RESEARCH PAPER

Phytochemical Isolation and Chromatographic Profiling Of Bioactive Constituents from Myxopyrum Serratulum A.W. Hill

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ABSTRACT

Medicinal plants continue to be an important source of bioactive compounds with significant therapeutic potential. In this context, the present study focuses on the isolation and characterization of phytoconstituents from Myxopyrum serratulum A.W. Hill using standard extraction and chromatographic techniques. M. serratulum, a climbing shrub belonging to the family Oleaceae, is traditionally used in indigenous systems of medicine for the treatment of wounds, inflammation, scabies, infections, and metabolic disorders. In the present work, the dried and powdered aerial parts of the plant were subjected to extraction using 70% ethanol, followed by successive solvent extraction with solvents of increasing polarity. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, phenolic compounds, terpenoids, and glycosides. Isolation of phytoconstituents was carried out using column chromatography, and the collected fractions were monitored by thin-layer chromatography (TLC). Iridoid glycosides were isolated using authenticated herbarium leaf material, while a pentacyclic triterpenoid, ursolic acid, was successfully isolated and purified through recrystallization. The isolated compounds were characterized and identified using sophisticated instrumental analytical techniques. High-performance thin-layer chromatography (HPTLC) fingerprinting was performed to determine the Rf values of the isolated compounds and to compare them with those of reference standards, confirming their identity and purity.The results of this study provide scientific support for the traditional medicinal uses of Myxopyrum serratulum and highlight its potential as a promising source of pharmacologically active phytoconstituents. Further studies on structural elucidation and biological evaluation of the isolated compounds may contribute to the development of novel therapeutic agents.

Keywords: *Myxopyrum serratulum A.W. Hill; Phytochemical isolation; Column chromatography; Iridoid glycosides; HPTLC fingerprinting*

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INTRODUCTION

Medicinal plants serve as a valuable source of bioactive compounds that play a crucial role in drug discovery and development[1]. Phytoconstituents such as alkaloids, flavonoids, phenolics, and terpenoids are known to exhibit diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects[2]. Systematic isolation and characterization of these compounds are essential for validating the therapeutic potential of traditional medicinal plants. *Myxopyrum serratulum* A.W. Hill (family: Oleaceae) is a lesser-explored medicinal plant native to the Indian subcontinent. Traditionally, various parts of the plant have been used in folk medicine for managing fever, wounds, inflammatory conditions, and metabolic disorders[3]. Despite its ethnomedicinal significance, scientific reports on the isolation of its phytoconstituents are limited. Therefore, the present study focuses on the isolation of phytochemical constituents from *M. serratulum* using solvent extraction and chromatographic techniques to support its medicinal relevance[(4)].

Natural products obtained from diverse natural sources represent an invaluable reservoir for the discovery of novel chemical entities in the drug development process[5]. The pharmacological activities of medicinal plants are largely attributed to the presence of phytoconstituents, particularly secondary metabolites. Medicinal herbs produce a wide array of structurally diverse compounds, commonly referred to as secondary metabolites, which differ from primary metabolites that are essential for plant growth and development. Over the past few decades, studies in chemical ecology have demonstrated that these secondary metabolites play crucial roles in plant–environment interactions[6]. Throughout human history, natural products have significantly influenced human civilization by contributing to physiological well-being and serving as therapeutic agents in traditional and modern medicine[7].

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Secondary metabolites such as terpenoids, steroids, fatty acid derivatives, alkaloids, phenylpropanoids, non-ribosomal peptides, and enzyme cofactors are commonly isolated from medicinal plants. Chromatographic techniques, particularly column chromatography, play a pivotal role in the isolation and purification of these phytoconstituents, where separation is based on differential adsorption of compounds onto a solid stationary phase. Structural characterization of isolated compounds is achieved using sophisticated analytical instrumentation[8]. Ultraviolet (UV) spectroscopy is employed for the analysis of organic compounds containing chromophoric groups, while infrared (IR) spectroscopy provides information on functional groups through characteristic vibrational frequencies[9]. Nuclear magnetic resonance (NMR) spectroscopy offers detailed insights into molecular structure and nuclear environments, and mass spectrometry enables accurate determination of molecular mass. Additionally, high-performance thin-layer chromatography (HPTLC) fingerprinting serves as a reliable tool for compound identification and quality assessment[10].

