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Isolation and Characterization of polyphenolic compounds from Ethanolic Extract of *Hibiscus syriacus*

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ABSTRACT
Background: Polyphenolic compounds are known for their structural diversity and potential therapeutic applications. *Hibiscus syriacus*, a plant traditionally used in herbal medicine, contains a rich profile of phytoconstituents, including polyphenols. This study aimed to isolate and structurally characterize polyphenolic compounds from the ethanolic extract of *H. syriacus* flowers using advanced spectroscopic techniques.
Methods: Flowers of *H. syriacus* were collected, shade-dried, powdered, and extracted using Soxhlet extraction with ethanol. The crude extract was partitioned and subjected to column chromatography and Sephadex LH-20 for fractionation. Final purification was achieved using reversed-phase semi-preparative high-performance liquid chromatography (HPLC). Isolated compounds were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Proton and Carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR), and Mass Spectrometry (MS) for detailed structural elucidation.
Results: Two distinct polyphenolic compounds (Compound 1 and Compound 2) were successfully isolated and identified. FTIR analysis confirmed the presence of key functional groups such as hydroxyl, aromatic rings, and carbonyl groups. NMR spectra provided detailed insights into the structural framework, revealing substitution patterns consistent with known polyphenolic structures. Mass spectrometry determined the molecular weights of Compound 1 (MW 164.16; m/z 165.05) and Compound 2 (MW 194.18; m/z 195.06), confirming their purity and identity.
Conclusion: The study successfully demonstrated the extraction, isolation, and spectroscopic characterization of two polyphenolic compounds from *Hibiscus syriacus* flowers. These findings contribute to the phytochemical profiling of the species and provide a foundation for future research into their potential biological or pharmaceutical applications.

Introduction

Polyphenolic compounds represent one of the most extensively researched groups of natural products due to their structural diversity and widespread occurrence in the plant kingdom. These compounds are secondary metabolites characterized by the presence of multiple phenol units and are commonly classified into flavonoids, phenolic acids, tannins, stilbenes, and lignans. They are typically involved in plant defense mechanisms against environmental stressors such as ultraviolet radiation, pathogens, and herbivores, and have been extensively studied for their potential applications in pharmaceuticals, nutraceuticals, cosmetics, and food industries.

The structural features of polyphenols, particularly the number and position of hydroxyl groups, contribute significantly to their chemical reactivity and biological potential. The growing interest

in these compounds has led to an increased emphasis on isolating and characterizing individual polyphenols from various plant species. Accurate isolation and structural elucidation are essential for understanding the role of these molecules and for evaluating their potential for future applications.

The process of isolating polyphenolic compounds from plant sources generally begins with solvent extraction. Ethanol is widely used as a solvent due to its efficiency in dissolving a broad spectrum of phenolic compounds while maintaining environmental safety and compatibility with food and pharmaceutical applications. Following extraction, various chromatographic techniques, such as column chromatography and high-performance liquid chromatography (HPLC), are employed to purify and isolate individual compounds. These procedures are critical for separating closely related phytochemicals and obtaining sufficient quantities for analytical characterization.

Advanced spectroscopic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) are indispensable tools for the structural analysis of isolated polyphenols. FTIR spectroscopy provides information about the functional groups present in a molecule by analyzing characteristic vibrational frequencies. NMR spectroscopy, both proton (^{1}H) and carbon (^{13}C), allows for detailed insight into the molecular framework, hydrogen bonding patterns, and substitution arrangements. Mass spectrometry complements these techniques by offering molecular weight determination and fragmentation patterns, which are essential for confirming the molecular identity.

Hibiscus syriacus Linn., a deciduous shrub of the Malvaceae family, is commonly known as Rose of Sharon. It is native to East and Southeast Asia and widely cultivated for ornamental purposes due to its attractive flowers. In addition to its aesthetic value, *H. syriacus* has been traditionally used in various systems of herbal medicine, particularly in Korean, Chinese, and Japanese traditions. The flowers, leaves, bark, and roots have been employed in folk remedies for their presumed therapeutic benefits, including anti-inflammatory, astringent, and demulcent properties.

Previous phytochemical investigations of *Hibiscus syriacus* have revealed the presence of numerous bioactive constituents, including flavonoids, anthocyanins, triterpenoids, and fatty acids. Among these, the polyphenolic constituents have garnered particular interest due to their structural richness and diversity. Despite its traditional usage and known phytochemical profile,

systematic isolation and detailed characterization of individual polyphenolic compounds from *H. syriacus*, particularly from its flowers, remain relatively underexplored.

