Research Article

# Pharmacognostic And Phytochemical Evaluation Of Tinospora Cordifolia, Tephrosia Purpurea And Cassia Occidentalis As A Standardized Herbal Source

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**ABSTRACT:**

This study presents a comprehensive phytochemical and pharmacognostic evaluation of Tinospora cordifolia (root), Cassia occidentalis (root), and Tephrosia purpurea (aerial part). The dried plant materials were assessed for moisture, ash, and extractive values, revealing that C. occidentalis exhibited the highest moisture and total ash content, while T. purpurea showed the lowest. Methanolic extracts yielded the richest profiles of secondary metabolites, including alkaloids, flavonoids, phenolics, and glycosides, highlighting their polar and water-soluble nature. Preliminary TLC and spectroscopic techniques (UV, FTIR, NMR, and MS) facilitated the structural elucidation of key phytochemicals such as tetrahydroxy benzapyranobenzopyran dione (T. cordifolia), pentahydroxyflavone (C. occidentalis), and trihydroxybenzoic acid (T. purpurea). These findings underscore the medicinal potential of these plants, particularly due to their antioxidant and polyphenolic content, and provide a strong foundation for further pharmacological exploration and therapeutic development.

## INTRODUCTION:

Around the world, traditional healthcare systems have made extensive use of herbal remedies. Because of its accessibility, affordability, and historical legitimacy, plant-based treatments have seen resurgence in popularity worldwide in recent years. Medicinal herbs remain the foundation of primary healthcare, especially in places such as Asia, Africa, and South America. There is a rising focus on scientifically verifying traditional herbal medicines to guarantee their efficacy, safety, and consistency as contemporary pharmacology increasingly acknowledges the benefits of phytotherapy. (1)

Three medicinal plants—Tinospora cordifolia, Tephrosia purpurea, and Cassia occidentalis are frequently used in Indian traditional systems like Ayurveda and Siddha to treat metabolic diseases, inflammatory conditions, and liver disorders. The presence of bioactive Phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds is primarily responsible for these plants' well-known Hepatoprotective properties.

Tinospora cordifolia, often known as "Guduchi" or "Amrita," is well known for its Hepatoprotective, Anti-inflammatory and Immunomodulatory qualities. (2) Tephrosia purpurea Known as "Sharpunkha," It has long been utilized as a blood purifier, liver tonic, and treatment for bronchitis, skin conditions, and urinary issues. (3) Commonly found in trash and untamed places, Cassia occidentalis, also known as "Kasamarda," is used in traditional medicine to treat liver conditions, urinary tract infections, and general detoxification.(4).

To establish Tinospora cordifolia, Tephrosia purpurea, and Cassia occidentalis as standardized herbal sources, the current study is to conduct a thorough Pharmacognostic and Phytochemical examination of these plants. Using contemporary methods, this involves morphological characterization, physicochemical analysis, first phytochemical screening, and chromatographic fingerprinting. To create herbal formulations with repeatable therapeutic effectiveness that have been scientifically confirmed, such rigorous assessment is necessary.

## MATERIALS AND METHODS

### 1. Collection, Identification and Drying of plant material

The roots of plant Tinospora cordifolia and Cassia occidentalis, aerial part of Tephrosia purpurea were collected from local area. The selected plants were also authenticated Botanical Survey of India, Pune, Maharashtra . After authentication of

plants, its root and aerial parts were dried at room temperature until they turn out to be free from dampness. Plants materials were powdered and through sifter #40, and subjected to institutionalization with diverse parameter.

**2. Pharmacognostic evaluation**

**Standardization of plant material** :

Standardization of roots and aerial part powder were done to evaluate the quality and purity of the drug. Various Standardization parameters like Loss on drying (Moisture content), Ash values (Acid insoluble ash and water soluble ash), Extractive values (Petroleum ether, chloroform, Hydroalcoholic and Methanol) were carried out. Physicochemical evaluation of herbal drug help in establishment of standard diagnostic values which can be useful in authentic determination of purity and quality of herbal raw material[(5)].

**A) Determination of Loss on drying:**

An excess of water in medicinal plant material will lead to deterioration through microbial growth or enzyme mediated hydrolysis in glycoside containing plants. Loss on drying is the loss in weight in % w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions[(5)].

**Loss on drying (%) = Loss in weight × 100/W/ W= weight of drug in gm.**

**B) Determination of Ash value**

An ash value indicates total inorganic matter present in crude drug which always remains constant and hence considered as important diagnostic parameter in determination of purity and correct identity of crude drugs. While preparing ash, it is necessary to remove all traces of black organic material inferring in an analysis of total inorganic elements.[(5, 6)]

**i) Determination of total Ash Value:**

The 2 gm of the air dried of powder of root of plant Tinospora cordifolia was transferred to a silica crucible and incinerated at a temperature not exceeding $45^0$C until free from carbon, cooled and weighed. The percentage of total ash value was calculated with reference to the air-dried material. Similar procedure was also carried out for root of plant Cassia occidentalis and aerial part of plant Tephrosia purpurea

**ii) Determination of Acid-insoluble Ash Value:**

Acid insoluble ash value is actually part of total ash insoluble in dilute hydrochloric acid. Acid insoluble ash indicates adhering dirt, sand as well as variation caused by calcium oxalate. The total ash was transferred in a beaker containing 25 mL of 2M hydrochloric acid and boiled for 5 min. The above mixture was filtered through gooch crucible, washed with hot water, ignited, cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air - dried material.

**iii) Determination of Water soluble Ash Value:**

The water extract of crude drugs possesses various biological activities and hence it is possible that water extracted means exhausted powder may be used as adulterant for original drug. Therefore water-soluble ash value can be quite reliable parameter which should be investigated to judge such type of adulteration.

**Water soluble ash = Total ash - Water insoluble ash**

**3. Extraction Methodology :**

Hot continuous counter-current extraction was performed using the Soxhlet apparatus. The powdered plant materials roots of Tinospora cordifolia and Cassia occidentalis, and aerial parts of Tephrosia purpurea were separately pulverized and sieved (#60–80 mesh). Each sample was placed in a cellulose thimble within the Soxhlet extractor. The solvent in a round-bottom flask was heated to reflux, allowing continuous evaporation, condensation, and percolation through the plant material. The extract was periodically siphoned back into the flask, and the cycle was repeated multiple times to ensure exhaustive extraction.

