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Isolation And Characterization of Quercetin-3-O-Glucoside from *Terminalia Chebula* Retz Seeds

N. Abinaya[1], S. Angala Parameswari*[1], S.S Rajendran[2], M. Alagusundharam[3], Neelaveni Thangavel [2]
[1]Department of Pharmaceutical Analysis, RVS college of pharmaceutical sciences, Coimbatore, Tamil Nadu, India.
[2]Department of Pharmaceutical chemistry, RVS college of pharmaceutical sciences, Coimbatore, Tamil Nadu, India.
[3]Department of Pharmaceutics, RVS college of pharmaceutical sciences, Coimbatore, Tamil Nadu, India.

Correspondence: Dr. S. Angala Parameswari
Department of pharmaceutical Analysis, Rvs college of pharmaceutical sciences,
Coimbatore -641 402, Tamil Nadu, India.

ABSTRACT

Objective: The investigation was undertaken to isolate and characterize the bioactive flavonoid quercetin-3-glucoside from the seeds of *Terminalia chebula* Retz., an important medicinal plant widely recognized in traditional systems of medicine.

Methods: *Terminalia chebula* Retz seed extract were prepared by Soxhlet extraction with ethanol. The extracts were subjected to preliminary phytochemical screening and structural elucidation The ethanol extract, identified as the richest in flavonoids and possessing suitable polarity for broad phytoconstituent recovery, was fractionated through column chromatography to facilitate the systematic isolation of bioactive secondary metabolites. The obtained fractions were monitored by thin layer chromatography (TLC) using silica gel G as the stationary phase. Several solvent systems were tested, of which methanol: water (1:9) gave the best resolution. A single prominent fluorescent spot with an Rf value of 0.8 was observed under UV light (365 nm), indicating the presence of a distinct polar phytoconstituent. Fractions with similar Rf values were pooled, recrystallized in methanol, and purified crystals were obtained. Structural elucidation of the isolated compound was carried out using UV–Visible spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, Proton Nuclear Magnetic Resonance (^{1}H NMR), Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR), and Mass spectroscopy.

Results: Ethanol extract revealed a significant number of flavonoids compared with other solvent extracts. TLC and column chromatography confirmed the presence of a single major phytoconstituent. Spectral data (UV–Vis, FT-IR, ^{1}H NMR, ^{13}C NMR, and MS) confirmed that the isolated compound was quercetin-3-glucoside.

Conclusion: The study successfully demonstrated the extraction, isolation, and structural characterization of quercetin-3-glucoside from *Terminalia chebula* seeds, thereby providing a scientific basis for its phytochemical richness and potential applications in natural product research.

KEYWORDS: *Terminalia chebula* Retz, Phytochemical screening, Column chromatography, Spectroscopic characterization, Quercetin-3-glucoside

INTRODUCTION

Terminalia chebula Retz., commonly known as “Haritaki,” belongs to the family Combretaceae and is widely distributed in India, Nepal, China, and Southeast Asian countries. It is considered one of the most important medicinal plants in Ayurvedic and Unani systems of medicine and has been traditionally used for centuries due to its broad therapeutic value. [1] The seeds and fruits of Terminalia chebula Retz are consumed both as food supplements and as medicinal preparations in many Asian countries.

The plant is a rich source of bioactive constituents including tannins, flavonoids, glycosides, and phenolic compounds.[2] Pharmacological studies have reported that Terminalia chebula Retz exhibits a wide range of biological activities such as antimicrobial, antiviral, antitumor, anti-inflammatory, hepatoprotective, and antioxidant properties.[3,4,5] It has also been employed in traditional practices for digestive health, wound healing, and as an immune booster. [6]

Previous reports suggest that the seeds and fruits of Terminalia chebula Retz contain diverse phytochemicals including gallic acid, chebulagic acid, chebulinic acid, ellagic acid, and flavonoids. [7,8] Among these, flavonoids such as quercetin and its glycosides are considered important due to their strong antioxidant and pharmacological potential. Despite the multiple bioactive properties attributed to Terminalia chebula Retz, most studies have emphasized its fruit, whereas detailed investigation on the seed-derived flavonoids is relatively limited. [9]