The current study focuses on the isolation and structural characterization of polyphenolic compounds from the ethanolic extract of *Hibiscus syriacus* flowers. The flowers were collected, shade-dried, and powdered prior to extraction using Soxhlet apparatus with ethanol as the solvent. The resulting crude extract was concentrated and subjected to fractionation using liquid-liquid partitioning and silica gel column chromatography. Subsequent purification was achieved using Sephadex LH-20 and reversed-phase semi-preparative HPLC.

The isolated compounds were characterized using a suite of analytical tools. FTIR spectroscopy was employed to identify characteristic functional groups such as hydroxyl (-OH), carbonyl (C=O), and aromatic ring systems typically present in polyphenols. ^{1}H-NMR and ^{13}C-NMR spectra provided detailed insights into the hydrogen and carbon frameworks, including coupling constants, substitution patterns, and chemical environments. These data were essential for determining the backbone structure and side-chain configurations of the polyphenolic compounds. Additionally, mass spectrometry was used to determine the molecular masses and fragmentation patterns, offering confirmatory evidence for the structural elucidation.

This study is significant in several ways. First, it contributes to the phytochemical mapping of *H. syriacus* by isolating and identifying individual polyphenolic constituents. Second, it establishes a reliable method for extraction, purification, and analysis that can be used for other bioactive plant materials. Third, the structural characterization of these compounds lays the groundwork for future studies related to their potential applications in pharmaceutical, nutraceutical, or cosmetic formulations.

Materials and methods

Plant Material

The flowers of *H.syriacus* was collected at Indore, Madhya Pradesh, India and was confirmed taxonomically by Dr. S. N. Dwivedi, Professor, Janta PG College, APS university Rewa, M.P. Dated 25/03/2022. A voucher specimen (H0123) has been deposited at the herbarium of the J/Bot/2022/HSL-012 AND J/Bot/2022/HSF-013.

Figure 1 :H.syriacus

Soxhlet Extraction

Plant flowers were dried in hot air oven for 72 h at 60℃ and powdered by using electric mill. 25 g of the powdered material was wrapped in filter paper and impregnated with solvent. Then it was extracted with 250 mL of ethanol analytical grade for a period of 24hrs. The extract was then filtered through Whatmann No 1 filter paper. Then it was evaporated in a vacuum rotary evaporator under reduced pressure at 40℃ until the filtrate was reduced to one-third of the starting filtrate volume. After the concentrate was freeze-dried and stored at 4℃ for further analysis.[22]

Figure 2 :Ethanolic Extraction of H.syriacus

Isolation of Ethanol Extract

The final extract, approximately 5g, was divipartitioned between ethyl acetate and water. A 2.25 g quantity of ethyl acetate extract was treated to column chromatography (CC) on silica gel with dichloromethane-methanol (100:1 to 0:100, V/V) to give four fractions (Fr. 1 to Fr. 4). Fr.4 (1.23 mg) was purified by sephadex LH-20 with Methanol. Reversed-phase (RP) semi-preparative HPLC (Shimadzu, Japan) was used with an SPD-20A UV detector, LC-20AT binary pump, and a YMC-Pack ODS preparative column (5 μm, 250 × 20.0 mm) with 30% Acetonitril/water to give compound 1 (0.512 mg) and compound 1 (0.364 mg).[23 - 25]

Characterization using Fourier transform infrared spectroscopy (FTIR)

A Perkin-Elmer Spectrum Two FTIR spectrometers (Norway, CT, USA) equipped with a Gladi ATR from Pike Technologies (Wisconsin, USA) were used to acquire transmittance spectra of the substances indicated above. Measurements were taken from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and averaged across 64 scans. [26]

Characterization by 1H and 13C NMR spectroscopy

The 1H- and 13C-NMR spectra were recorded at room temperature using a Mercury 300 spectrometer (Varian/Agilent, Palo Alto, CA, USA) with an automated triple broadband (ATB) probe. Sample concentrations were 1 mg/mL in DMSO-d6. The relaxation delay was 1 second following a 45-degree pulse with an acquisition duration of 1042 seconds. The 1H-NMR spectrum was scanned 256 times, whereas the 13C coupled spectra was scanned 6800 times. Both 13C spectra were collected using the nuclear overhauser effect (NOE). The spectral width was 18,832 Hz, with 39,248 points captured and zero-filled to 256K points before Fourier processing to provide a digital resolution of 0.14 Hz. No apodization was applied.[26-28]

Mass Spectrum

The mass spectra were collected using a Shimadzu LCMS 2020 instrument in an acetonitrile solvent.[29-32]

Results and Discussion

The chemical constituents were isolated from ethanol extract of *H. syriacus* by column chromatography four fractions (Fr1 to Fr 4) were identified as shown in the figure - 3.