The final extract was collected in the round-bottom flask after completion of the extraction cycles [(7, 8, 9)].

**i. Thin Layer Chromatography (TLC) of Plant extracts** .

Thin layer chromatography (TLC) was performed to optimize the mobile phase for subsequent column chromatographic separation of phytoconstituents. Methanolic extracts of Tinospora cordifolia, Cassia occidentalis, and Tephrosia purpurea were analysed.

Pre-coated silica gel-G aluminium plates (Merck, Germany) were used as the stationary phase. Plates were activated at 105 °C for 30 min prior to use. Samples were applied as spots 2 cm above the base using capillary tubes and allowed to dry. The TLC chamber was saturated with the mobile phase for 30 min before development.

Chromatographic separation was carried out using the following solvent systems: (a) toluene:ethyl acetate:methanol:formic acid (5:4:1:1) and (b) (4:4:2:1). Plates were developed up to a distance of 9–9.5 cm, ensuring the solvent front did not disturb the sample application point.

After development, plates were air-dried and visualized under UV light (254 nm and 366 nm), followed by derivatization using ammonical vapors and methanolic ferric chloride solution to detect phytoconstituents [6, 10].

**ii. High Performance Thin Layer Chromatography (HPTLC) Fingerprinting of the extract of Tinospora cordifolia, Cassia occidentalis and Tephrosia purpure** .

The dried plant extract residues were reconstituted in chromatographic grade methanol (1 mL) and subjected to HPTLC analysis. Methanolic extracts of Tinospora cordifolia, Cassia occidentalis, and Tephrosia purpurea were applied on pre-coated silica gel 60 $F_{254}$ aluminum plates (Merck).Sample application was performed using a CAMAG Linomat 5 applicator, controlled by Vision CATS software. Extracts were applied in duplicate as 8 mm bands using a Hamilton syringe (10 µL per application) on 20 × 10 cm plates. Chromatographic development was carried out in a twin-trough glass chamber saturated for 20 min with the mobile phase. The optimized solvent systems used were toluene:ethyl acetate:methanol:formic acid in the ratios of (5:4:1:1) and (4:4:2:1), which provided satisfactory resolution of phytoconstituents.After development, plates were air-dried and visualized under UV light at 366 nm. Densitometric scanning was performed using a CAMAG TLC scanner, and Rf values along with fingerprint profiles were recorded using Vision CATS software [6, 10].

**iii. Chromatographic separation by Column chromatography** [12, 13].

Methanolic extracts of Tinospora cordifolia, Cassia occidentalis, and Tephrosia purpurea were subjected to column chromatography for isolation of phytoconstituents. Silica gel (60–120 mesh) was used as the stationary phase after activation at 105 °C. The extract was adsorbed onto silica gel, dried to obtain a free-flowing powder, and loaded onto the column.

Elution was carried out using gradient solvent systems comprising toluene, toluene:ethyl acetate, and ethyl acetate:methanol. Fractions were collected at a controlled flow rate (7–10 drops/min) and monitored by TLC. Fractions exhibiting similar chromatographic profiles were pooled, while those containing mixed or unresolved compounds were further purified or discarded.

**iv. Structural elucidation of isolated phytoconstituents of roots of plant Tinospora cordifolia and Cassia occidentalis, aerial part of Tephrosia purpurea**

Isolated phytoconstituents were characterized using spectroscopic techniques:

- **UV–Visible Spectroscopy:** Analysis was performed in the range of 300–1000 nm using a UV–Vis spectrophotometer after appropriate dilution and filtration to determine absorption maxima.
- **FT-IR Spectroscopy:** Functional groups were identified using FT-IR (4000–400 $cm^{-1}$) by preparing KBr pellets of the isolated compounds.
- **$^{1}H$ and $^{13}C$ NMR Spectroscopy:** Spectra were recorded using a Bruker 500 MHz NMR spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts (δ, ppm) and coupling constants (J, Hz) were used for structural interpretation.
- **Mass Spectrometry (ESI-MS/MS):** Molecular weight and fragmentation patterns were determined using Q-TOF mass spectrometry under negative ionization mode.

**RESULT:**

1. **Pharmacognostic Study**

   Pharmacognostic analysis of selected plant parts of Cassia occidentalis, Tinospora cordifolia, and Tephrosia purpurea revealed significant variations in moisture content, ash values, and extractive yields. Cassia occidentalis showed the highest moisture content (10.54 ± 0.19% w/w) and total ash value (13.90 ± 0.07% w/w), whereas Tephrosia purpurea exhibited the lowest moisture (7.85 ± 0.23% w/w) and ash content (9.42 ± 0.06% w/w), indicating lower inorganic matter. Among extractive values, methanol extracts showed the highest yield in all three plants, suggesting a

predominance of polar phytoconstituents. Hydroalcoholic and aqueous solvents also demonstrated higher extractive values compared to petroleum ether and chloroform, indicating the abundance of water-soluble compounds.

**Table 2.1: Pharmacognostic Evaluation of Different Plant Parts**

| Sr. No. | Parameter Study | Tinospora cordifolia (Root) (% w/w) | Cassia occidentalis (Root) (% w/w) | Tephrosia purpurea (Aerial part) (% w/w) |
|---|---|---|---|---|
| 1 | **Loss on Drying** | 9.90 ± 0.21 | 10.54 ± 0.19 | 7.85 ± 0.23 |
| 2 | **Ash Values** | | | |
| | Total Ash | 12.10 ± 0.32 | 13.90 ± 0.07 | 9.42 ± 0.06 |
| | Acid Soluble Ash | 2.80 ± 0.05 | 3.28 ± 0.03 | 2.14 ± 0.04 |
| | Water Soluble Ash | 0.28 ± 0.02 | 0.36 ± 0.01 | 0.25 ± 0.03 |
| 3 | **Extractive Values** | | | |
| | Petroleum Ether | 6.00 | 7.00 | 6.50 |
| | Chloroform | 7.00 | 8.50 | 7.50 |
| | Hydro Alcoholic | 11.50 | 12.50 | 11.50 |
| | Methanol | 13.50 | 14.00 | 13.50 |

Values are represented as Mean± SEM (n=3)

**3.2 Preliminary Phytochemical Investigations**

The preliminary Phytochemical screening of the extracts of plant of Tinospora cordifolia, Cassia occidentalis and Tephrosia purpurea was done to assess the presence of bioactive components. The presence of various phytoconstituents like alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds was carried out for extracts of plant of Tinospora cordifolia, Cassia occidentalis and Tephrosia purpurea .

