

In Vitro Callus Induction and Metabolite Fingerprinting of *Chassalia curviflora* (Wall.) Thwaites

Bashidha Banu S¹* and Bojasa A Rosy²

¹ Research Scholar (Reg No.: 23113042262009),

² Assistant professor,

Department of Botany and Research Centre,

Holy Cross College (Autonomous), Nagercoil,

Affiliated to Manonmaniam Sundaranar University, Abishekapatti, 627 012,

Tirunelveli, Tamil Nadu, India.

*Corresponding Author: bashidhabanu@gmail.com

DOI: [https://doi.org/10.63001/tbs.2026.v21.i04.S.I\(4\).pp18-35](https://doi.org/10.63001/tbs.2026.v21.i04.S.I(4).pp18-35)

KEYWORDS

Chassalia curviflora,
Callus induction,
secondary metabolites,
GC-MS, FT-IR,
HPLC.

Article Info

Received: 14/08/2026

Accepted :20/09/2026

Published: 01/10/2026

Abstract

An efficient invitro callus induction protocol was developed for the first time in *Chassalia curviflora* (Wall.) Thwaites, which is employed in folk medicine for the management of inflammatory conditions, wounds, infections and other ailments. The present study focused on callus induction from leaf explants and characterized the phytochemical constituents of the regenerated callus using GC-MS, FTIR and HPLC analyses. Callus induction was evaluated on half-strength Murashige and Skoog medium supplemented with different concentrations using various growth regulators. Among the treatments, 1.0 mg/L 2, 4-D + 0.5 mg/L BAP failed to induce callus. The highest callus biomass of 21.00 ± 0.42 g was obtained on $\frac{1}{2}$ MS medium containing 4.0 mg/L 2, 4-D alone after 45 days. GC-MS analysis of the callus extract identified twenty phytoconstituents belonging to diverse chemical classes, including terpenoids, sterols, long-chain hydrocarbons, fatty acid derivatives, phenolic derivatives and nitrogen-containing compounds. FTIR analysis revealed functional groups associated with hydroxyl, amine, alkene, amide and halogen-containing compounds. HPLC analysis demonstrated relatively simple phenolic profile comprising chlorogenic acid. This study represented the detection and identification of different bioactive compounds from the callus extract. Thus, these findings revealed that *C. curviflora* callus can serve as a promising source of diverse phytoconstituents and provide a useful system in the pharmaceutical industry.

Introduction

Medicinal plants served as an important source of bioactive compounds with significant therapeutic potential. These naturally occurring

substances contribute to the development of pharmaceutical agents and play a vital role in human healthcare (Greenwell & Rahman, 2015). Among the medicinally important members of Rubiaceae, *C. curviflora* is an erect

shrub characterized by white to pale pink flowers and glossy black fruits and is commonly found in evergreen forest ecosystems (Anonymous, 1986). It is distributed from India to South China and the Philippines. In India, the species occurs extensively in Sikkim, Darjeeling, Meghalaya, Maharashtra, Karnataka, the Andaman Islands and the Western Ghats. It is referred locally as Vellakurinji, Yamari or Mundanchedi in Malayalam. The plant has been widely utilized in traditional medicine across several Asian regions. In Peninsular Malaysia, root decoctions are traditionally administered for the treatment of malaria, cough, rheumatism, pneumonia and excessive phlegm accumulation (Chopra *et al.*, 1956).

Plant cell and tissue culture has been a powerful tool for rapid propagation and biomass production of valuable species. One of the major advantages of callus culture is that cellular processes can be studied under highly controlled conditions, minimizing the influence of external environmental factors. These characteristics makes it valuable for understanding plant physiology and developmental processes (Efferth, 2019). In vitro callus induction and indirect plant regeneration by plant growth regulators (PGRs) accelerate the proliferation of plantlets all over the year overcoming the seasonal barrier (Kumlay and Ercisli 2015). The escalating need for phytochemicals has

refocused attention on tissue culture methods as a viable tool for enhancing the production of phytochemicals. When compared to traditional procedures, in vitro technologies have produced a huge number of medicinal plants and their metabolites in a very small space of time and are less toxic and more effective in controlling the development of microorganisms (Chandran *et al.*, 2020).

Structural elucidation of secondary metabolites is commonly performed through chromatographic and spectroscopic methods such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC–MS) and other modern analytical approaches (Wangkheirakpam, 2018). These techniques facilitate the identification, quantification and characterization of biologically active compounds present in plant extract. Fourier transform infrared (FT-IR) spectrometry measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic “fingerprint” of the sample. By attaining IR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites (Mc Cann *et al.*, 1992). The characteristics functional groups are responsible for the medicinal properties of plant which are confirmed by FTIR analysis (Muruganatham *et al.*, 2009).

