

Journal of Molecular Science

www.jmolecularsci.com

ISSN:1000-9035

Extraction, Isolation, and Characterization of Key Bioactive Compounds
from *Tinospora cordifolia* and *Centella asiatica*Abhishek Sharma^{*1}, Dharmendra Ahuja², Akash Pareek³^{*1}Research Scholar, Department of Pharmaceutical Science, Jayoti Vidyapeeth Women's University (JVWU), Jaipur, Rajasthan, India²Professor, Department of Pharmaceutical Science, Faculty of Education and Methodology, Jayoti Vidyapeeth Women's University (JVWU), Jaipur, Rajasthan, India³Vice President Quality, Amol Pharmaceutical Pvt. Ltd. (APPL), Jaipur, Rajasthan, India

Corresponding author Email: life4abhishek@gmail.com.

Article Information

Received: 13-08-2025

Revised: 22-08-2025

Accepted: 03-09-2025

Published: 19-09-2025

Keywords

Tinospora cordifolia, *Centella asiatica*, phytochemical, UV, FTIR, NMR, mass spectrometry etc

ABSTRACT

Tinospora cordifolia and *Centella asiatica* are well-known medicinal plants widely used in Ayurvedic and folk medicine for their diverse therapeutic properties. The present study aimed to isolate and characterize bioactive phytoconstituents from the stem of *T. cordifolia* and the leaves of *C. asiatica*. Petroleum ether, ethyl acetate, and methanolic extracts were prepared by Soxhlet extraction, followed by qualitative and quantitative phytochemical analysis. The extracts revealed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids, and phenolic compounds. Total phenolic content (TPC) was highest in methanolic extract of *T. cordifolia* (219.30 mg/g GAE), while total flavonoid content (TFC) was highest in methanolic extract of *T. cordifolia* (407.76 mg/g RE). Thin-layer chromatography (TLC) and column chromatography guided the isolation of bioactive fractions, which were further subjected to spectroscopic characterization. UV, FTIR, NMR, and mass spectrometry confirmed the presence of an alkaloid, Berberine derivative ($C_{20}H_{18}NO_4$, m/z 336.1308) from *T. cordifolia* and a phenolic acid, Ferulic acid derivative ($C_{10}H_{10}O_4$, m/z 194.0918) from *C. asiatica*. The findings provide scientific validation of the phytochemical richness and therapeutic potential of these plants, supporting their traditional use and encouraging further pharmacological investigations..

INTRODUCTION:

Throughout history, humans have shown great interest in naturally occurring compounds derived from prebiotic, microbial, plant, and animal sources. Extracts from various plant parts have long been used in folk medicine, perfumes, food flavoring, and preservation, and continue to play a significant role in the treatment of both common and chronic diseases (Rasul et al., 2018). Globally, more than 80% of the population depends on traditional medicine for primary healthcare, with plant extracts forming the core of such practices (Sandhya et al., 2006). Medicinal plants represent the richest bio-resources for traditional systems of medicine, nutraceuticals, food supplements, pharmaceutical intermediates, and serve as sources for both crude and synthetic drugs (Pandey and Tripathi, 2014).

©2025 The authors

This is an Open Access article distributed under the terms of the Creative Commons Attribution (CC BY NC), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers. (<https://creativecommons.org/licenses/by-nc/4.0/>)

Tinospora cordifolia is a widely recognized medicinal plant traditionally used to treat various ailments. Commonly known as Amrita or Guduchi, it belongs to the family Menispermaceae (Sharma et al., 2019). Distributed across the Indian subcontinent and parts of China, this plant is extensively employed in both folk and Ayurvedic medicine, either alone or in combination with other herbs. Owing to its therapeutic significance, *T. cordifolia* has been the focus of intensive research over the past four decades, leading to the identification of diverse bioactive compounds such as alkaloids, sesquiterpenoids, diterpenoids, phenolics, steroids, aliphatic compounds, and polysaccharides. These constituents contribute to its broad pharmacological properties, including immunomodulatory, anticancer, hepatoprotective, and hypoglycemic effects (Singh and Chaudhuri, 2017).