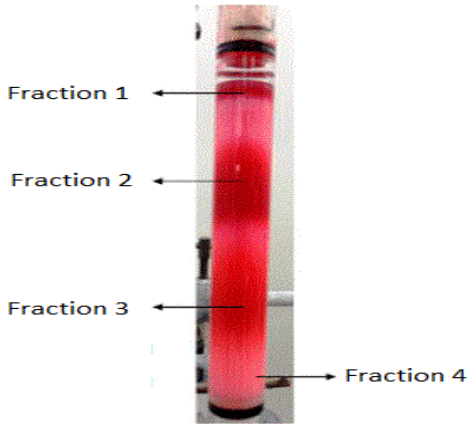
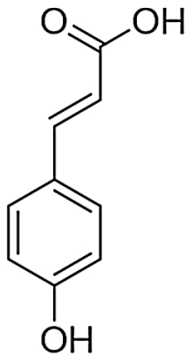
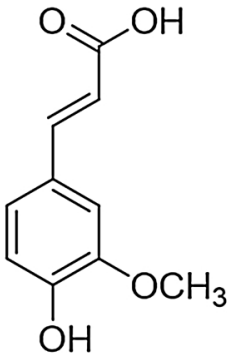


Figure 3: Isolation of Compound from ethanol extract of H. syriacus by column chromatography

The fraction 4 was further purified with semipreparative HPLC and it yielded Two Compound (Compound1 and Compound 2). From further characterization by IR, Mass and NMR the isolate were identified as two polyphenols as shown in the figure .4[38].



Compound 1



Compound 2

Figure 4: Isolated of Compound from ethanol extract of H. syriacus by semipreparative HPLC

FTIR spectral analysis

The functional groups of the isolated compounds (Compound 1 and Compound 2) were identified using IR spectral analysis. Figure 5& 6is the FTIR spectra of the extract while summarizes the different bands obtained and their assignation.

