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Research Article

Preliminary successive solvent extraction, phytochemical screening and HPLC-PDA fingerprinting of *Terminalia chebula* Retz. fruit pulp

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Abstract

Background. The dried fruit pulp of *Terminalia chebula* contains chemically diverse secondary metabolites. This preliminary study determined the successive solvent extractive values, qualitative phytochemical profiles and HPLC-PDA fingerprint of one authenticated fruit-pulp batch.

Methods. Mature fruits collected in September 2020 from Sathyamangalam forest, Tamil Nadu, were authenticated, dried and powdered. A 500 g sample underwent successive hot percolation with petroleum ether, chloroform, acetone and 95% ethanol (72 h each), followed by 72 h aqueous-stage maceration with 10 mL chloroform added as preservative. One extraction series was performed (n = 1). Qualitative phytochemical tests were recorded as positive or negative. The aqueous extract was fingerprinted by HPLC-PDA at 270 nm.

Results. The extractive yields for petroleum ether, chloroform, acetone, ethanol and the aqueous stage were 2.8%, 1.5%, 2.1%, 7.2% and 13.8% w/w, respectively. The aqueous extract gave the highest recovery (69.0 g), and the ethanol extract showed the broadest positive screening profile (nine operational test categories). Phenolic reactions were positive in acetone, ethanol and water-stage extracts, although the separately recorded tannin tests showed discordant results. Molisch reactions in the glycoside screen were non-specific and did not confirm glycosides. A major HPLC-PDA peak at 7.569 min was tentatively assigned to gallic acid, and five further peak regions were tentatively assigned to hydrolysable tannin and phenolic acid markers.

Conclusion. This single-run study provides preliminary descriptive evidence of polarity-dependent extractive recovery and heterogeneous qualitative phytochemical profiles. The aqueous extract gave the highest yield, whereas the ethanol extract showed the broadest screening profile. Molecular identities, glycoside status and constituent concentrations were not confirmed; replicate extractions and comparison with authenticated reference standards are required.

Keywords: *Terminalia chebula*; successive extraction; phytochemical screening; exploratory study; HPLC-PDA; fruit pulp; aqueous extract.

1. Introduction:

Successive solvent extraction fractionates plant material according to polarity, allowing the distribution of constituent classes among fractions to be characterized before a single extract is selected for pharmacological study. A single-

solvent extract does not indicate whether an activity observed subsequently is associated with lipid constituents, mid-polarity nitrogenous compounds or the galloyl esters that predominate in *Terminalia chebula* fruit pulp. Qualitative phytochemical screening of successive fractions therefore remains a standard preliminary step before chromatographic analysis.[1,2]

Terminalia chebula Retz. (Combretaceae) is a deciduous tree whose ribbed drupes, as dried fruit pulp, are known as haritaki or kadukkai.[3,4] Commercial fruits vary in size and maturity. Ethanolic extracts of the galls have also been investigated, but galls and fruit pulp are distinct materials.[5] The species is distributed across South and South-East Asia.[4,6] Published chemical profiles indicate that the content of tannins and their hydrolysis products varies with source and preparation conditions.[7,8]

In traditional medicine, the fruit pulp is used chiefly for its astringent and digestive actions.[3,9] Aqueous, alcoholic and hydroalcoholic extracts have been reported to exhibit antioxidant, antimicrobial, hepatoprotective, antidiabetic, anti-inflammatory, anti-ulcer, wound-healing and cytoprotective activities.[6,10–26] Most of these activities have been attributed to hydrolysable tannins and phenolic acids, whereas lipid-rich fractions differ substantially in composition; fractionation of the pulp is therefore warranted before a single extract is selected for pharmacological evaluation. Extraction practice nevertheless varies widely. Single-solvent methanolic, ethanolic, aqueous and hydroalcoholic extracts predominate,[27–31] and defatting with petroleum ether is common, although its potential to remove phenolic constituents is not always considered. Successive extraction followed by classical qualitative phytochemical screening provides an established pharmacognostic approach to such preliminary fractionation.[1,2,32–35]

Prior chromatographic work has reported multiple hydrolysable tannins and related phenolic acids in *Terminalia chebula*, including gallic acid, chebulic acid, corilagin, ellagic acid, chebulagic acid and chebulinic acid.[7,8] Reviews additionally describe flavonoids, triterpenoids, sterols and other chemical classes.[36–38] These reports guide interpretation of the present data but do not establish the identity of compounds in the present extracts. Nutritional constituents such as carbohydrates and amino acids can contribute to non-specific colour reactions.[39]

Solvent polarity determines which constituents are recovered in each fraction. Petroleum ether and chloroform extract triglycerides and some sterols but extract gallotannins poorly; acetone and ethanol extract polymeric ellagitannins and many flavonoid glycosides; and water extracts free sugars, amino acids, saponins, gallic acid and part of the more hydrophilic tannin fraction, as in traditional decoction.[27,28] In successive extraction, the same marc is extracted with solvents of increasing polarity, so that each fraction contains material not removed by the preceding solvents. Prolonged heating may hydrolyse chebulagic and chebulinic acids to gallic and ellagic acids, consistent with reports on ellagitannin hydrolysis;[7,8,40] however, the successive scheme ensures that all five fractions are derived from a single batch.

The present study therefore aimed to determine the extractive values, qualitative phytochemical profiles and chromatographic fingerprint of an authenticated batch of *Terminalia chebula* fruit pulp using successive solvent extraction, qualitative phytochemical screening and HPLC-PDA fingerprinting, and to establish the basis for selecting the aqueous extract for subsequent pharmacological studies. The study was exploratory and was not designed to estimate reproducibility, assay precision, biological efficacy or constituent concentrations.

2. Materials and methods

2.1 Plant material

Mature fruits of *Terminalia chebula* Retz. were collected in September 2020 from the Sathyamangalam forest, Erode district, Tamil Nadu, India. The material was identified and

authenticated by Dr Chandrashekar R (Figure 1). A voucher specimen (TCMA-1; reference A.N.16-A/2020, dated 5 October 2020) was deposited in the Department of Pharmacology, Vinayaka Mission's Kirupananda Variyar Medical College, Salem, for future reference. Only fruits matching the ribbed drupe of this species were retained; leaves, bark and seed kernels were not extracted.

