

Isolation and Structural Elucidation of Bioactive Phytoconstituents from Selected Medicinal Plants

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Abstract

Medicinal plants are an important source of novel chemical entities that possess diverse therapeutic activities and contribute significantly to the prevention and treatment of various diseases in living organisms. These plants are rich in secondary metabolites including alkaloids, flavonoids, polyphenols, glycosides, tannins and saponins which exhibit a wide range of pharmacological activities. These bioactive phytoconstituents interact with various biological pathways and molecular targets, thereby producing a wide range of therapeutic effects and contributing to the discovery and development of effective drug candidates. Owing to their diverse pharmacological properties, phytoconstituents provide substantial therapeutic benefits and continue to play a significant role in modern medicine. They function as potent antioxidants that neutralize free radicals which protects cells from damage and lowering the risk of chronic conditions like cardiovascular disease, neurological disease, cancer and diabetes. Many of these compounds display strong anti-inflammatory, antimicrobial and even anticancer properties by regulating cell growth and boosting immune responses. Thus, the present study involves extraction, isolation and characterisation of bioactive constituents of selected medicinal plants. The dried root powder was used for preparation of extracts of *Delphinium denudatum* wall. and *Berberis aristata* DC. Isolation of individual phytoconstituents was achieved through column chromatographic separation of the extracts. The isolated fractions were subsequently subjected to chromatographic and spectroscopic analyses to determine their chemical identity and structural characteristics.

Introduction:

In the contemporary era, rapid urbanization, industrialization and technological advancement have significantly altered human lifestyles. Alteration in lifestyle includes many factors such as sedentary behaviour, unhealthy dietary habits, excessive consumption of processed foods, environmental pollution, psychological

stress, smoking, alcohol intake and exposure to various toxic chemicals have contributed substantially to the increasing prevalence of chronic and degenerative diseases worldwide. These lifestyle-associated disorders including cardiovascular diseases, diabetes mellitus, obesity, cancer, neurodegenerative disorders and

inflammatory conditions represent a major challenge to global healthcare systems¹.

Although numerous synthetic drugs are available for the treatment and management of these diseases, their long-term use is often associated with several limitations. Adverse drug reactions, drug resistance, organ toxicity, altered hematological parameters, hepatotoxicity, nephrotoxicity and harmful drug-drug interactions are frequently reported². In many cases, therapeutic agents may produce undesirable physiological responses that deviate from normal biological mechanisms, thereby compromising treatment efficacy and patient safety. Furthermore, the rising cost of healthcare and pharmaceutical products has increased the demand for safer, affordable and more effective therapeutic alternatives³.

The process of drug discovery is continuously growing to identify novel therapeutic targets and bioactive molecules capable of overcoming the limitations of conventional medicines. Natural products have played vital role serving as an important source of structurally diverse compounds with significant pharmacological activities⁴. Historically, medicinal plants have provided lead molecules for the development of novel chemical entity. Their therapeutic potential is primarily attributed to the presence of

secondary metabolites such as alkaloids, flavonoids, phenolic compounds, terpenoids, glycosides, tannins and saponins which is binding with many protein targets and ligands to show a therapeutic potential⁵.

Plant-derived phytoconstituents exhibit a broad spectrum of biological activities including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, cardioprotective, neuroprotective, immunomodulatory and anticancer effects. These compounds can modulate oxidative stress, regulate inflammatory mediators, inhibit microbial growth, protect cellular structures from damage and influence signaling pathways involved in disease progression⁶. Consequently, medicinal plants have gained increasing attention as promising candidates for the discovery and development of novel therapeutic agents with enhanced efficacy and reduced toxicity.

Despite extensive research on many medicinal plants, numerous bioactive constituents remain unexplored or inadequately characterized. Detailed phytochemical investigations involving extraction, isolation, purification and structural elucidation are essential to identify novel compounds and establish their therapeutic significance. Such studies not only contribute to the scientific validation of

traditional medicinal systems but also facilitate the development of new drug candidates for the prevention and treatment of various human diseases⁷.

Therefore, the exploration of medicinal plants for the identification and characterization of bioactive phytoconstituents remains an important area of pharmaceutical and phytochemistry research. The discovery of new natural compounds may provide safer and more effective therapeutic alternatives which contribute to modern drug development and help to address the growing burden of chronic diseases associated with modern lifestyles⁸. In the present study, two medicinally important plants such as *Delphinium denudatum* Wall. (Family: *Ranunculaceae*) and *Berberis aristata* DC. (Family: *Berberidaceae*) were selected for phytochemical investigation. These species are widely distributed in the Himalayan regions of India and have been extensively utilized in traditional systems of medicine for the treatment of various ailments. Both plants are known to be rich sources of diverse bioactive phytoconstituents including alkaloids, flavonoids, phenolic compounds, glycosides, tannins and other secondary metabolites that are responsible for their broad spectrum of pharmacological

activities. Previous studies have reported several therapeutic properties of these plants, including antioxidant, anti-inflammatory, antimicrobial, neuroprotective, hepatoprotective, and antidiabetic activities⁹.

Materials and Methods: The fresh roots of *Delphinium denudatum* wall and *Berberis aristata* DC were collected from the Udham Singh Nagar district (Kichha), Uttarakhand, India.

Chemicals and Reagent: All chemicals and reagents of analytical grade were procured from New Neeta Chemicals, Pune, India.