The phytochemical analysis of Terminalia chebula Retz seeds revealed the presence of flavonoids and polyphenolic compounds with potent antioxidant activity, which could serve as a natural source for therapeutic applications.[10] The growing interest in naturally derived compounds, particularly flavonoids, highlights the importance of developing effective methodologies for their extraction, isolation, and structural characterization. In this study, an attempt was made to isolate and characterize quercetin-3-glucoside, a major flavonoid glycoside, from the seeds of Terminalia chebula Retz.

MATERIALS AND METHODS

Plant collection and sample preparation

The seeds of *Terminalia chebula* Retz. were purchased from Pooja Store, Sulur, Coimbatore, Tamil Nadu, India. The collected material was authenticated at the Botanical Survey of India, Southern Regional Centre, Coimbatore. The specimen was identified as *Terminalia chebula* Retz. (Family: Combretaceae) and an official authentication certificate was issued (Certificate No: BSI/SRC/5/23/2025-26/Tech/604, dated 14.01.2025). The authenticated seeds were shade-dried, cleaned to remove dust and foreign matter, and ground to a fine powder using a mechanical grinder. The powder was passed through a 100# sieve to ensure uniform particle size and stored in airtight containers to maintain phytochemical integrity until further analysis.

Preparation of Ethanolic seed extract

A total of 200 g of powdered seed material was subjected to Soxhlet extraction using ethanol (boiling point 78 °C), chosen for its suitable polarity to extract a broad range of bioactive compounds. The extraction was carried out continuously for 28 hours to ensure maximum recovery of phytoconstituents. After extraction, the solvent was evaporated under reduced pressure using a rotary evaporator to obtain the crude ethanolic extract. The extract was stored in amber-coloured containers for subsequent phytochemical screening and analytical studies.

Preliminary phytochemical screening

The crude ethanolic extract of Terminalia chebula seeds was analysed for the presence of major phytoconstituents, including carbohydrates, tannins, saponins, Phenolic Compounds flavonoids, glycosides. Standard qualitative procedures as described by Harborne [11]Thimmaiah SK [12] and Sadasivam S [13] were employed.

Thin Layer Chromatography (TLC)

TLC was performed to monitor the separation and purity of phytoconstituents from the ethanolic seed extract of *Terminalia chebula* Retz. Pre-coated silica gel G (100–200 mesh size) plates were used as the stationary phase. Different solvent systems including chloroform, toluene, and methanol in various ratios were initially tested. Methanol: Water (1:9) was selected as the optimal developing solvent due to its ability to achieve the best resolution. The ethanolic extract showed a single prominent fluorescent spot under UV light at 365 nm, with an Rf value of 0.8, indicating a distinct phytoconstituent. Fractions corresponding to this spot were collected and recrystallized in methanol to obtain purified crystals, which were preserved for further structural and analytical characterization. The clear and distinct separation of phytoconstituents confirmed successful extraction of polar compounds, consistent with the polarity of the solvent system used.

Rf = Distance travelled by the solute/Distance travelled by the solvent

Column Chromatography

Column chromatography was performed using silica gel (200 mesh) as the stationary phase. The column was packed by wet slurry method to ensure uniform packing and prevent air gaps. The crude ethanolic extract (2 g) was carefully adsorbed onto a small portion of silica gel and introduced into the packed column. Elution was initiated with100% petroleum ether to remove non-polar impurities, followed sequentially by100% chloroform and methanol in increasing polarity. This gradient elution yielded ten fractions; however, complete separation of compounds was not achieved. To improve resolution, further elution was carried out using methanol: water (1:9) mixtures followed by a 100% water, which produced thirty fractions. The polar eluates afforded a single purified compound (50 mg), which was preserved for subsequent analytical and structural characterization. The combined application of TLC and column chromatography confirmed the efficient isolation of polar bioactive constituents from *Terminalia chebula* Retz seeds, validating the effectiveness of the chosen chromatographic procedure for phytochemical separation. Spectroscopic and Analytical Studies

