RESEARCH PAPER

Extraction, Phytochemical Characterization, and Isolation of Coumarin from the Carnivorous Plant *Utricularia stellaris* L

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Abstract:
Background: *Utricularia stellaris* L. (Lentibulariaceae), an aquatic carnivorous plant, is traditionally employed in folk medicine for various ailments, including inflammation and malaria. Despite its ethnopharmacological significance, a comprehensive phytochemical and pharmaceutical characterization of this species remain lacking. **Objective:** The goal of this study was to do a full phytochemical study of *Utricularia stellaris* L. (Lentibulariaceae) and to find its bioactive constituent, coumarin, in order to support its traditional medicinal usage with scientific proof. **Methods:** The whole plant was dried, ground up, and then extracted with solvents such as water, ethanol, ethyl acetate, and binary mixtures of ethanol and ethyl acetate (60:40 and 30:70). A qualitative phytochemical study was done to find the main secondary metabolites. We employed the systematic isolation procedure to separate coumarin from the raw ethanol extract. We used high-performance liquid chromatography (HPLC), alkaline tests, thin-layer chromatography (TLC), UV–Vis spectroscopy, liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS), proton nuclear magnetic resonance (^{1}H-NMR), and Fourier-transform infrared (FTIR) spectroscopy to identify compound. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, glycosides, and terpenoids. A 60:40 ethanol:ethyl acetate solvent system proved optimal for secondary metabolite extraction. The isolation protocol yielded 5.3% w/w of a black crystalline compound, confirmed as coumarin ($C_9H_6O_2$) with a purity of approximately 90%. Characterization data included: TLC Rf value of 0.28, UV-Vis λmax at 240 and 300 nm, HPLC retention time of ~16.7 minutes, and a prominent LC-ESI-MS ion at *m/z* 144.0412 $[M+H]^+$. Structural elucidation was further confirmed by FTIR and ^{1}H-NMR spectroscopy. **Conclusion:** This study successfully demonstrates that *U. stellaris* is a promising natural source of high-purity coumarin. The optimized extraction and isolation workflow provides a foundation for the large-scale production of this bioactive compound. Our findings provide scientific validation for the plant's traditional uses and establish a solid groundwork for future pharmacological, toxicological, and preclinical investigations into the therapeutic applications of *U. stellaris*-derived coumarin.

Keywords: *Utricularia stellaris,* carnivorous plant, coumarin, phytochemical screening, LC-ESI-MS, ^{1}H-NMR, and traditional medicine.
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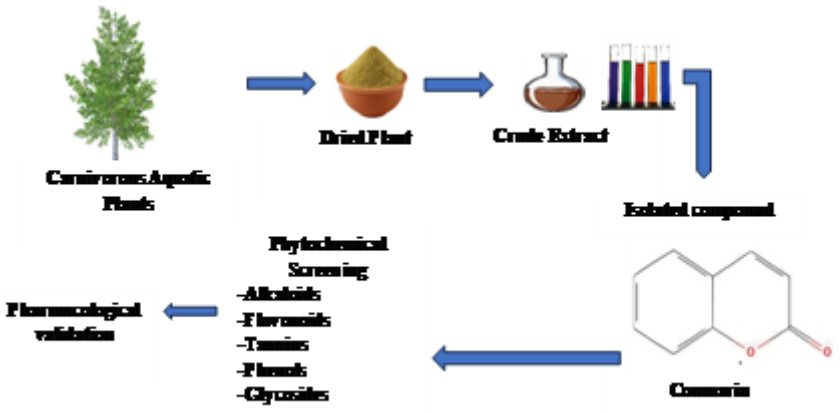
Graphical Abstract:



The graphical abstract shows how compounds were taken from a carnivorous aquatic plant, identified, and then separated into coumarin. extracting plant material, extracting the crude drug out, isolating coumarin, and completing physiochemical assays and confirmation are the primary steps.