Methodology:

Extraction:

Based on polarity principle, successive solvent extraction of *Delphinium denudatum* Wall. and *Berberis aristata* DC. was performed using solvents of increasing polarity. Dried stem and root samples were separately crushed, powdered and subjected to extraction. Briefly, 1 kg of each powdered sample was packed in muslin cloth bags and placed in separate Soxhlet apparatus. Initially, extraction was carried out with petroleum ether (60-80°C) to remove lipophilic constituents such as fats, sterols and other non-polar compounds. The extraction process was repeated three times and the resulting extracts were concentrated under reduced pressure using a rotary vacuum

evaporator. The defatted marc obtained after petroleum ether extraction was subsequently subjected to successive extraction with acetone, ethanol and water in order of increasing polarity¹⁰.

Isolation of Bioactive compounds:

Based on the phytochemical screening and qualitative thin layer chromatography profiling, the ethanol extracts were selected for the further isolation method¹⁰. Column chromatography was carried out using silica gel as the stationary phase for the separation and isolation of phytoconstituents from the extracts. A clean glass column was firmly clamped in a vertical position and partially filled with the initial solvent system to prevent air entrapment. A small cotton plug was carefully placed at the bottom of the column to support the adsorbent bed. The column was packed by the wet slurry method, wherein silica gel was mixed with a suitable solvent to form a homogeneous slurry free from air bubbles. The slurry was gradually transferred into the column through a funnel, and the column was gently tapped to ensure uniform packing and to remove trapped air. Excess solvent was allowed to drain through the stopcock resulting in a compact and even adsorbent bed. The concentrated extract sample was dissolved in a minimum volume of the packing solvent and carefully loaded

onto the top of the silica gel bed without disturbing the surface. Elution was performed using an appropriate solvent system, and fresh solvent was added continuously to maintain a constant solvent level above the sample. The eluate was collected sequentially in sterile glass tubes as individual fractions. All collected fractions were analysed by thin-layer chromatography (TLC) using the solvent systems previously standardized during phytochemical screening. Fractions exhibiting similar chromatographic profiles and identical R_f values were scraped from thin layer of glass plates and concentrated under reduced pressure using a rotary vacuum evaporator. The resulting concentrated fractions were dried to obtain purified isolated compounds for further characterization and analysis¹¹.

Characterisation of isolated compounds:

Characterization is the process where unknown structure is elucidated by interpreting the spectra of analytical techniques. All four fractions were isolated and collected through chromatographic technique. Out of that, two isolated compounds such as DD2 and BA2 are subsequently analysed using spectroscopic methods to determine their chemical composition and structural features. These two isolated compounds were analysed by

FT-IR, H-NMR and Mass spectrometric techniques for the interpretation of number of hydrogen atoms, carbon atoms, molecular formula and functional groups present in medicinal extract.

Fourier Transform Infrared (FT-IR) Spectroscopy

The isolated compounds were analysed by using FT-IR spectra to identify the functional groups present in compound. The sampling preparation was done by KBr pellet method in which 1 mg of the isolated compound was added into 100 mg of dry KBr (1:100 ratio) and grinded to obtain a homogeneous mixture. The mixture was then compressed under a hydraulic pressure of 8-10 tons to form a transparent pellet. These pellets were scanned in $4000\text{--}400\text{ cm}^{-1}$ and the frequency of bands were observed to confirm the specific functional groups and molecular vibrations¹².

Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy was employed for detailed arrangement of structure of the isolated compounds. This technique provides valuable information regarding the number of proton and carbon signals, number of protons and carbons, and chemical environment within a molecule. Approximately 10 mg of

the isolated compound was dissolved in an appropriate deuterated solvent and kept under magnetic field using tetramethylsilane (TMS) as an internal reference standard. The spectra were studied by chemical shifts values, signal multiplicities, coupling constants and integration values to determine the molecular structure of the isolated compounds¹³.

Mass Spectrometry (MS)

Mass spectrometry was performed to determine the molecular mass and structural characteristics of the isolated compounds. The technique involves ionization of the analyte to detect molecular ion and its fragment ions to measure their mass-to-charge (m/z) ratios. For analysis, the isolated compounds were dissolved in a highly pure volatile solvent, and introduced into the instrument through an autosampler. The obtained mass spectra were studied and recorded¹³.

Results and Discussion:

Extraction:

Successive solvent extraction of *Delphinium denudatum* wall. and *Berberis aristata* DC. stem and root materials was carried out using solvents of increasing polarity to obtain bioactive phytoconstituents. The extracts obtained with petroleum ether, acetone, ethanol and water were concentrated and

their practical percentage yields were calculated. Among all the solvents employed, the ethanolic extract exhibited the highest extraction yield in both plant species indicating its greater efficiency in extracting a broad spectrum of phytochemical constituents. Preliminary phytochemical screening of the various extracts was performed and it was revealed that the ethanolic extracts contained a wider range of secondary metabolites compared to the petroleum ether, acetone and aqueous extracts. The ethanolic extracts showed the presence of important phytoconstituents such as alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins and other bioactive constituents. The abundance and diversity of these phytochemicals suggested that ethanol is an effective solvent for the extraction of both moderately polar and polar compounds.