Uv visible spectroscopy

UV-Visible spectra of the isolated compounds were recorded using a Shimadzu UV-1800 double beam spectrophotometer in the wavelength range of 200–800 nm. Methanol was used as a solvent, and quartz cuvettes of 1 cm path length were employed for measurements.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra were recorded on a Bruker Alpha TKBR spectrophotometer equipped with an ATR accessory. Samples were analysed in the range of 4000–400 cm^{-1} to identify the functional groups present in the isolated constituents.

Nuclear Magnetic Resonance (NMR)Spectroscopy

^1H and ^{13}C-NMR spectra were obtained on a Bruker AV NMR spectrometer operating at 500 MHz and 125 MHz, respectively, using 5 mm probes. Deuterated dimethyl sulfoxide (DMSO-d_6) was used as solvent, and tetramethylsilane (TMS) served as the internal standard.

Mass spectroscopy

Mass spectra were recorded on a Waters Q-TOF Micro mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ion mode. The obtained molecular ion peaks were used to confirm the molecular weight of the isolated compounds.

RESULTS AND DISCUSSION

Total Extraction Yield

The powdered seeds of *Terminalia chebula* Retz (200 g) were subjected to Soxhlet extraction using ethanol as solvent (b.p. 78 °C) for 28 h. After evaporation, a solid residue of 81.4 g was obtained, giving a yield of

40.7%. This high percentage yield indicates efficient extraction of ethanol-soluble phytochemicals. Extraction details were listed in Table 1.

Table 1. Extraction data of Terminalia chebula Retz seed powder

Parameter	Details
Plant material used	*Terminalia chebula* seed powder
Solvent used	Ethanol
Boiling point of solvent	78 °C
Extraction method	Soxhlet extraction
Duration of extraction	28 h
Weight of extract	81.4 g
Percentage yield	40.7%
Observation	Substantial recovery of phytoconstituents

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the ethanolic extract revealed the presence of flavonoids, tannins, and carbohydrates, while alkaloids, glycosides, steroids, proteins, saponins, and starch was absent. These results suggest that ethanol selectively extracts polyphenolic compounds, in agreement with previous reports on *Terminalia chebula* Retz.

Thin Layer Chromatography (TLC)

TLC was carried out using silica gel plates as stationary phase. Various solvent systems were tested, and methanol: water (1:9) gave the best separation, producing a distinct fluorescent spot with Rf value of 0.8 under UV (365 nm), which was shown in figure.1 suggesting the presence of a major phytoconstituent. [14]

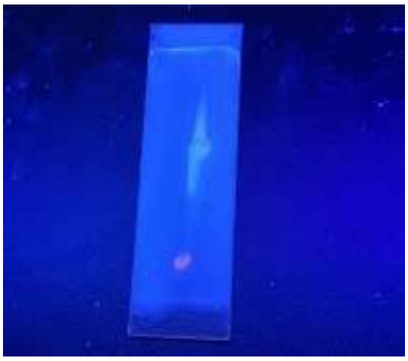

Fig.1. TLC of isolates

Isolation by Column Chromatography

The ethanolic extract was fractionated by column chromatography using silica gel (200 mesh). Initial elution with petroleum ether, chloroform, and methanol did not yield distinct compounds. Subsequent gradient elution with methanol: water systems (9:1 and 5:5) was also ineffective. Finally, methanol: water (1:9) followed by 100% water successfully eluted a polar fraction, resulting in isolation of ~50 mg of a pure compound, which was subjected to further characterization. Similar column fractionation techniques have been reported for the isolation of flavonoid glycosides from medicinal plant seeds.