Similarly, *Centella asiatica* (CA) is a highly valued medicinal herb traditionally used in Asian medicine (Gohil et al., 2010) and increasingly gaining popularity in Western practices (Chevallier et al., 1996). Known locally as Mandukparni, Indian pennywort, or Jalbrahmi, it has been an integral part of Ayurvedic medicine for centuries and is mentioned in the classical text *Sushruta Samhita* (Chopra et al., 1986; Diwan et al., 1991). By the nineteenth century, CA and its extracts were incorporated into the Indian pharmacopoeia, where they were recommended not only for wound healing but also for the treatment of various conditions such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, fever, amenorrhea, and disorders of the female genitourinary tract (Brinkhaus et al., 2000).

Both *T. cordifolia* and *C. asiatica* contain a wide range of phytoconstituents that account for their diverse pharmacological properties. The present study focuses on the isolation and structural elucidation of alkaloid and phenolic acid compounds from stem part of *Tinospora cordifolia* and leave part of *Centella asiatica*, using FTIR, NMR and mass spectrometry techniques.

MATERIALS AND METHODS:

Plant collection and authentication:

T. cordifolia was collected from the local area of Jaipur, Rajasthan, whereas *C. asiatica* was collected from the local area of Udaipur, Rajasthan. Voucher specimens (PRFH-23-18233 for *T. cordifolia* and PRFH-23-13781 for *C. asiatica*) were deposited at the Patanjali Research Foundation Herbarium, Uttarakhand, India. The stem part and leaves of *T. cordifolia* and *C. asiatica* were dried at 40°C in shade dried and finely powdered.

Extraction by Soxhlet method:

Dried and powdered stem part of *Tinospora cordifolia* and leave part of *Centella asiatica* were successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using a solvent system like ethyl acetate and methanol at a temperature of 40-60 °C on the heating mantle for 8-10 hours. The extract was evaporated to dryness in rotary vacuum evaporator at 40°C (Wan et al., 2011). Extraction yield of all extracts were calculated using the following equation below:
Formula of Percentage yield.

$$\frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

Phytochemical Estimation of Extracts:

Chemical tests were carried out on the *T. cordifolia* and *C. asiatica* extracts for the qualitative determination of phytochemical constituents as described by Harborne (1988), and Trease and Evans (1989).

Quantitative Phytochemical estimation:

Total phenolic content was determined using Folin-Ciocalteu reagent as previously described (McDonald et al., 2001). Total phenol value was expressed as mg/g gallic acid equivalent.

Total flavonoid contents were determined spectrophotometrically in samples according to the German Pharmacopoeia (Deutsches Arzneibuch, 1996) method, measuring the flavonoids in AlCl₃-complex form.

Preliminary Thin layer chromatography:

In the preliminary TLC of *Tinospora cordifolia* and *Centella asiatica* extracts, the solvent systems that produced the maximum number of visible spots were Toluene: Ethyl acetate: Methanol: Acetic acid (6:8:2:0.4) and Toluene: Ethyl acetate: Acetic acid (6:4:0.4), respectively, when used with standard alkaloid (Berberine) and phenolic compound (Ferulic acid). Therefore, Toluene: Ethyl acetate: Methanol: Acetic acid (6:8:2:0.4) was selected as the mobile phase for *Tinospora cordifolia*, and Toluene: Ethyl acetate: Acetic acid (6:4:0.4) was selected as the mobile phase for *Centella asiatica* in column chromatography.

Column chromatography:

The extracts were subjected to silica gel column chromatography for the isolation of the alkaloid (Berberine) from *Tinospora cordifolia* and the phenolic compound (Ferulic acid) from *Centella asiatica* extracts. A gradient elution technique was employed, wherein the column was eluted with Toluene and Ethyl acetate, followed by Methanol in increasing polarity. The collected fractions were concentrated and analyzed by TLC on silica gel 60

F254 plates using Toluene: Ethyl acetate: Methanol: Acetic acid (6:8:2:0.4) for *Tinospora cordifolia* and Toluene: Ethyl acetate: Acetic acid (6:4:0.4) for *Centella asiatica*. The presence of the target compounds was confirmed by TLC (Revathy *et al.*, 2011).

Spectroscopic characterization

The isolated fraction (F) of TC and isolated fraction (G) of CA extracts were scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) (Patel *et al.*, 2022; Picollo *et al.*, 2019). FT-IR spectroscopy was performed using perkin Spectrum BX spectrophotometer. Infrared spectra were recorded in the region of 400 - 4,000 cm^{-1} (Moraes *et al.*, 2008; Dutta, 2017). JNM EC-500 NMR spectroscopy for this purpose was Nuclear Magnetic Resonance spectroscopy (Zia *et al.*, 2019; Marion, 2013). Mass spectrometry

converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of isolated fraction (F) of TC and isolated fraction (G) of CA extracts was recorded on mass spectrometer instrument micrOTOF-Q 228888.10348 (Wiley *et al.*, 1995; Torgerson *et al.*, 1974).

