commonly employed as a positive-control inhibitor. Importantly, assay interpretation should account for sample colour, turbidity and blank correction because crude plant extracts can interfere with spectrophotometric or fluorometric measurements.⁷

For *T. catappa* specifically, previous work on leaf extracts has demonstrated inhibitory activity against α -amylase and α -glucosidase, with the activity associated with a chemically diverse mixture of phenolics, terpenes and related metabolites. Such findings provide biological plausibility for evaluating additional plant parts, including the roots.³

Compared with leaves and bark, the roots of *T. catappa* are less represented in the literature. The present study therefore focused on the phytochemical and in vitro evaluation of root extracts, with emphasis on preliminary chemical screening, elemental safety, chromatographic fingerprinting, mass-spectrometric profiling and α -amylase inhibitory activity.

The aim of the present work was to characterize the root extracts of *Terminalia catappa* Linn. and to evaluate their in vitro α -amylase inhibitory activity. The study was designed to prepare ethanolic and aqueous extracts of *T. catappa* roots and determine their extraction yields, followed by preliminary qualitative phytochemical screening to identify the major classes of secondary metabolites present in the extracts. The ethanolic root extract was further evaluated for selected toxic heavy metals to assess its elemental safety profile. Chromatographic fingerprinting was established using HPTLC at 254 and 366 nm, while LC-MS and GC-MS analyses were performed to characterize the ethanolic extract and record tentative phytochemical assignments. Finally, the concentration-dependent in vitro α -amylase inhibitory activities of the ethanolic and aqueous extracts were evaluated using acarbose as the reference inhibitor.

MATERIALS AND METHODS

Plant material and extraction

Roots of *Terminalia catappa* Linn. were collected, cleaned to remove extraneous material, dried and pulverized to a coarse powder. The powdered root material was used to prepare ethanolic and aqueous extracts by standard solvent-extraction procedures, after which the extracts were concentrated and preserved for subsequent analyses. Percentage yield was calculated on a weight-by-weight basis relative to the dried root material.

Preliminary phytochemical screening

Both EETC and AETC were subjected to conventional qualitative phytochemical screening for carbohydrates, proteins, alkaloids, tannins, phenols, flavonoids, saponins, glycosides, terpenoids and steroids. Formation of the characteristic colour or precipitate for each qualitative reaction was recorded as a positive test; absence of the characteristic response was recorded as negative.

Elemental analysis

The ethanolic root extract was examined for mercury (Hg), arsenic (As), cadmium (Cd) and lead (Pb). The laboratory method used for these analytes had a stated detection limit of 0.05 ppm. Results were recorded as detected or not detected relative to this analytical threshold.

HPTLC fingerprinting

The ethanolic extract was subjected to high-performance thin-layer chromatography (HPTLC). Developed plates were scanned at 254 and 366 nm and densitometric profiles were processed using WINCATS Planar Chromatography Manager software. The number of resolved peaks and corresponding R_f values were used to describe the chromatographic fingerprint of the extract.

LC-MS and GC-MS characterization

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Liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS) were used for additional phytochemical characterization of the ethanolic root extract. LC retention times, relative peak areas and major m/z signals were recorded. GC–MS fragment ions were interpreted by comparison with characteristic fragmentation patterns. Compound names reported from these spectra were treated as tentative assignments rather than definitive identifications.

In vitro α -amylase inhibitory assay

EETC and AETC were evaluated at 12.5, 25, 50 and 100 $\mu\text{g/mL}$, with acarbose used as the reference inhibitor. α -Amylase inhibition was determined using a conventional starch-hydrolysis/enzyme-inhibition approach. The use of acarbose as a positive control and careful blank correction are standard features of natural-product α -amylase screening.^{5,7}

Results were expressed as percentage inhibition of α -amylase activity relative to the control. The values are presented as mean $\pm$ standard deviation (SD). The concentration-response pattern of each extract was compared descriptively with acarbose.

Data presentation

Qualitative phytochemical and elemental data are presented categorically. HPTLC, LC and mass-spectrometric data are summarized in tables and figures. In vitro enzyme-inhibition values are reported as mean $\pm$ SD at each tested concentration.

RESULTS

Extraction yield

The ethanolic extract produced a higher percentage yield than the aqueous extract. EETC yielded 14.35% w/w, whereas AETC yielded 9.64% w/w. The difference in recovery suggests that the ethanol-based extraction recovered a greater mass of soluble constituents under the extraction conditions used.

Table 1. Percentage yield of Terminalia catappa root extracts.