1. Introduction:

Plants have been a cornerstone of traditional medicine for millennia and continue to be an invaluable resource for modern drug discovery, providing a vast and chemically diverse pool of bioactive molecules [1]. The genus Utricularia and the family Lentibulariaceaecomprising carnivorous aquatic and terrestrial plants, has garnered interest not only for its unique ecology but also for its ethnopharmacological significance [2].*Utricularia stellaris* L. commonly known as the Star Bladderwort and locally referred to as Jol-Jhanjhi, is a free-floating aquatic plant found in the wetlands of Asia, including the Murshidabad and North 24 Parganas districts of West Bengal, India [3].

People regularly use it in traditional medicine, and it is known as Jol-Jhanjhi or Jhanjhi. Different cultures have used this herb for a long time to cure wounds, burns, inflammation, malaria, renal disease, and trouble urinating [3]. This old use shows how important it is in ethnobotany and how traditional medicine is linked to the plants that grow well in a certain area. We still don't know much about the specific phytochemicals in the plant that people use to treat themselves and that make it a medicine. This indicates a potential avenue for additional investigation that may yield significant insights into the plant's prospective applications in modern medicine and its mechanisms of action [4].

1,2-benzopyrones, often known as coumarins, are found in plants all over the world. They are a type of lactonic chemical. People know that these substances have a lot of impacts on the body, such as being antibacterial, anti-inflammatory, anticoagulant, and antimalarial. They also come in many distinct shapes [5-6]. These positive results are especially interesting because we have used *U. stellaris* in herbal therapy before [7]. Also, keep in mind that the polarity of the solvents employed in the extraction process has a huge effect on how well these bioactive chemicals may be extracted out of plants [8]. This is a very essential step because picking the correct solvent could make the coumarins function better in therapy by making them more bioactive and easier to produce [9].Plants all throughout the world contain 1,2-benzopyrones, which are also called coumarins. They belong to the lactonic family of chemicals. People know that these things have a multitude of effects on the body, such killing bacteria, reducing inflammation, stopping blood clots, and fighting malaria. They also come in a lot of different shapes [6]. These good outcomes are especially interesting because we have employed *U. stellaris* in herbal medicine before [7]. Also, remember that the polarity of the solvents used to extract these bioactive compounds from plants has a big effect on how well they work [8].

Coumarin is a naturally occurring benzopyrone widely recognized for its diverse pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, anticoagulant, and anticancer effects. Beyond its biological activities, coumarin possesses significant pharmacogenomic relevance because its metabolism in humans is largely mediated by cytochrome P450 enzymes, particularly CYP2A6. Genetic polymorphisms in CYP2A6 substantially influence coumarin biotransformation, resulting in marked interindividual variability in therapeutic efficacy and toxicity [4-9].

Several studies have demonstrated that individuals carrying reduced-function CYP2A6 alleles exhibit slower coumarin metabolism and altered pharmacokinetic profiles. Such genetic variability highlights the importance of considering personalized therapeutic strategies when developing coumarin-based interventions. Therefore, identification of coumarin from medicinal plants such as *Utricularia stellaris* may provide a valuable natural source for future investigations focused on genotype-guided therapies and precision medicine [5-9].

The primary objectives of this study were to: (1) systematically evaluate the phytochemical profile of *U. stellaris* whole-plant extracts using a gradient of solvent polarities to identify the most effective extraction conditions; (2) develop a simple and effective protocol for the isolation of coumarin from the crude extract; and (3) employ a comprehensive suite of advanced analytical techniquesincluding TLC, HPLC, LC-ESI-MS, FTIR, and NMRfor the unequivocal structural characterization and purity assessment of the isolated compound [9-10]. This research aims to provide a solid scientific rationale for the traditional use of *U. stellaris* and to establish a pharmaceutical foundation for its potential development as a source of therapeutic agents.

Although the present study primarily focuses on phytochemical isolation and characterization, the identification of coumarin establishes a scientific basis for future pharmacogenomic and personalized medicine research.