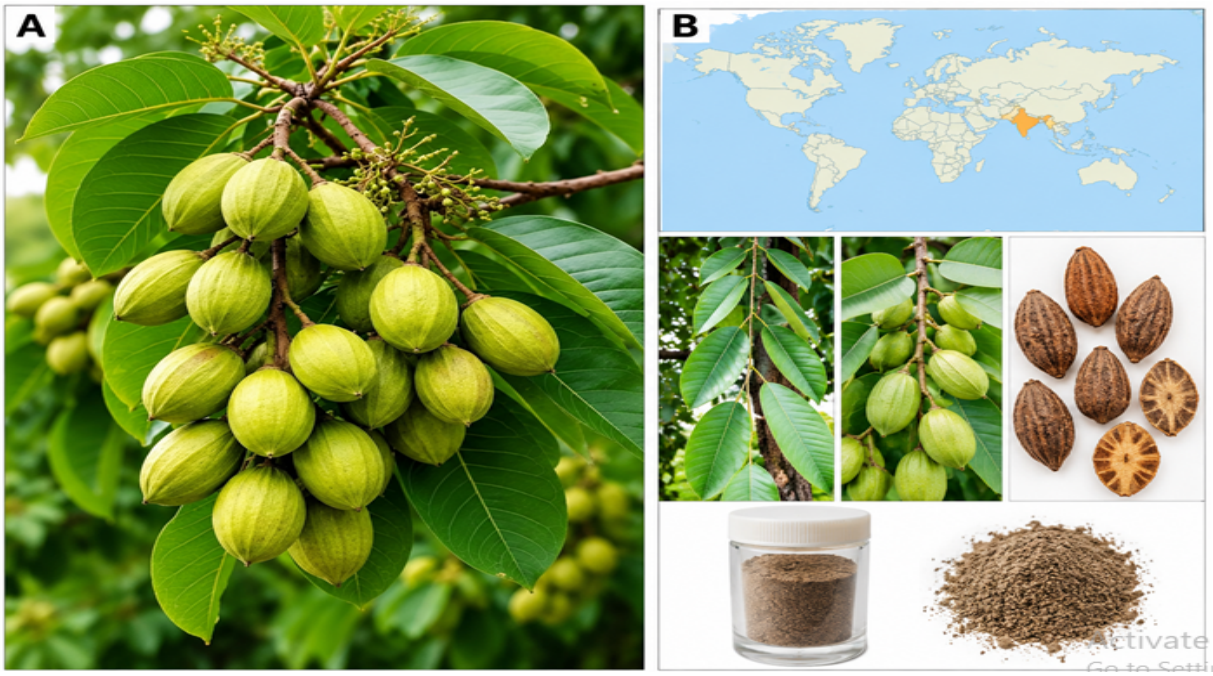


Figure 1. Botanical illustration of Terminalia chebula: (A) ribbed immature fruits on a leafy branch; (B) geographic and morphological overview, dried fruits and powdered fruit material.

2.2 Drying and powdering

Fruits were shade-dried at room temperature for 10 days, and then tray-dried at 40 °C for 48 h to complete drying of the pulp. The dried pulp was separated, coarsely ground in a mechanical grinder and passed through sieve No. 60. The powder was stored in an airtight container until extraction. A 500 g portion of the powder was used for successive extraction. Solvents were purified before use (SDFCL, SD Fine-Chem Limited, India). The solvents, in order of use, were petroleum ether (boiling range 60–80 °C), chloroform (59.1–61.5 °C), acetone (55.5–56.5 °C) and ethanol 95% v/v (75–78 °C), followed by distilled water.

2.3 Successive extraction

The powder was extracted successively with each organic solvent by continuous hot percolation in a Soxhlet apparatus for 72 h (Figure 2).[2] Each extract was filtered, concentrated and dried. The marc was dried between solvents; no quantitative residual-solvent endpoint was determined. Prolonged exposure to heat could alter hydrolysable tannins; the design does not distinguish extraction effects from any resulting chemical degradation.

After the four organic steps, the dried marc underwent maceration with distilled water for 72 h in a 2 L flask. Chloroform (10 mL) was added as a preservative, and the resulting extract was processed to a dry residue; this preparation is designated the aqueous-stage extract of *Terminalia chebula* (AETC; Figure 2B). Because chloroform was added, the extract is not a purely aqueous preparation. Residual chloroform was not measured, and clearance before later biological use was not verified. Extractive yield (% w/w) was calculated as (dry residue mass/500 g) × 100. One extraction run (n = 1) was performed.

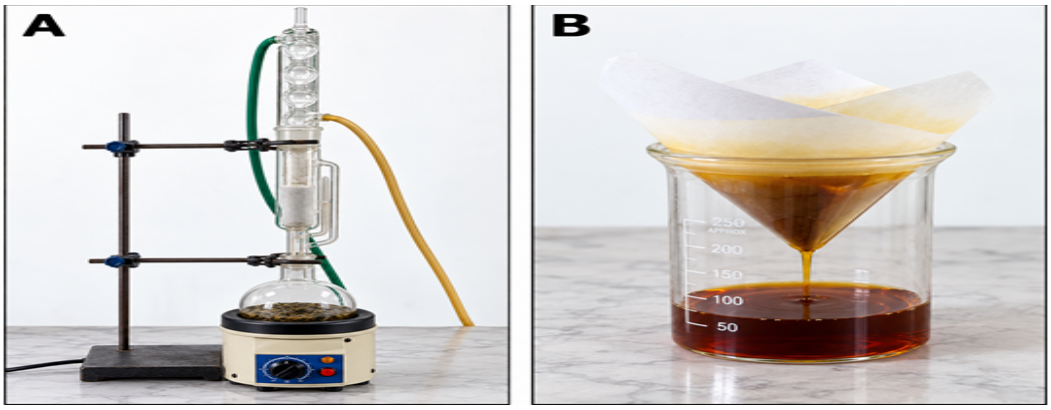


Figure 2. Illustrative extraction and filtration procedures: (A) Soxhlet apparatus and (B) filtration arrangement. Both panels are illustrations rather than experimental photographs.

2.4 Phytochemical tests

All five dry extracts were subjected to qualitative screening assays using standard reagent procedures.[1] A positive entry denotes only the stated colour, precipitate or foam endpoint, not chemical identity or concentration. The matrix is a set of binary observations; reaction intensity and between-run variability were not assessed.

Alkaloid-reagent screening. Acidified extract was treated separately with Dragendorff, Hager, Wagner and Mayer reagents, recording orange-red, yellow, brown or pale precipitates, respectively, as positive endpoint reactions. Concordance among the four precipitation tests was recorded. These reagents are not specific enough to confirm molecular alkaloids in a complex tannin-rich matrix, and reference controls were not included.

Sterols. In the Liebermann–Burchard test, 2 mg of dry extract in acetic anhydride was heated, cooled, and layered with 1 mL of concentrated sulphuric acid; green was positive. In the Salkowski test, 2 mg was shaken with chloroform and sulphuric acid was added to form a lower layer; red in the chloroform or at the interface was positive.

Carbohydrates. Two milligrams were shaken with 10 mL of water, filtered, and the filtrate was concentrated. Anthrone (2 mL) gave green or blue if positive. Benedict's solution (5 mL), boiled for 5 min, gave a brick-red precipitate if reducing sugar was present. Equal parts of Fehling's A and B, boiled briefly, gave the same precipitate. Molisch's test used two drops of 20% ethanolic α-naphthol and 2 mL of concentrated sulphuric acid layered beneath; a red-violet ring, lost on adding excess alkali, was positive. The four tests indicate carbohydrate but do not identify the sugar.

Fixed oils. Extract pressed between filter papers left a persistent translucent stain if positive. A second tube was heated 1–2 h with 0.5 N alcoholic potassium hydroxide and phenolphthalein; partial neutralization, indicating saponification, supported the presence of esterified fatty acids. Only the spot-test result is reported.

Phenolic screening. Ferric chloride (5%), gelatin (1% in 10% sodium chloride) and lead acetate (10%) were evaluated as separately recorded phenolic reactions. A second ferric chloride/gelatin series was performed separately as tannin screening. The extract concentrations, pH and reagent conditions were not consistently matched across the two series; the two series are therefore reported independently, and discordant results are not combined into a single tannin assignment.

Total phenolic and flavonoid content. Total phenolic content of the aqueous-stage extract was determined by the Folin–Ciocalteu method and expressed as gallic-acid equivalents, with absorbance measured at 765 nm after 30 min. Total flavonoid content was determined by aluminium-chloride colorimetry and expressed as

quercetin equivalents, with absorbance measured at 415 nm after 40 min. Individual absorbance readings, calibration tables, replicate counts and the statistical definition of the ± values are not reported; these assays are therefore treated as descriptive measurements, not as a validated tannin-specific assay or as evidence of extraction reproducibility.

Protein- and amino-group reactions. Millon, ninhydrin and biuret tests were recorded individually. Ninhydrin indicates accessible amino groups, biuret indicates peptide-associated bonding under appropriate conditions, and Millon detects certain phenolic amino-acid-related reactions; none, alone, measures total protein mass. These endpoints are reported together but are not treated as indicators of a single chemically specific constituent.