RESULTS AND DISCUSSION:

Phytochemical Estimation of Extracts:

Qualitative screening of phytochemical compounds in the petroleum ether, ethyl acetate, and methanolic extracts of *Tinospora cordifolia* (TC) and *Centella asiatica* (CA) revealed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids, and phenolic compounds, among others.

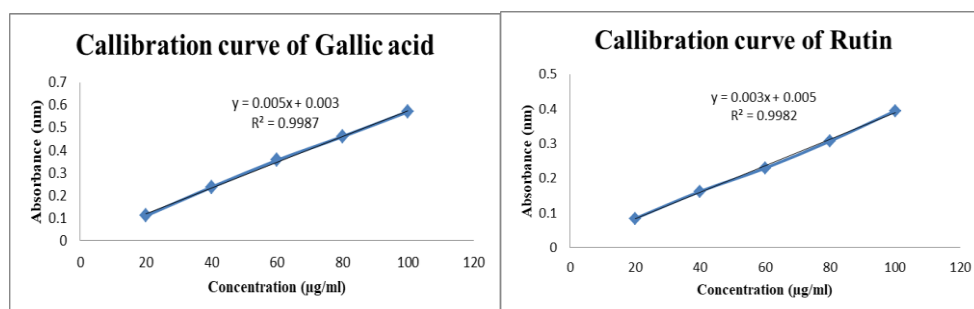


Figure 1 Graph represent standard curve of gallic acid and Rutin

The total phenolic (TPC) and flavonoid contents (TFC) of the extracts were determined from their respective calibration curves (TPC: $Y = 0.005x + 0.003$, $R^2 = 0.998$; TFC: $Y = 0.003x + 0.005$, $R^2 = 0.998$) prepared using gallic acid and rutin standards (Figure 1), and expressed as mg gallic acid equivalents (GAE) and mg rutin equivalents (RE) per gram of extract. The total phenolic content of the ethyl acetate and methanolic extracts of *T. cordifolia* (stem) and *C. asiatica* (leaves) was expressed as mg of gallic acid equivalents per mg/g dry weight of the

sample. It was found to be 49.74 and 219.30 mg/g for TC, and 40.08 and 52.08 mg/g for CA, respectively.

The total flavonoid content of the ethyl acetate and methanolic extracts of *T. cordifolia* (stem) and *C. asiatica* (leaves) was expressed as a percentage of rutin equivalents per mg/g dry weight of the sample. The content was 169.60 and 407.76 mg/g for TC, and 260.53 and 264.40 mg/g for CA, respectively.

Thin layer chromatography:

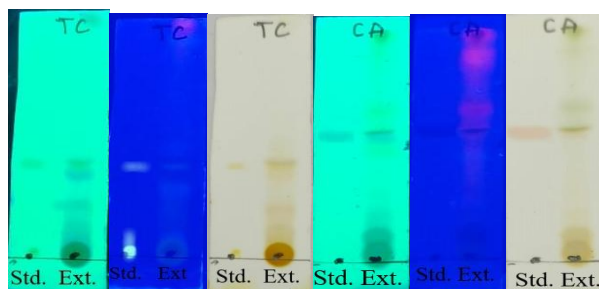


Figure 2 TLC Estimation of *Tinospora cordifolia* (TC) with Standard Alkaloid (Berberine) and *Centella asiatica* (CA) with Standard Phenolic Compound (Ferulic acid)

TLC of *Tinospora cordifolia* (TC) and *Centella asiatica* (CA) extracts was performed using different solvent systems, selected on the basis of a literature

survey. The solvent system Toluene: Ethyl acetate: Methanol: Acetic acid (6:8:2:0.4) was used for TC, and Toluene: Ethyl acetate: Acetic acid (6:4:0.4)

was used for CA. Distinct bands of the extracts were observed along with the standard alkaloid (Berberine) and phenolic compound (Ferulic acid). The R_f value of TC with standard Berberine was found to be 0.42, and that of CA with standard Ferulic acid was 0.56.

Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of *Tinospora cordifolia* and *Centella asiatica* extracts were tested for the detection of various phyto compounds using TLC. The collected fractions/elutes were taken properly and do the UV spectrum.