Isolation of Bioactive compounds:

The ethanolic extracts of both plants were selected for isolation of phytoconstituent through column chromatography. *Delphinium denudatum* wall. and *Berberis aristata* DC ethanolic extracts were loaded in silica gel packed column with the developed mobile phase petroleum ether: ethyl acetate (8:2) and ethyl acetate: hexane (6:4) respectively as eluting solvent. After all the

bands were separated, various fractions were collected based on increasing polarity. Each fraction was subjected for chemical test and TLC analysis in the same mobile phase. Those showed similar spots were scraped and collected for further study.

Characterisation of isolated compounds:

The isolated compounds such as DEL2 and BER2 of ethanolic extract of *Delphinium denudatum* wall and *Berberis aristata* DC respectively were analysed using multiple spectroscopic techniques to elucidate their structural identity.

Characterisation of Isolated compound (DEL2):

1. FTIR:

The FTIR spectra of DEL2 were obtained as shown in figure 1. DEL2 displayed characteristics absorption of H-bonded hydroxyl group at 3381.57 cm^{-1} . two peaks at 2961.16 cm^{-1} and 2933.2 cm^{-1} were observed to C-H stretching of aliphatic chains. A characteristic band appeared at 1713.44 cm^{-1} attributed to C=O stretching suggesting carbonyl group present in compound. The distinct band at 1619.91 cm^{-1} showed aromatic C=C stretching confirmed aromatic ring. Additionally, band at 1453.1 were resulted to $-\text{CH}_3$ bend

and 1265.07 cm^{-1} and 1080.91 cm^{-1} were attributed to C-O stretching

vibrations confirmed alcohols, esters, ethers or phenolic group.

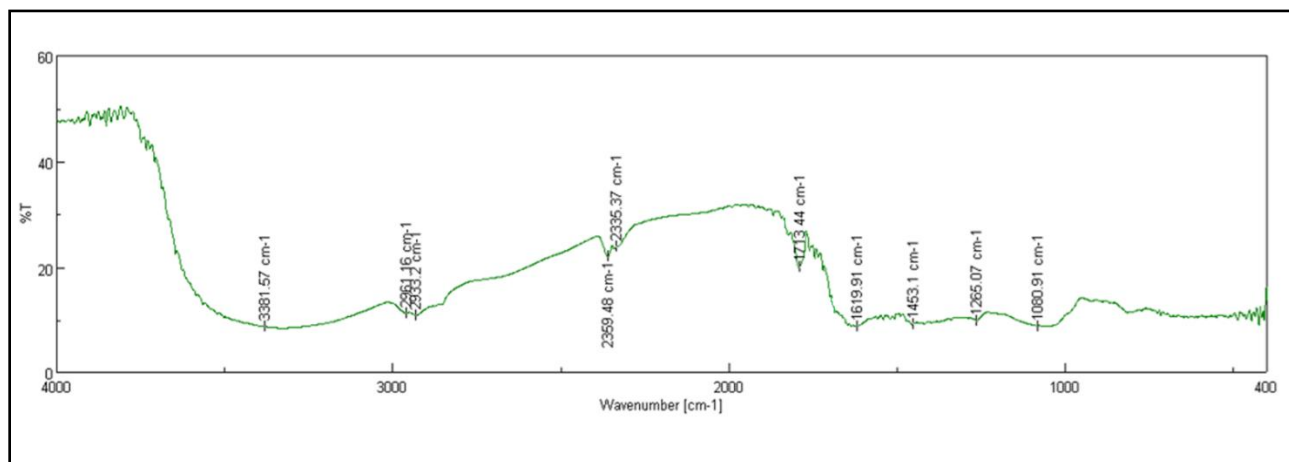
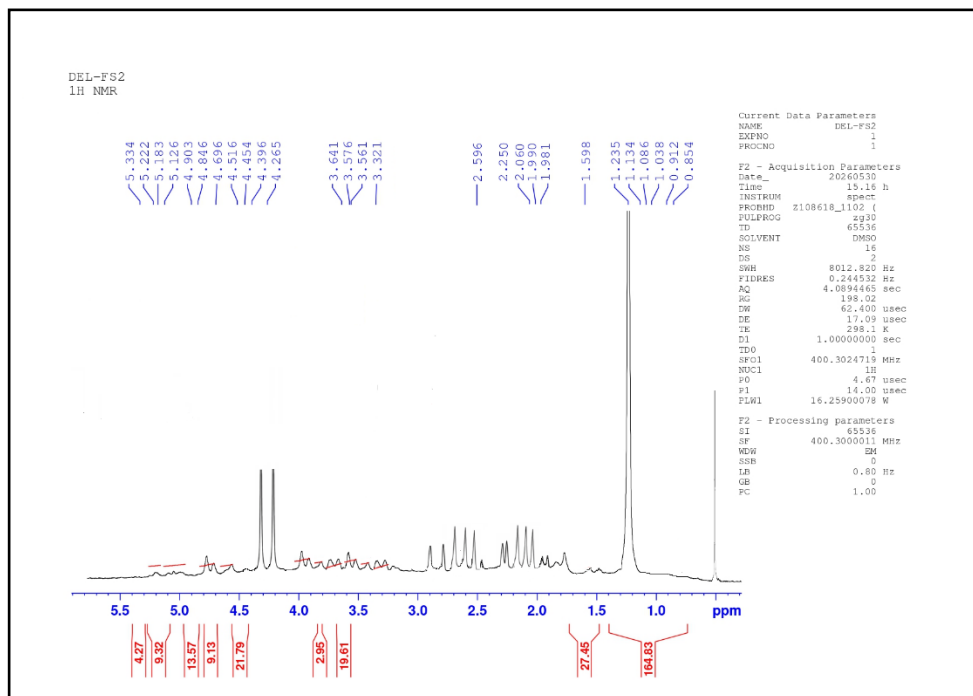
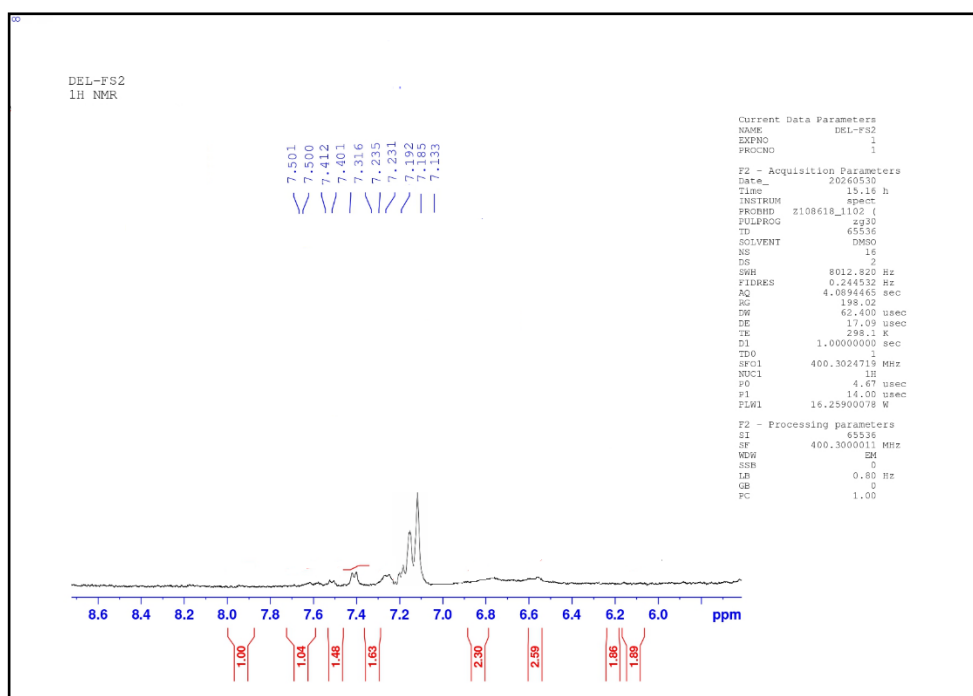