Foam (saponin-screening) test. Extract solution was shaken with sodium bicarbonate and observed for a persistent froth over 3 min. A persistent foam was recorded as a positive response. No haemolysis or specific saponin characterization was undertaken; foam formation is interpreted as an operational screen only.

Tannin-series screening. Gelatin–salt and ferric chloride were tested and scored independently of the phenolic-series tests. Because the two series were not confirmed to have been performed at matched sample concentration and pH, discordant results are reported as observed. A validated protein-precipitation assay or chromatographic comparison would be needed for further characterization.

Gums and mucilages. Extract was stirred into 25 mL of absolute ethanol and filtered. The air-dried precipitate was examined for swelling in water. Swelling was taken to indicate gum or mucilage; the test does not identify a polysaccharide.

Flavonoids. Two milligrams in 5 mL of ethanol, ten drops of dilute hydrochloric acid and a piece of magnesium (Shinoda) gave pink, red or brown if positive. Concentrated sulphuric acid in a second tube gave the classical yellow-orange, yellow or orange-to-crimson colours associated with anthocyanins, flavones and flavanones. No subclass was assigned from that colour alone.

Molisch screening for glycosides. The red-violet ring obtained with Molisch reagent was scored in the glycoside row. This is a general carbohydrate response and cannot establish that sugar is glycosidically bound. No selective hydrolysis, aglycone isolation or glycoside-specific confirmatory assay was performed.

Assay controls. Positive and negative reference controls, reagent blanks, replicate colour readings and exactly matched extract concentrations were not included. Positive/negative calls are therefore reported as preliminary reactions rather than validated identifications.

2.5 Scope of phytochemical identification

The colour, precipitation, foam and Molisch reactions were used for class-oriented screening only. They cannot establish compound identity, abundance or the absence of a constituent below an assay's detection capability. The non-specific and sometimes discordant responses were interpreted separately from the HPLC-PDA fingerprint.

Illustrations of selected reaction appearances and the original binary outcome matrix are presented with the Results (Section 3.3). The reaction-tube figure is illustrative and does not depict the study extracts.

2.6 HPLC-PDA fingerprint recording

The HPLC-PDA trace was recorded for the aqueous-stage extract (AETC) under the following conditions: a Shimadzu Prominence system, a Phenomenex C18 column (250 × 4.6 mm, 5 µm) at 25 °C, solvent A (0.1% phosphoric acid in water) and solvent B (acetonitrile), 1.0 mL/min flow and 20 µL injection. Samples were prepared from 10 mg dried extract in 10 mL methanol, with sonication and 0.45 µm membrane filtration. Detector output was recorded at 270 nm (4 nm bandwidth) over approximately 60 min. The full gradient time/%B schedule, injection sequence, equilibration conditions, system suitability,

reference-standard chromatograms, full PDA spectra and peak integrations are not reported. Annotated compounds are therefore regarded as tentative peak labels, rather than standard-confirmed identifications; no concentration is calculated.

2.7 Descriptive analysis

A single extraction series (n = 1) was summarized as percentage yield and the calculated mass of each dried residue. No replicate extracts were prepared; standard deviations, coefficients of variation, confidence intervals, inferential tests and between-solvent significance comparisons are therefore not reported. Qualitative assay results were tabulated as observed, without statistical inference.

3. Results

3.1 Plant material

The fruit pulp used for extraction was obtained from authenticated Terminalia chebula, characterized by longitudinally ribbed drupes (Figure 1). Following shade drying and powdering, the material passed through sieve No. 60. The voucher specimen is TCMA-1 (A.N.16-A/2020).

The successive Soxhlet arrangement and filtration procedure are illustrated in Figure 2A and Figure 2B, respectively.

3.2 Physical characteristics and extractive values

The extracts differed in colour and extractive recovery (Table 1; Figure 3A). The sequential extraction and the progression from petroleum ether to aqueous maceration are depicted in Figure 3B. Extract mass values calculated from a 500 g charge are given in Table 1.

Table 1. Successive extractive values of dried fruit pulp of Terminalia chebula (500 g charge).

Solvent	Method and conditions	Colour of residue	Yield (% w/w)	Mass (g)
Petroleum ether (60–80 °C)	Hot percolation, 72 h	Dark yellow	2.8	14.0
Chloroform (59–62 °C)	Hot percolation, 72 h, on dried marc	Dark greenish brown	1.5	7.5
Acetone (56–57 °C)	Hot percolation, 72 h, on dried marc	Dark brown	2.1	10.5
Ethanol 95% (75–78 °C)	Hot percolation, 72 h, on dried marc	Dark brown	7.2	36.0
Distilled water	Cold maceration, 72 h, 2 L flask*	Brown	13.8†	69.0†
Combined recovery	—	—	27.4†	137.0†

*The aqueous stage included 10 mL of chloroform as preservative. Yields are percentages of a single 500 g extraction run (n = 1), and masses are calculated from those percentages. The calculated aqueous-stage yield is 13.8% w/w (69.0 g), whereas the recorded residue mass corresponds to 13.28% w/w (66.4 g); the calculated value is used throughout, and the weighed residue mass is the determining value.

†Calculated value; the recorded residue mass corresponds to 13.28% w/w (66.4 g), giving a combined recovery of 26.88% (134.4 g).

From the 500 g starting material, the petroleum ether and chloroform extracts together accounted for 4.3% w/w (21.5 g). Acetone yielded 2.1% w/w (10.5 g), ethanol yielded 7.2% w/w (36.0 g) and the aqueous stage yielded 13.8% w/w (69.0 g), the largest single recovery. As these are descriptive results for one batch, variability and statistical differences among solvents were not assessed.

The recorded aqueous-stage residue mass corresponds to 13.28% w/w (66.4 g), compared with the calculated value of 13.8% w/w (69.0 g). The plotted values and calculated totals use 13.8%; because the definitive yield depends on the weighed residue mass, the difference between the two values is a limitation of this single-run determination.

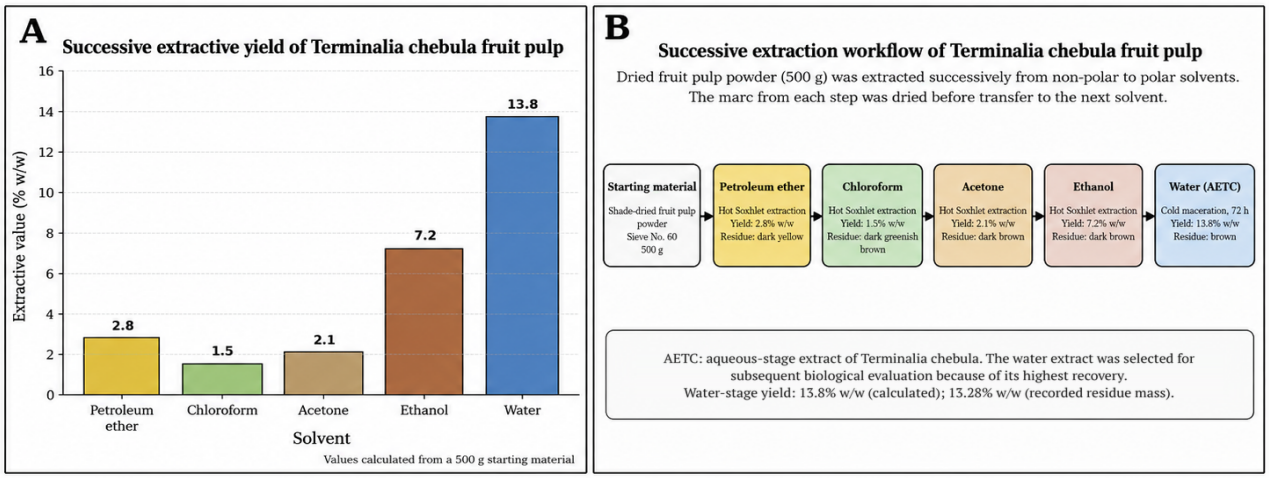


Figure 3. Successive extraction of Terminalia chebula fruit pulp. (A) Extractive yields (% w/w) of five solvent fractions. (B) Sequence of successive extraction from 500 g dried fruit pulp. Yields are from a single extraction run (n = 1). The aqueous-stage bar shows the calculated value (13.8% w/w); the value corresponding to the recorded residue mass (13.28% w/w) is given in panel B and Table 1.