MATERIALS AND METHODS

Good collection practices was followed and fresh aerial parts of *Myxopyrum serratulum* were collected in September 2012 from Tirunelveli, Tamil Nadu, and India. Authentication was done by Botanist Prof. Jayaraman, Director, Plant Anatomical Research Centre, and Chennai. A voucher Document has been deposited in our college for further reference. (PARC/2013/3604). The medicinal herb were shade dried and coarsely powdered (Sieve No 40) .Column was carried out using silica gel 60-120 mesh while TLC was performed by S. gel 60 F_{254}..Melting points were recorded by means of Sunbim melting point apparatus. UV was recorded using Perkin Elmer UV spectrophotometer.IR spectra were done on Perkin Elmer FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL spectrometer using DMSO as solvent. Mass techniques were recorded by using Agilent MSD VL mass spectrometer.

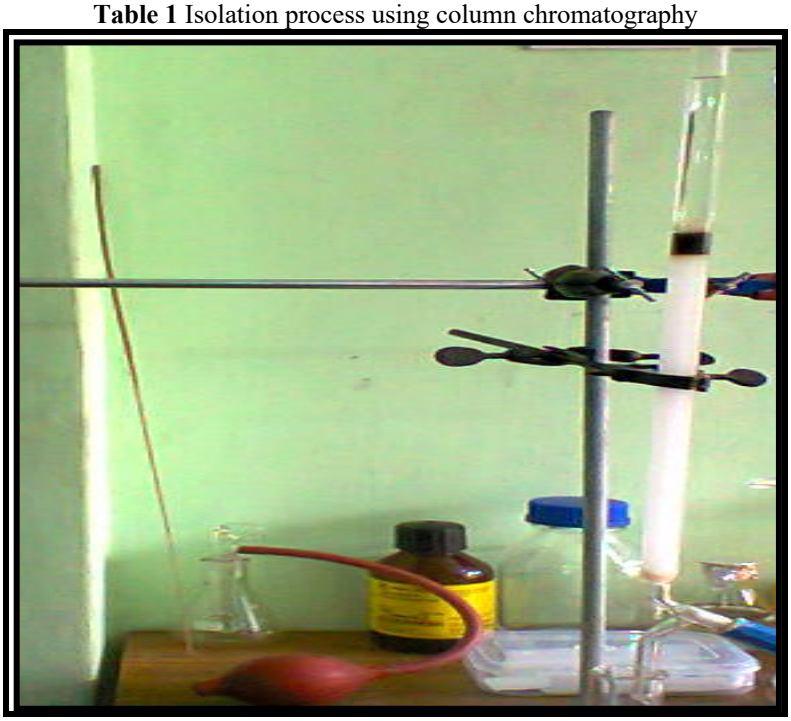
Extraction of plant materials:

Extraction procedure was done in about (800 g) of Powdered material using 70 % ethanol. The obtained extract was concentrated and the residue was suspended in water[11]. Partition was done with petroleum ether and ethyl acetate. The presence of terpenoids was identified in ethyl acetate fraction using TLC with corresponding spray reagents.

Isolation of Compound

Adsorbent is suitably moistened with solvent and packed in the column with a cotton at the bottom. Components to be separated is positioned on the top of packed adsorbent with a second cotton in between.(Fig:1) Solvent system is poured into the column over the sample mixture. To collect the fractions a collecting beaker is placed at the bottom of column .In column, ethyl acetate fraction was suspended on s/phase using chloroform-methanol (10: 8) as the eluting solvent to produce thirty five fractions (F_1-F_{35}). Fractions between (F_{16}-F_{24}) contained the major compound with a few minor constituents. The similar fractions were combined and the resultant product was washed with hexane. Recrystallization was done using methanol. Compound obtained was white powder (65 mg) with the melting point of about 282-284ºC.

Table 1 Isolation process using column chromatography



TLC PROFILE

Sample : 70 % Ethanol extract

Stationary phase : Silica gel 60 mesh

Mobile phase : Toluene: Ethyl acetate: Formic acid (9: 1: 0.5)

R_f value : 0.7

Detecting Agent : Vanillin in sulphuric acid

Chemical Test

Test for triterpenoids: Take 1 ml of sample in a clean test tube to it add tin (one bit) and thionyl chloride. Presence of triterpenoids is indicated by presence of pink color.