Extract	Yield (% w/w)
EETC	14.35
AETC	9.64

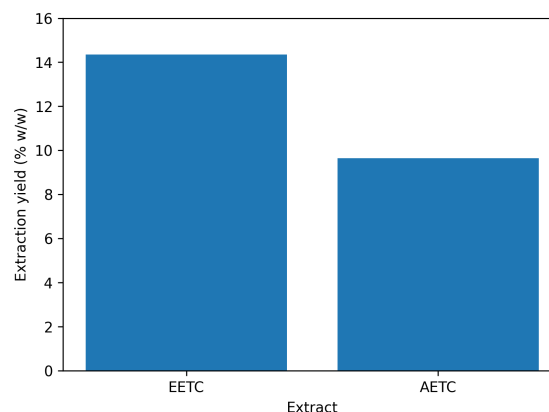


Figure 1. Percentage extraction yield of ethanolic (EETC) and aqueous (AETC) Terminalia catappa root extracts.

Preliminary phytochemical profile

Both extracts showed the same qualitative profile in the preliminary screening. Carbohydrates, tannins, phenols, flavonoids and saponins were detected, whereas proteins, alkaloids, glycosides, terpenoids and steroids were not detected by the corresponding qualitative tests. The positive phenolic and flavonoid reactions are relevant because these classes are frequently associated with antioxidant and enzyme-modulating properties in Terminalia species.

Table 2. Preliminary phytochemical screening of Terminalia catappa root extracts.

Phytochemical constituent	EETC	AETC
Carbohydrates	+	+
Proteins	–	–
Alkaloids	–	–
Tannins	+	+
Phenols	+	+
Flavonoids	+	+
Saponins	+	+
Glycosides	–	–
Terpenoids	–	–
Steroids	–	–

Note: “+” = detected; “–” = not detected.

Elemental analysis

Mercury, arsenic, cadmium and lead were reported as not detected in the ethanolic extract at a method detection limit of 0.05 ppm for each analyte. These findings indicate that none of the four tested toxic elements exceeded the analytical detection threshold in the tested extract.

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Table 3. Elemental analysis of the ethanolic root extract.

Element	Method detection limit	Result
Mercury	0.05 ppm	ND
Arsenic	0.05 ppm	ND
Cadmium	0.05 ppm	ND
Lead	0.05 ppm	ND

HPTLC fingerprint at 254 nm

HPTLC analysis at 254 nm produced distinct multi-peak profiles across all four tracks. Track 1 contained five peaks, Track 2 contained six peaks, and Tracks 3 and 4 each contained eight peaks. Rf values near 0.03, 0.04, 0.50 and 0.96 recurred across several tracks, demonstrating reproducible chromatographic zones within the ethanolic extract.

Table 4. HPTLC peak distribution at 254 nm.

Track	No. of peaks	Rf values
Track 1	5	0.03, 0.04, 0.20, 0.50, 0.96
Track 2	6	0.03, 0.04, 0.36, 0.50, 0.64, 0.96
Track 3	8	0.03, 0.04, 0.20, 0.36, 0.50, 0.77, 0.88, 0.96
Track 4	8	0.03, 0.04, 0.10, 0.35, 0.50, 0.77, 0.88, 0.96

HPTLC fingerprint at 366 nm

At 366 nm, Track 1 contained four peaks, Track 2 contained three peaks, and Tracks 3 and 4 each contained six peaks. Recurrent zones at approximately Rf 0.01 and 0.10 were observed in several tracks. Tracks 3 and 4 showed identical reported Rf series (0.01, 0.05, 0.10, 0.51, 0.86 and 0.94), supporting the reproducibility of the fluorescent fingerprint.

Table 5. HPTLC peak distribution at 366 nm.

Track	No. of peaks	Rf values
Track 1	4	0.01, 0.05, 0.10, 0.65
Track 2	3	0.01, 0.10, 0.53

Track 3	6	0.01, 0.05, 0.10, 0.51, 0.86, 0.94
Track 4	6	0.01, 0.05, 0.10, 0.51, 0.86, 0.94

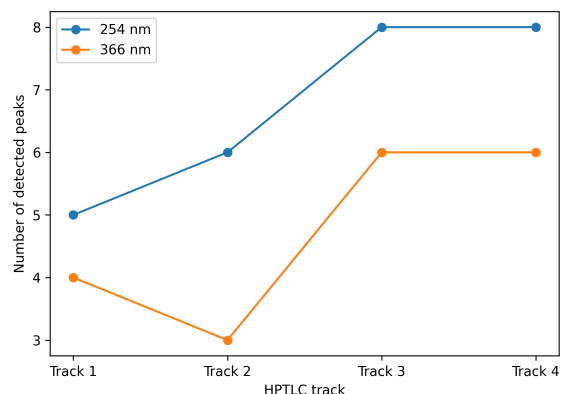


Figure 2. Number of HPTLC peaks detected in four tracks at 254 and 366 nm.