3.3 Phytochemical screening

Table 2 presents the individual qualitative assay endpoints across the five extracts. The reactions are illustrated schematically in Figure 4, while Figure 5 summarizes the binary reactions and Table 3 lists positive operational screening categories. A positive category is not confirmation of a specific phytochemical compound.

Table 2. Recorded preliminary qualitative reaction endpoints in successive *Terminalia chebula* fruit-pulp extracts (single run).

Class	Test	Petroleum ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	Mayer	−	+	+	+	−
Alkaloids	Dragendorff	−	+	+	+	−
Alkaloids	Hager	−	+	+	+	−
Alkaloids	Wagner	−	+	+	+	−
Sterols	Liebermann–Burchard	−	−	+	+	+
Sterols	Salkowski	−	−	+	+	+
Carbohydrates	Molisch	+	+	+	+	+
Carbohydrates	Fehling	+	+	+	+	+
Carbohydrates	Benedict	+	+	+	+	+
Carbohydrates	Anthrone	+	+	+	+	+
Fixed oils	Spot test	+	+	−	−	−
Phenolics	Ferric chloride	−	−	+	+	+
Phenolics	Gelatin–salt	−	−	+	+	+
Phenolics	Lead acetate	−	−	+	+	+
Proteins and amino acids	Biuret	−	+	+	+	+
Proteins and amino acids	Ninhydrin	−	+	+	+	+
Proteins and amino acids	Millon	−	+	+	+	+
Saponins	Foam	−	+	−	+	+
Tannins	Gelatin–salt	−	+	−	−	−

Tannins	Ferric chloride	−	+	+	+	−
Gums and mucilages	Ethanol precipitation	+	+	−	−	−
Flavonoids	Shinoda	−	−	+	+	+
Flavonoids	Concentrated sulphuric acid	−	−	+	+	+
Molisch glycoside screen*	Molisch (non-specific)	−	−	+	+	+

Symbols: +, recorded positive endpoint; −, recorded negative endpoint. The separately performed phenolic and tannin series had discordant ferric chloride/gelatin responses. *The final Molisch row is not a glycoside-specific test. Discordant entries are retained and summarized in Table 4.

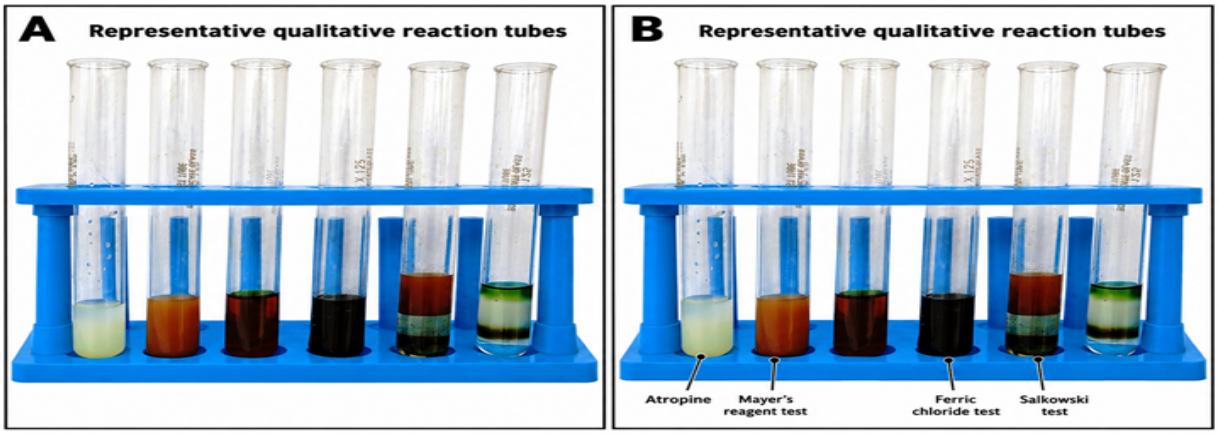


Figure 4. Illustration of representative reagent reaction appearances: (A) unlabelled and (B) annotated for assays described in Section 2.4. The figure is illustrative rather than an experimental photograph; the tube labelled atropine is a comparator and does not indicate atropine in the extracts.

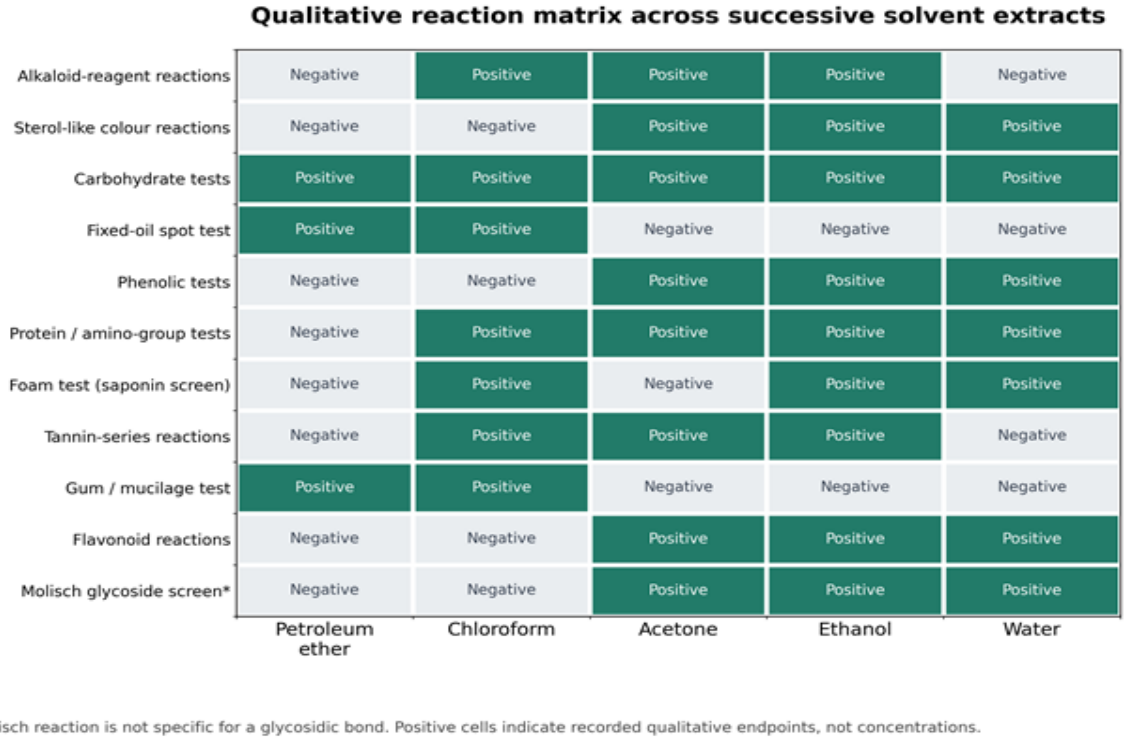


Figure 5. Category-level summary of positive and negative qualitative reactions in Table 2 (a category is positive when any associated assay was positive). Molisch screening does not establish glycosides; colours indicate observed endpoints, not analyte concentration.