2. Materials and Methods

2.1. Plant Material and Extraction

The plantswere collected from the freshwater in the West Bengal, India, districts of North 24 Parganas and Murshidabad.The plant was authenticated (Voucher Specimen No: MS-03) and cleaned it very carefully. The material was shade-dried at a temperature below 50°C to keep chemicals that are sensitive to heat safe. Then, a machine ground it up. The powder was combined with 100 cc of five different solvents for 24 hours. Ethyl acetate, ethanol, water, and two combinations of ethanol and ethyl acetate (60:40, v/v and 30:70, v/v) were used as solvents. The mixture was shaken from time to time [11]. We filtered the extracts and then

put the filtrates under low pressure to make them thicker so we could see them better [12].

2.2. Qualitative and Quantitative Phytochemical Analysis

We used standard chemical tests to find alkaloids (Wagner's test), flavonoids (Alkaline reagent test), tannins and phenols (Alkaline reagent), glycosides (Concentrated H_2SO_4 test), proteins (Xanthoproteic test), carbohydrates (Benedict's test), amino acids (Ninhydrin test), fats (Stain test), and terpenoids (Salkowski test).

We utilized the Folin-Ciocalteu method to determine the total phenolic content, the Vanillin-HCl method to find flavonoids, the Folin-Denis method to find tannins, the Ninhydrin method to identify proteins, and the Ninhydrin method to find amino acids, lipids, and glycosides. The results were assessed in mg/g equivalents of the standards that were utilized, namely gallic acid and leucine.

2.3. Isolation and Purification of Coumarin

To get rid of the volatile phenolic components, the crude ethanol extract was roasted in a hot air oven at 60°C for 2 to 3 hours [13]. The dark residue was mixed with 10 ml of distilled water for 30 minutes to get rid of water-soluble contaminants. Then, it was dried again by letting the water evaporate. The last amount of residue was ground up in a mortar and pestle with 40% ethanol, which converted it into black coumarin crystals. The crystals were gathered, and the amount of powder utilized (0.265 g from 5 g) was figured out.

2.4. Characterization of Isolated Coumarin

2.4.1. Biochemical Test for Coumarin Detection

We utilized a standard alkaline hydrolysis assay to check for coumarin or coumarin-like metabolites in the culture of bacteria. After 72 hours of incubation, the culture broth was spun at 8,000 × g for 15 minutes to get rid of the cells. 1 ml of the cell-free supernatant was put into a clean test tube. We added two to three drops of a 10% (w/v) NaOH solution to the supernatant. The liquid was thoroughly mixed with a vortex and then left at room temperature. The appearance of a pale-yellow color within 10 minutes was identified as a significant indicator of coumarin derivatives [14].

2.5. Analysis of Thin-Layer Chromatography (TLC)

We utilized TLC to separate the coumarin portions of the crude extract and see for the first time that they were there [15].

2.5.1 Stationary and Mobile Phase

We did TLC on silica gel plates that were already coated (Silica gel 60 F_{254}, 0.25 mm thick). The mobile phase was made up of 40% chloroform and 60% absolute ethanol. We made a novel solvent system and put it in a rectangular glass TLC chamber that was lined with filter paper to fill the air with it [16].

2.5.2 Sample Application and Development

We used a capillary tube to put the crude extract and a reference coumarin solution (1 mg/mL in methanol) on the TLC plate as separate bands 1.5 cm from the bottom edge [17]. They put the plate in the pre-saturated chamber, making sure that the solvent level was below the line where it would be used. The development continued until the solvent front was 8 cm away from where it originated [18].

2.5.3. Visualization and Calculation

The solvents on the plate went away when we let it dry in a room with fragrances. When ultraviolet (UV) light was used at 254 nm and 365 nm, it was feasible to see the split bands. To find the Retention factor (Rf) value for each band we had solved, we used the formula Rf = (Distance travelled by the solute) / (Distance travelled by the solvent front). We compared the sample bands' Rf values and fluorescence characteristics to those of the real coumarin standard.