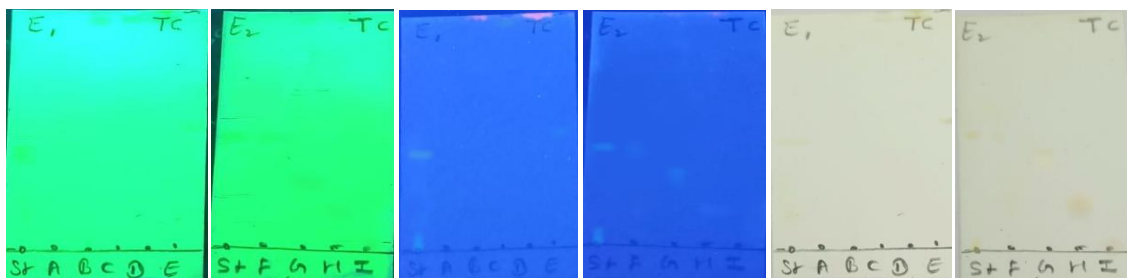


Figure 3 TLC of fractions of TC after column chromatography with Std. Alkaloid (Berberine)

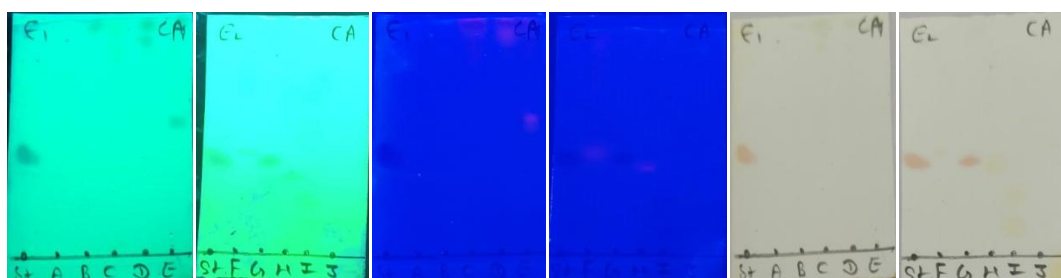


Figure 4 TLC of fractions of CA after column chromatography with Std. phenolic compound (Ferulic acid)

Ten fractions (A to J) were collected from both *Tinospora cordifolia* (TC) and *Centella asiatica* (CA) extracts. TLC analysis confirmed that fraction

F of TC and fraction G of CA exhibited R_f values corresponding to their respective standards.

Spectroscopic characterization: By UV-Spectroscopy



Figure 5 UV-Spectra of Isolated Fraction F of *Tinospora cordifolia* and Fraction G of *Centella asiatica*

UV spectra of the isolated fractions (F and G) from *Tinospora cordifolia* (TC) and *Centella asiatica* (CA) extracts were recorded using a Shimadzu 1700 double-beam UV-VIS spectrophotometer. The spectra were obtained in Toluene: Ethyl acetate: Methanol: Acetic acid (6:8:2:0.4) for TC and Toluene: Ethyl acetate: Acetic acid (6:4:0.4) for CA, over a scanning range of 200–800 nm, and the λ_{max} values of the isolated compounds were determined.

The UV spectrum of fraction F from the TC extract showed three peaks at 348, 265, and 228 nm, while fraction G from the CA extract showed three peaks at 322, 229, and 209 nm.

By FTIR – Spectroscopy

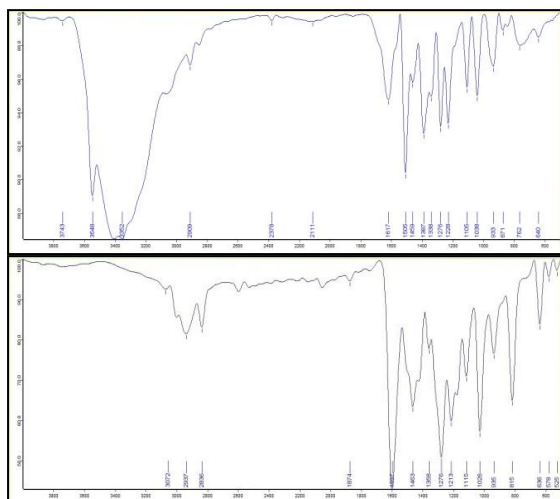


Figure 6 IR spectra of the isolated fraction F of *Tinospora cordifolia* and Fraction G of *Centella asiatica*