LC chromatographic profile

The LC profile contained five reported peaks at retention times of 1.332, 1.498, 1.548, 31.740 and 38.966 min. The peak at 31.740 min had the largest reported relative area (58.6425), substantially exceeding the other detected peaks. This dominant chromatographic feature may be useful as a marker region for future extract standardization, although compound identity cannot be assigned from retention time alone.

Table 6. LC chromatographic peaks of the ethanolic root extract.

Peak	Retention time (min)	Relative area
1	1.332	3.7075
2	1.498	18.7124
3	1.548	9.1067
4	31.740	58.6425
5	38.966	9.8310

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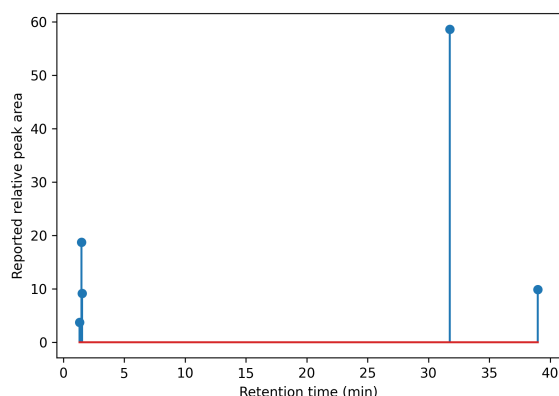


Figure 3. LC retention-time profile showing the reported relative peak areas of the ethanolic root extract.

Tentative LC–MS and GC–MS characterization

Mass-spectrometric data indicated several candidate constituents. LC–MS signals were tentatively assigned to gallic acid (m/z 170.10), ellagic acid (m/z 301.75), catechin (m/z 290.90) and β -sitosterol (m/z 414.00). In the GC–MS spectrum, prominent fragment ions at m/z 83.0579, 85.0478 and 87.1060 were reported as compatible with a γ -sitosterol-like fragmentation pattern. These identifications remain tentative and require confirmation using authentic standards and complete spectral matching.

Table 7. Mass-spectrometric signals and tentative phytochemical assignments.

Analysis	Signal/feature	Tentative assignment	Interpretive status
LC–MS	m/z 170.10	Gallic acid	Tentative
LC–MS	m/z 301.75	Ellagic acid	Tentative
LC–MS	m/z 290.90	Catechin	Tentative
LC–MS	m/z 414.00	β -Sitosterol	Tentative
GC–MS	m/z 83.0579, 85.0478, 87.1060	γ -Sitosterol	Tentative; confirmation required

In vitro α -amylase inhibitory activity

EETC and AETC produced a progressive increase in α -amylase inhibition over the concentration range of 12.5–100 $\mu\text{g/mL}$. EETC showed numerically higher inhibition than AETC at each tested concentration. Acarbose produced the highest inhibition throughout the concentration range. At

12.5 $\mu\text{g/mL}$, inhibition was $47.86 \pm 1.65\%$ for EETC, $43.26 \pm 1.31\%$ for AETC and $56.90 \pm 1.02\%$ for acarbose. At 100 $\mu\text{g/mL}$, the respective values increased to $77.50 \pm 1.25\%$, $74.52 \pm 1.02\%$ and $86.00 \pm 1.12\%$.

Table 8. In vitro α -amylase inhibitory activity of Terminalia catappa root extracts and acarbose.

Concentration ($\mu\text{g/mL}$)	EETC (% inhibition)	AETC (% inhibition)	Acarbose (% inhibition)
12.5	47.86 ± 1.65	43.26 ± 1.31	56.90 ± 1.02
25	58.86 ± 1.41	53.16 ± 1.05	65.30 ± 1.53
50	68.14 ± 1.29	64.43 ± 0.80	76.63 ± 1.16
100	77.50 ± 1.25	74.52 ± 1.02	86.00 ± 1.12

Values are expressed as mean $\pm$ SD.