Liebermann Burchard Test: To the sample and add chloroform mix well.Then add acetic anhydride followed by sulphuric acid. Presence of terpenoids is indicated by presence of pink color

HPTLC analysis

It was carried out on a 10 x 10 cm pre activated silica gel 60GF 254 plate. Extract were applied to the plate as 6 mm wide band with an automatic TLC applicator Linomat. Densitometric scanning was carried out on CAMAG scanner. Prewashing of the plates were done by methanol and activation process was done at 60°C. Scanning speed was employed of 20 mm/s was employed[12]. The mobile phase was fixed by running various solvent system (Toluene, toluene: ethyl acetate and ethyl acetate: methanol). Ascending development was carried out (20 x 10 cm) twin glass chamber equilibrated with the mobile phase at 60°C [3] and represented in (Table 1).

Chromatographic Condition

Table 2 chromatographic condition of HPTLC

Standard	Ursolic acid (Sigma Aldrich, Chennai)
Stationary phase	Silica gel GF_{254}
Solvent system	CHCl3: CH3OH(9.5 : 0.5)
Derivatizing agent	5 % sulphuric acid in methanol
Scanning wavelength	510 nm
Sample concentration	*Myxopyrum serratulum* (50 mg/ml Isolated compound (2 mg/ml) Ursolic acid (0.1 mg/ml)
Development mode	Ascending mode

RESULTS AND DISCUSSION

Terpenoids represent one of the largest and most structurally diverse classes of secondary metabolites, with more than 30,000 identified compounds, including steroids. These compounds are widely distributed in medicinal plants and are known to exhibit a broad spectrum of biological activities, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Terpenoids play a crucial role in plant defense mechanisms and contribute significantly to the therapeutic potential of herbal medicines. Several clinically important drugs have been derived from this class of compounds, notably the anticancer agent paclitaxel (Taxol) and the antimalarial drug artemisinin, which are among the most recognized terpene-based pharmaceuticals[14]. In the present study, the isolation of a pentacyclic triterpenoid, ursolic acid, from *Myxopyrum serratulum* further supports the medicinal relevance of the plant. Ursolic acid has been reported to possess multiple pharmacological activities, including anti-inflammatory, hepatoprotective, antidiabetic, and anticancer effects. The presence of such bioactive terpenoids may contribute to the traditional use of *M. serratulum* in the treatment of wounds, inflammation, and related disorders[15]. These findings reinforce the importance of phytochemical investigations in identifying bioactive constituents that can serve as potential leads for drug development[16].

Examination of Isolated Compound

The 70 % ethanolic extract was subjected to column chromatography. From the collective fractions, a white powder was isolated and re-crystallized.

Description - White powder

Solvent system - Toluene: Ethyl acetate: Formic acid (9:1:0.5)

Detecting Agent - Vanillin-Sulphuric acid

Melting Point - 282-284°C

R_f Value - 0.7

Characterization of the isolated compound

Characterization of the isolated compound was carried out using sophisticated analytical techniques. The structural features and functional groups were elucidated through UV–Visible, FT-IR, NMR, and mass spectrometric analyses, which collectively confirmed the identity and purity of the isolated phytoconstituents.

UV spectroscopy

Isolated sample was dissolved in methanol and scanned between 200-400 nm. Spectrum of the isolated sample was given in the Figure 2.