UV-Visible Spectroscopy

The isolated compound exhibited prominent absorption peaks at 294 nm (Abs 2.770), characteristic of $\pi \rightarrow \pi^*$ transitions in aromatic flavonoids. Additional peaks at 266 nm and 402 nm suggested extended conjugation were listed in Table 2, Fig.2. Such UV absorbance patterns are in agreement with literature reports and its glycosides, confirming the flavonoid nature of the isolated compound. [15,16]These findings support the presence of flavonoid-type compounds.

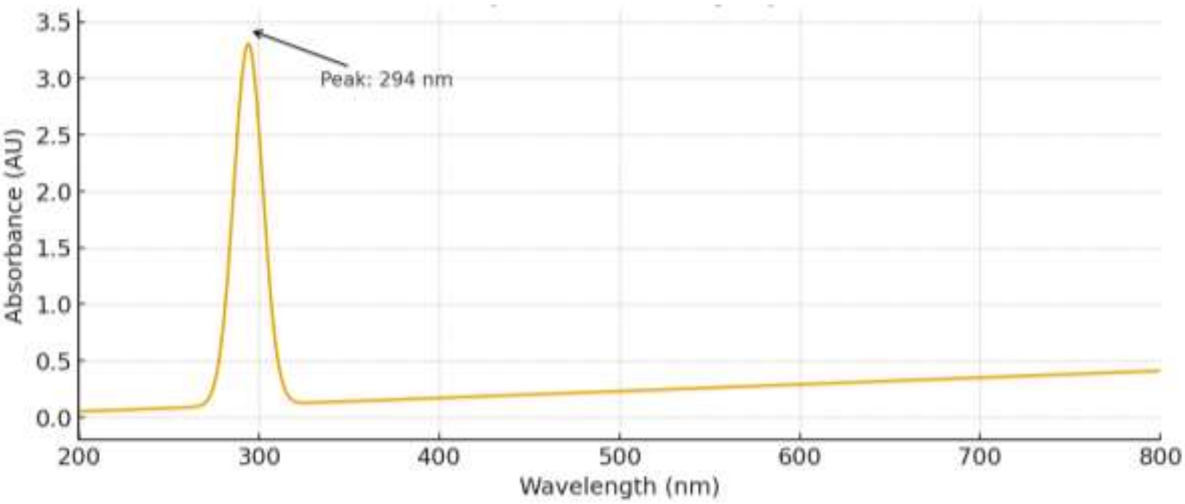

Fig. 2: UV–Visible spectrum of Terminalia chebula Retz isolates

Table 2. UV–Visible spectral data of isolated compound

Wavelength (nm)	Absorbance	Interpretation
294.00	2.770	π→π* transitions of aromatic rings (flavonoids)
266.00	1.641	Conjugated systems (tannins, phenolic acids)
402.00	0.401	Extended conjugation (flavonoids)

Fourier Transform Infrared (FT-IR) Analysis

The FT-IR spectrum revealed strong absorption bands at 3852–3670 cm^{-1} (O–H stretching), 1696 cm^{-1} (C=O stretching of conjugated systems), and 1053 cm^{-1} (C–O stretching), along with peaks for aliphatic C–H and aromatic structures were listed in Table 3; Fig. 3. These functional groups are consistent with a flavonoid glycoside such as quercetin derivative. Similar FT-IR spectral features have been observed in previously characterized glucoside compounds.[17,18]