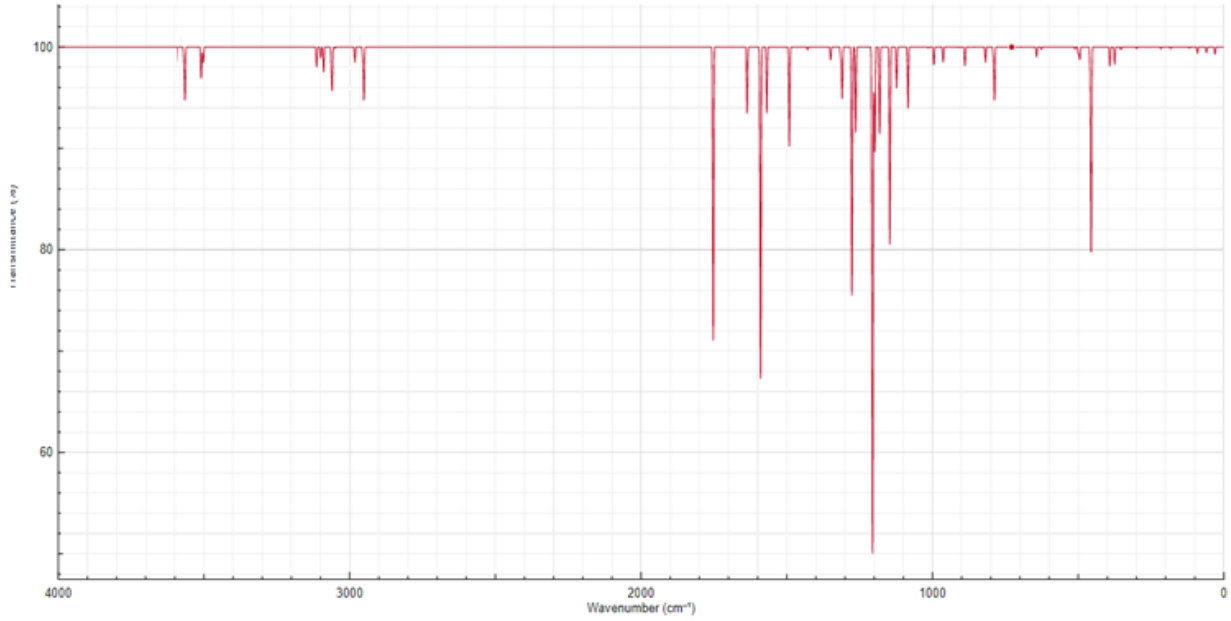


Figure 5 FTIR of Compound 1

FTIR of Compound 1

IR (KBr) (ν, cm-1): 3572,3465(OH), 3080(CHarom), 2950(CHaliph), 1720(C=O), 1205(C-O)

ESI-MS

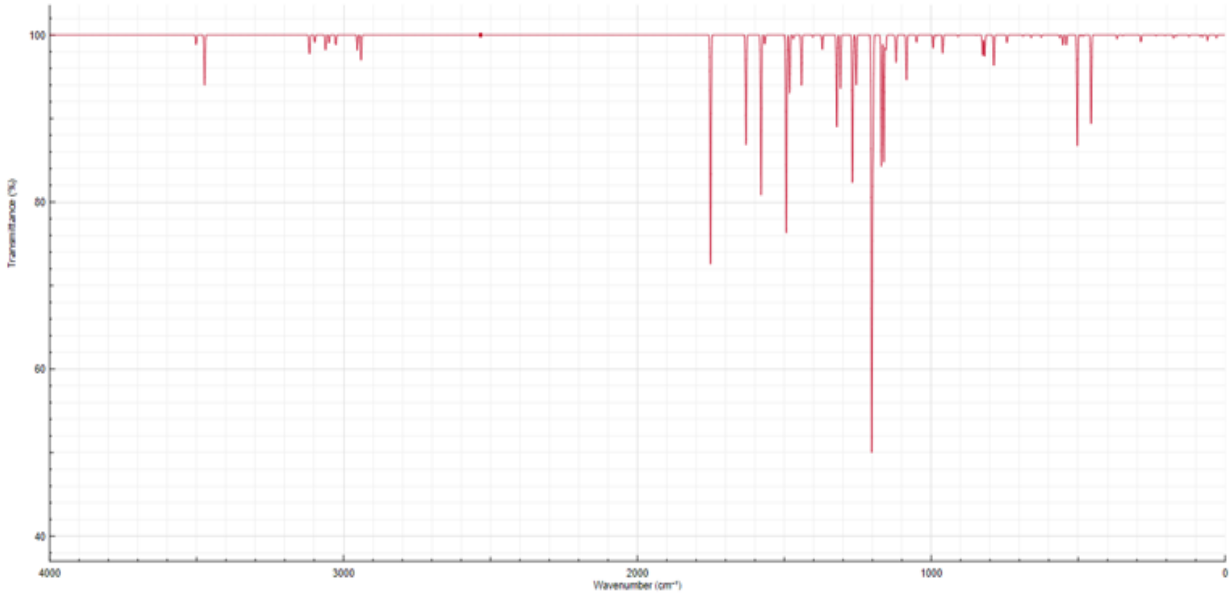


Figure 6 FTIR of Compound 2

FTIR of Compound 2

IR (KBr) (ν, cm-1): 3521,3486(OH), 3072(CHarom), 2966(CHaliph), 1734(C=O), 1190(C-O)

ESI-MS

NMR Spectrum

In the ^{1}H -NMR spectrum of Compound 1, ^{1}H NMR : δ 9.83 (s,1H, –COOH), 7.91 (s, 1H, –OH), 7.87-7.84 (m, 2H, phenyl), 7.64-7.61(m, 2H, phenyl), 6.13 (s, 2H, -C=CH)

were absorbed and recorded in figure. 7.