(A) IR spectra of the isolated fraction (F) of TC extract

The IR spectrum of the isolated fraction (F) from the *Tinospora cordifolia* (TC) extract showed characteristic peaks corresponding to various functional groups. N–H stretching bands appeared as medium peaks at 3548 and 3352 cm^{-1} . A C–H stretching peak of alkanes was observed at 2909 cm^{-1} , while a C–H bending peak of aromatic compounds was noted at 1617 cm^{-1} . An N–O stretching peak appeared at 1505 cm^{-1} . C–H bending peaks of alkanes and methyl groups were observed at 1459 and 1387 cm^{-1} , respectively. C–O stretching bands of ethers were present at 1276 and 1228 cm^{-1} , while C–N stretching peaks of amines were recorded at 1105 and 1038 cm^{-1} . Additionally, C=C bending peaks of substituted compounds were observed at 871 and 762 cm^{-1} , along with a strong C=C bending peak of alkenes at 640 cm^{-1} .

(B) IR spectra of the isolated fraction (G) of CA extract

The IR spectrum of the isolated fraction (G) from the *Centella asiatica* (CA) extract showed characteristic absorption peaks corresponding to different functional groups. A weak, broad O–H stretching band appeared at 3072 cm^{-1} . C–H stretching peaks of alkanes were observed at 2937 and 2836 cm^{-1} , while a C–H bending peak of aromatic compounds was noted at 1874 cm^{-1} . A C=C stretching peak of cyclic alkenes was observed at 1597 cm^{-1} . An O–H bending band of alcohols appeared at 1358 cm^{-1} . C–O stretching bands were observed at 1276 cm^{-1} (aromatic ester), 1213 cm^{-1} (ether), and 1115 and 1026 cm^{-1} (alcohol). A C=C bending peak of substituted compounds was present at 935 cm^{-1} , while medium and strong C=C bending bands of alkenes appeared at 815 and 636 cm^{-1} , respectively.

¹H NMR – Spectroscopy

¹H NMR spectra of the isolated fractions (F and G)

from *Tinospora cordifolia* (TC) and *Centella asiatica* (CA) extracts were recorded using an NMR spectrometer. Tetramethylsilane (TMS) was used as the internal standard. The proton signals were assigned with the notations: s (singlet), d (doublet), t (triplet), and m (multiplet).

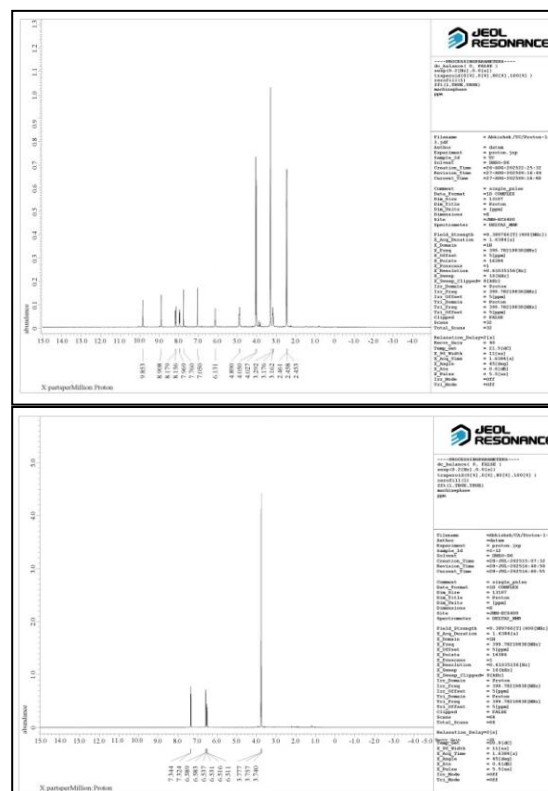


Figure 7 ¹H-NMR spectra of the isolated fraction F from *Tinospora cordifolia* (TC) and fraction G from *Centella asiatica* (CA)

(A) ¹H NMR spectra of the isolated fraction (G) of TC

In the ¹H-NMR spectrum of the isolated fraction (F) from *Tinospora cordifolia*, the following proton signals were observed: H-3 protons appeared at 2.453–2.461 (s) ppm, H-2 proton appeared at 3.162–3.176 (d) ppm, H-1 proton appeared at 3.292 (d) ppm, H-2 proton appeared at 4.027–4.050 (dd) ppm, H-2 proton appeared at 4.890 (d) ppm, H-1 proton appeared at 6.131 (dd) ppm, H-1 protons appeared at 7.050 (s) ppm, H-1 proton appeared at 7.760 (d) ppm, H-1 protons appeared at 7.969 (dd) ppm, H-2 protons appeared at 8.156–8.179 (dd) ppm, H-1 proton appeared at 8.908 (d) ppm and H-1 proton appeared at 9.853 (d) ppm.