Table 3. Solvent-resolved summary of positive screening categories from Table 2; counts are operational and not numbers of confirmed chemical classes.

Solvent	Yield (% w/w)	Positive screening categories*	Recorded positive assay categories	Interpretation
Petroleum ether	2.8	3	Carbohydrate; fixed oil; gums/mucilage	Alkaloid, sterol, phenolic, protein, saponin, tannin, flavonoid and glycoside rows negative
Chloroform	1.5	7	Alkaloid; carbohydrate; fixed oil; protein/amino acid; saponin; tannin; gums/mucilage	The tannin row is positive but all three phenolic-row assays are negative
Acetone	2.1	8	Alkaloid; sterol; carbohydrate; phenolic; protein/amino acid; tannin; flavonoid; non-specific Molisch screen	Tannin response rests on ferric chloride only; foam negative
Ethanol (95%)	7.2	9	Alkaloid; sterol; carbohydrate; phenolic; protein/amino acid; saponin; tannin; flavonoid; non-specific Molisch screen	Widest qualitative profile; tannin gelatin test negative
Water	13.8†	7	Sterol; carbohydrate; phenolic; protein/amino acid; saponin; flavonoid; non-specific Molisch screen	Phenolic row positive yet tannin row negative; Molisch reaction is not glycoside-specific

*One category counted positive when any associated assay gave a positive endpoint. Counts include the non-specific Molisch glycoside screen and do not represent numbers of molecular classes identified. †Calculated value; the recorded residue mass corresponds to 13.28% w/w (see Table 1).

3.4 Alkaloids

Mayer, Dragendorff, Hager and Wagner reagents all gave positive precipitation endpoints in chloroform, acetone and ethanol and negative endpoints in petroleum ether and water (Table 2). The concordant reagent pattern is a preliminary screening result. Matrix effects and non-alkaloid precipitation cannot be excluded without authenticated controls or compound-specific analysis; individual alkaloids were not identified.

3.5 Sterols

Liebermann–Burchard and Salkowski colour responses were negative in petroleum ether and chloroform but positive in acetone, ethanol and the aqueous stage (Table 2). These are sterol-like assay reactions; they do not distinguish free sterols, their conjugates or other colour-producing constituents. No individual sterol was established.

3.6 Carbohydrates

Molisch, Fehling, Benedict and anthrone assays were recorded as positive for all five extracts (Table 2). The recorded responses suggest carbohydrate-reactive material, but they neither identify individual sugars nor establish that sugars were genuinely extracted into the non-polar fractions. Carryover or matrix interference remains possible and was not evaluated experimentally.

3.7 Fixed oils and gums

Fixed-oil spot tests and ethanol precipitation/swelling tests were positive in the petroleum ether and chloroform fractions and negative after those steps. Such responses are compatible with lipophilic residue and precipitable, water-swelling material, respectively; their identity and solvent-partition mechanism cannot be confirmed from these qualitative endpoints alone.

3.8 Phenolic compounds

The separately designated phenolic series (ferric chloride, gelatin–salt and lead acetate) was negative in petroleum ether and chloroform and positive in acetone, ethanol and the aqueous-stage extract. The positive colour/precipitation pattern is consistent with phenolic-reactive material, but cannot quantify total phenolics or establish a specific tannin, because assay responses depend on matrix and test conditions.

3.9 Proteins and free amino acids

Biuret, ninhydrin and Millon reactions were negative for the petroleum ether fraction and positive for the four remaining fractions. These assays probe different amino- or peptide-related chemical features and cannot be used as interchangeable estimates of total protein. The findings are therefore treated as assay-specific responses rather than proof of a defined protein fraction.[39]

3.10 Saponins

The recorded three-minute foam response was positive in chloroform, ethanol and water-stage extracts and negative in petroleum ether and acetone. Foam persistence depends on concentration and matrix effects; the irregular pattern does not identify specific saponins or prove a solvent-dependent saponin mechanism. No replicate foam observations or confirmatory assays were performed.

3.11 Tannins

Tannin-series gelatin precipitation was recorded as positive only for chloroform, whereas tannin-series ferric chloride reactions were positive in chloroform, acetone and ethanol. By contrast, the separately labelled phenolic series recorded negative reactions for chloroform and positive reactions for the aqueous stage. These discrepancies are preserved in Table 2 and summarized in Table 4, without assigning an untested cause.

Because matched extract concentrations, pH, reagent blanks and reference controls were not established for the two series, the discrepancies preclude reliable positive/negative claims for specific tannin constituents. The aqueous stage was phenolic-screen positive and tannin-series negative; chloroform showed the reverse pattern. Additional matched-condition assays and a validated tannin measurement would be required to reconcile the two series.

Table 4. Discordant qualitative responses and recommended confirmatory analyses.

Observation	Recorded results	Meaning for interpretation	Recommended confirmatory analysis
Phenolics versus tannins—chloroform	Phenolic FeCl3, gelatin and lead acetate: all negative; tannin FeCl3 and gelatin: both positive	The same nominal reagents gave different responses in the two series; no chemically specific attribution is possible	Repeat both series at matched concentration and pH, with positive and negative controls; quantitative total phenolic and tannin assays
Phenolics versus tannins—water	All three phenolic assays positive; both tannin-row assays negative	Supports a general phenolic response but does not establish high-molecular-weight tannins in water	Repeat ferric chloride and gelatin at defined concentration; quantitative tannin assay
Acetone and ethanol tannin tests	Tannin-row ferric chloride positive, gelatin negative	Ferric chloride detects phenolic groups but does not confirm protein-precipitating tannin	Protein-precipitation assay and chromatographic markers
Molisch repeated as glycoside test	Carbohydrate Molisch positive for all five extracts; glycoside-row Molisch negative in the first two extracts	Molisch responds to carbohydrates and cannot alone prove glycosidic bonding	Selective hydrolysis with aglycone/sugar identification
Saponins by foam alone	Chloroform, ethanol and water positive; petroleum ether and acetone negative	Foam is indicative and susceptible to matrix effects; no saponin assigned	Confirm chromatographically or with validated orthogonal assay

FeCl3, ferric chloride. The phenolic and tannin series were not confirmed as performed under matched sample concentrations and conditions; the discordances are reported as observed limitations of the qualitative screening, without assigning an untested cause.

Total phenolic and flavonoid content of AETC. Additional colorimetric assays gave a total phenolic content of 72.12 ± 0.54 mg gallic acid equivalents/g dry extract and a total flavonoid content of 32.13 ±

0.25 mg quercetin equivalents/g dry extract. The dispersion statistic represented by "±", the individual readings and the calibration data are not reported. These assays provide supporting colorimetric context, but do not quantify tannins and do not resolve the discordance between the phenolic and tannin-screening series.

3.12 Flavonoids

Shinoda and concentrated sulphuric acid tests were recorded as negative in the first two solvent fractions and positive in acetone, ethanol and the aqueous stage. These reactions are consistent with flavonoid-like material but do not specify luteolin, quercetin or any other flavonoid.[36,37]

3.13 Glycosides

Molisch reactions recorded in the glycoside screen were negative for petroleum ether and chloroform and positive for acetone, ethanol and the aqueous stage. Carbohydrate-row Molisch reactions, however, were positive for every fraction. Because Molisch responds to carbohydrates whether free or glycosidically bound, the glycoside-row reactions are interpreted as non-specific sugar-related observations; the presence or absence of glycosides was not established.