GC-MS is a hyphenated analytical technique that combines the separation properties of gas liquid chromatography with the detection feature of mass spectrography to identify and determine the organic compounds from complex mixtures. This technique was proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids (Xie *et al.*, 2013). HPLC is a versatile, robust and widely used chromatographic technique used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of a mixture. The use of HPLC is to characterise and quantify the secondary metabolites in plant extracts. It is the primary choice for fingerprinting research for herbal plant quality control (Barbosa *et al.*, 2014). Therefore the current study used GCMS, FTIR and HPLC to profile the bioactive compounds in ethanol extracts of *C.curviflora* callus.

Materials and Methods

Collection of Plant Material and Explant Preparation

Leaves of *C. curviflora* were collected from healthy, mature plants in Kerala. They were authenticated properly and used as explants for in vitro culture. The explants were washed under running tap water for 5–7 min, treated with Tween-20 for 10 min and rinsed three times with distilled water. They were

subsequently treated with Bavistin (100 mg 100 mL⁻¹) for 15–20 min, followed by 2% sodium hypochlorite for 7–8 min and 0.1% (w/v) HgCl₂ for 1–2 min. The explants were thoroughly rinsed with sterile distilled water, then treated with 70% ethanol and rinsed again with sterile distilled water. The cut ends were trimmed using a sterile scalpel and the explants were inoculated aseptically onto the culture medium.

Culture Medium and Callus Induction

Murashige and Skoog (MS) medium was prepared using standard macronutrient, micronutrient and vitamin stock solutions. The medium was supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar and the pH was adjusted to 5.7 using 0.1 N NaOH or HCl. The medium was dispensed into culture vessels and sterilized by autoclaving under standard conditions. Half-strength MS medium was used for callus induction. Half-strength MS medium containing 3% (w/v) sucrose was supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP) namely 1.0 mg L⁻¹ 2,4-D + 0.5 mg L⁻¹ BAP, 2.0 mg L⁻¹ 2,4-D + 0.5 mg L⁻¹ BAP, and 4.0 mg L⁻¹ 2,4-D alone. The inoculated explants were maintained at 25 °C under 16 h photoperiod and 8h dark period. Callus induction percentage and morphological characteristics were recorded periodically.

GC-MS Analysis

The ethanol callus extract of *C. curviflora* was analysed using an Agilent 7820A GC system coupled to an Agilent 5977E mass selective detector and fitted with a DB-5 capillary column. Helium was employed as the carrier gas at a flow rate of 1.2 mL min⁻¹. The oven temperature was programmed at 80 °C for 5 min, followed by an increase from 100 to 270 °C at 10 °C min⁻¹. An aliquot of 0.01 µL of the callus extract was manually injected using an Agilent micro syringe. The mass spectral data obtained were compared with reference spectra in the NIST and Wiley mass spectral libraries for tentative identification of the detected compounds. Compound identification was based on spectral matching with library reference spectra together with comparison of the available retention-index data. The identified compounds were characterized based on their corresponding molecular masses and reported structural information.

Fourier Transform Infrared Spectroscopy Analysis

FT-IR is one of the most effective tools for determining the types of chemical bonds present in compounds. Chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum. Approximately 10 mg of each dried callus extract was thoroughly mixed with approximately 100 mg of spectroscopic-grade potassium bromide (KBr) and compressed to form translucent pellets. The

prepared pellets were analysed using an FTIR spectrometer (Shimadzu, Japan) over the spectral range of 400–4000 cm⁻¹ at a resolution of 4 cm⁻¹. The spectra obtained were used to identify characteristic absorption bands corresponding to different chemical bonds and functional groups.

High-Performance Liquid Chromatography Analysis

HPLC analysis was performed using a Shimadzu HPLC system (CBM-20 A lite controller, LC- 10AT pump and SPD-M20 A PDA detector (Shimadzu, Japan). The samples were diluted to a final volume of 1 mL and an injection volume of 20 µL was used for analysis. Separation was carried out using a reverse – phase C18G column (250mm * 4.6 mm). The mobile phase consisted of solvent A (water containing 0.1% acidifier) and solvent B (acetonitrile or methanol). The flow rate was maintained at 1.0 mL min⁻¹. The chromatographic data were acquired at 25°C over a 20 min analysis period.

Results and Discussion

Callus Induction

Callus culture serves as a model system for studying cellular processes, genetic variability and the effects of various plant growth regulators and stress conditions on plant cells. In the present study, the leaf explants of *C. curviflora* were used for callus induction under controlled

in vitro conditions. Callus induction and growth varied significantly with the concentration of plant growth regulators and culture duration. In treatments T1, explants were cultured on $\frac{1}{2}$ MS medium supplemented with 1.0mg/L 2,4D and 0.5 mg/L BAP in the observation period of 15, 30 and 45 days. The treatment combination of T1 failed to initiate callus formation at all observation periods under this hormonal combination. The absence of callus formation in T1 indicated that lower auxin concentrations even in combination with cytokinin are insufficient to trigger cellular dedifferentiation. This findings supported the general view that callus induction is highly dependent on adequate auxin levels and appropriate hormonal balance (Ali *et al.*, 2017).