(B) ¹H NMR spectra of the isolated fraction (G) of CA

In the ¹H-NMR spectrum of the isolated fraction (G) from *Centella asiatica*, the following proton signals were observed: ¹H-3 protons appeared at 3.740–3.777 (s) ppm, ¹H-1 proton appeared at 6.511 (d)

ppm, ^1H -1 proton appeared at 6.516-6.531 (dd) ppm, ^1H -1 proton appeared at 6.537 (dd) ppm, ^1H -2 protons appeared at 6.583-6.589 (dd) ppm and ^1H -2 protons appeared at 7.324-7.344 (d) ppm.

Mass – Spectroscopy:

Mass spectra of the isolated fraction (F) from *Tinospora cordifolia* (TC) and fraction (G) from *Centella asiatica* (CA) were recorded using a Bruker micrOTOF-Q mass spectrometer.

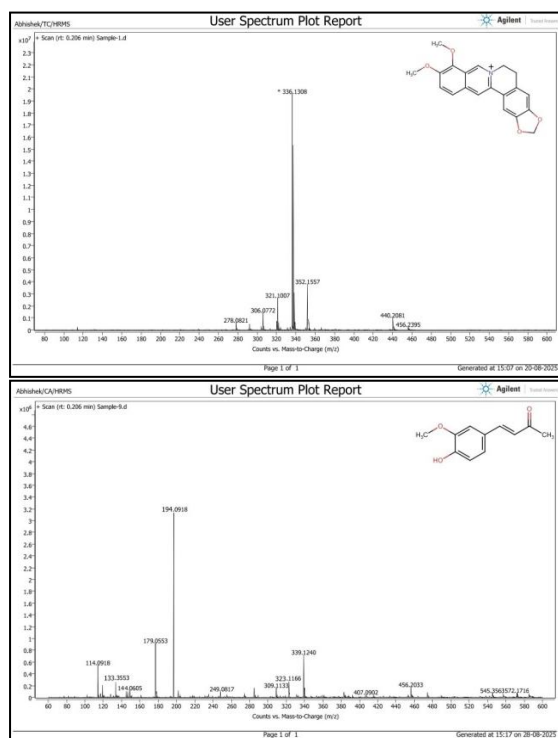


Figure 8 Mass spectra of the isolated fraction (F) of TC and (G) of CA

(A) Mass spectra of the isolated fraction (F) of TC-

The mass spectrum of the isolated fraction (F) from *Tinospora cordifolia* (TC) showed a molecular ion peak [M^+] at m/z 336.1308, corresponding to the compound 9,10-dimethoxy-5,6-dihydrodioxolo[4,5-g]isoquinolo[3,2-a]isoquinolin-7-ium. The molecular composition indicated the presence of 20 carbon atoms (C), 18 hydrogen atoms (H), one nitrogen atom (N), and four oxygen atoms (O), resulting in the molecular formula $\text{C}_{20}\text{H}_{18}\text{NO}_4$. This identification was further supported by characteristic fragment ions observed at m/z 278, 306, 321, and 352.

(B) Mass spectra of the isolated fraction (G) of CA-

The mass spectrum of the isolated fraction (G) from *Centella asiatica* (CA) showed a molecular ion peak [M^+] at m/z 194.0918, corresponding to the compound (E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid. The molecular composition indicated the presence of 10 carbon atoms (C), 10

hydrogen atoms (H), and 4 oxygen atoms (O), resulting in the molecular formula $\text{C}_{10}\text{H}_{10}\text{O}_4$. This identification was further supported by characteristic fragment ions observed at m/z 114, 133, 144, 179, 249, 309, 323, and 339.