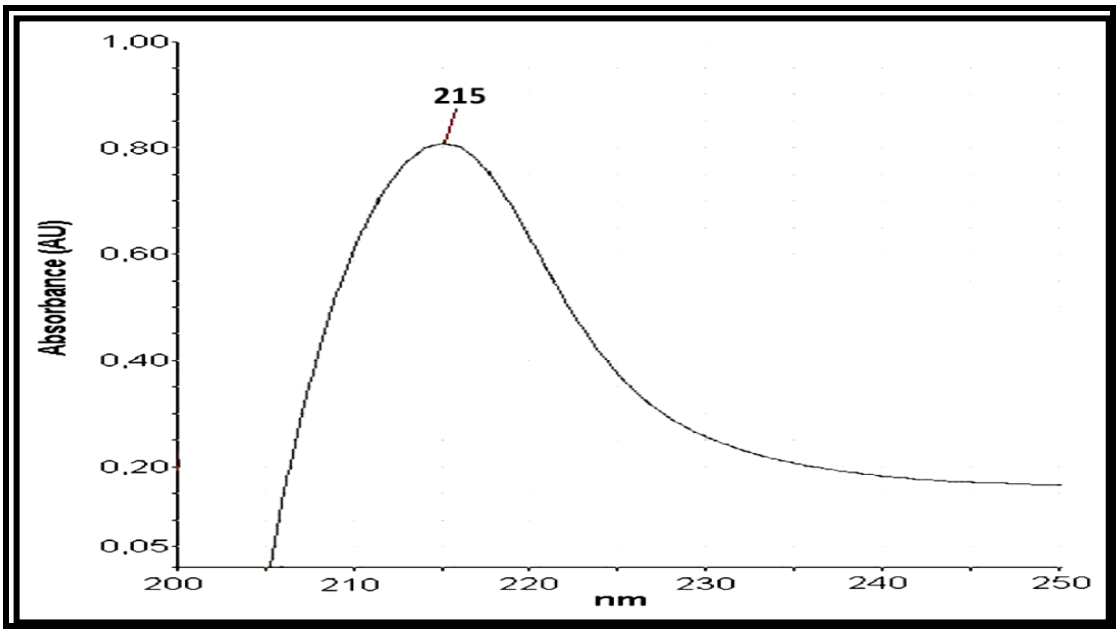


Figure 2 : UV Spectra of isolated

Infrared Spectroscopy

The isolated compound showed the following absorption peaks, 3434 cm^{-1} which corresponds to OH stretching, 2931 & 1694 cm^{-1} corresponds to carboxylic acid (carbonyl group), 2931 cm^{-1} corresponds to CH (Str) Superimposed on OH (Str), 1385 cm^{-1} corresponds to Gem dimethyl group $C(CH_3)_2$, 1043 cm^{-1} corresponds to the C-O (Str), 1043 & 998 cm^{-1} corresponds to C-OH band and 998 & 663 cm^{-1} corresponds to OH (def) it is represented in Figure 3.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR : δ 5.13 (t, J=3.5Hz, 1 H), 4.36 (t, J=5 Hz, 1 H), 4.30 (d, J=5.5 Hz, 1 H), 3.47-3.39 (m, 1 H), 3.02-2.98 (m, 1 H), 2.11 (d, J= 11Hz, 1H), 1.92 (qd, J=13, 4.5Hz, 1H), 1.89-1.77 (m, 3 H), 1.60-1.43 (m, 10 H), 1.34-1.25 (m, 4 H), 1.08-0.99 (m, 5 H), 0.95-0.87 (m, 11 H), 0.82 (d, J=6.5Hz 1 H), 0.75 (s, 3 H), 0.68 (s, 3 H) represented in (Figure 4).

^{1}H NMR spectrum of the sample showed one proton broad signal at δ 5.12 assigned to Vinylic proton. One proton singlet at δ 2.5 attributed to OH of COOH. One proton doublet at δ 2.1 represents the presence of hydroxyl group. The signals below indicate the presence of 7 methyl protons in the structure.

^{13}C-NMR : δ ppm: 178.99 (C-28); 138.58 (C-13); 125.21 (C-12); 77.31 (C-3); 55.24 (C-5); 52.84 (C-18); 47.48 (C-9); 47.29 (C-17); 42.11 (C-14); 40.47 (C-8); 39.97 (C-20); 39.81 (C-19); 39.64 (C-4); 39.56 (C-1); 38.70 (C-10); 36.99 (C-22); 33.17 (C-15); 30.65 (C-7); 28.72 (C-23); 28.00 (C-21); 2745 (C-2); 24.36 (C-11); 23.73 (C-27); 23.33 (C-16); 21.53 (C-30); 18.47 (C-6); 17.47 (C-29); 17.38 (C-24); 16.54 (C-25); 15.68 (C-26)

In ^{13}C-NMR, a peak at δ 178.99 attributed to carboxylic group at the position C-28. The chemical shift value at δ 125.21 and δ 138.58 represented resonance olefinic carbons in C-12 and C-13. The chemical shift value at δ 77.31 represented carbinol carbon in the structure (Figure 5).