**3.3 Thin Layer Chromatographic profile of Methanolic extracts of Tephrosia purpurea (TP), Cassia occidentalis (CO) and Tinospora cordifolia (TP)**

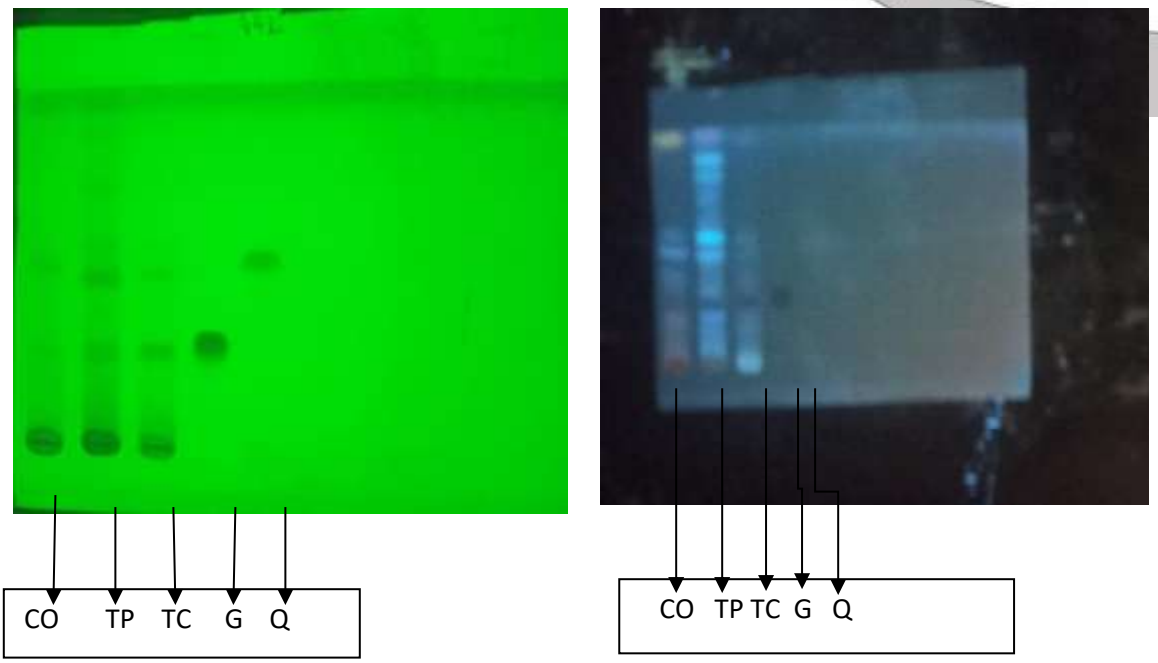
Rf value of plant extracts was evaluated using following formula.

$$\text{Rf Value} = \frac{\text{Distance travelled by sample from baseline}}{\text{Distance travelled by solvent from baseline}}$$

**C) Thin Layer Chromatographic profile of Methanolic extracts of plants Tephrosia purpurea, Cassia occidentalis and Tinospora cordifolia with standard marker drugs**

**i) Mobile Phase-** Toluene: Ethyl acetate: Methanol: Formic acid (4:4:2:1)

UV Cabinet at 254 nm UV Cabinet at 366 nm




G- Gallic acid Q- Qurecetin

**Figure 3.3.3: Thin Layer Chromatographic profile of Methanolic extracts of plants Tephrosia purpurea, Cassia occidentalis and Tinospora cordifolia with standard marker drugs (4:4:2:1)**

**ii) Mobile Phase-** Toluene: Ethyl acetate: Methanol: Formic acid (5:4:1:1)

UV Cabinet at 254 nm UV Cabinet at 366 nm

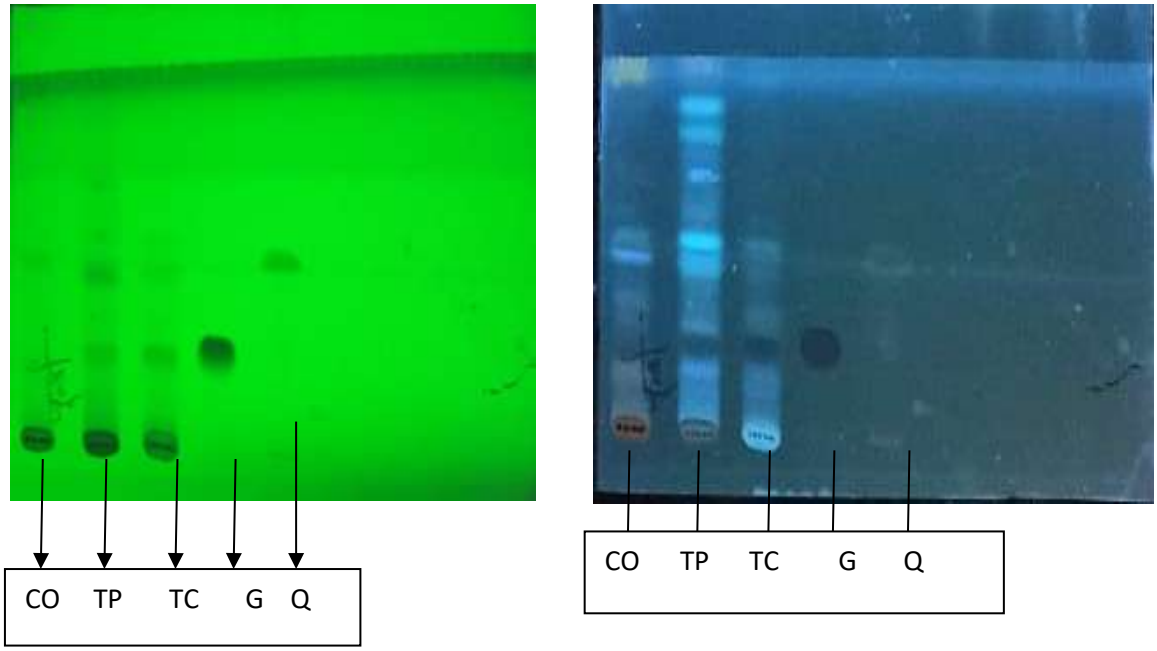
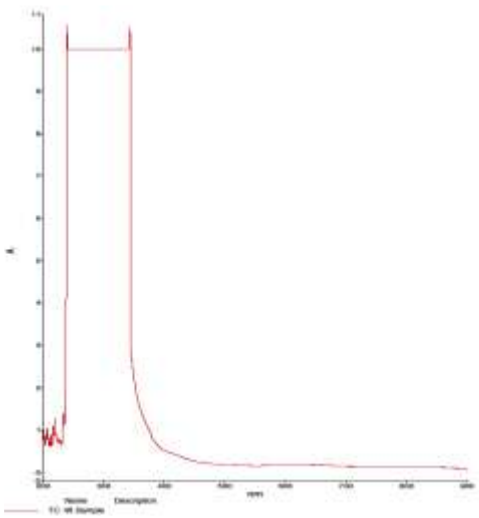