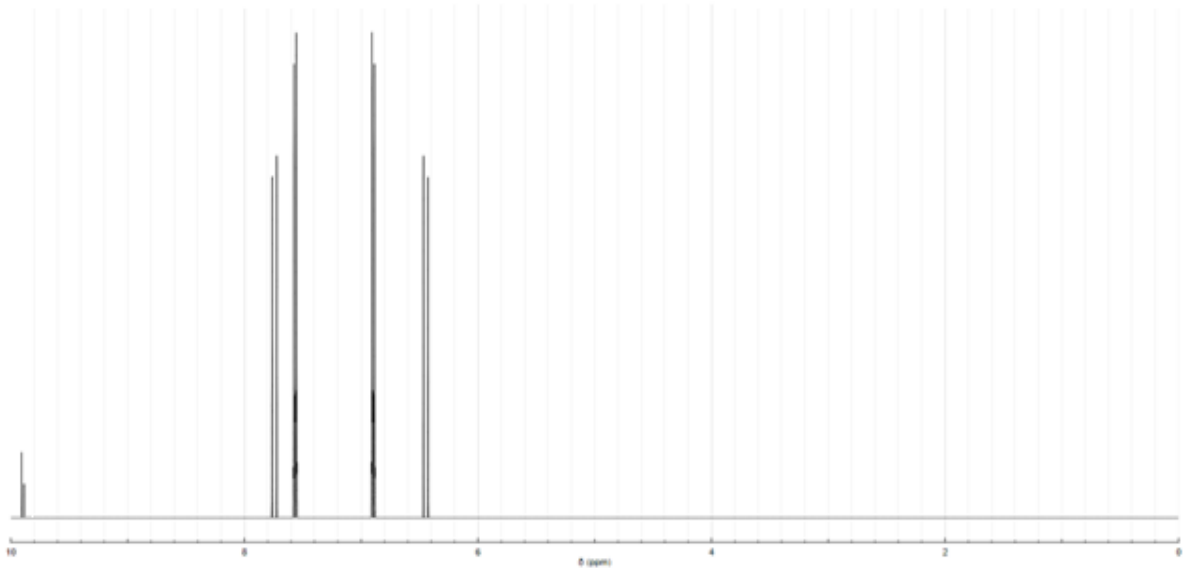


Figure 7 [1]HNMR of Compound 1

In ^{13}C-NMR spectrum of compound 1 ^{13}C NMR: δ 115.5 (2C, s), 120.6 (1C, s), 128.3 (2C, s), 134.8 (1C, s), 144.3 (1C, s), 157.2 (1C, s), 167.7 (1C, s). (Figure 8).

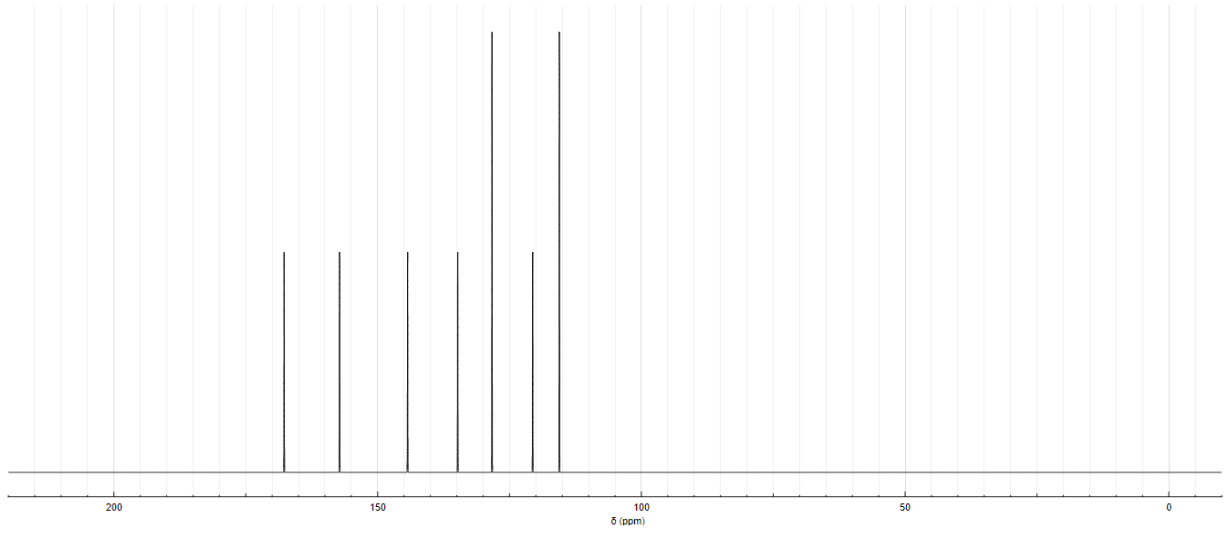


Figure 8 [13]C NMR of Compound 1

In the 1H-NMR spectrum of Compound 2, 1H NMR : δ 9.73 (s,1H, –COOH), 7.84 (s, 1H, –OH), 7.68-7.63 (m, 2H, phenyl), 6.96-6.92(m, 2H, phenyl), 6.51 (s, 2H, -C=CH) 3.93(s, 3H, –OCH3)