2.6. Ultraviolet-Visible (UV-Vis) Spectroscopy

We used the UV-Vis absorption spectra of the purified molecule to figure out what kind of chromophore it had. The study was finished with a Shimadzu UV-2600 spectrophotometer. To get a solution that was about 10 µg/mL, we combined the sample with 100% ethanol. We used a quartz cuvette with a 1 cm path length to scan the spectra between 200 and 400 nm. The blank just had ethanol in it.

2.7. High-Performance Liquid Chromatography (HPLC)

We utilized HPLC to see how pure the isolated chemical was and how well it held onto items relative to a true standard [19]. We used an Agilent 1260 Infinity II system and a diode array detector (DAD) to do the analysis. The samples were separated using a reversed-phase C18 column (ZORBAX Eclipse Plus, 4.6 x 150 mm, 5 µm) that was maintained at 25°C. The mobile phase was 0.1% Trifluoroacetic acid (TFA) in HPLC-grade methanol, and it moved at a rate of 1.0 mL/min [20]. We put the sample in methanol and gave it 20 µL. It was possible to see at 275 nm. We quickly compared how long it took for the sample peak to stay in place with how long it took for a real coumarin standard (Sigma-Aldrich) to stay in position when we tested them both the same manner.

2.8. Liquid Chromatography-Electrospray Ionization Mass Spectrometry (LC-ESI-MS)

We employed high-resolution mass spectrometry to find the exact molecular weight and make sure the structure was right [21]. We used a Waters XEVO G2-XS Q-Tof mass spectrometer with an Electrospray Ionization (ESI) source to do the research. To do a direct infusion, the purified sample was combined with a solution of methanol and water (1:1, v/v) that had 0.1% formic acid in it. Then, it was injected at a rate of 10 µL/min. The mass spectrometer worked in positive ion mode.

The mass spectrometer was set to positive ion mode. The standard source featured a capillary voltage of 3.0 kV, a source temperature of 120°C, a desolvation temperature of 350°C, and a flow of desolvation gas (N_2) of 800 L/h. We collected data with a mass-to-charge ratio of 50–1200 Da and found the $[M+H]^+$ ion.

2.9. Nuclear Magnetic Resonance (NMR) Spectroscopy

We used ^{1}H-Nuclear Magnetic Resonance (NMR) spectroscopy to gain a comprehensive image of the structure [22]. At 25°C, the Bruker Ascend 800 MHz spectrometer took the spectra. The cleaned product weighed around 2 mg and was inserted into a 5 mm NMR tube after being combined with 0.6 mL of deuterated chloroform ($CDCl_3$). The changes in chemicals (δ) are measured in parts per million (ppm) against the $CDCl_3$ residual solvent signal, which is 7.26 ppm.

2.10. Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was employed to identify the molecule's distinctive functional groups. We used a PerkinElmer Spectrum Two FTIR spectrometer with a Universal ATR (Attenuated Total Reflectance) adapter to get the infrared spectrum. A little bit of the solid, purified sample was put directly on the diamond crystal, and pressure was used to make sure they touched. There were 32 scans of the spectra between 4000 and 450 cm^{-1}, and each scan had a resolution of 4 cm^{-1}. Before looking at the samples, background spectra were gathered.

3. Results and Discussion

3.1. Phytochemical Screening

The qualitative analysis (Table 1) showed that the ethanol: ethyl acetate (60:40) extract was positive for all of the main phytochemicals, except for terpenoids. There are also a number of different parts in ethanol and water extracts. Ethyl acetate worked well to remove fats and lipids from items.