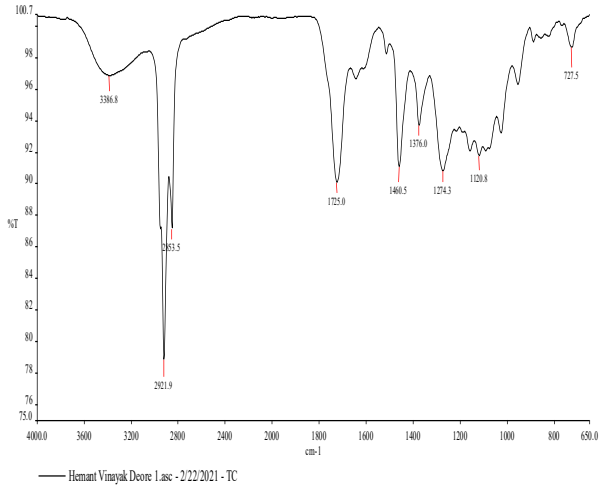

**Figure 3.3.4: Thin Layer Chromatographic profile of Methanolic extracts of plants Tephrosia purpurea, Cassia occidentalis and Tinospora cordifolia with standard marker drugs (5:4:1:1)**

Thin layer chromatography of methanolic extracts of Cassia occidentalis, Tephrosia purpurea, and Tinospora cordifolia was performed using two solvent systems: toluene:ethyl acetate:methanol:formic acid (5:4:1:1) and (4:4:2:1) (v/v). In the (5:4:1:1) system, multiple phytoconstituent spots were observed under UV light (366 nm), with Tephrosia purpurea showing the highest number of spots (8), followed by Cassia occidentalis (5 spots) and Tinospora cordifolia (4 spots). At 254 nm, fewer spots were detected across all extracts.In the (4:4:2:1) solvent system, a similar trend was observed, with Tephrosia purpurea exhibiting a higher number of resolved spots compared to the other extracts.The Rf values of several spots were found to be comparable with standard compounds, where gallic acid (Rf ≈ 0.25–0.26) and quercetin (Rf ≈ 0.50–0.55) served as reference markers, indicating the possible presence of phenolic and flavonoid constituents.
Visualization was carried out under UV light (254 nm and 366 nm) and after derivatization using anisaldehyde–sulphuric acid reagent. The TLC profiles demonstrated good resolution of phytoconstituents and confirmed the suitability of the selected solvent systems for further chromatographic separation.

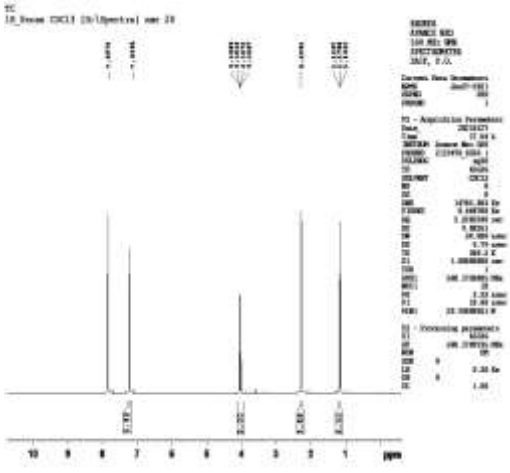
**A) Characterization of isolated phytoconstituents of Tinospora cordifolia (TC) by Spectroscopic study**

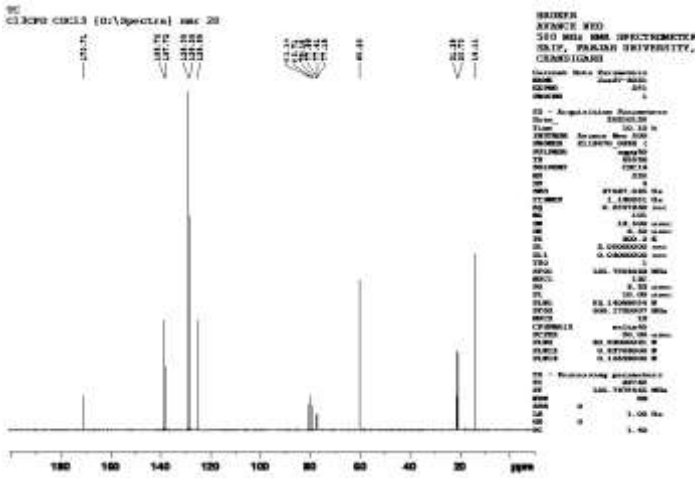
UV spectra: 256-364 nm.