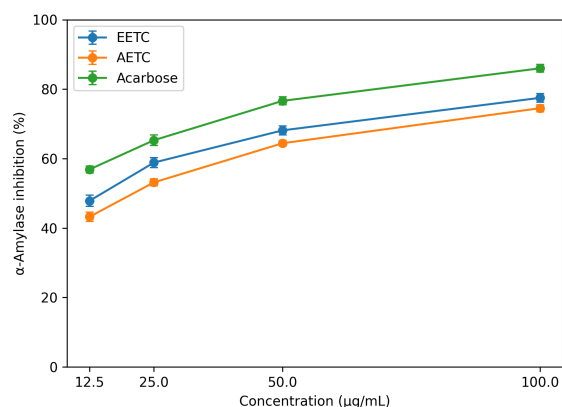


Figure 4. Concentration-dependent in vitro α -amylase inhibitory activity of EETC, AETC and acarbose. Error bars represent SD.

DISCUSSION

The root extracts of *T. catappa* displayed a phytochemical pattern dominated by phenolic/tannin, flavonoid and saponin reactions. This finding is broadly compatible with the chemically rich profile described for other parts of *T. catappa* and with the general prominence of polyphenolic metabolites in *Terminalia* species.^{1,4}

The higher extraction yield of EETC than AETC indicates that ethanol recovered a greater total mass of extractable material from the root under the conditions used. This does not by itself identify which chemical class was preferentially recovered, but the difference is relevant when comparing downstream bioactivity because extraction solvent

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strongly affects the relative abundance of polar and moderately polar constituents.

The absence of detectable mercury, arsenic, cadmium and lead at the reported analytical threshold provides a useful quality-control observation for the tested ethanolic extract. Heavy-metal screening does not establish comprehensive toxicological safety, but it addresses an important contamination parameter in herbal-material evaluation.

HPTLC produced complex and reproducible fingerprints at both 254 and 366 nm. Recurrent Rf zones across replicate tracks support the value of the fingerprint for identity and batch-comparison purposes. The greater number of peaks in Tracks 3 and 4 at 254 nm and the reproducible six-peak pattern in Tracks 3 and 4 at 366 nm further indicate that the extract contains multiple UV-absorbing and fluorescent constituents.

The tentative LC–MS assignments of gallic acid and ellagic acid are chemically plausible within the genus *Terminalia*, in which tannin-related phenolic markers have been demonstrated by validated chromatographic methods. Nevertheless, m/z agreement alone is insufficient for definitive identification, and authenticated standards or tandem mass-spectral confirmation would be required for a conclusive assignment.⁴

The in vitro α -amylase results provide the clearest functional finding of the present study. Both extracts showed monotonic concentration-dependent inhibition, and the ethanolic extract consistently exceeded the aqueous extract by approximately 3–6 percentage points across the tested range. This pattern parallels the higher extraction yield of EETC and may indicate that the constituents responsible for enzyme inhibition were more effectively recovered in ethanol.

The magnitude and direction of the α -amylase effect are also consistent with previous evidence that *T. catappa* extracts can inhibit enzymes related to carbohydrate digestion. In leaf extracts, α -amylase and α -glucosidase inhibition has been linked to a diverse mixture of phenolics and terpenoid constituents. However, direct comparison of potency between studies is limited by differences in plant part, extraction procedure, assay format, enzyme source, concentration units and blank-correction strategy.^{3,7}

Acarbose remained the strongest inhibitor at every tested concentration, as expected for a reference α -amylase inhibitor. The root extracts therefore should not be interpreted as equivalent to acarbose; rather, their activity identifies them as candidates for further bioassay-guided fractionation. Follow-up work should quantify total phenolic and flavonoid content, determine IC₅₀ values using a

suitable concentration range, perform α -glucosidase testing, incorporate rigorous sample blanks, and confirm the chemical identity of active constituents.

Overall, the combination of qualitative phytochemical screening, HPTLC fingerprinting, LC–MS/GC–MS profiling and enzyme inhibition gives a coherent in vitro characterization of *T. catappa* root extracts. The data support continued investigation of the root as a source of enzyme-modulating phytochemicals while keeping the interpretation within the scope of the in vitro evidence.

CONCLUSION

Ethanolic and aqueous root extracts of *Terminalia catappa* Linn. contained carbohydrates, tannins/phenolic compounds, flavonoids and saponins and were free of detectable mercury, arsenic, cadmium and lead at the stated analytical detection limit. HPTLC demonstrated reproducible multi-peak fingerprints, while LC–MS and GC–MS provided tentative evidence for several phenolic and sterol-related constituents. Both extracts showed concentration-dependent in vitro α -amylase inhibition, with EETC consistently more active than AETC and acarbose remaining the strongest reference inhibitor. These findings establish an in vitro phytochemical and enzyme-inhibition profile for *T. catappa* root and provide a basis for future fractionation, compound confirmation and expanded antidiabetic enzyme studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable to the present manuscript because it reports phytochemical and in vitro experimental work only.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author on reasonable request.

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