Table 1:A qualitative phytochemical analysis of the carnivorous plant *Utricularia stellaris* Leaves' Extracts

Sr No	Test	Water	Ethanol	Ethyl Acetate	EtOH :EA (60:40)	EtOH: EA (30:70)
1	Flavonoid	Yes	Yes	No	Yes	Yes
2	Fat	Yes	Yes	Yes	Yes	Yes
3	Tannin and Phenol	Yes	No	No	Yes	Yes
4	Glycosides	Yes	Yes	No	Yes	Yes
5	Alkaloids	Yes	Yes	Yes	Yes	Yes
6	Protein	Yes	Yes	No	No	Yes
7	Carbohydrate	Yes	No	Yes	Yes	Yes
8	Amino Acid	Yes	No	No	No	No
9	Lipid (Steroid)	Yes	No	Yes	No	No
10	Terpenoid	Yes	Yes	No	No	No

The extract of ethanol and ethyl acetate (60:40) had the highest flavonoids (OD = 1.523), while the extract of ethanol had a lot of phenolics.

3.2. Isolation and Identification of Coumarin

The isolation process took 5 grams of plant material and turned it into 0.265 grams of black coumarin crystals, which is a yield of 5.3% w/w. Fig. 1 illustrates a diagram of the methods used to clean and isolate coumarin.

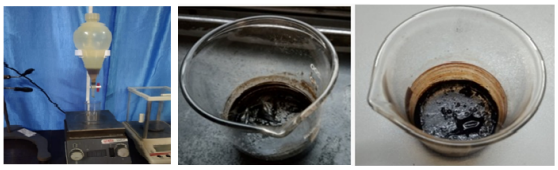


Figure:1: A graphic showing how to isolate and purify coumarin.

3.3. Biochemical Test for Coumarin

The biochemical test for coumarin was positive. The results confirmed the presence of coumarin since the reaction mixture turned a clear yellow or light-yellow tint. Figure 2 displays the biochemical test for coumarin.

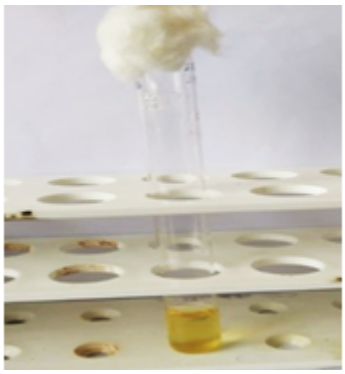


Figure:2: Test for coumarin in the body.

3.4. Thin Layer Chromatography (TLC) Analysis

For the TLC test, we utilized a stationary phase with a mesh size of 60–120 and a mobile phase that was 40% chloroform and 60% ethanol. One part of the TLC plate had a retardation factor (Rf) value of 0.28. The computer test of the spot showed that the chemical was 89.2% pure. The TLC profile of the isolated chemical (Fig. 3) showed one clear spot with an Rf value of 0.28, which means that the compound was present as a predominant constituent.

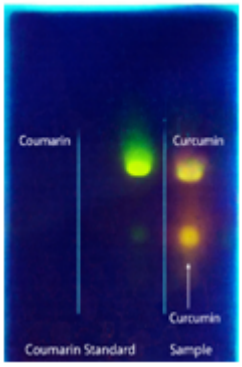


Figure3: The TLC profile of the isolated drug displays one clear spot with an Rf value of 0.28.

3.5. UV-Spectroscopy

The chemical's UV/Vis absorption spectra showed two clear absorption maxima (λmax): one at 300 nm with an absorbance of 0.35 and another at 240 nm with an absorbance of 0.25.

3.6. Chromatographic Analysis: Standard and Sample

We used a reversed-phase C18 column with a mobile phase of 0.1% TFA in methanol that flowed at 1.0 mL/min to separate the compounds.

We detected a single, clear, and symmetrical peak that lasted for 16.786 minutes when we looked at the standard chemical at two separate wavelengths (254 nm and 290 nm). The shape of the top and how far apart it is from the others suggest that it was done successfully, considering the circumstances. Figure 4 shows the HPLC chromatogram of the coumarin standard. It has a peak that lasts for 16.786 minutes.