3.14 Selection of the aqueous extract

The operational screening-category count was three for petroleum ether, seven for chloroform, eight for acetone, nine for ethanol and seven for the aqueous stage (Table 3; Figure 5). Ethanol therefore had the broadest recorded set of positive categories, without demonstrating the greatest concentration of any molecule. The aqueous-stage extract produced the largest mass (69.0 g, 13.8% w/w; Table 5). The aqueous extract was selected for subsequent pharmacological studies on the basis of its recovery and the relevance of aqueous preparations; however, chloroform was added during maceration, and residual-solvent suitability for biological use was not measured in this study.

Table 5. Mass balance of the successive extraction (500 g charge).

Solvent	Yield (% w/w)	Mass (g)	Share of total (%)	Cumulative yield (% w/w)	Cumulative mass (g)
Petroleum ether	2.8	14.0	10.2	2.8	14.0
Chloroform	1.5	7.5	5.5	4.3	21.5
Acetone	2.1	10.5	7.7	6.4	32.0
Ethanol 95%	7.2	36.0	26.3	13.6	68.0
Distilled water (AETC)	13.8†	69.0†	50.4	27.4	137.0
Total	27.4	137.0	100.0	—	—

Shares of total recovery and cumulative masses are computed from the calculated aqueous-stage value of 13.8%: 137.0 g (27.4%) total recovery. The recorded residue mass for that stage corresponds to 13.28%, giving 134.4 g (26.88%) total recovery from 500 g. Both are summaries of the same single run, not independent experiments, and no replicate variance is available. Shares are rounded to one decimal place. †Calculated value; see Table 1 footnote.

The remaining four solvent yields sum to 13.6% w/w (68.0 g), and the water fraction remained the largest contributor to the total extractive recovery.

3.15 Reference structures of major phenolic constituents

Selected literature-reported reference structures, including chebulagic acid, chebulinic acid, gallic acid and ellagic acid, are shown in Figure 6.[7,41] The structures are provided for chemical reference. Tentative peak assignments for the aqueous extract, based on the HPLC-PDA chromatogram obtained in this study, are reported in Section 3.16.

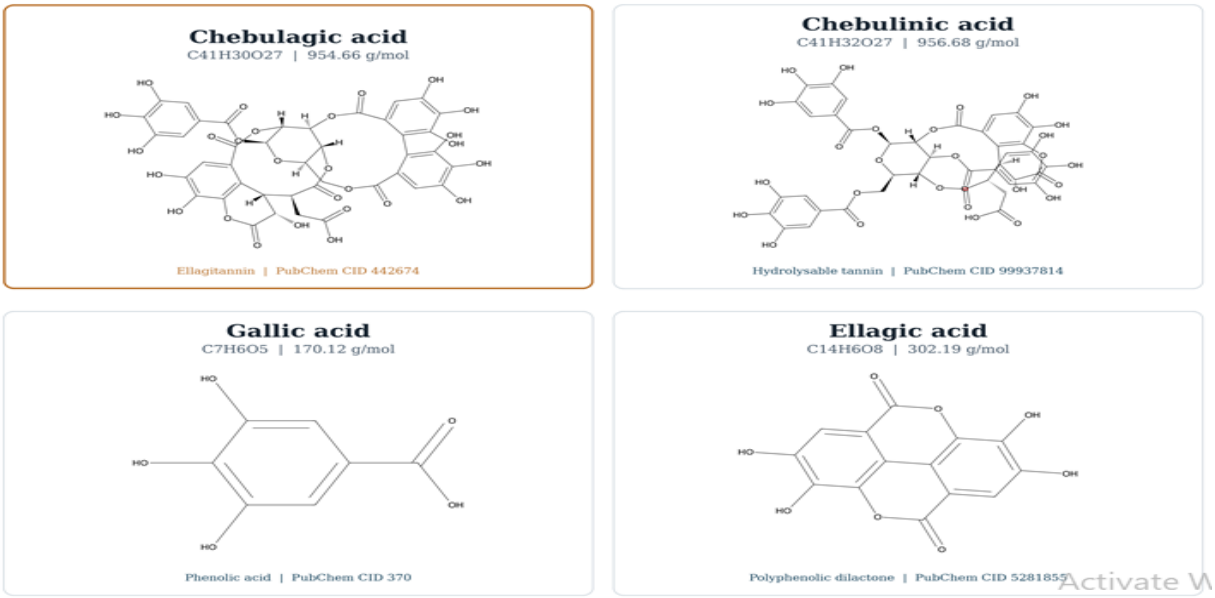


Figure 6. Reference molecular structures of selected literature-reported phenolics from Terminalia chebula: chebulagic acid (top left), chebulinic acid (top right), gallic acid (bottom left) and ellagic acid (bottom right). The structures are shown for reference only and do not represent isolated compounds, study identifications or abundance measurements.

3.16 Exploratory HPLC-PDA fingerprint of the aqueous-stage extract

The HPLC-PDA chromatogram of the aqueous extract showed a prominent peak at 7.569 min, tentatively assigned to gallic acid, with additional annotations for punicalagin, chebulic acid, chebulagic acid, chebulinic acid and ellagic acid (Figure 7; Table 6). Some labels correspond to crowded retention-time regions rather than unequivocally resolved peaks, particularly the region marked chebulagic acid (approximately 25.8–28.9 min). Because authentic-standard matches, peak-purity data, full PDA spectra and integrated areas are not reported, these are tentative chromatographic annotations only. No concentration, relative abundance or between-sample change was estimated.[7]

Figure 7. HPLC-PDA fingerprint of the aqueous extract of *Terminalia chebula*

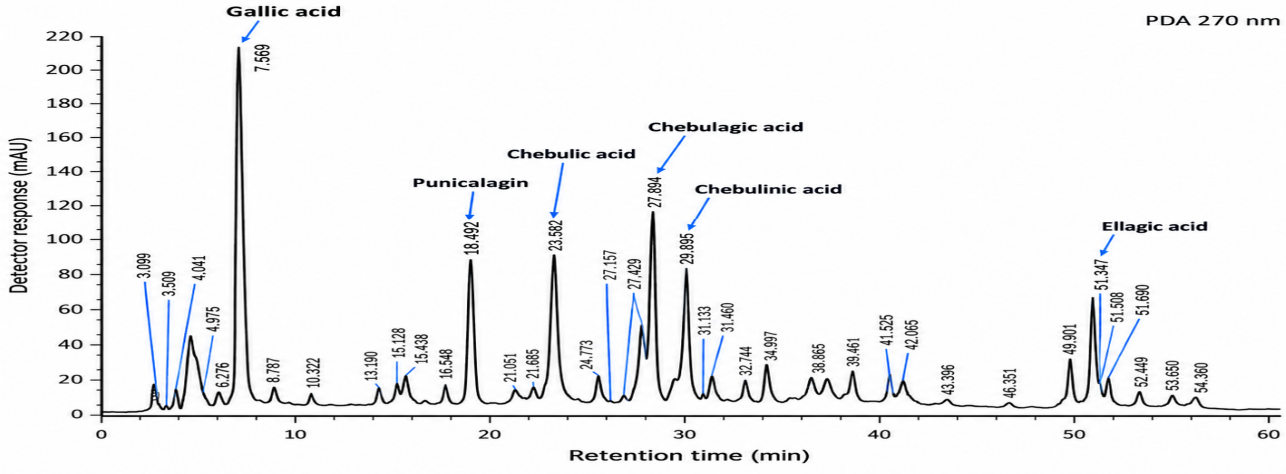


Figure 7. HPLC-PDA fingerprint recorded for the aqueous-stage extract of Terminalia chebula. Detection was at 270 nm (4 nm bandwidth); compound names are tentative assignments, not standard-confirmed identities. See Table 6.