. Were absorbed and recorded in figure. 9

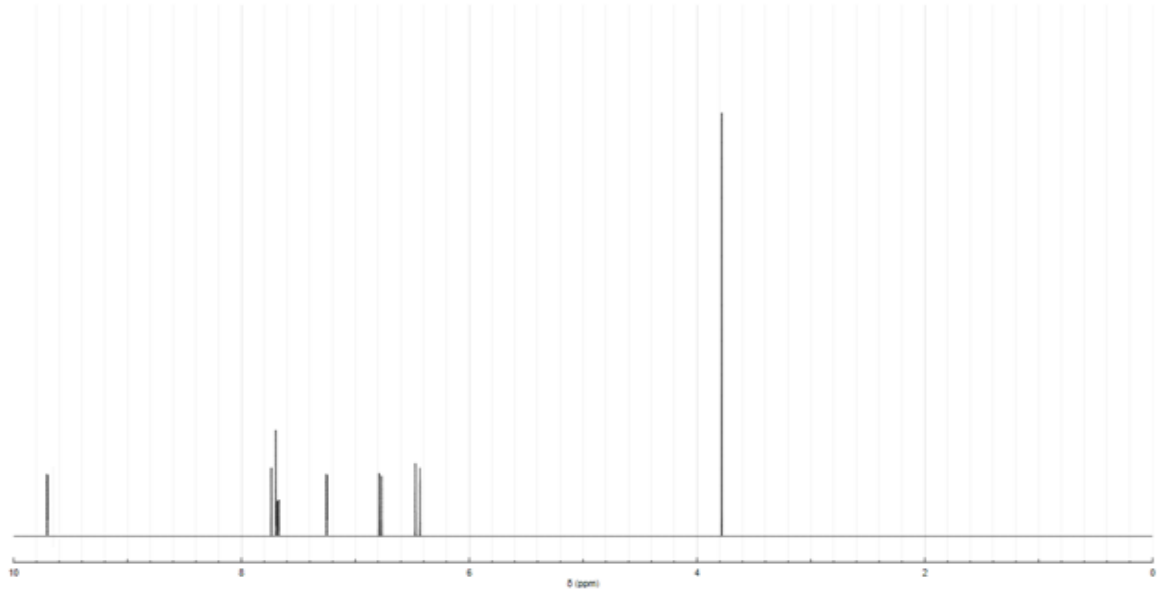


Figure 9 ^{1}H NMR of Compound 2

In ^{13}C-NMR spectrum of compound 2 13C NMR: δ 55.9 (1C, s), 110.1 (1C, s), 115.9 (1C, s), 120.6 (1C, s), 128.3 (1C, s), 129.2 (1C, s), 144.3 (1C, s), 147.1 (1C, s), 147.8 (1C, s), 167.7 (1C, s). (Figure 10).