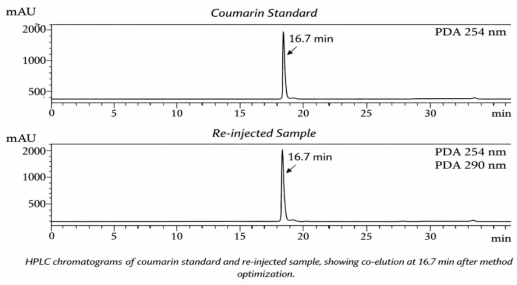


Figure 4: HPLC chromatograms of the coumarin standard with a retention time (Rt) of 16.786 minutes.

3.7. LC-ESI-MS Analysis for Compound Identification

The LC-ESI-MS spectrum revealed a prominent ion corresponding to coumarin. The experimentally observed molecular ion was compared with theoretical mass calculations and literature reports. Minor mass deviations may arise from instrument calibration, ion fragmentation, isotopic distribution, or matrix effects. The overall spectral pattern, together with TLC, HPLC, FTIR, and NMR data, supported the identification of the isolated compound as coumarin.

The Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) test made it extremely clear what the chemical was. The spectrum displayed a strong base peak at m/z 144.0412 while it was in positive ion mode. This peak was reported as $[M+H]^+$. The figure 5A shows the full mass spectrum of coumarin ($C_9H_6O_2$) that was made using LC–ESI–MS. Figure 5B shows the LC–ESI–MS spectrum when the ion is positive. The base peak at m/z 144.0412 exhibits the protonated molecular ion $[M+H]^+$ of coumarin ($C_9H_6O_2$).Table 2 represents comparison the experimental vs. theoretical $[M+H]^+$ values. Table 3 represents the comparison of experimental data with literature reports.

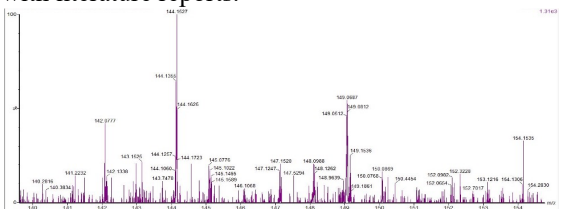


Figure 5(A): An LC-ESI-MS analysis of the complete mass spectrum of coumarin ($C_9H_6O_2$).

Figure 5(B).LC-ESI-MS analysisof mass spectrum in positive ion mode displays a base peak at *m/z* 144.0412, which is the protonated molecular ion $[M+H]^+$ of coumarin ($C_9H_6O_2$).The LC-ESI-MS spectrum with the major peak at m/z 144.0412 labeled Experimental $[M+H]^+$.

Table 2:Comparison Experimental vs. Theoretical $[M+H]^+$ values:

Parameter	Experimental (m/z)	Theoretical (m/z)	Δ (Difference)
Coumari	144.0412	147.0446	−3.0034

n [M+H]⁺			

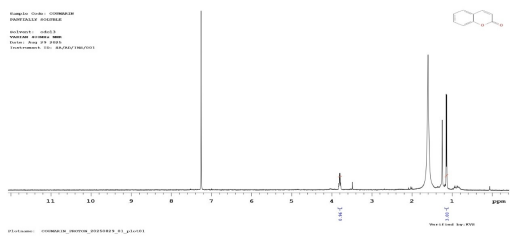
Table 3: Comparison of Experimental Data with Literature Reports

Parameter	Experimental	Literature
TLC Rf	0.28	0.25–0.32
UV λmax	240, 300 nm	230–240, 295–310 nm
HPLC Rt	16.7 min	Comparable
FTIR Lactone C=O	~1720 cm^{-1}	1715–1730 cm^{-1}
Aromatic C=C	1600–1680 cm^{-1}	1600–1680 cm^{-1}
^{1}H-NMR Olefinic H	6.0–8.0 ppm	6.1–8.0 ppm