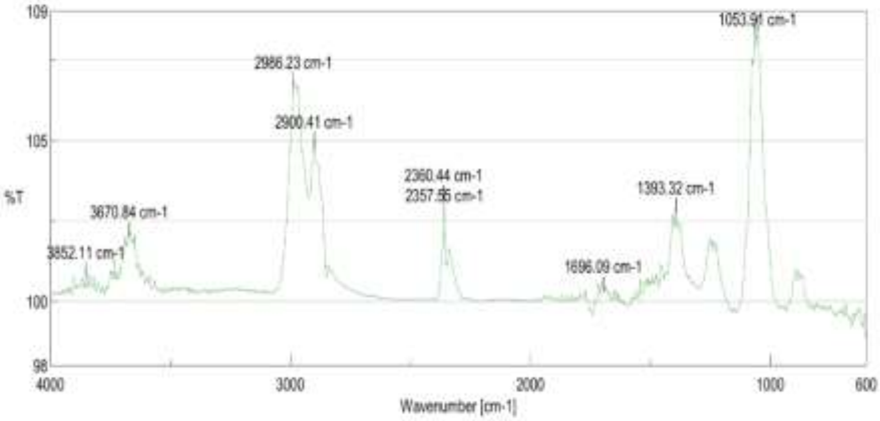

Fig. 3: FT-IR spectrum of isolated compound

Table 3. FT-IR data of isolated compound

Wavenumber (cm^{-1})	Type of vibration	Phytochemical relevance
3852.11, 3670.84	O–H stretching	Phenolic hydroxyl groups
2986.23, 2900.41	O–H stretching	Aliphatic CH_2
1696.09	C=O stretching	Conjugated carbonyl (flavonoids)
1393.32	C–H bending	Alkanes
1053.91	C–O stretching	Flavonoid glycosides

^1H NMR Analysis

The ^1H-NMR spectrum (MeOD) displayed signals characteristic of a flavonoid skeleton. Peaks at δ 6–8 ppm corresponded to aromatic protons, while downfield singlets at δ 9.77 ppm indicated phenolic hydroxyl protons. Additional signals at δ 1.36 (methyl) and δ 4.21 (methylene) suggested hydroxymethyl substituents were listed in Table 4; Fig. 4. The data strongly suggest a polyhydroxy flavonoid such as quercetin. [14]

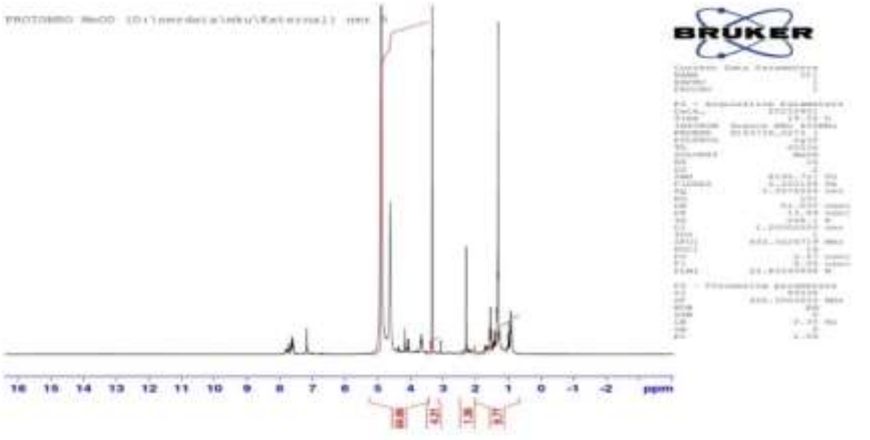

Fig. 4: ^1H NMR spectrum of isolated compound

Table 4. ^1H NMR spectral data of isolated compound

δ (ppm)	Proton type	Interpretation
1.36	CH_3 (methyl)	Aliphatic region
4.21	CH_2OH (methylene)	Hydroxymethyl protons
6–8	Aromatic protons	Flavonoid aromatic rings
9.77	Deshielded OH/CHO	Phenolic or aldehydic protons

^{13}C NMR Analysis

The ^{13}C-NMR spectrum showed 15 characteristic carbon signals. A strong resonance at δ 177.3 ppm was assigned to the C-4 carbonyl carbon of the flavanol nucleus. The signal at δ 133.1 ppm (C-3) confirmed O-glycosidic substitution, while δ 104.4 ppm corresponded to the anomeric carbon of glucose, confirming β-D-glucoside linkage. Additional glucose carbons resonated between δ 78–71 and δ 62.5 ppm were listed in table 5; Fig. 5. These assignments confirm the structure as quercetin-3-O-glucoside. These NMR features are in good agreement with reported data for quercetin-3-O-glucoside.[19,20]