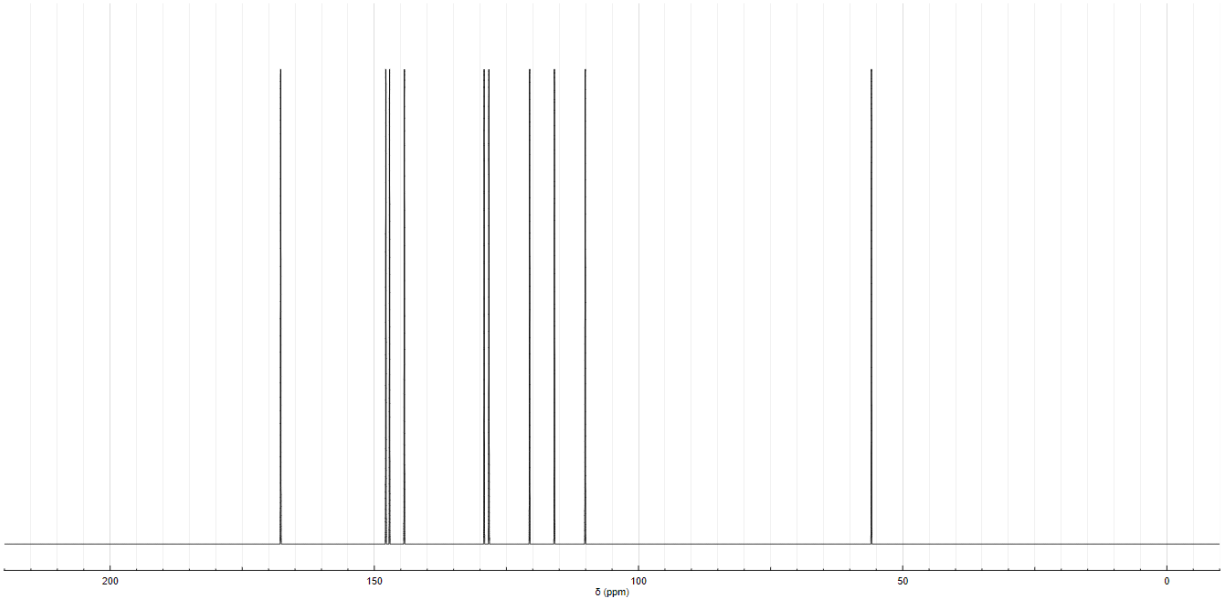


Figure 1013C NMR of Compound 2

Mass Spectrum

The mass spectra of Compound 1 shows 7 fragmentation peaks with the molecular weight of 164.16. with m/z at 165.05. (Figure 11)

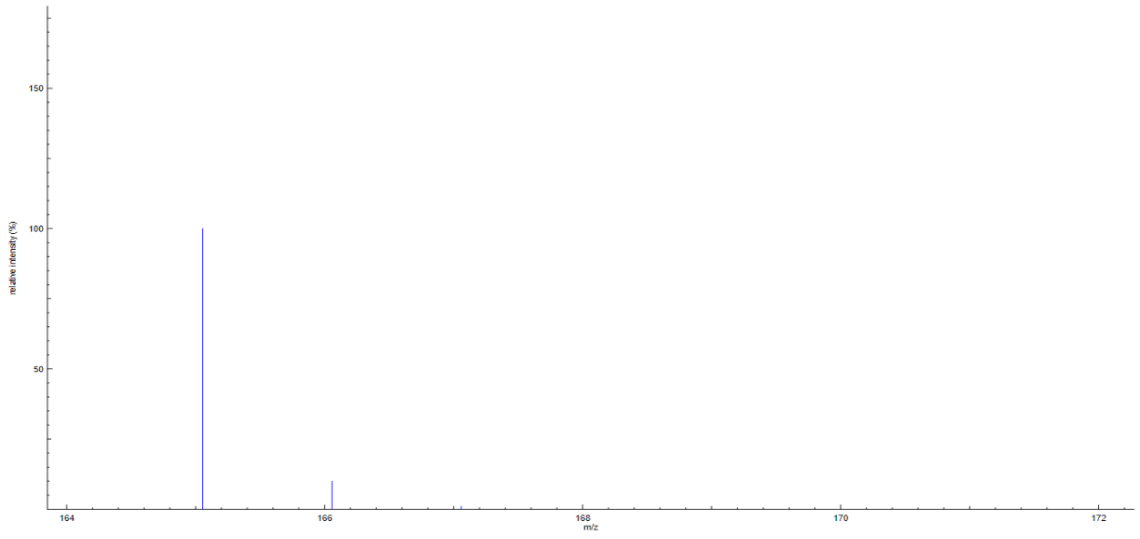


Figure 11: Mass Spectrum of Compound 1

The mass spectra of Compound 2 shows 7 fragmentation peaks with the molecular weight of 194.18. with m/z at 195.06. (Figure 12)