The treatment T2 containing $\frac{1}{2}$ MS medium with 2.0mg/L 2,4D and 0.5mg/L BAP resulted in moderate callus induction and callus biomass increased progressively with time which indicated a positive effect of increased 2,4D concentration on callus proliferation. Similar observations have been reported in various plant species, where 2,4-D in combination with low cytokinin levels supports callus formation but does not maximize biomass production (Osman *et al.*, 2013). The highest callus induction and growth were recorded in treatment T3 where explants cultured on $\frac{1}{2}$ MS medium supplemented with 4.0mg/L 2,4D alone. Callus weight increased significantly

from 5.00 ± 0.21 g at 15 days to 12.00 ± 0.35 g at 30 days and reaching a peak of 21.00 ± 0.42 g after 45 days. It is well established that 2, 4-D is one of the most effective auxins for cellular reprogramming and induction of callus formation (Feher, 2005). Its effectiveness is attributed to its ability to regulate endogenous auxin metabolism, influence DNA methylation and induce specific proteins involved in cell division and differentiation (Pan *et al.*, 2010).

From the three different treatment methods, T3 treatment proved to be the most effective for callus induction and biomass accumulation (Table 1; Figure 1). The higher concentrations of 2,4 D in the absence of BAP enhanced callus induction and growth, and prolonged culture duration further promoted callus biomass accumulation. These findings also align with the classical concept proposed by Skoog and Miller (1957), which states that the ratio of auxin to cytokinin determines morphogenic responses in plant tissue culture. In the present study, the higher auxin-to-cytokinin ratio, particularly in T3 where cytokinin was absent, favored extensive callus proliferation rather than organ differentiation. Additionally, high auxin and low cytokinin conditions are generally associated with enhanced callus formation due to increased 'mitotic activity (Dangesh *et al.*, 2015).

Table-1. Effect of plant growth regulator concentrations on callus induction from leaf explants of *C. curviflora* on half-strength MS medium

Treatments	Medium	Concentrations of Growth Regulators (mg/L)		Culture Duration (days)	Callus weight (g)	Callus Induction Response	% of Callus Induction
		2,4-D	BAP				
T1	½ MS + Sucrose	1.0	0.5	15	0	No response	0%
				30	0	No response	
				45	0	No response	
T2	½ MS + Sucrose	2.0	0.5	15	0.50±0.04	Slight response	60%
				30	0.75±0.06	Minimum response	
				45	0.95±0.07	Moderate response	
T3	½ MS + Sucrose	4.0	-	15	5.00±0.21	High response	90%
				30	12.00±0.35	Very high response	
				45	21.00±0.42	Maximum response	

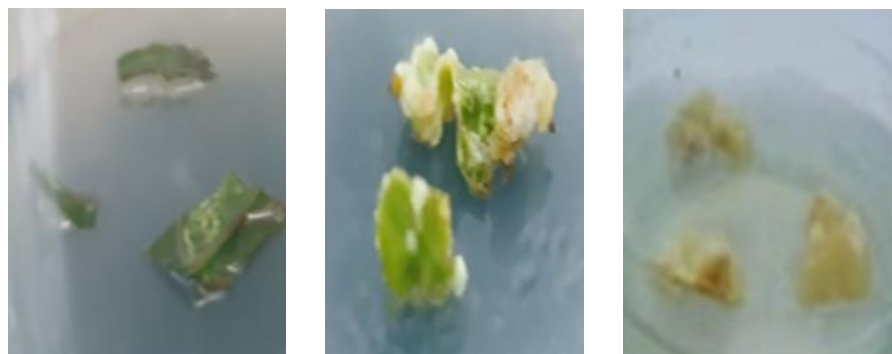


Fig-1(a): Explant Inoculated Fig-1(b): Callus Initiation Fig-1(c): Callus formation



Fig-1(d): Callus growth Fig-1(e): Callus Proliferation Fig-1(f): Callus Expansion

Figure 1. Different stages of callus induction from leaf explants of *C. curviflora* cultured on half-strength MS medium

GC-MS analysis

GC-MS is a reliable and efficient technique for identifying a wide range of phytochemicals in plant extracts (Sampathkumar *et al.*, 2011). Bioactive compounds present in ethanol callus extract obtained from *C. curviflora* are shown in Table 2. Twenty phytoconstituents comprising terpenoids, sterols, long-chain hydrocarbons, fatty acid esters, phenolic derivatives and nitrogen-containing compounds were identified with the retention time between 19.41-33.78 was presented in Figure 2.