**UV spectra of Tinospora cordifolia (TC)** **FT-IR Spectra of Tinospora cordifolia (TC)**





**$^{1}$ H NMR Spectra of Tinospora cordifolia** **$^{13}$ H NMR Spectra of Tinospora cordifolia**

**Mass Spectra of Fraction of Tinospora cordifolia (TC)**

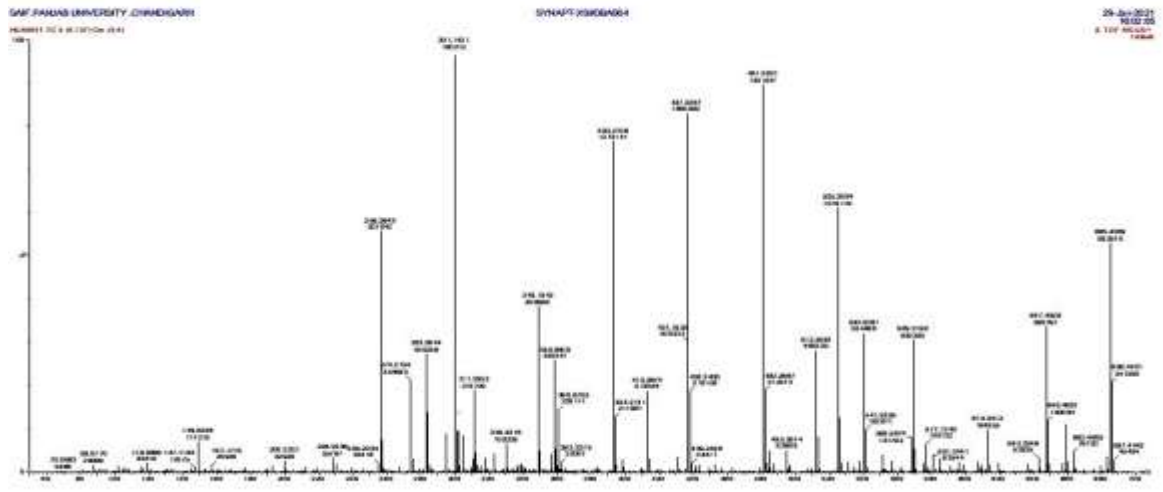
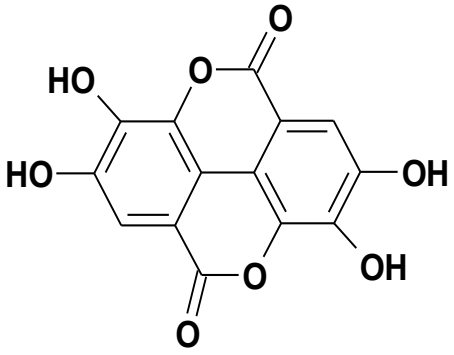


**Figure: 3.6.5 Mass spectra of Tinospora cordifolia (TC)**

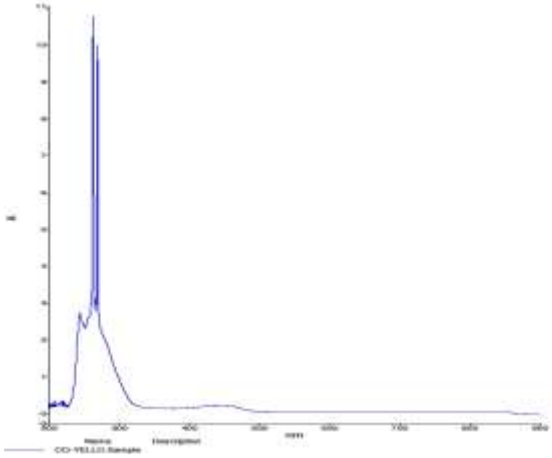
**Highest Mass Spectra-302.19**

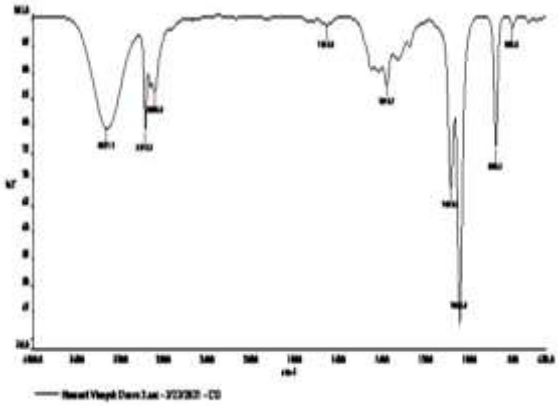
Possible structure of phytoconstituents fraction Tinospora cordifolia (TC) isolated from Column chromatography of Methanolic extract of plant Tinospora cordifolia root From UV, FT-IR, $^{1}H$ NMR, $^{13}C$ NMR and Mass spectral Data, the possible structure may be Tetrahydroxy benzapyrano benzopyran-dione. Molecular Formula is $C_{14}H_6O_8$