Figure 1 FTIR Spectra of DEL2

2. $^1\text{H-NMR}$:

The $^1\text{H-NMR}$ spectrum (400 MHz, DMSO) of DEL2 (Figure 2 & 3) showed aromatic region ($\delta 7.5 - \delta 7.1$ ppm), $\delta 7.501$ ppm (m, 1H), $\delta 7.412$ ppm (m, 1H), $\delta 7.401$ ppm (m, 1H), $\delta 7.316$ ppm (m, 1H), $\delta 7.235$ ppm (m, 1H), $\delta 7.192$ ppm (m, 1H), $\delta 7.133$ ppm (m, 1H). Oxygenated methine/methylene protons ($\delta 5.32 - \delta 3.3$ ppm), $\delta 4.590$ ppm (d, $J=6\text{Hz}$,

1H), $\delta 4.396$ ppm (t, $J=5\text{Hz}$, 1H), $\delta 4.265$ ppm (t, $J=5\text{Hz}$, 1H), $\delta 3.321$ ppm (m, 1H), Aliphatic region ($\delta 2.6 - \delta 2.2$ ppm) exhibited $\delta 2.596$ ppm (d, $J=11\text{Hz}$, 1H), $\delta 2.250$ ppm (d, $J=11\text{Hz}$, 1H), $\delta 2.060$ ppm (qd, $J=4.5\text{Hz}$, 1H), $\delta 1.990$ ppm (m, 1H), $\delta 1.981$ ppm (m, 1H), $\delta 1.235$ ppm (m, 1H), $\delta 1.134$ ppm (m, 1H) corresponds to multiple methyl/methylene group.

Figure 2 ^1H -NMR spectra of DEL2Figure 3 ^1H -NMR spectra of DEL2

3. ^{13}C -NMR:

The ^{13}C NMR spectra of DEL2 (Figure 4) showed $\delta 169.46$ ppm (C-7) (C=O), $\delta 166.32$ ppm (C-12), $\delta 132.66$ ppm (C-19), $\delta 132.56$ ppm (C-16,17,18), $\delta 131.46$ ppm (C-15), $\delta 124.16$ ppm (C-14) exhibited aromatic C-H carbon, $\delta 77.702$ ppm (C-2) oxygenated carbon,

$\delta 77.021$ ppm (C-5), $\delta 70.425$ ppm (C-10) aliphatic methine carbon, $\delta 46.915$ ppm (C-20) and $\delta 44.995$ ppm (C-9) attributed to aliphatic methine carbon. $\delta 43.782$ ppm (C-21) aliphatic methylene carbon, $\delta 40.182$ ppm (C-4) aliphatic methylene carbon, $\delta 26.695$ ppm (C-1) corresponded simple aliphatic methyl carbon.