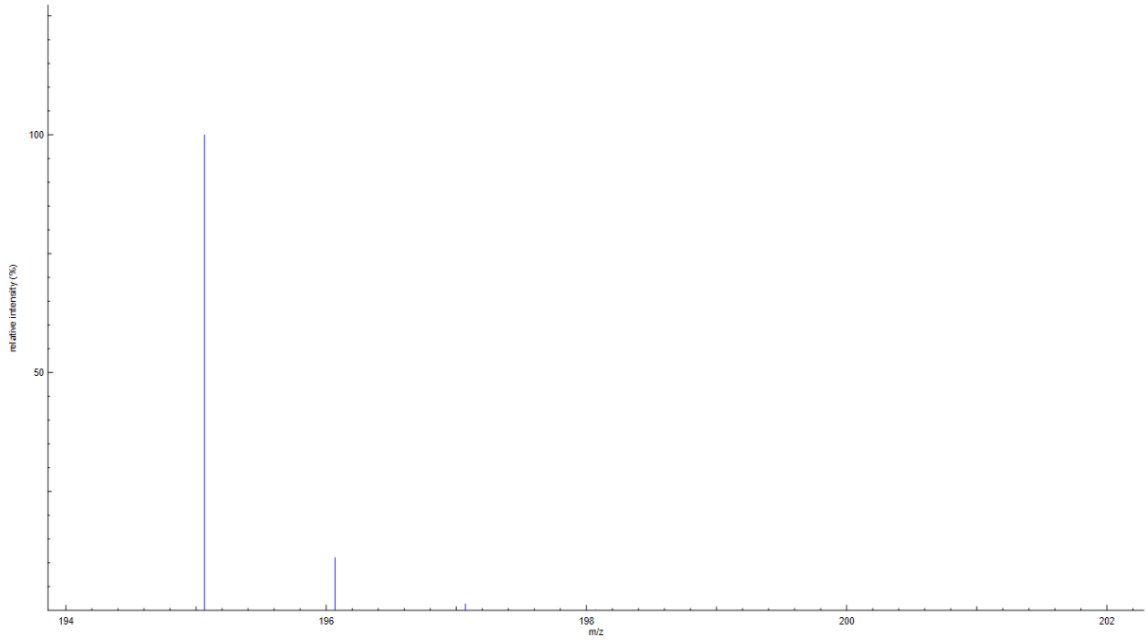


Figure 12: Mass Spectrum of Compound 2

Conclusion

The present study provides a comprehensive analysis of the isolation and structural elucidation of two polyphenolic compounds from the ethanolic extract of *Hibiscus syriacus* flowers. The research was motivated by the increasing scientific interest in natural polyphenols due to their structural complexity, abundance in medicinal plants, and diverse functional properties. Through a carefully designed methodological approach involving solvent extraction, chromatographic separation, and advanced spectroscopic techniques, we successfully identified and characterized two distinct polyphenolic compounds, referred to as Compound 1 and Compound 2.

The ethanolic extract of *H. syriacus* was obtained through Soxhlet extraction, which proved to be effective in isolating a rich fraction of polyphenols. Subsequent purification using column chromatography and Sephadex LH-20 enabled the separation of individual fractions, among which the final purification through reversed-phase semi-preparative HPLC yielded two chemically distinct compounds in purified form. The strategic use of ethanol as an extraction

solvent, combined with gradient elution systems during chromatographic steps, was crucial in obtaining the targeted polyphenolic fractions.

Fourier Transform Infrared Spectroscopy (FTIR) provided initial confirmation of functional groups commonly present in polyphenolic structures, including hydroxyl (-OH) stretching, aromatic ring vibrations, and carbonyl (C=O) groups. These findings were corroborated with high-resolution Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) data, which revealed detailed substitution patterns and ring structures. The NMR spectra showed characteristic signals corresponding to aromatic protons, double bond configurations, and methoxy or hydroxyl substitutions—features typical of bioactive polyphenolic compounds. Mass spectrometric analysis further validated the structural assignments by confirming the molecular weights of Compound 1 (MW 164.16) and Compound 2 (MW 194.18), with respective m/z values of 165.05 and 195.06 in positive ion mode.

The structural information derived from this multi-spectroscopic analysis not only confirms the identity of the isolated compounds as polyphenols but also demonstrates the reliability of combining chromatographic and spectral techniques for plant-based compound characterization. This integrated approach ensures high precision in natural product research and helps in the accurate identification of minor yet biologically significant phytoconstituents.

This investigation enriches the existing phytochemical knowledge of *Hibiscus syriacus*, a plant already known for its ethnomedicinal applications. While previous reports have indicated the presence of polyphenols in various parts of the plant, the isolation and detailed structural elucidation of specific compounds from its flower extract had remained largely unreported. By identifying these constituents, this study sets the stage for further exploration into their functional roles, stability, and interactions with biological systems.

The successful isolation and comprehensive characterization of two polyphenolic compounds from *H. syriacus* flower extract underscores the plant's rich phytochemical potential. The findings not only affirm the value of traditional botanical resources in modern scientific research but also open new avenues for the development of polyphenol-based natural products. Future studies could focus on quantitative analysis, bioavailability assessments, and evaluation of these compounds for antioxidant, anti-inflammatory, or other therapeutic activities.

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