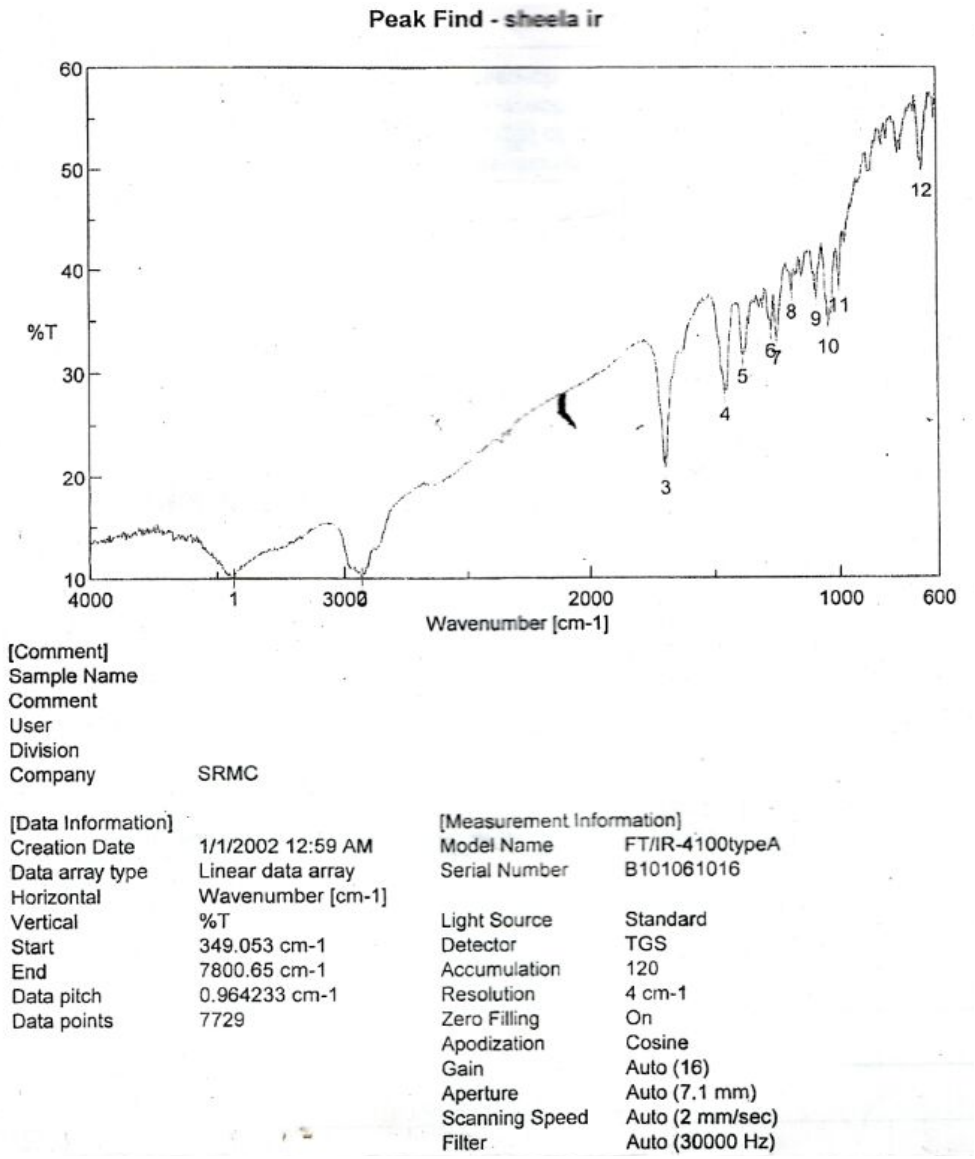


Figure 3 IR Spectra of Isolated Compound

Table 3 wave numbers and it key groups

WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUP
3434	OH (Str)
2931 & 1694	Carboxylic acid(carbonyl group)
2931	CH (Str) Superimposed on OH (Str)
1385	Gem dimethyl group, $C(CH_3)_2$
1043	C-O (Str)
1043 & 998	C-OH Band
998 & 663	OH (def)