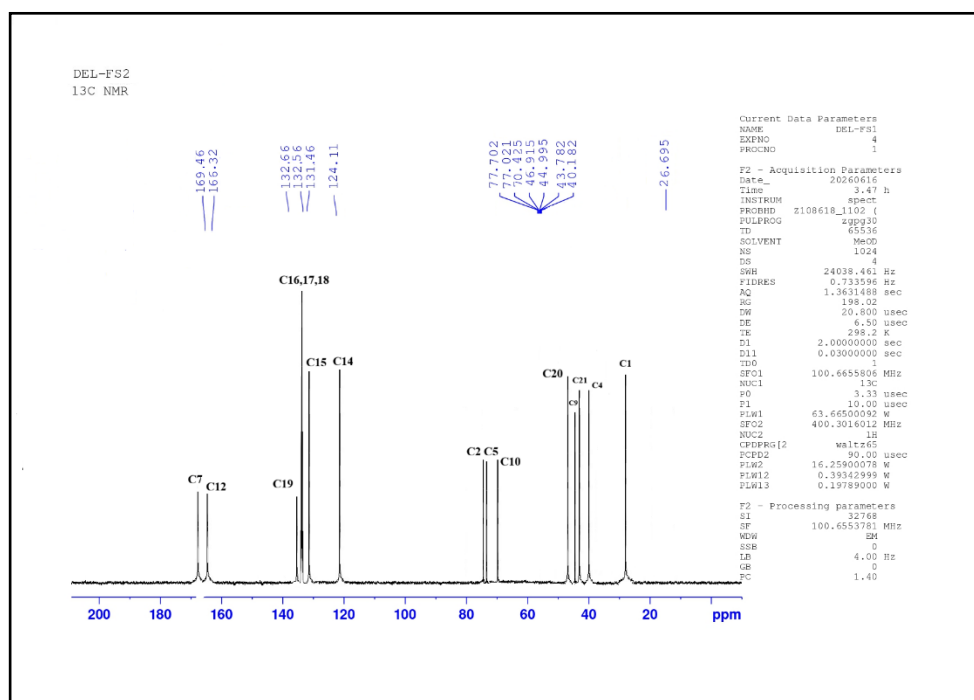
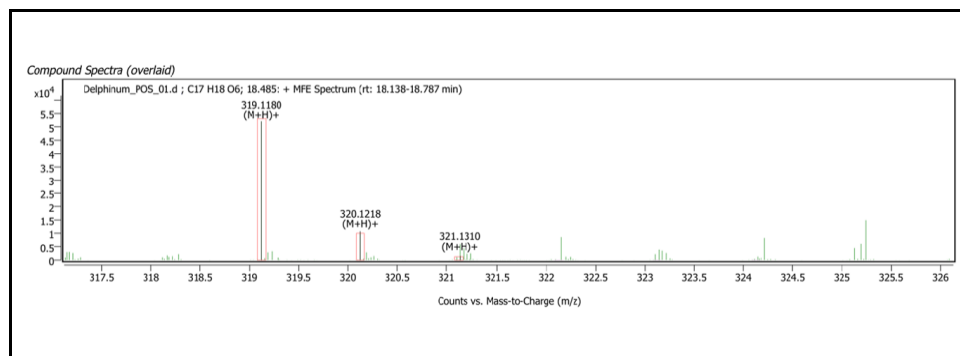


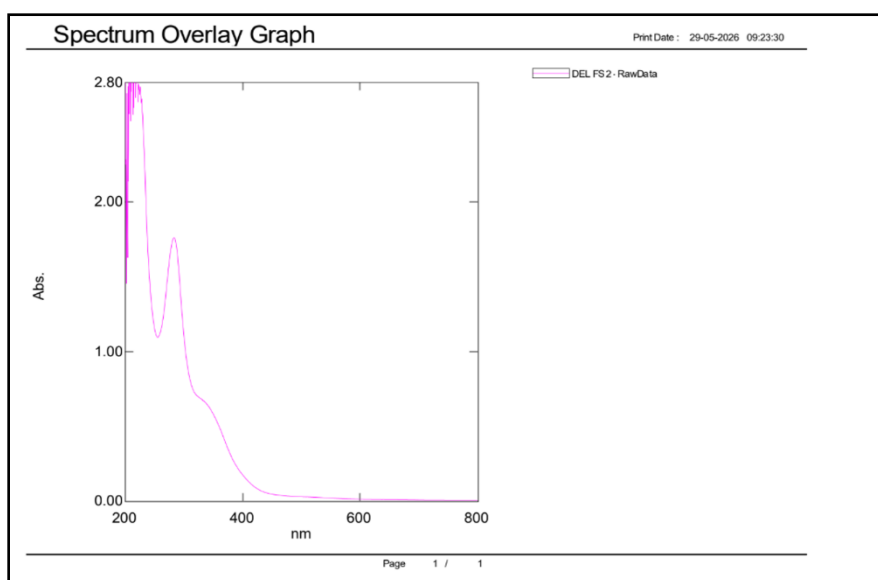
Figure 4 ^{13}C -NMR spectra of DEL FS2

4. MS Spectra:

The MS spectrum of DEL FS2 exhibited a prominent protonated molecular ion peak at m/z 319.1180 $[\text{M}+\text{H}]^+$ corresponding to a molecular weight of 318.11 Da and the molecular formula $\text{C}_{17}\text{H}_{18}\text{O}_6$. The isotopic peaks observed at m/z 320.1218 and 321.1310.