Neophytadiene recorded the highest peak area (22.31%) in chromatogram and the

lowest peak area is recorded in Tetracosane and 9,19 -Cyclolanost-24-en-3-ol, acetate(3B) (0.75%). n-hexadecanoic acid, which has been previously proved to have anti-inflammatory, antioxidant, hypocholesterolemic, aromatic, pesticide, and antiandrogenic activities (Henry *et al.*, 2002). Several authors have shown that natural compounds possess important biological activities such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, estrogenic and antibacterial activities (Kumar *et al.*, 2010 & Rizvi *et al.*, 2016). Terpenoids such as neophytadiene and squalene are widely reported to possess antimicrobial, antioxidant and anti-inflammatory activities. These compounds are

known for defensive role in plants against pathogenic organisms and their biological and Neelamegam, 2012). Similar findings have been reported in other plant species, where terpenoids and hydrocarbons contribute significantly to pharmacological properties (Balahbib *et al.*, 2021).

The identification of long-chain alcohols and fatty acid esters, such as hexadecanoic acid derivatives and octacosyl acetate supported the presence of lipid-based bioactive compounds. Comparable results have been reported by Srinivasan *et al.*, (2016), who identified n-hexadecanoic acid as a major constituent along with other fatty acids and terpenoid derivatives. Similarly, the occurrence of n-hexadecanoic acid and octadecanoic acid in *G. sylvestre* leaves has been previously documented, corroborating the present findings (Parinala and Ramasubramaniraja, 2010). The detection of

activity which is largely influenced by their lipophilic nature and functional groups (Ezhilan hydrocarbons such as tetracontane and hexatriacontane supported earlier reports that volatile and semi-volatile compounds contribute to biological activities particularly antimicrobial effects (Balahbib *et al.*, 2021). Steroidal and triterpenoid compounds such as β -sitosterol acetate, stigmasta derivatives and cyclolanostane-type molecules identified in the callus extract are known for their diverse pharmacological properties including anti-inflammatory, anticancer and cytoprotective activities (Jaradat *et al.*, 2017). The presence of phenolic compounds further enhanced the therapeutic potential of the extract, as phenolics are well known for their antioxidant and antimicrobial properties (Okunowo *et al.*, 2013).

Peak	Retention Time	Area %	Molecular weight(g/mol)	Name of the compound	Nature of the compound	Biological activity
1	19.418	22.31	278.52	Neophytadiene	Diterpene hydrocarbon	Anti-inflammatory, antimicrobial, antioxidant activities
2	19.703	4.14	296.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Diterpene alcohol	Anticancer, anti-inflammatory, antioxidant and antimicrobial activities
3	21.099	2.19	284.48	Hexadecanoic acid, ethyl ester	Fatty acid ester	Antioxidant, anti-inflammatory and hypocholesterolemic activities
4	23.015	4.26	338.57	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	Diterpene ester	Antimicrobial, antioxidant activities
5	23.311	19.81	228.29	Phenol, 4,4'-(1-methylethylidene)bis-	Phenolic compound	Antioxidant and antimicrobial activity
6	25.692	8.36	390.56	Bis(2-ethylhexyl) phthalate	Phthalate ester	Antimicrobial, antifouling and cytotoxic activities
7	26.173	5.44	323.43	2-((2H-benzotriazo-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol	Phenolic compound	Antioxidant, UV protective and antimicrobial activities
8	26.848	0.75	562.07	Tetracontane	Long chain alkane	Antimicrobial and anti-inflammatory activities
9	27.685	0.89	410.73	Squalene	Triterpene hydrocarbon	Antioxidant, anticancer, immunomodulatory and cardio protective activities
10	28.242	1.78	562.07	Tetracontane	Long chain alkane	Antimicrobial and protective bioactivity

11	29.020	0.94	480.84	Triacetyl acetate	Long chain fatty alcohol	Antimicrobial and anti-inflammatory activities
12	29.586	1.40	506.99	Hexatriacontane	Long chain alkane	Antimicrobial, antioxidant activities
13	29.777	0.92		.beta.-Sitosterol acetate	Phytosterol ester	Anticancer, Anti-inflammatory, cholesterol lowering activities
14	30.506	2.75	452.79	Octacosyl acetate	Long chain fatty alcohol ester	Antimicrobial and anti-inflammatory activities
15	31.271	1.13	562.07	Tetracontane	Long chain alkane	Antimicrobial activity
16	32.426	13.01	456.70	9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E)-	Triterpenoid	Anticancer, anti-inflammatory, hepatoprotective activities
17	32.551	2.10	452.79	Octacosyl acetate	Long chain fatty alcohol ester	Antimicrobial activity
18	33.101	4.84	396.69	Stigmasta-3,5-diene	Phytosterol hydrocarbon	Anticancer, antioxidant activities
19	33.429	2.22	412.69	gamma.-Sitostenone	Phytosterol ketone	Anticancer, anti-inflammatory activities
20	33.783	0.75	468.75	9, 19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	Triterpenoid acetate	Anticancer and Antioxidant activities

Table 2. Bioactive compounds identified in the ethanol callus extract of *C. curviflora*