CONCLUSION:

This study successfully isolated and characterized key bioactive compounds from *Tinospora cordifolia* and *Centella asiatica*, demonstrating their richness in phenolic and flavonoid constituents. The methanolic extracts of both plants exhibited comparatively higher TPC and TFC, highlighting the efficiency of polar solvents in extracting secondary metabolites. Spectroscopic analysis confirmed the identity of an alkaloid (Berberine derivative) in *T. cordifolia* and a phenolic compound (Ferulic acid derivative) in *C. asiatica*, both of which are known for their pharmacological relevance. These results not only validate the traditional medicinal applications of these plants but also emphasize their potential as sources of lead compounds for drug discovery. Further *in vivo* and clinical studies are warranted to establish their therapeutic efficacy and safety profiles.

REFERENCES:

1. Arzneibuch, D. (1996). Amtliche Ausgabe (DAB 10).
2. Brinkhaus, B., Lindner, M., Schuppan, D., & Hahn, E. G. (2000). Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. *Phytomedicine*, 7(5), 427-448.
3. Chevallier, A. (1996). The encyclopedia of medicinal plants.
4. Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1986). Glossary of Indian medicinal plants (including the supplement). *Council of scientific and industrial research, New Delhi*, 89, 77-100.
5. Diwan, P. V., Karwande, I., & Singh, A. K. (1991). Anti-anxiety profile of manduk parni (*Centella asiatica*) in animals.
6. Dutta, A. (2017). Fourier transform infrared spectroscopy. *Spectroscopic methods for nanomaterials characterization*, 73-93.
7. Gohil, K. J., Patel, J. A., & Gajjar, A. K. (2010). Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian journal of pharmaceutical sciences*, 72(5), 546.
8. Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media.
9. Marion, D. (2013). An introduction to biological NMR spectroscopy. *Molecular & Cellular Proteomics*, 12(11), 3006-3025.
10. McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food chemistry*, 73(1), 73-84.
11. Moraes, L. G. P., Rocha, R. S. F., Menegazzo, L. M., Aratijo, E. B. D., Yukimito, K., & Moraes, J. C. S. (2008). Infrared spectroscopy: a tool for determination of the degree of conversion in dental composites. *Journal of Applied Oral Science*, 16, 145-149.
12. Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and phytochemistry*, 2(5).
13. Patel, S., Raulji, A., Patel, D., Panchal, D., Dalwadi, M., &

- Upadhyay, U. (2022). A review on UV visible spectroscopy. *Int. J. Pharm. Res. Appl*, 7(10), 1144-1151.
14. Picollo, M., Aceto, M., & Vitorino, T. (2019). UV-Vis spectroscopy. *Physical sciences reviews*, 4(4), 20180008.
15. Rasul, M. G. (2018). Extraction, isolation and characterization of natural products from medicinal plants. *Int. J. Basic Sci. Appl. Comput*, 2(6), 1-6.
16. Revathy, S., Elumalai, S., & Antony, M. B. (2011). Isolation, purification and identification of curcuminoids from turmeric (*Curcuma longa* L.) by column chromatography. *Journal of Experimental sciences*, 2(7).
17. Sandhya, B., Thomas, S., Isabel, W., & Shenbagarathai, R. (2006). Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (reserved forest), Tamilnadu, India-a pilot study. *African Journal of Traditional, Complementary and Alternative Medicines*, 3(1), 101-114.
18. Sharma, P., Dwivedee, B. P., Bisht, D., Dash, A. K., & Kumar, D. (2019). The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. *Heliyon*, 5(9).
19. Singh, D., & Chaudhuri, P. K. (2017). Chemistry and pharmacology of *Tinospora cordifolia*. *Natural product communications*, 12(2), 1934578X1701200240.
20. Torgerson, D. F., Skowronski, R. P., & Macfarlane, R. D. (1974). New approach to the mass spectroscopy of non-volatile compounds. *Biochemical and Biophysical Research Communications*, 60(2), 616-621.
21. Trease, G. E., & Evans, W. C. (1989). Trease and Evans' pharmacognosy. (No Title).
22. Wan, C., Yu, Y., Zhou, S., Tian, S., & Cao, S. (2011). Isolation and identification of phenolic compounds from *Gynura divaricata* leaves. *Pharmacognosy magazine*, 7(26), 101.
23. Wiley, W. C., & McLaren, I. H. (1955). Time-of-flight mass spectrometer with improved resolution. *Review of scientific instruments*, 26(12), 1150-1157.
24. Zia, K., Siddiqui, T., Ali, S., Farooq, I., Zafar, M. S., & Khurshid, Z. (2019). Nuclear magnetic resonance spectroscopy for medical and dental applications: a comprehensive review. *European journal of dentistry*, 13(01), 124-128.