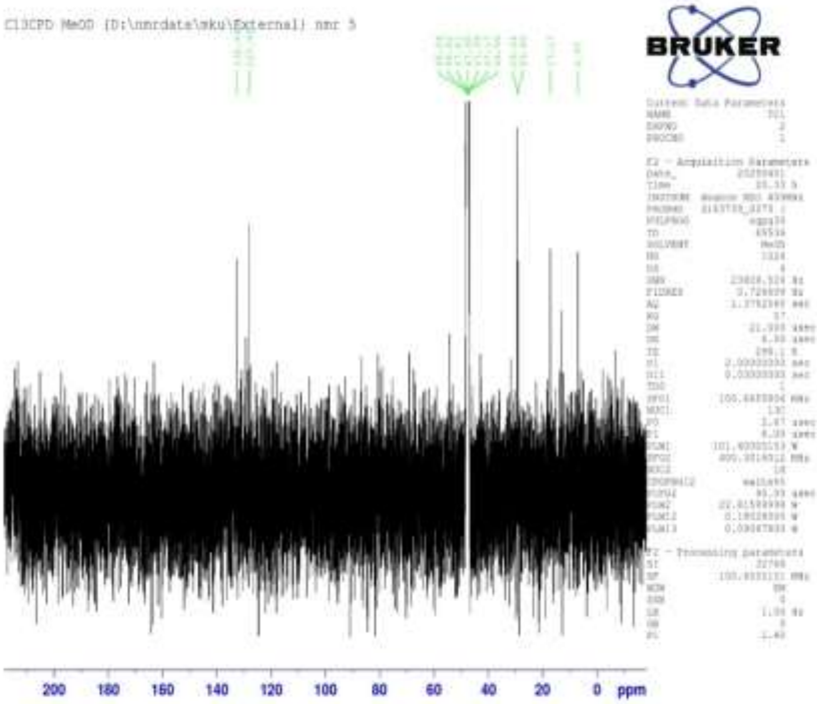

Fig. 5: ^{13}C NMR spectrum of isolated compound

Table 5. ^{13}C NMR spectral data of isolated compound

δ (ppm)	Carbon Type	Interpretation
177.3	C-4 (carbonyl)	Flavanol ketone present
133.1	C-3 (Cq–O–Glc)	Sugar attached at C-3
104.4	C-1″ (anomeric)	β-D-glucose anomeric carbon
78–71 & 62.5	C-2″–C-5″ (CH) & C-6″ (CH_2)	Complete glucose carbon set

Mass Spectrometry

The ESI-MS spectrum exhibited a molecular ion peak at m/z 465.5 $[M+H]^+$, consistent with quercetin-3-O-glucoside (MW~464 g/mol). Fragment ions at m/z 301.4 and 303.6 corresponded to the aglycone quercetin unit, while additional fragments at m/z 174.4 and 214.4 indicated partial glucose cleavage. A smaller fragment at m/z 121.4 reflected B-ring cleavage, characteristic of catechol-type flavonoids (Table 6; Fig. 6). This fragmentation pattern corroborates previous literature reports for quercetin-3-glucoside .[21,22,23]