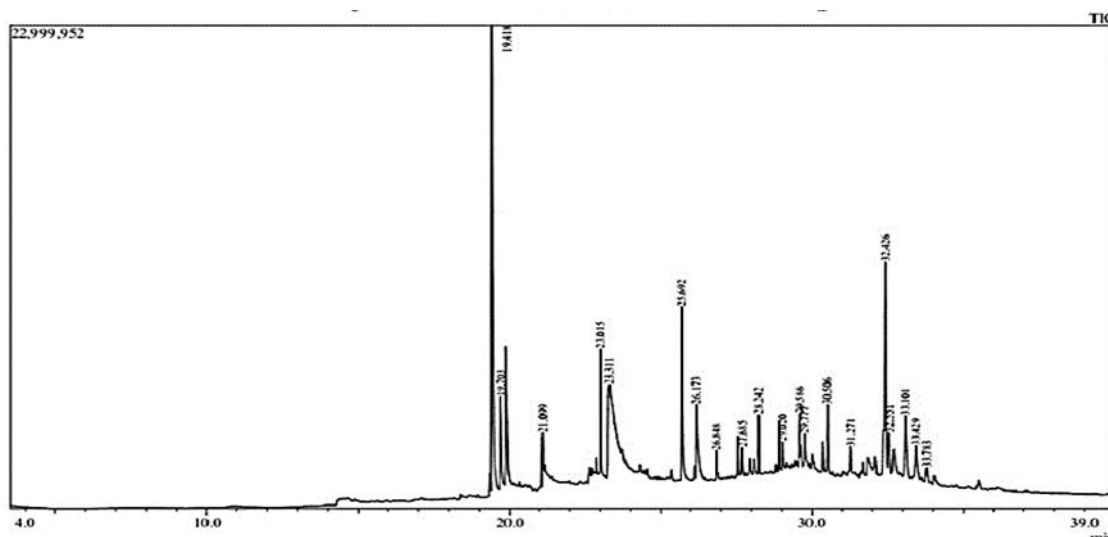


Figure 2. GC–MS profile of the ethanol callus extract of *C. curviflora* showing the identified phytoconstituents

FT-IR Analysis

Spectroscopic techniques have evolved into a valuable technique for secondary metabolite profiling as well as qualitative and quantitative assessment. FT-IR technique allows the implication of different functional groups and host molecules by analysing the significant changes in size and position of the absorbance bands. When the callus extract of *C.curviflora* was allowed to FT-IR, the functional groups of the components were separated based on its peaks ratio. The frequency range and the functional group from absorption spectra are presented in Table 3.

The absorption peak at 435.91 cm^{-1} corresponds to inorganic or halogenated compounds (Meenambal *et al.*, 2012). Similarly, the band at 534.28 cm^{-1} confirmed C–Br

stretching of alkyl halides. These findings are consistent with earlier FT-IR studies where halogenated functional groups were detected in plant extracts (Yamunadevi *et al.*, 2012). The absorption peak at 1637.56 cm^{-1} corresponded to C=C stretching or amide I vibrations may be associated with unsaturated compounds and amide-containing biomolecules such as proteins or peptides. A prominent broad band at 3448.72 cm^{-1} was assigned to O-H/N-H stretching, showed the presence of hydroxyl- or amine-containing compounds particularly polyphenols, alcohols and amines. The peak at 3765.05 cm^{-1} was characteristic of free O-H stretching with the presence of free hydroxyl groups associated with alcohols and phenolic compounds. This observation is in line with studies on other medicinal plants, where similar peaks were associated with bioactive and structural

biomolecules (Srinivasan *et al.*, 2015).

The FT-IR analysis confirmed the presence of hydroxyl, amine, alkene, amide and halogen-associated functional groups in the callus extract. These functional groups are indicative of polyphenolic compounds, which are known for their antioxidant and therapeutic properties (Ashok Kumar and Ramaswamy,

2014). All these compounds belong to secondary plant metabolites as per Researcher explanation Skoog *et al.*, 2017. Fourier transform infrared transmission is very useful in plant characterization because of the presence of inorganic and organic compounds in plants which serves as an indicator of different medicinal properties.

Table 3. FTIR spectral peak values of the ethanol callus extract of *C. curviflora*

SI. No	Wavelength(cm-1)	Possible Functional Group	Bond	Compound type
1	435.91	Metal-O/ Alkyl halides	M-o or C-I stretching	Metal –Ligand complexes/Inorganic or halogenated compounds
2	534.28	Alkyl halides	C-Br stretching	Halogenated organic compounds/metal-oxygen bonded compounds
3	1637.56	Alkenes/Amides	C=C stretching/ Amide I	Unsaturated compounds/ amide – containing biomolecules (proteins, peptides)
4	3448.72	Alcohols/Phenols/Amines	O-H or N-H stretching	Polyphenols, alcohols, amines (hydroxyl-rich bioactive compounds)
5	3765.05	Hydroxyl groups	Free O-H stretching	Free hydroxyl- containing compounds (alcohols, phenols)