Table 6. Exploratory interpretation of six labelled peak regions in Figure 7; retention times are observational and molecular assignments remain tentative.

Assigned marker / group	Apparent RT (min)	Chromatographic observation	Interpretation / required confirmation
Gallic acid Phenolic acid	7.569	Dominant, narrow peak with labelled apex. A single trace supports position, not identity.	Tentative; matching authentic standard RT and PDA spectrum, plus peak purity/integration, required.
Punicalagin Ellagitannin	~16.5–18.5	Smaller peak group near punicalagin label; multiple local signals may include related isomers.	Tentative region; authenticated standard/isomer separation and orthogonal LC–MS/MS required.
Chebulic acid Phenolic acid derivative	~23.6; 23.582 labelled nearby	Annotated middle retention-time region with adjacent minor peaks.	Tentative assignment; standard/PDA comparison and peak integration required.
Chebulagic acid Ellagitannin	~25.8–28.9	Crowded region with labelled positions near 25.834, 27.157, 27.894 and 28.984 min. No unique apex resolved.	Tentative; no increase or concentration claim. Needs standard spike, peak-purity analysis and integration.
Chebulinic acid Hydrolysable tannin	~29.9; 29.895 nearby	Prominent central-region signal next to overlapping labelled peaks.	Tentative; an authentic standard, spectral concordance and LC–MS/MS are needed.
Ellagic acid Phenolic dilactone	~49.5–51.7	Late signal group with multiple peaks; exact arrow-to-apex pairing unclear.	Tentative; standard and PDA/LC–MS/MS confirmation plus area integration required.

Table note. RT = retention time; approximate values are estimated from the chromatogram. PDA response at 270 nm was used for fingerprint description only. Peak areas, replicate integrations, calibration, system-suitability data, validated resolution and concentration results are not reported. LC–MS/MS and authenticated standards would be needed for definitive molecular assignment.

3.17 Literature-reported chemical constituents

Table 7 lists 30 named constituents from the published species-level inventory of Riaz and colleagues, and Figure 8 presents selected compounds by general chemical category.[41] These are literature-reported compounds, not 30 analytes detected in this study. Tentative assignments for the present extract are given only in Figure 7 and Table 6.

Table 7. Thirty previously reported species-level chemical constituents of Terminalia chebula, adapted from Riaz et al. (2017);[41] a literature inventory, not compounds identified in the present study.

No.	Chemical constituent	Review ref.*
1	Punicalagin (2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose)	[13]
2	Terflavin A	[13]
3	Terchebulin	[13]
4	Terchebin (1,3,6-trigalloyl glucose)	[14]
5	Terflavin B	[13]
6	Terflavin C	[13]
7	Terflavin D	[13]
8	Punicalin	[13]
9	Neo-chebulic acid	[15]
10	1,6-di-O-galloyl-D-glucose	[16]
11	Gallic acid (3,4,5-trihydroxybenzoic acid)	[16]
12	Casuarinin	[16]
13	Chebulanin	[16]
14	Corilagin	[16]
15	Ellagic acid (2,3,7,8-tetrahydroxychromeno[5,4,3-cde]chromene-5,10-dione)	[16]
16	Chebulagic acid	[16]

17	Chebulinic acid (1,3,6-Tri-O-galloyl-2,4-chebuloyl-β-D-glucopyranoside)	[16]
18	1,2,3,4,6-penta-O-galloyl-D-glucose	[16]
19	2,3,4,6-tetra-O-galloyl-β-D-glucose	[17]
20	Ethyl gallate (ethyl 3,4,5-trihydroxybenzoate)	[17]
21	Methyl gallate (methyl 3,4,5-trihydroxybenzoate)	[17]
22	Chebulaginic acid	[14]
23	4-O-methylgallic acid	[18]
24	Methyl (S)-flavogallonate	[18]
25	Methyl neochebulagate	[18]
26	Eugenol	[19]
27	Ascorbic acid	[19]
28	Triethyl chebulate	[20]
29	Tannic acid [2,3-dihydroxy-5-({[(2R,3R,4S,5R,6R)-3,4,5,6-tetrakis({3,4-dihydroxy-5-[(3,4,5-trihydroxyphenyl)carbonyloxy]phenyl}carbonyloxy)oxan-2-yl]methoxy}carbonyl)phenyl 3,4,5-trihydroxybenzoate]	[20]
30	2,4-Chebulyl-β-D-glucopyranose	[13]

Table note. An additional entry designated "X+" in the cited review has no definable compound name and is omitted. *Bracketed numbers in the right-hand column refer to the bibliography of the cited review.

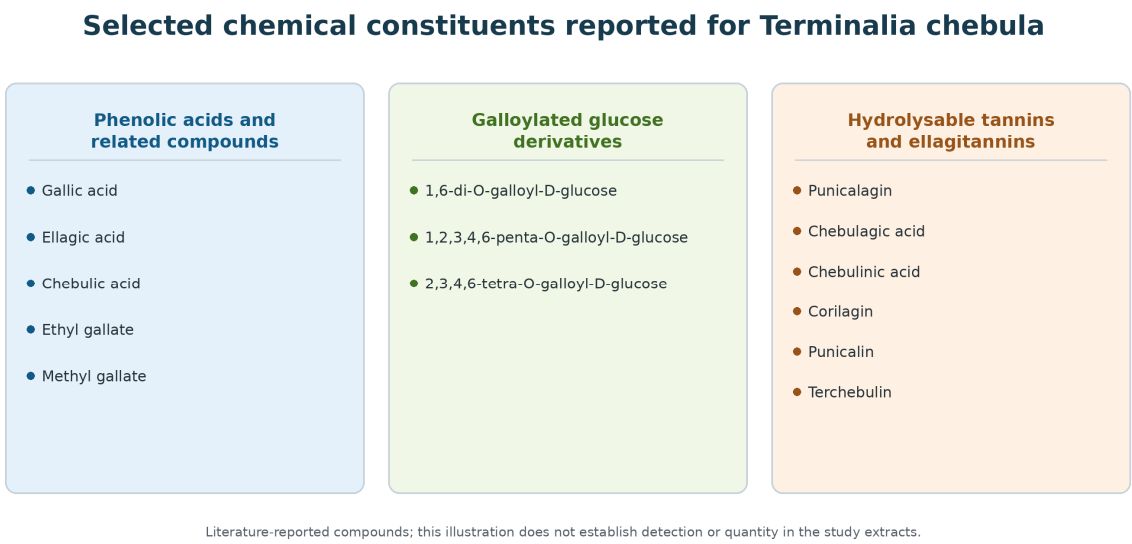


Figure 8. Previously reported species-level chemical constituents of Terminalia chebula grouped by broad chemical category. The diagram is a nomenclature and classification aid derived from published species chemistry; these constituents are not study identifications and are distinct from the six tentative labels in Figure 7 and Table 6. Chebulic acid, which is not listed in Table 7, is included from earlier chromatographic reports.[7,8,41]

4. Discussion

4.1 Extractive yields

The two most polar extraction stages accounted for 21.0% w/w of the 27.4% w/w combined recovery, while the first three stages contributed 6.4% w/w. This pattern is compatible with substantial polar-soluble material in the pulp, in line with published phenolic chemistry,[3,7,8] but an extraction-yield percentage is not a direct measurement of tannins, sugars or any particular compound. Because only one successive run was performed, solvent effects cannot be distinguished

from the effects of extraction order, residence time or batch variability.