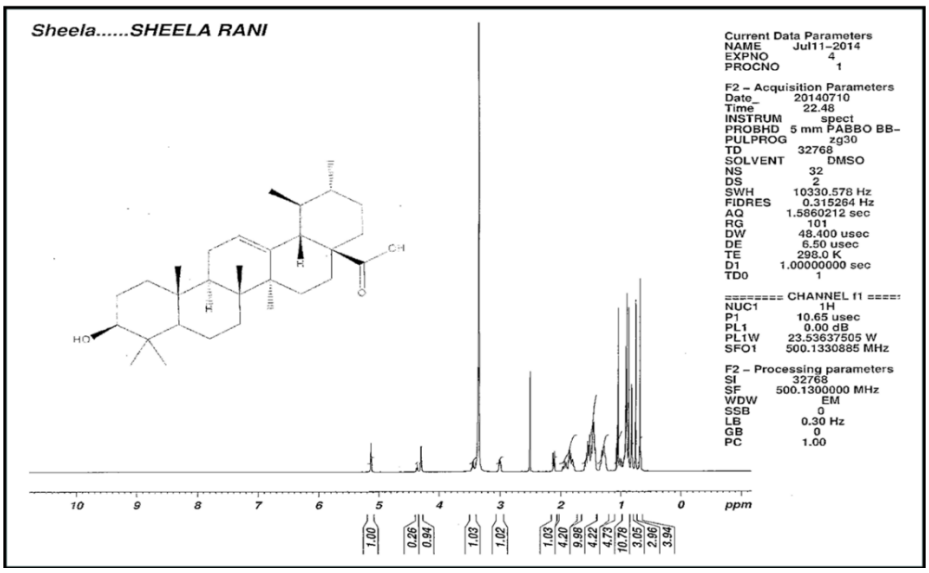


Figure 4 1H NMR of Isolated Compound

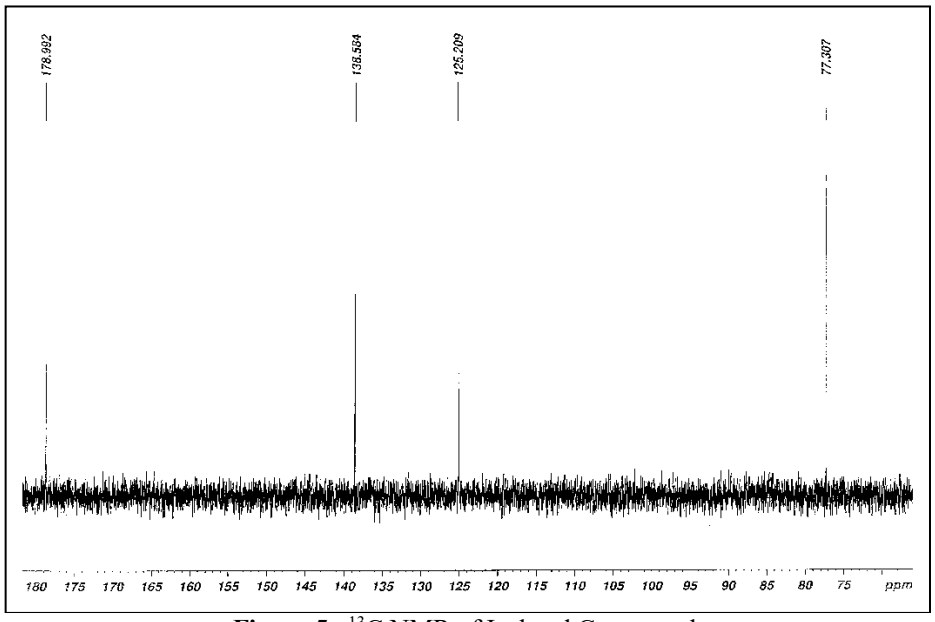


Figure 5 ^{13}C NMR of Isolated Compound

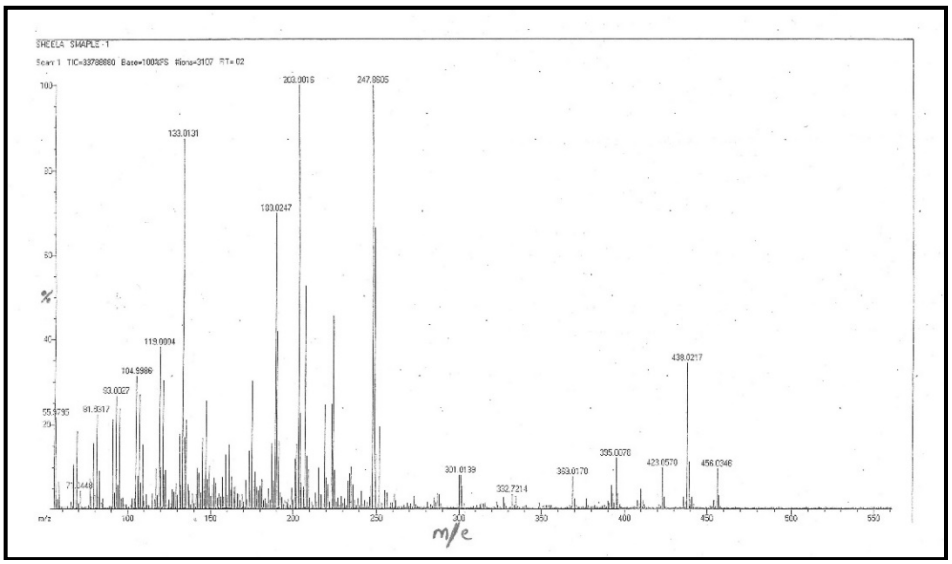


Figure 6 Mass Spectra of Isolated Compound

Mass spectrometry

Mass spectra of isolated sample was shown in Figure 6. Mass spectrum showed the M+ peak at m/e 456.03 that corresponds to molecular formula $C_{30}H_{48}O_3$ and fragmented peaks at 438.02, 423.05, 247.86, 203.00, 189.02 and 133.01. Structure of isolated constituent was found out by spectroscopic techniques. Characterization indicates that the compound is a pentacyclic triterpenoid, Ursolic acid.

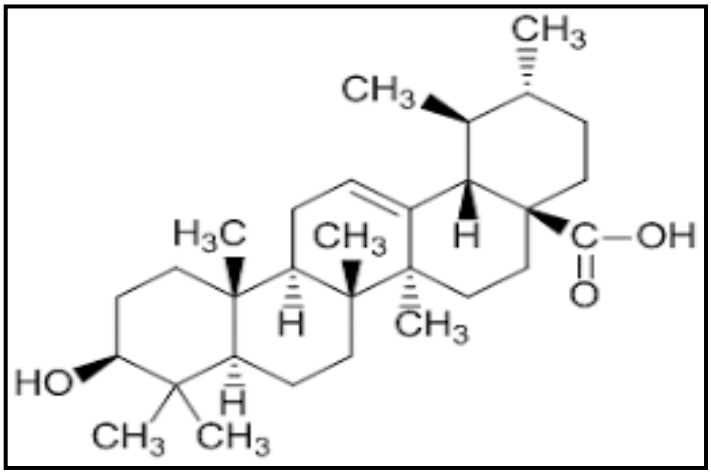


Figure 7 Structure of Isolated Compound - Ursolic acid

Molecular Formula	IUPAC NAME
C_{30} H_{48} O_3	3β-Hydroxy-urs-12-ene-28-oic acid

HPTLC finger printing of standard, isolated compound and extract

HPTLC finger print of Ursolic acid was taken and the R_f value was found to be 0.4. Similarly the HPTLC finger print of 70 % ethanolic extract & isolated sample were taken. The chromatogram of 70 % ethanolic extract showed 8 peaks in the range of R_f value 0.12 to 0.76. Among the peaks the 6th peak with R_f value of 0.4 with percentage area of 69.3 confirms the occurrence of Ursolic acid in ethanolic extract. HPTLC finger print of isolated sample showed 4 peaks with the range of R_f values from 0.16 to 0.77. Among the peaks the 3rd peak at R_f value 0.42 corresponds to the standard Ursolic acid (R_f value - 0.4) with percentage area of 86.55. It confirmed that the isolated compound is Ursolic acid (Table 4).

Table 4 HPTLC profile of extract and isolated compound

Track	Peak	Start R_f	Start Height	Max R_f	Max Height	Height %	End R_f	End Height	Area	Area %
4	1	0.35	7.9	0.4	225.1	100	0.45	0.4	6770.7	100

7	6	0.33	6.5	0.4	243.9	62.5	0.48	1	9322	69.3
8	3	0.35	19.6	0.42	263.7	84.5	0.47	7	11087.6	86.55