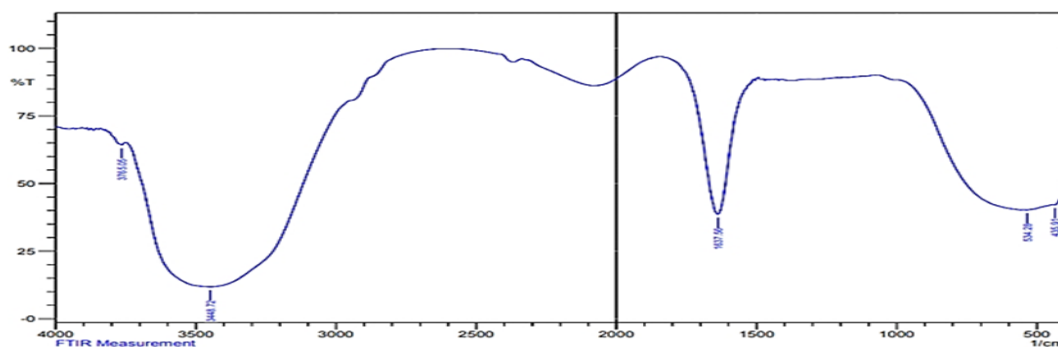


Figure 3. FTIR spectrum of the ethanol callus extract of *C. curviflora*

HPLC Analysis

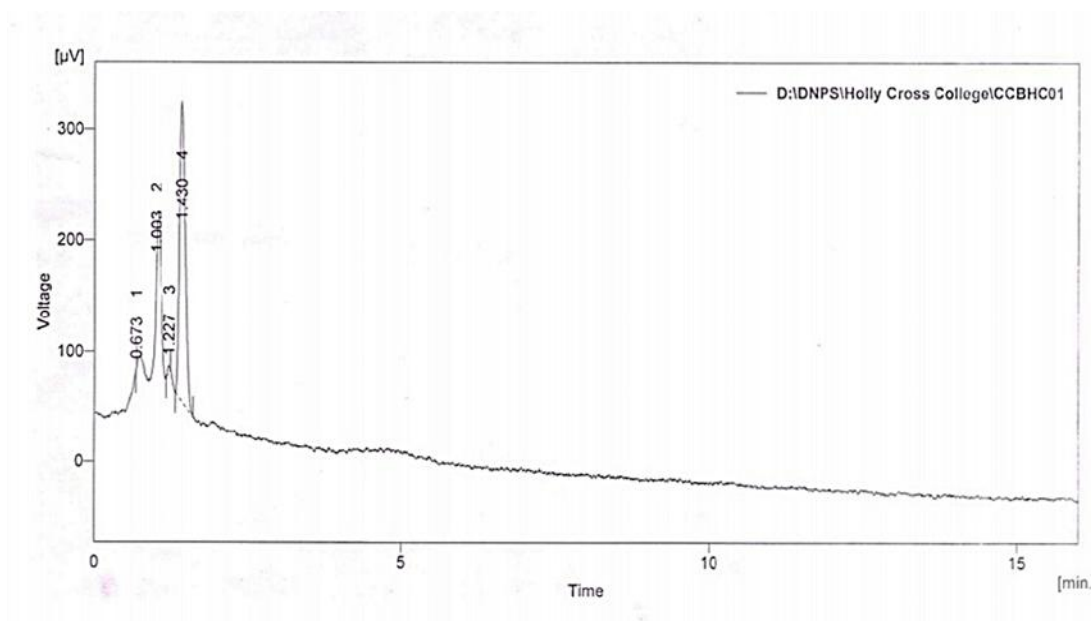
The compounds in the callus extract were estimated using HPLC analysis. HPLC has gained popularity in fingerprinting study as well as in characterization and quantification of secondary metabolites. The chromatograms can be used to identify the unknown compounds in comparison with the chromatograms of known compounds reported in literature (Pandey *et al.*, 2013). The HPLC analysis of the ethanol callus extract revealed comparatively simpler phenolic profile which consisting of four identified compounds (Table 4; Figure 4) among which chlorogenic acid was predominant with a significantly higher peak area percentage and concentration. This marked dominance of chlorogenic acid indicated an enhanced biosynthetic accumulation of this metabolite in callus tissue under in vitro conditions. These findings align with previous reports highlighting

chlorogenic acid as a major bioactive compound with therapeutic potential (Mukherjee *et al.*, 2022).

The presence of caffeic acid, gallic acid and catechol in lower concentrations in the callus extract reflected the reduced diversity of phenolic compounds due to metabolic alterations during dedifferentiation and in vitro culture. Nevertheless, the detection of chlorogenic and caffeic acids corroborates earlier studies reporting these compounds in aerial plant parts (Orhan *et al.*, 2012). Similar enrichment of phenolic compounds has been reported in solvent fractionation studies, where ethyl acetate fractions exhibited high concentrations of phenolics including chlorogenic and caffeic acids (Ramchoun *et al.*, 2009).