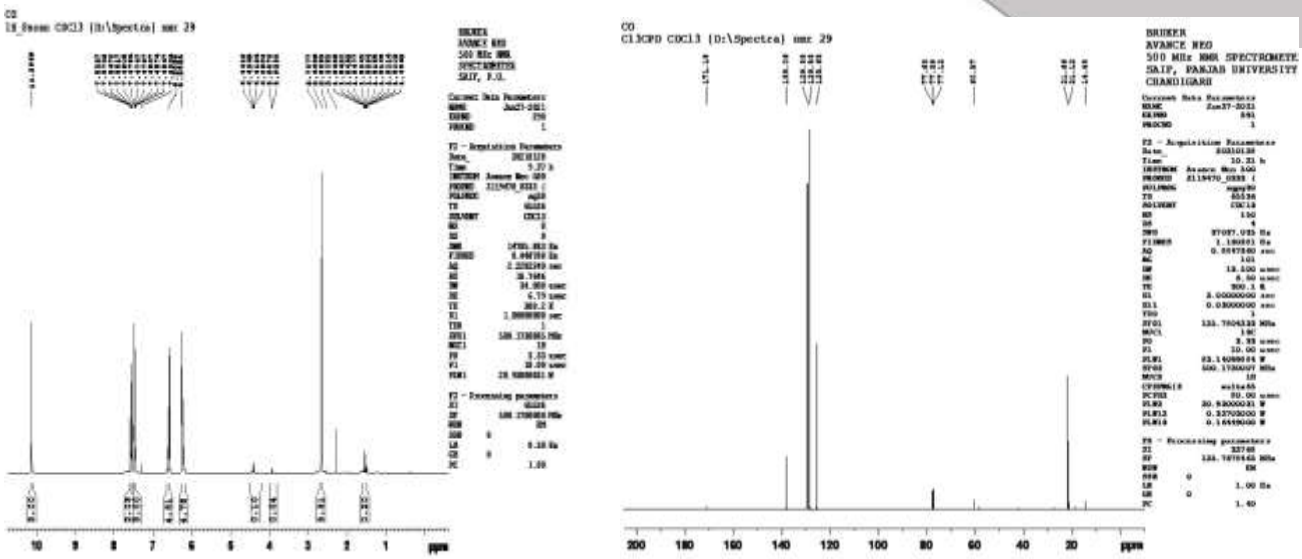

**Tetrahydroxy benzapyrano benzopyran-dione**

**B) Characterization of isolated phytoconstituents of Cassia occidentalis (CO) by Spectroscopic study**



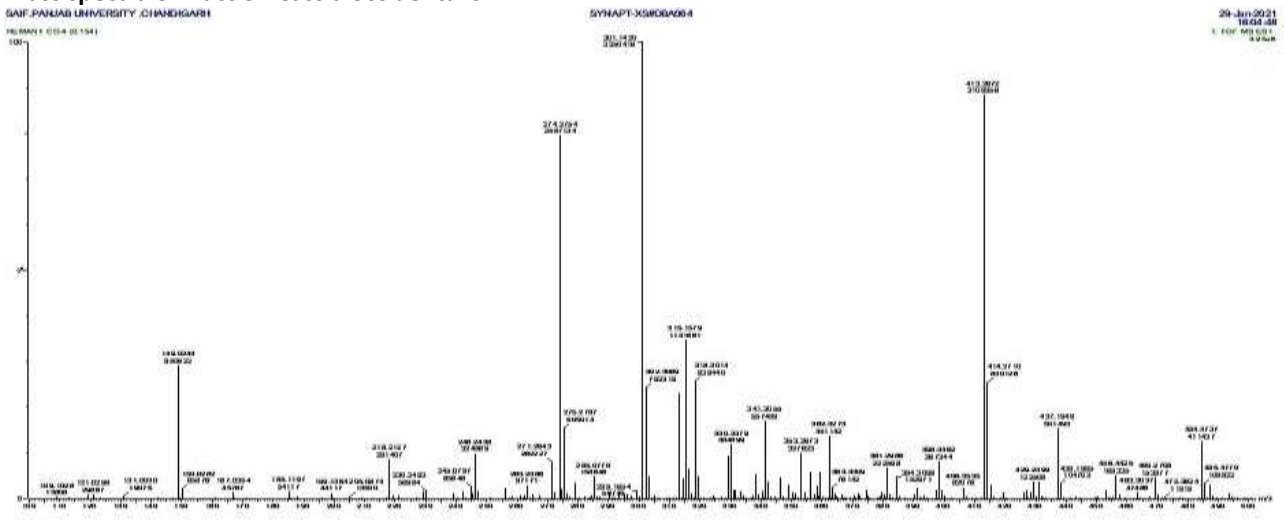


**UV Spectra of Cassia occidentalis** **FT-IR Spectra of Cassia occidentalis**



**$^{1}$H NMR Spectra of Cassia occidentalis** **$^{13}$C NMR Spectra of Cassia occidentalis**

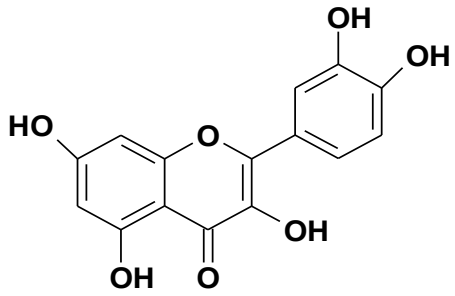
**Mass spectra of fraction Cassia occidentalis**



**Mass spectra of fraction Cassia occidentalis (CO)**

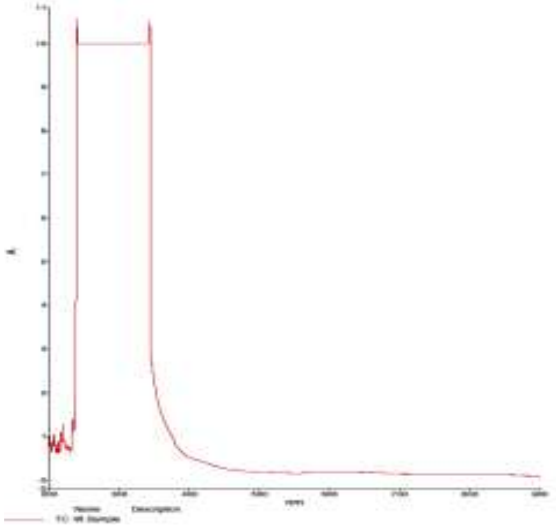
**Highest Mass spectra- 301.14**

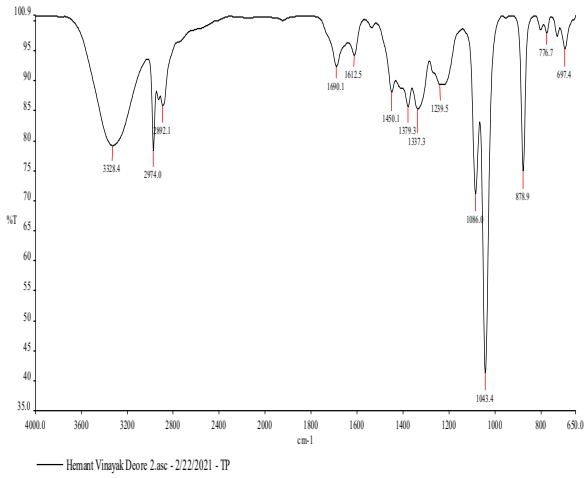
Possible structure of Phytoconstituents Fraction of Cassia occidentalis isolated from Column Chromatography of Methanolic extract of plant Cassia occidentalis root From UV, FT-IR, $^{1}$H NMR, $^{13}$C NMR and Mass Spectral Data, the possible structure may be 3,3',4',5,7-Pentahydroxyflavone. Molecular Formula is $C_{15}H_{10}O_7$