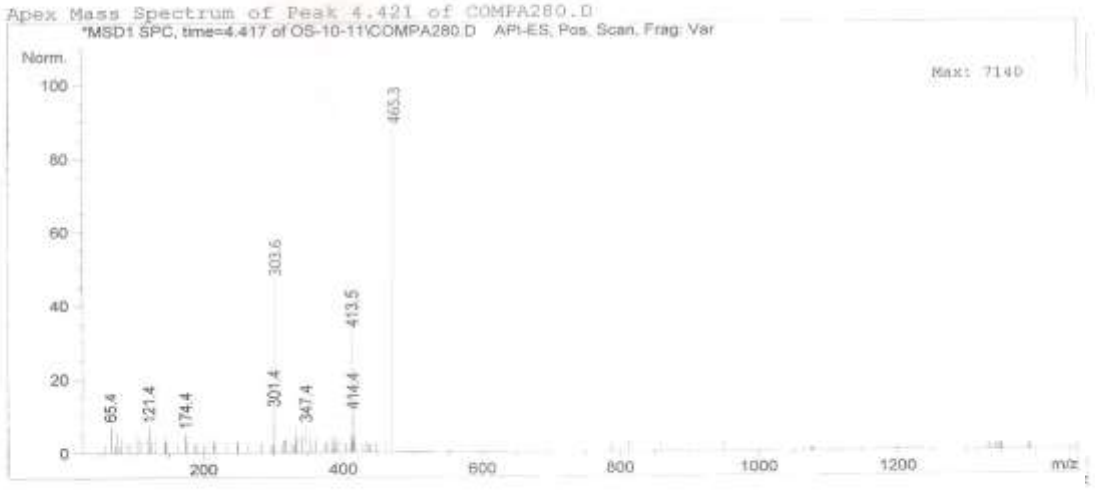

Fig. 5: Mass spectra of isolated compound

Table 6: Mass Spectral Data of the Isolated Compound

m/z(Observed)	Probable Fragment	Interpretation
65.4	Low-mass fragment	Possibly a sugar-related or ring cleavage fragment
121.4	$C_7H_5O_2^+$ or similar	Characteristic fragment from B-ring cleavage (catechol-type)
174.4	Sugar fragment ($C_6H_{10}O_5^+$ or similar)	Glucose fragmentation
214.4	$C_9H_{10}O_6^+$ or similar	Sugar + partial ring fragment
301.4	$[M–H]^-$ of Quercetin aglycone	Base peak for quercetin ($C_{15}H_{10}O_7$), characteristic ion
303.6	$[M+H]^+$ of Quercetin aglycone	Protonated quercetin

Structural Confirmation

The combined spectroscopic data (UV, FT-IR, NMR, and MS) confirmed the isolated compound as **quercetin-3-O-glucoside**, a glycosylated flavonoid. Its spectral features, glycosidic linkage at C-3, and fragmentation pattern matched literature reports. The glucose moiety enhances solubility and bioavailability, while the quercetin aglycone contributes to effective antioxidant, anti-inflammatory, and antimicrobial agent.

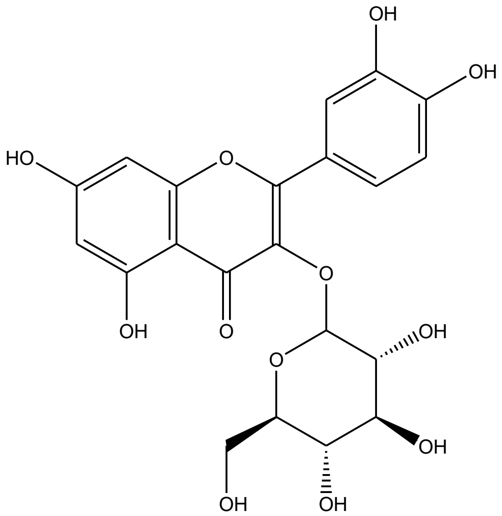

Fig.6.Structure of Quercetin-3-Glucoside

CONCLUSION

The present investigation successfully demonstrated the extraction, isolation, and characterization of bioactive compounds from the seeds of *Terminalia chebula* Retz. Ethanol proved to be an effective solvent, yielding a higher percentage of phytoconstituents compared to other extraction approaches. Preliminary phytochemical screening confirmed the presence of flavonoids, tannins, and carbohydrates. TLC and chromatographic studies facilitated the isolation of a major compound, which was further characterized using UV–Visible, FT-IR, ^{1}H and ^{13}C NMR, and mass spectrometry. Spectral analysis confirmed the isolated compound as quercetin-3-O-glucoside, a flavonoid glycoside of significant pharmacological importance. The findings highlight *Terminalia chebula* Retz as a rich source of flavonoids and provide a scientific basis for its traditional use. This work establishes a reliable and efficient approach for the extraction and structural elucidation of bioactive flavonoids from medicinal plants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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