Table 4. HPLC profile of the ethanol callus extract of *C. curviflora*

Peak no.	Retention Time[min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	0.673	8.941e-05	0.001	4.970e-03	0.2
2	1.003	0.004	4.023e-04	0.2	0.1
3	1.227	0.018	0.008	1.0	2.8
4	1.430	1777	0.269	98.8	96.8

**Figure 4. HPLC chromatogram of the ethanol callus extract of *C. curviflora* showing the identified phenolic compounds**

Summary and Conclusion

It is the first report of invitro callus induction in *C. curviflora* and its integrated metabolite approach for the identification of individual phytochemicals. Phytochemical profiling of the callus extract using GC–MS, FT-

IR and HPLC revealed a diverse range of secondary metabolites and functional groups. GC–MS identified 20 phytoconstituents, with neophytadiene as the major compound, while FT-IR indicated hydroxyl, amine, alkene, amide and halogen-associated functional groups.

HPLC confirmed chlorogenic acid as the predominant phenolic compound. Due to the identification of large number of useful compounds from the callus extract of this species, it is considered phytopharmaceutically important for drug discovery and development.

Acknowledgements

The author wishes to thank Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, Tamil Nadu.

References

- Ali, M., Mujib, A., Tonk, D., & Zafar, N. (2017). Plant regeneration through somatic embryogenesis and genome size analysis of *Coriandrum sativum* L. *Protoplasma*, 254, 343–352.
- Anonymous. (1986). *The useful plants of India*. Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, p. 119.
- Ashokkumar, R., & Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, 3(1), 395–406.
- Balahbib, A., Omari, N. E., Hachlafi, N. E., Lakhdar, F., Menyiy, N. E., Salhi, N., & Bouyahya, A. (2021). Health-beneficial and pharmacological properties of p-cymene. *Food and Chemical Toxicology*, 153, 1–5.
- Barbosa-Filho, V. M., Waczuk, E. P., Kamdem, J. P., *et al.*, (2014). Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum*). *Industrial Crops and Products*, 55, 280–288.
- Chandran, H., Meena, M., Barupal, T., & Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnology Reports*, 26, e00450.
- Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). *Glossary of Indian medicinal plants*. Council of Scientific and Industrial Research, New Delhi, p. 60.
- Dangash, A., Ram, M., Niranjana, R., Bharillya, A., Misra, H., Pandya, N., & Jain, C. (2015). In vitro selection and hormonal regulation in cell culture of *Artemisia annua* L. *JSM Cell Developmental Biology*, 3, 1013–1019.
- Dahiya, P., & Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug-resistant bacteria from clinical isolates. *Indian Journal Sciences*, 74(5), 443.

- Efferth, T. (2019). Biotechnology applications of plant callus cultures. *Engineering*, 5, 50–59.
- Ezhilan, B. P., & Neelamegam, R. (2012). GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. *Pharmacognosy Research*, 4(1), 11–14.
- Fehér, A. (2005). Why somatic plant cells start to form embryos. In *Somatic embryogenesis*. Berlin: Springer.
https://doi.org/10.1007/7089_019
- Greenwell, M., & Rahman, P. K. (2015). Medicinal plants: Their use in anticancer treatment. *International Journal of Pharmaceutical Sciences Research*, 1(6), 4103.
- Henry, G. E., Momin, R. A., Nair, M. G., & Dewitt, D. L. (2002). Antioxidant and cyclooxygenase activities of fatty acids found in food. *Journal of Agricultural and Food Chemistry*, 50(8), 2231–2234.
- Jaradat, N., Adwan, L., Kaibni, S., Zaid, A. N., Shtaya, M. J. Y., Shraim, N., & Assali, M. (2017). Variability of chemical compositions and antimicrobial and antioxidant activities of *Ruta chalepensis* leaf essential oils from three Palestinian regions. *BioMed Research International*, 2017, 2672689.
- Kumar, P. P., Kumaravel, S., & Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *African Journal of Biochemistry Research*, 4(7), 191–195.
- Kumlay, A. M., & Ercisli, S. (2015). Callus induction, shoot proliferation and root regeneration of potato (*Solanum tuberosum* L.) stem node and leaf explants under long day conditions. *Biotechnology & Biotechnological Equipment*, 29(6), 1075–1084.
- McCann, M. C., Hammouri, M., Wilson, R., Belton, P., & Roberts, K. (1992). Fourier transform infrared micro spectroscopy is a new way to look at plant cell walls. *Plant Physiology*, 100, 1940–1947.
- Meenambal, M., Pughalendy, K., Vasantharaja, C., Prapakaran, S., & Vijayan, P. (2012). Phytochemical information from FTIR and GC-MS studies of methanol extract of *Delonix elata* leaves. *International Journal of Chemical and Analytical Science*, 3(6), 1446–1448.
- Mukherjee, P. K., Singha, S., Kar, A., Chanda, J., Banerjee, S., Dasgupta, B., Haldar, P. K., & Sharma, N. (2022). Therapeutic importance of Cucurbitaceae: A medicinally important family. *Journal of Ethnopharmacology*, 282, 114599.
- Muruganatham, S., Anbalagan, G., & Ramamurthy, N. (2009). FTIR and SEM-EDS comparative analysis of medicinal plants. *Eclipta alba* HASSK and *Eclipta prostrata* Linn. *Romanian Journal of Biophysics*, 19(4), 285–294.