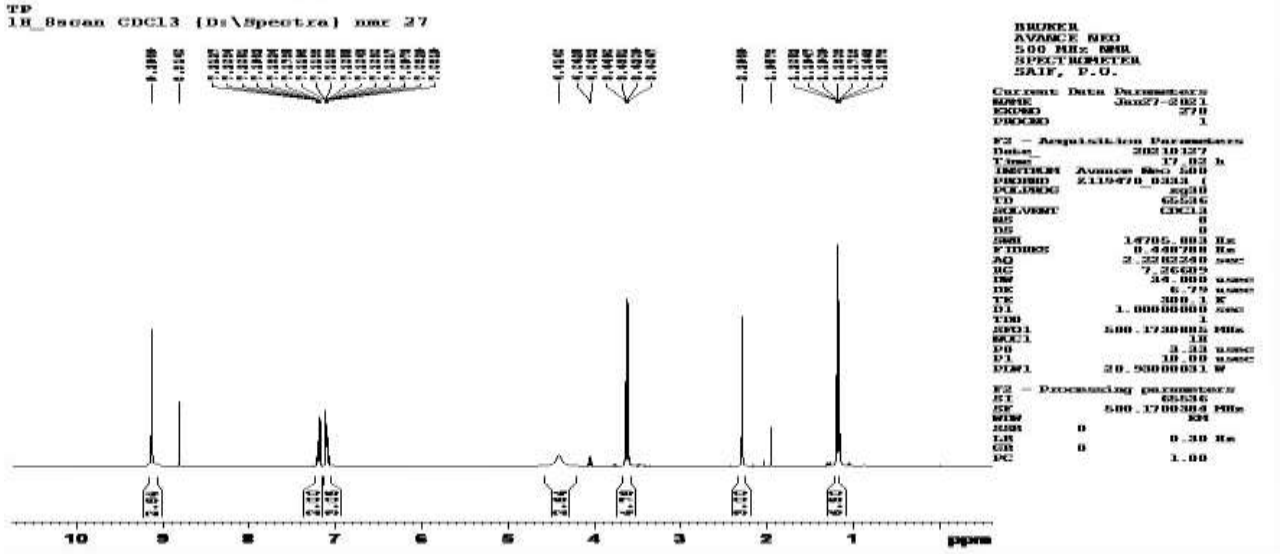

**3, 3’,4’,5,7-Pentahydroxyflavone**

**C) Characterization of isolated phytoconstituents of Tephrosia purpurea (TP) by Spectroscopic study**

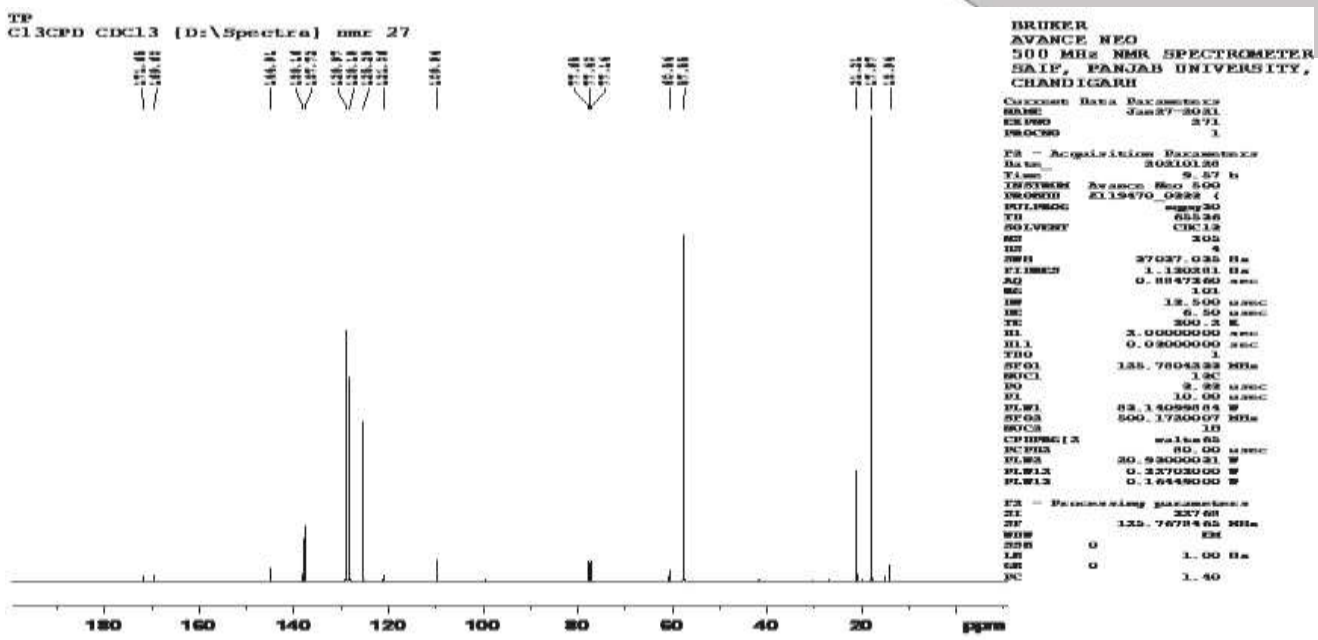





**UV Spectra of Tephrosia purpurea** **FT-IR Spectra of Tephrosia purpurea**




**$^{1}$H NMR spectra of Tephrosia purpurea**




**[13] C NMR spectra of Tephrosia purpurea**

**Mass Spectra of Fraction of Tephrosia purpurea**

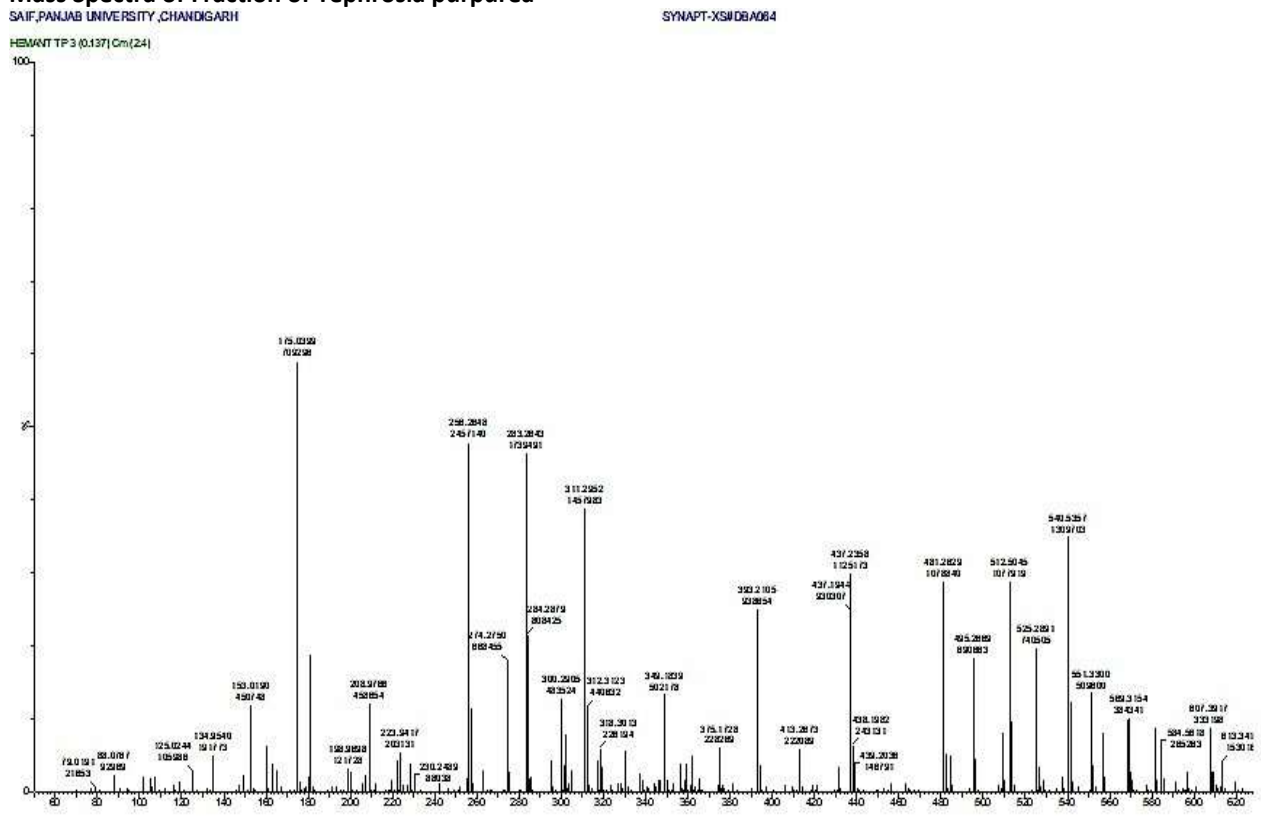
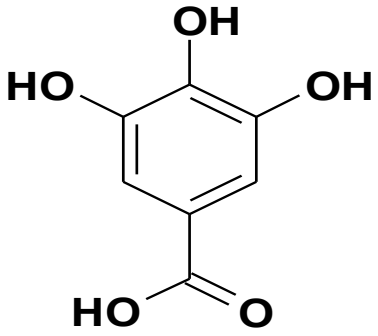


**Figure: 3.6.15 Mass spectra of Tephrosia purpurea Highest Mass Spectra- 175.03**

Possible structure of phytoconstituents fraction of Tephrosia purpurea (TP) isolated from Column chromatography of Methanolic extract of plant Tephrosia purpurea aerial part from UV, FT-IR, $^{1}H$ NMR, $^{13}C$ NMR and Mass spectral data, the possible structure may be 3, 4, 5 Tri hydroxy benzoic acid. Molecular Formula is $C_{10}H_{12}O_5$




**3, 4, 5 Tri hydroxy benzoic acid**

## DICUSSION:

The harvested parts of the plants roots of Tinospora cordifolia and Cassia occidentalis with fresh aerial part of Tephrosia purpurea were verified and dried in a controlled environment to capture active chemical compounds. For basic quality assurance in the drying of plant materials, moisture (by loss on drying), and ash (total, acid soluble, water soluble), and extractive values were determined. Total moisture obtained during loss on drying for the plant parts demonstrated that on average, the dried portions of the plants with the highest moisture content was Cassia occidentalis root (10.54 ± 0.19 % w/w) and had the highest total ash (+13.90 ± 0.07% w/w) indicating a significant inorganic component, while Tephrosia purpurea aerial part had the lowest moisture (7.85 ± 0.23%), and total ash content (9.42 ± 0.06% w/w) based on ash value analysis. The extractives yield values were high and the yields were higher in methanol solution (13.5 to 14%) and hydroalcohol solution (11.5 to 12.5%) stated that the majority of the phytoconstituents were polar and water soluble, as was hypothesized. Phytochemical screening revealed that the methanolic extracts consistently contained the richest profiles of alkaloids, flavonoids, phenolics and glycosides, whereas petroleum ether and chloroform extracts contained fewer and more varied constituents. Notably, Tinospora cordifolia extracts showed presence of alkaloids, flavonoids, steroids, carbohydrates, glycosides, and phenolics. Cassia occidentalis and Tephrosia purpurea methanol fractions similarly showed maximal levels (+ + +) of these compounds, with Cassia occidentalis also showing proteins/amino acids.