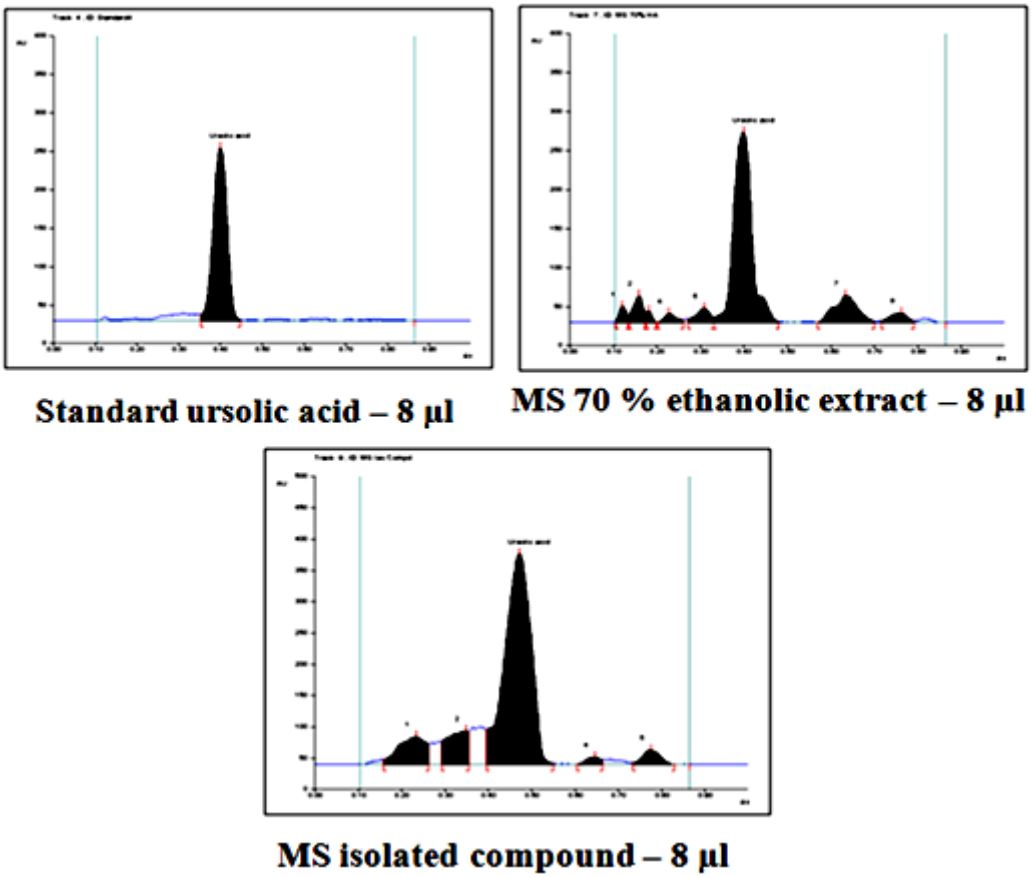


Figure 8 HPTLC chromatogram of standard, isolated compound and extract

DISCUSSION

The successful isolation of ursolic acid from the ethanolic extract of *Myxopyrum serratulum* using column chromatography underscores the effectiveness of ethanol as a suitable solvent for extracting bioactive triterpenoids[17]. Spectral characterization confirmed the identity of the isolated compound, validating the reliability of the applied analytical techniques. The presence of ursolic acid, a pentacyclic triterpenoid with well-documented pharmacological properties including anti-inflammatory, antitumor, antioxidant, hypoglycemic, antiulcer, and antihyperlipidemic activities, provides scientific justification for the traditional medicinal applications of the plant [18-20]. Furthermore, the isolation of such a therapeutically important compound highlights *M. serratulum* as a promising natural source of pharmacologically active phytoconstituents. This study lays a foundation for further investigations focusing on quantitative estimation, detailed biological evaluation, and formulation development, which may contribute to the discovery of novel plant-based therapeutic agents [21-23].

CONCLUSION

The present study successfully isolated and characterized phytoconstituents from the ethanolic extract of *Myxopyrum serratulum* A.W. Hill using column chromatography. Spectral analyses confirmed the identity of the isolated compound as ursolic acid, a pentacyclic triterpenoid. The effective extraction and isolation highlight ethanol as a suitable solvent for recovering bioactive constituents. The presence of ursolic acid supports the traditional medicinal use of *M. serratulum* due to its reported pharmacological activities. Overall, the findings indicate that *Myxopyrum serratulum* is a promising source of therapeutically relevant natural compounds.

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Author Contribution

Mahesh Kumar performed the experimental work and drafted the manuscript. T. Sheelarani supervised the study and reviewed the manuscript. Sangeetha Mani contributed to data interpretation and critical revision. All authors approved the final version of the manuscript.

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