- Okunowo, W. O., Oyedele, O., Afolabi, L. O., & Matanmi, E. (2013). Essential oil of grapefruit (*Citrus paradisi*) peels and its antimicrobial activities. *American Journal of Plant Sciences*, 4, 1–9.
- Orhan, N., Orhan, D. D., Aslan, M., Şukuroglu, M., & Orhan, I. E. (2012). UPLC–TOF-MS analysis of *Galium spurium* towards its neuroprotective and anticonvulsant activities. *Journal of Ethnopharmacology*, 141, 220–227.
- Osman, N. I., Awal, A., Sidik, N. J., & Abdullah, S. (2013). Callus induction and somatic embryogenesis from leaf and nodal explants of *Lycium barbarum* L. (Goji). *Biotechnology*, 12, 36–45.
- Pandey, P., Mehta, R., & Upadhyay, R. (2013). Physico-chemical and preliminary phytochemical screening of *Psoralea corylifolia*. *Archives of Applied Science Research*, 5, 261–265.
- Pan, Z., Zhu, S., Guan, R., & Deng, X. (2010). Identification of 2, 4-D-responsive proteins in embryogenic callus of Valencia sweet orange (*Citrus sinensis* Osbeck) following osmotic stress. *Plant Cell, Tissue and Organ Culture*, 103, 145–153.
- Parimala, B. D., & Ramasubramaniam, R. (2010). Pharmacognostical and antimicrobial screening of *Gymnema sylvestre* R. Br. and evaluation of Gurmar herbal tooth paste and powder, composed of *Gymnema sylvestre* R. Br. extract in dental caries. *Journal of Pharma & Bio Sciences*, 1(3), 1–16.
- Ramchoun, M., Harnafi, H., Alem, C., Benlyas, M., Elrhaffari, L., & Amrani, S. (2009). Study on antioxidant and hypolipidemic effects of polyphenol-rich extracts from *Thymus vulgaris* and *Lavendula multifida*. *Pharmacognosy Research*, 1, 106–112.
- Rizvi, S. M. D., Shaikh, S., Sharma, S. K., *et al.*, (2016). Combating multi-drug resistance in *E. coli* and *S. aureus* with methanolic flower extracts of *Spilanthes oleracea* and estimating its phytochemical constituents. *World Journal of Pharmaceutical Research*, 4(8), 1867–1887.
- Sampathkumar, S., & Ramakrishnan, N. (2011). Chromatographic fingerprint analysis of *Naringicrenulata* by HPTLC technique. *Asian Pacific Journal of Tropical Biomedicine*, S195–S198.
- Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). *Principles of instrumental analysis* (7th ed.). Cengage Learning.
- Skoog, F., & Miller, C. (1957). Chemical regulation of growth and organ formation in plant tissue cultured in vitro. *Symposia of the Society for Experimental Biology*, 11, 118–130.
- Srinivasan, J., Liu, C., & Kelly, R. (2016). Geometric evolution of flux from a corroding

one-dimensional pit and its implications on the evaluation of kinetic parameters for pit stability. *Journal of the Electrochemical Society*, 163, C694.

Srinivasan, P., Dineshbabu, J., Manimekalai, K., & Priya, D. D. (2015). Spectroscopic analysis and antibacterial efficacy of bioactive compounds from *Limonia acidissima* L. fruit extract against clinical pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(3), 383–389.

Surewicz, W. K., Mantsch, H. H., & Chapman, D. (1993). Determination of protein secondary structure by Fourier transform infrared spectroscopy: A critical assessment. *Biochemistry*, 32(2), 389–3.

Wangkheirakpam, S. (2018). Traditional and folk medicine as a target for drug discovery. In *Natural Products and Drug Discovery*, 29–56.

Xie, Z., Liu, Q., Liang, Z., Zhao, M., Yu, X., Yang, D., & Xu, X. (2013). The GC/MS analysis of volatile components extracted by different methods from *Exocarpium Citri Grandis*. *Journal of Analytical Methods in Chemistry*, 2013, 918406.

Yamunadevi, M., Wesely, E. G., & Johnson, M. (2012). FTIR spectroscopic studies on *Aerva lanata* (L.) Juss. ex Schult. *Asian Journal of Pharmaceutical and Clinical Research*, 5(2), 82–86.