**Figure 5 Figure 60 Mass spectra of DEL2****5. UV-visible spectra:**

DEL2 sample recorded in the range of 200–400 nm. It showed the absorption maxima at λ_{max} 283 nm (Figure no. 6).

**Figure no. 6 UV spectra of DEL2****Characterisation of Isolated compound (BER2):****1. FTIR:**

The FTIR spectra of BER2 (figure 7) showed absorption characteristics at 3429.78 cm^{-1} for presence of hydroxyl group (-OH). The distinct bands appeared at 2957.3 cm^{-1} ,

2926.45 cm^{-1} and 2853.17 cm^{-1} resulting to C-H stretching. An absorption peak observed at 1633.41 cm^{-1} of C=C stretching in aromatic ring. Distinct bands were observed at 1455.99 cm^{-1} and 1418.39 cm^{-1} for -

CH₂ bend or -CH₃ bend. The absorption at 1338.36 cm⁻¹, 1275.68

cm⁻¹ and 1126.22 cm⁻¹ showed C-O stretching vibrations.

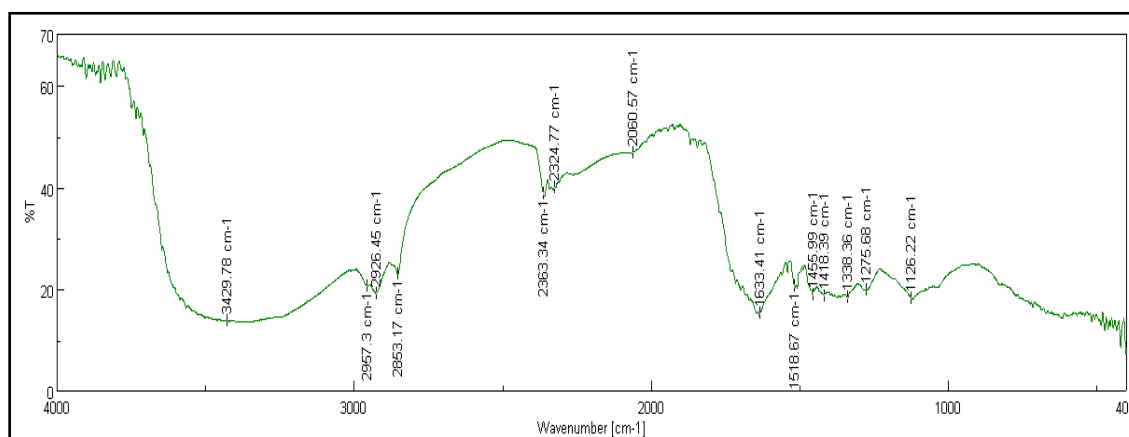


Figure 7 FTIR Spectra of BER2

2. ¹H-NMR:

The ¹H-NMR spectrum (400 MHz, DMSO) of BER2 (Figure 8) exhibited (δ4.4–δ3.3 ppm) an oxygenated methylene or methyl protons, δ4.454 ppm (t, J=5.5Hz, 1H), δ4.222 ppm (d, J= 6Hz, 1H), δ3.952 ppm (dd, J=4.5Hz, 1H), δ3.687 ppm (d, J=7Hz, 1H), δ3.344 ppm (m, 1H), δ3.214 ppm, (m, 1H), δ3.085 ppm (m, 1H). Benzylic -CH₂ group in the region (δ2.85 – δ2.20 ppm), δ2.852 ppm (d,

J=11Hz, 1H), and δ2.712 ppm (d, J=11Hz, 1H), benzylic -CH₂, δ2.653 ppm (m, 1H), δ2.504 ppm (m, 1H), δ2.200 ppm (m, 1H), δ2.181 ppm (d, J=11Hz, 1H), Aliphatic chain system (δ1.64 – δ0.85 ppm), δ1.640 ppm (m, 1H), δ1.625 ppm (s, 1H), δ1.475 ppm (m, 1H), δ1.235 ppm (m, 1H), δ1.187 ppm (m, 1H) consistent with aliphatic -CH₂, δ0.930 ppm (t, J=7Hz, 3H), δ0.912 ppm (t, J=7Hz, 3H), δ0.894 ppm (t, J=7Hz, 3H), δ0.853 ppm (t, J=7Hz, 3H).