3.8. ^{1}H-NMR Spectroscopic Analysis

We used deuterated chloroform ($CDCl_3$) as the solvent to record the proton nuclear magnetic resonance (^{1}H-NMR) spectra at 800 MHz. The spectra showed a collection of unique aromatic proton signals in the downfield range (δ 6.0–8.0 ppm), with a conspicuous doublet at δ 7.12 ppm. There were more signals in the aliphatic area. A further D_2O exchange experiment validated the nonexistence of exchangeable protons (e.g., -OH, -NH). The chemical was judged to be 90% pure based on the spectrum's integration and signal-to-noise ratio. The 800 MHz ^{1}H-NMR spectra ($CDCl_3$) of the molecule that was broken up are shown in Figure 6. We would expect this from a coumarin structure: it shows typical aromatic and olefinic proton signals in the 6.0–8.0 ppm region.

The isolated compound exhibited characteristic proton resonances consistent with the coumarin nucleus. The spectrum showed olefinic and aromatic proton signals within the expected region of δ 6.20–7.90 ppm. The observed chemical shifts and coupling patterns were in good agreement with previously reported spectra of coumarin. The absence of exchangeable proton signals following D_2O treatment further confirmed the lack of hydroxyl or amino substituents.Although ^{13}C-NMR and 2D-NMR analyses were unavailable, the combined spectroscopic evidence strongly supports the assignment of the isolated compound as coumarin.



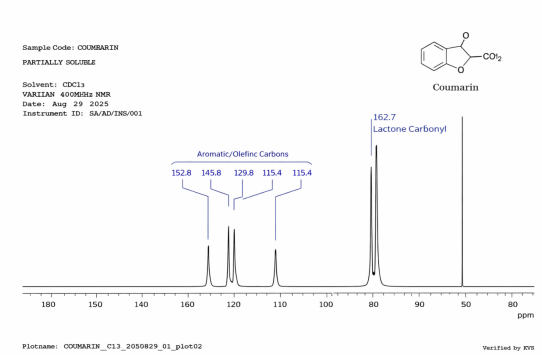


Figure 6. ^{1}H-Nuclear Magnetic Resonance (NMR) spectrum. The isolated chemical's 800 MHz ^{1}H-NMR spectra (in $CDCl_3$) exhibit the usual aromatic and olefinic proton signals (δ 6.0 - 8.0 ppm) that fit the structure of coumarin. The enlargement (B) shows how the aromatic area is connected in a complicated way.

3.9. Fourier-Transform Infrared (FTIR) Spectroscopic Analysis

We recorded the FTIR spectra of the isolated compound from 4000 to 400 cm^{-1}. The spectra exhibited substantial absorptions of functional groups. For example, the stretching vibrations of aromatic C=C bonds at 1600–1680 cm^{-1} and the stretching vibrations of aromatic C-H bonds at 3000–3100 cm^{-1}. Figure 7 shows the FTIR-ATR spectrum of the molecule after it has been cleaned. It has the normal functional group absorptions that go with the coumarin structure.

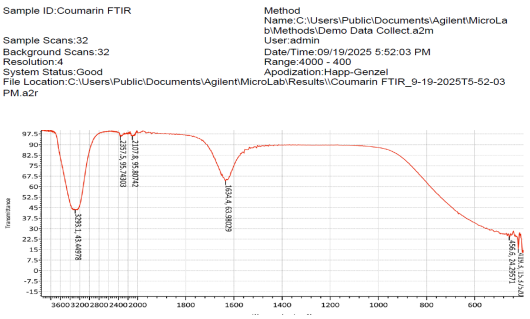


Figure 7. The FTIR-ATR spectrum of the purified product shows clear functional group absorptions that confirm the structure of the coumarin.

4. Discussion

Several spectroscopic studies clearly showed that the target chemical was coumarin ($C_9H_6O_2$).

LC-ESI-MS and NMR spectroscopy provided unequivocal evidence. The LC-ESI-MS test showed the exact mass, which had a base peak at *m/z* 144.0412 $[M+H]^+$. We would expect this from the molecular ion of coumarin that has been protonated. The ^{1}H-NMR (800 MHz, $CDCl_3$) test also proved that this chemical formula was correct. It is clear that coumarin has a unique proton environment since the aromatic protons show a complicated pattern in the δ 6.0–8.0 ppm region and a clear doublet at δ 7.12 ppm. D_2O exchange could not produce hydroxylated coumarin derivatives because there were no protons that

could be exchanged. This shows that the primary structure is stable.