Water- and alcohol-based preparations of *Terminalia chebula* are described in extraction studies,[27] although their methods and material may differ. The aqueous (13.8%) and ethanolic (7.2%) yields obtained here are descriptive values rather than comparative estimates of extraction efficiency or phenolic recovery.

4.2 Non-polar fractions

Positive fixed-oil and gum/mucilage responses in the petroleum ether and chloroform fractions suggest lipophilic and polysaccharide-like material, respectively. The detection of gum- or mucilage-like material in non-polar fractions may reflect physical carryover or other matrix effects. No fraction-specific molecular characterization or carryover measurement was performed, and the assay reactions do not constitute definitive proof of fixed oil or gum composition.

4.3 Alkaloid-reagent reactions

Four classical alkaloid reagents gave positive precipitates in three intermediate solvent fractions. Although the four reagents gave concordant responses, this concordance does not establish molecular identity or exclude interference by other constituents. No alkaloid was chromatographically isolated, and no authenticated control was included; the result is therefore described as alkaloid-reagent positivity.

4.4 Phenolic and flavonoid reactions

Phenolic- and flavonoid-screening reactions were recorded as positive in the three later extracts. Figure 6 depicts four literature-reported reference structures, and Figure 7 contains tentative chromatographic annotations for several reported *Terminalia chebula* phenolics.[7,8,22] Neither the colour tests nor the annotated trace provides validated quantification or definitive assignment of an individual molecule. The results therefore describe a phenolic-reactive profile without establishing the identity of individual constituents.

4.5 Discordance between phenolic and tannin screening

The mismatch between phenolic-series and tannin-series ferric chloride/gelatin results is one of the principal analytical limitations. Even when the same nominal reagent was used, the series were not confirmed as concentration- or pH-matched. It is therefore not possible to attribute the pattern to a specific tannin size, solubility change or heat-induced hydrolysis. Such mechanisms remain to be investigated.[7,8,40]

The aqueous-stage fraction was positive in the phenolic series and negative in the tannin series, whereas chloroform exhibited the converse pattern. Both findings are reported as observed, without assigning a cause. Matched-concentration positive and negative controls, total phenolic content and a suitable protein-precipitation tannin assay would be needed to determine whether analytical conditions explain the discordance.

The AETC colorimetric results (72.12 ± 0.54 mg GAE/g and 32.13 ± 0.25 mg QE/g) are consistent with detectable phenolic- and flavonoid-reactive material, but do not indicate the amount of protein-precipitating tannin or explain the contradictory individual screening reactions. A matched-condition confirmatory assay would be required to resolve this question.

4.6 Saponins and sterols in the polar extracts

Foam and sterol-colour endpoints indicate preliminary assay responses, not purified or chemically confirmed saponins or sterols. The positive chloroform foam test and negative acetone foam test may reflect matrix effects, but this explanation was not tested. Similarly, the later appearance of sterol-like colour reactions does not identify a sterol glycoside or prove a solvent-driven conversion.[36,42]

4.7 Carbohydrate, amino-acid and glycoside reactions

The carbohydrate-row Molisch and reducing-sugar reactions are consistent with sugar-reactive material, but the separate Molisch response in the glycoside screen cannot establish a glycosidic

bond. Glycoside identification would require a chemically selective assay, hydrolysis with aglycone characterization or an orthogonal molecular method. These unresolved observations are therefore not counted as confirmed compound identifications.

Ninhydrin, Millon and biuret probe related but distinct chemical features. Their positive responses do not represent a measured protein fraction or a known peptide concentration.[39] Likewise, the positive phenolic colour reactions do not show that the full mass of the aqueous-stage extract consists of polyphenols.[13,28,29]

4.8 Comparison with published extraction studies

Earlier studies have used aqueous, methanolic, ethanolic and other preparations for phytochemical or biological endpoints.[10,12,14,43,44] The five-solvent successive scheme used here additionally characterizes the constituent classes remaining after each extraction step. Its single-run nature, unmeasured processing endpoints and non-specific colour reactions limit direct comparisons with other extraction methods. Consequently, no mechanistic explanation can be offered for atypical fraction profiles.[28–30]

4.9 Rationale for selecting the aqueous extract

The aqueous-stage extract was selected for subsequent research because it gave the greatest recovered dry mass and showed several positive screening categories. Ethanol, however, had the largest number of positive categories. The addition of chloroform during the final maceration means the material may not have the same residual-solvent profile as a strictly water-only preparation. Residual chloroform and safety qualification were not assessed; separate residual-solvent analysis is required before biological use.[7,43]

4.10 Interpretation of HPLC-PDA assignments

The study chromatogram (Figure 7; Table 6) has a clearly labelled 7.569 min gallic-acid peak, while other labels are associated with adjacent or partially overlapping peaks. Assignment by the apparent retention-time region is exploratory. Authentic reference standards, PDA spectral matches, full gradient and system-suitability records and, ideally, LC–MS/MS would be required to confirm constituent identities. Without integrated peak areas or calibration, neither concentrations nor a relative increase in chebulagic acid can be inferred.[7,43]

Table 7 and Figure 8 summarize previously reported species-level constituents, which are distinct from the six tentative labels of the study fingerprint. Figure 6 shows reference molecular structures only.

Long hot percolation may affect heat-sensitive ellagitannins, but degradation products were not measured in these extractions.[7,8,40] Inter-step residual solvent was also not analytically measured. Chloroform was deliberately added at the final maceration stage, and removal from the resulting dry material was not verified. These methodological limitations should be considered before the preparation is compared with traditional aqueous decoctions or used in biological experiments.

4.11 Study limitations and reproducibility

The investigation represents a single botanical collection and one successive extraction series. Results were recorded as gravimetric percentages and binary reagent endpoints, without biological or analytical replication, matched assay controls, validated specificity, solvent-residue measurements or statistical comparisons. The full HPLC gradient, injection sequence, PDA peak spectra and integrated chromatographic data are not reported; hence the chromatogram is a preliminary fingerprint only. The proposed chemical mechanisms and molecular labels require confirmation in an independently repeated study.

5. Conclusion

In this preliminary single-batch study, successive extraction of 500 g dried *Terminalia chebula* fruit pulp yielded 2.8% petroleum ether, 1.5% chloroform, 2.1% acetone, 7.2% ethanol and

13.8% aqueous-stage residue. The aqueous stage yielded the greatest single mass, while ethanol showed the broadest positive qualitative screening profile. Discordant tannin and phenolic tests, non-specific Molisch results and alkaloid precipitation assays were retained as screening observations rather than definitive compound identifications. The HPLC-PDA fingerprint provided tentative marker assignments but no authenticated molecular identities or quantitative concentrations. Independent replicates and validated assays are required for reproducibility and chemical confirmation. The calculated aqueous-stage yield of 13.8% w/w differs from the 13.28% w/w corresponding to the recorded residue mass; because the definitive yield depends on the weighed residue mass, this difference is a limitation of the single-run determination.

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Funding and conflicts of interest

This research received no specific grant from any funding agency, and the authors declare no conflicts of interest. Biological experiments using related preparations are not included in the present study.

Ethics and plant collection

This study used harvested plant material phytochemical analysis and involved no experiments on humans or live animals. Forest-collection permit details are not reported.

Data availability

The extractive yield data, qualitative assay matrix and HPLC-PDA chromatogram are presented in the article. Original laboratory data and plant-authentication records are available from the corresponding author on reasonable request. Electronic instrument files, integrated peak reports, the complete gradient programme and system-suitability records are not available.

Use of artificial intelligence

Generative AI tools were used solely for language editing and grammar correction. No AI tools were used in study design, data collection, analysis, interpretation, or generation of scientific content. The authors take full responsibility for the final manuscript.

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