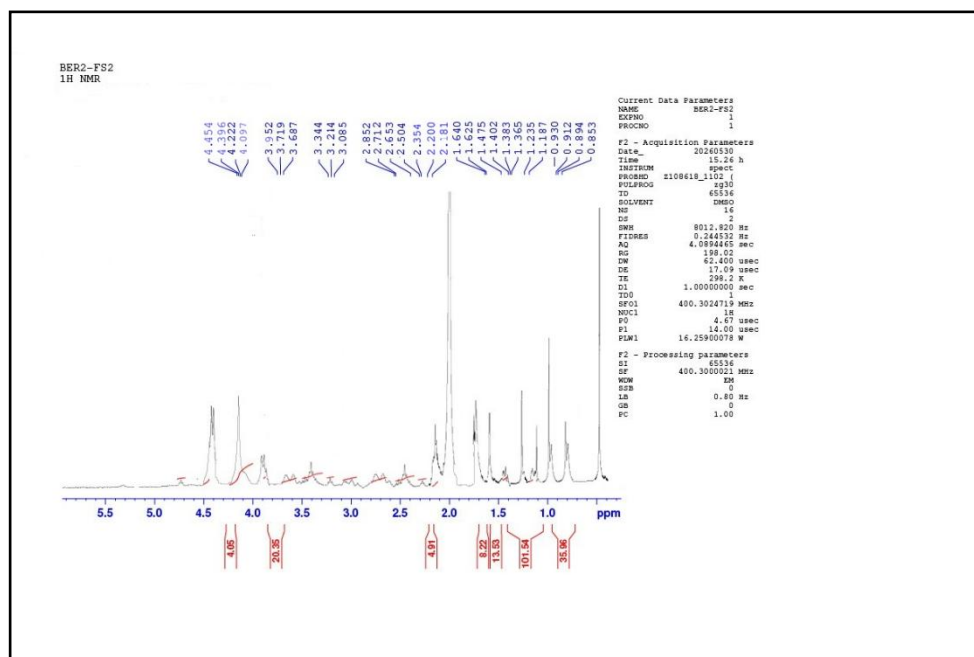


Figure 8 ^1H -NMR spectra of BER2

3. ^{13}C -NMR:

The ^{13}C NMR spectra of BER2 (Figure 9) showed δ 147.18 ppm (C-2) and δ 146.35 ppm (C-15), δ 127.18 ppm (C-3), δ 126.31 ppm (C-16) exhibited aromatic C-H, δ 80.470 ppm (C-11), δ 73.725 ppm (C-21) resulted oxygenated methine likely C-O-CH carbons, δ 67.318 ppm (C-14) and δ 58.421 ppm (C-17) -OCH₂ carbon, δ 49.208 ppm (C-6), δ 48.995 ppm (C-

10). δ 42.782 ppm (C-23) O-CH₃ substituents, δ 38.569 ppm (C-20) benzylic/aliphatic CH₂ δ 26.705 ppm (C-19) and δ 23.495 ppm (C-12), aliphatic CH₂ side chain of methylene, δ 21.705 ppm (C-9), δ 21.051 ppm (C-24, C-25), δ 20.695 ppm (C-1), δ 16.210 ppm (C-7) terminal CH₃ groups or methyl substituents.

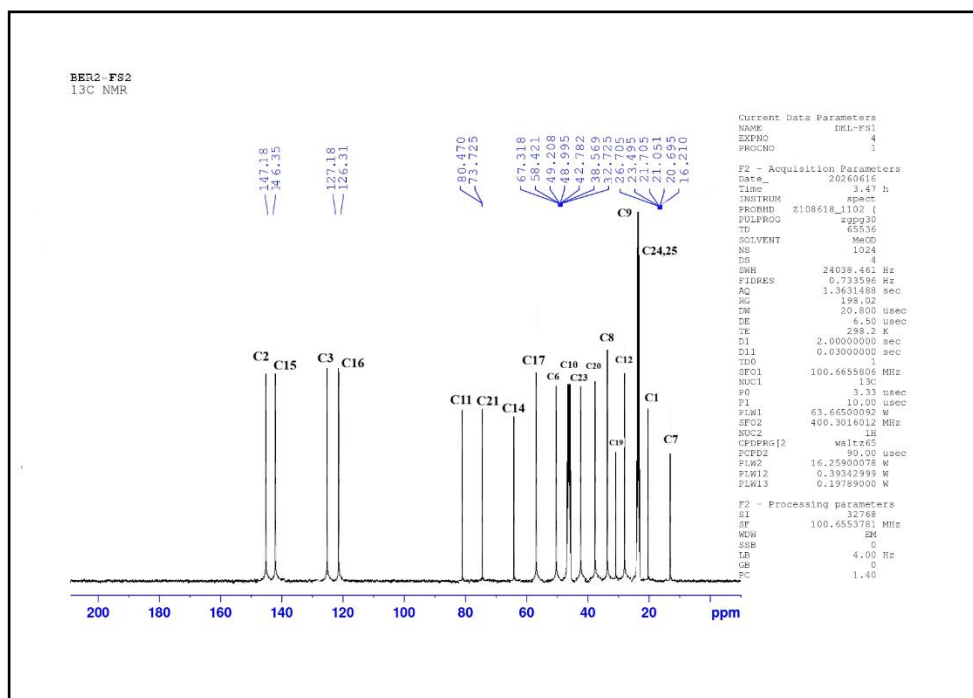


Figure 9 ^{13}C -NMR spectra of BER2

4. Mass spectra:

The mass spectrum of BER2 resulted molecular ion peak at m/z 370.2958 $[\text{M}+\text{NH}_4]^+$ with a molecular weight

of 352.26 Da. The isotopic peaks at m/z 371.2990 and 372.3017 with molecular formula $\text{C}_{21}\text{H}_{36}\text{O}_4$.