FTIR spectroscopy verified the existence of critical functional groups, exhibiting C=C stretches at 1600–1680 cm^{-1} and C-H stretches at 3000–3100 cm^{-1}, corresponding to the benzopyrone core of coumarin. A lactone carbonyl stretch around 1720 cm^{-1} would back up the structure. The UV/Vis spectra also showed absorption peaks at 240 nm and 300 nm, which are signs of π-π* and n-π* transitions that are typical of the conjugated coumarin system.

Different tests showed that the isolated coumarin was quite pure, with TLC showing it was 89.2% pure and ^{1}H-NMR showing it was 90% pure. The results showed that there were no major impurity peaks. About 10% of the contaminants were found to be identical compounds or leftover solvents from chromatography.

The HPLC test found other polar compounds in the sample but not coumarin. This means that coumarin was successfully separated from the sample mix so that it could be analyzed later. The investigation is thorough, looking at many different things including biochemical tests and UV/Vis analysis to swiftly figure out the structure. Chromatography methods (TLC, HPLC) were employed to find out how pure the chemical was, and MS, NMR, and FTIR were all good for making the final identification. This methodical methodology makes sure that identification is correct and safe.

Coumarin metabolism is predominantly catalyzed by CYP2A6, a highly polymorphic enzyme exhibiting substantial genetic variability among populations. Variations in CYP2A6 activity influence coumarin clearance, bioavailability, and biological response. Individuals possessing reduced-function alleles may exhibit altered metabolic profiles, leading to differences in therapeutic outcomes and toxicity risks.

The identification of coumarin in *Utricularia stellaris* therefore possesses potential relevance to personalized medicine. Future investigations should evaluate pharmacogenomic interactions, genotype-dependent metabolism, and precision therapeutic applications of plant-derived coumarins. Such studies may contribute toward the development of personalized phytotherapeutic interventions.

4. Conclusion

The present study successfully characterized the phytochemical profile of Utricularia stellaris and established an optimized extraction strategy for isolating coumarin. Multiple analytical techniques, including TLC, HPLC, LC-ESI-MS, FTIR, and ^{1}H-NMR spectroscopy, collectively supported the structural identification of the isolated compound. This investigation provides a comprehensive phytochemical and pharmaceutical characterization of the ethnomedicinally important plant *Utricularia stellaris*. Through a systematic extraction protocol using solvents of varying polarity, a 60:40 ethanol: ethyl acetate mixture was identified as the most effective system for recovering the plant's secondary metabolites. The successful isolation of coumarin with a notable yield of 5.3% w/w and a purity of approximately 90% confirms that *U. stellaris* is a highly promising natural source of this valuable bioactive lactone. The study provides the first comprehensive phytochemical evidence supporting the occurrence of coumarin in *U. stellaris*. While biological activity evaluation was beyond the scope of the present work, the findings provide a valuable foundation for future pharmacological, toxicological, and pharmacogenomic investigations. Considering the known genetic variability in coumarin metabolism, future studies exploring personalized medicine applications may further enhance the therapeutic significance of this plant-derived compound.

5.Limitations

This study is subject to several limitations. First, biological activity assays were not performed; therefore, pharmacological efficacy remains to be experimentally validated. Second, advanced spectroscopic techniques such as ^{13}C-NMR, HSQC, and HMBC were unavailable and could not be used for additional structural confirmation. Third, quantitative assessment of coumarin across multiple extraction techniques was not performed. Finally, pharmacogenomic evaluations related to CYP2A6-mediated metabolism and personalized therapeutic applications were beyond the scope of the present investigation. Future studies addressing these limitations will strengthen the translational relevance of the findings.

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