After performing thin layer chromatography (TLC) using two solvent systems at UV 254 nm and 366 nm the chromatograms exhibited variously distinct fingerprint patterns. For Cassia occidentalis methanolic extract depending on the condition, there were 4-6 visible spots, for Tephrosia purpurea there were 6–7 spots; for Tinospora cordifolia we sometimes observed 4–5 spots, indicating different levels of chemical complexity. Rf values for each species were diverse and were used to differentiate various levels of taxa.

Spectroscopic characterization of fractions isolated from column chromatography assigned probable structures:

Tinospora cordifolia fraction corresponded to tetrahydroxy benzapyranobenzopyran dione ($C_{14}H_6O_8$).

Cassia occidentalis fraction corresponded to 3,3',4',5,7 pentahydroxyflavone (a kaempferol derivative, $C_{15}H_{10}O_7$).

Tephrosia purpurea fraction corresponded to 3,4,5 trihydroxybenzoic acid ($C_{10}H_{12}O_5$).

Though, these assignments were further corroborated by UV visible spectra absorption ranges, functional group vibrations determined through FT IR (e.g., O–H, C=O, etc.), NMR chemical shift values, and mass spectral molecular ions. Taken together, these analyses substantiate the existence of known antioxidant and polyphenolic phytochemicals in plant extracts, reinforcing recorded health benefits associated with herbal use.

## CONCLUSION:

The work identifies three plant materials, Tinospora cordifolia, Cassia occidentalis , and Tephrosia purpurea , on the basis of their moisture, ash, and extractive values. The methanolic extracts contain an abundance of phytochemical constituents with high concentrations of significant secondary metabolites such as alkaloids, flavonoids, glycosides, and phenolics. Chemical signatures from species-specific TLC and spectroscopic studies identify the bioactive molecules such as

tetrahydroxy benzapyranobenzopyran dione, pentahydroxyflavone, and trihydroxybenzoic acid, which are most probably accountable for the medicinal virtues of the plants. They hold therapeutic significance, which will direct future isolation, pharmacological evaluation, and formulation research.

**CONFLICT OF INTEREST**

Authors are declared no any conflict of interest.

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