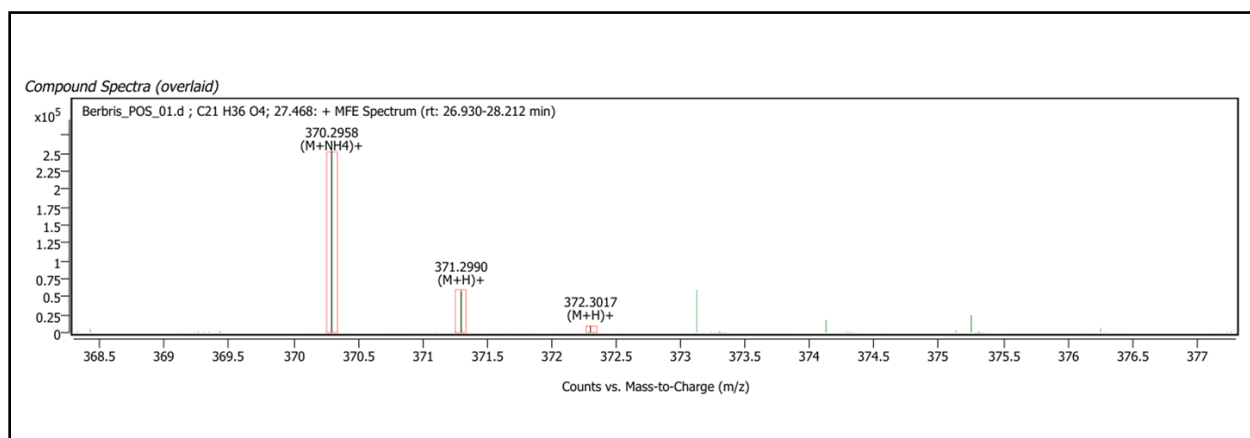


Figure 10 Mass spectra of BER2

5. UV-Visible spectroscopy:

The UV-visible spectra of BER2 displayed absorption maximum λ_{max}

at 281 nm indicating the presence of specific functionality within the sample.

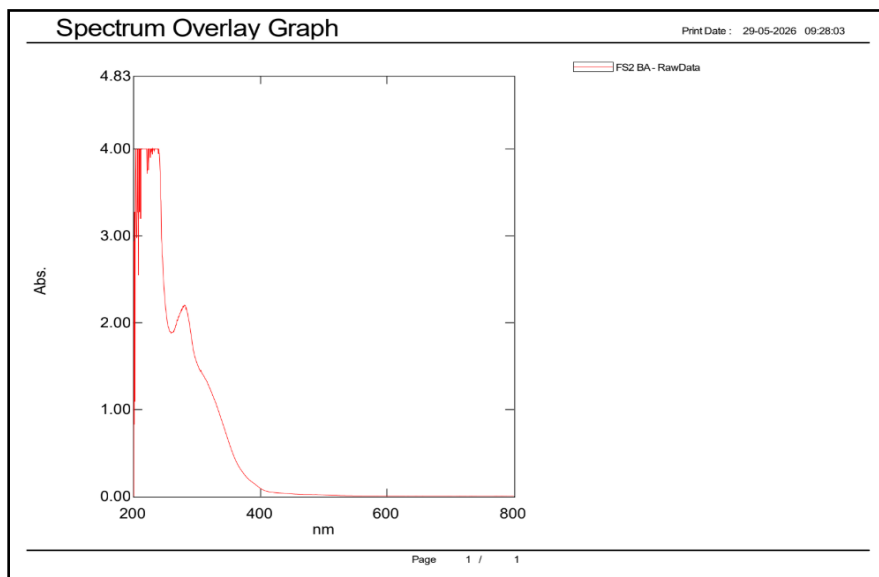


Figure 11 UV Spectra of BER2

Conclusion:

The present investigation successfully accomplished the extraction, isolation and characterization of bioactive phytoconstituents from the roots of *Delphinium denudatum* Wall. and *Berberis aristata* DC. Based on preliminary phytochemical screening and TLC profiling, the ethanolic extracts were selected for chromatographic separation leading to the isolation of two purified compounds designated as DEL2 and BER2. The isolated compounds were comprehensively characterized using FT-IR, UV-Visible

spectroscopy, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and Mass spectrometry which revealed the presence of hydroxyl, carbonyl, aromatic and ether functionalities with a protonated molecular ion peak at m/z 319.1180 corresponding to the molecular formula $\text{C}_{17}\text{H}_{18}\text{O}_6$ in DEL2. Similarly, BER2 exhibited characteristic hydroxyl, aromatic and oxygenated aliphatic groups and showed a molecular ion peak at m/z 370.2958 $[\text{M}+\text{NH}_4]^+$ indicating a molecular formula of $\text{C}_{21}\text{H}_{36}\text{O}_4$. The UV absorption maxima observed at 283 nm for DEL2 and 281 nm for BER2 further supported the presence of conjugated chromophoric systems such as [6-hydroxy-6-methyl-2,5-dioxo-3a,4,7,7a-tetrahydro-3H-

1-benzofuran-3-yl]methyl benzoate and 9-[3-hydroxy-6-(2-hydroxyethylidene)-2-methyloxepan-2-yl]-2,3,6-trimethylnon-3-en-5-one respectively within their structures. In conclusion, the study highlights *D. denudatum* and *B. aristata* as important sources of bioactive phytoconstituents with significant chemical diversity. The successful isolation and structural elucidation of DEL2 and BER2 enrich the phytochemical database of these medicinal plants and provide a scientific basis for their potential pharmacological applications and future drug discovery research.

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