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Gas Chromatography-mass Spectrometry Analysis, Antioxidant, and Anticancer Evaluation of *Tinospora cordifolia* Leaf Extract: Implications for Nutraceutical Development

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Abstract

This study investigates the phytochemical profile and *in vitro* bioactivities of a *Tinospora cordifolia* methanolic leaf extract (TCMLE) to evaluate its potential for drug development and functional food applications. Gas chromatography-mass spectrometry (GC-MS) analysis identified 37 distinct compounds, revealing a complex profile dominated by alkaloids, esters, and phenolic derivatives. Notably, 2 nitrogenous heterocycles-9-methoxycanthin-6-one (12.79%) and 6H-indolo[3,2,1-de][1,5]naphthyridine (10.46%) emerged as major β-carboline alkaloids, alongside significant levels of benzofuran, 2,3-dihydro (12.32%) and various fatty acid methyl esters. The extract demonstrated potent, dose-dependent antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with inhibition increasing from 8.75 ± 2.16% at 20 µg/mL to 81.20 ± 1.94% at 100 µg/mL, indicating a high concentration of redox-active phytochemicals. Furthermore, TCMLE exhibited significant cytotoxic effects against human cancer cell lines in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Against chronic myeloid leukemia (K562) cells, viability reduction ranged from 12.48 ± 2.36% at 25 µg/mL to 95.77 ± 3.04% at 200 µg/mL. A more potent effect was observed against cervical adenocarcinoma (HeLa) cells, where inhibition increased from 21.24 ± 1.92% at 25 µg/mL to 97.51 ± 2.17% at 200 µg/mL. The strong bioactivities correlate directly with the rich phytochemical composition, particularly the abundant alkaloids and phenolics. These findings scientifically validate the traditional use of *T. cordifolia* and highlight its leaves as a valuable source of bioactive compounds with dual potential: as leads for anticancer drug development and as multifunctional ingredients for antioxidant-rich nutraceuticals and functional foods.

Keywords: *Tinospora cordifolia*, Gas chromatography-mass spectrometry, Phytochemical profiling, Alkaloids, Antioxidant activity, Cytotoxic activity

Introduction

T. cordifolia (Willd.) Miers, commonly known as Guduchi or Giloy, is a perennial, climbing shrub deeply entrenched in the ayurvedic pharmacopoeia [1, 2]. Celebrated as a 'rasayana' or 'rejuvenator', it has been traditionally prescribed for centuries to treat a wide spectrum of ailments, including fevers, diabetes, jaundice, rheumatism, and immune disorders [1, 2]. The plant's purported therapeutic efficacy is attributed to its rich reservoir of diverse phytoconstituents, such as alkaloids, diterpenoid lactones, glycosides, steroids, and phenolics, which are known to confer antioxidants, anti-inflammatory, immunomodulatory, and hepatoprotective properties [3, 4]. In recent decades, the global shift towards

natural and plant-based therapeutics has spurred rigorous scientific investigation into such traditional herbs, aiming to validate their uses and isolate novel bioactive molecules for modern medicine and functional food products [5, 6].

Despite its historical significance, much of the research on *T. cordifolia* has disproportionately focused on its stem, which is the primary part used in classical ayurvedic formulations [7]. The leaves, however, represent an under-explored yet highly promising resource. Preliminary studies suggest that *T. cordifolia* leaves may harbor a distinct and potentially richer profile of volatile and semi-volatile bioactive compounds compared to the stem [8]. A comprehensive phytochemical fingerprint of the leaf extract is therefore essential to fully understand its therapeutic potential and to explore its viability as a sustainable source of nutraceutical ingredients, particularly as leaf harvesting is less detrimental to the plant than stem collection.

GC-MS has emerged as a powerful analytical tool for the qualitative and semi-quantitative profiling of complex plant extracts [9]. It enables the separation, identification, and relative quantification of volatile and thermally stable compounds, providing a chemical blueprint that can be directly correlated with biological activity [10]. While several GC-MS studies on *T. cordifolia* stem and root exist, a detailed analysis of the methanolic leaf extract, which can capture a broader range of mid-polarity compounds like many alkaloids and fatty acid derivatives, remains less documented. Such an analysis is a critical first step in bridging the gap between traditional knowledge and evidence-based application.

Oxidative stress, resulting from an imbalance between free radicals and the body's antioxidant defenses, is a fundamental pathological mechanism underlying chronic diseases such as cancer, diabetes, and neurodegeneration [11, 12]. The search for potent natural antioxidants from plant sources is a major focus in preventive healthcare and functional food development [13, 14]. Evaluating the free radical scavenging capacity of plant extracts using assays like the DPPH test provides a preliminary yet vital indicator of their potential to mitigate oxidative damage and support cellular health [15]. Concurrently, the exploration of plant-derived compounds with selective cytotoxicity towards cancer cells is a cornerstone of anticancer drug discovery [16]. *In vitro* cytotoxicity assays, such as the MTT test, are routinely employed to screen plant extracts for their antiproliferative effects against specific cancer cell lines [17]. Establishing a link between a detailed phytochemical profile and cytotoxic activity can help pinpoint the specific compounds responsible for the observed bioactivity, guiding subsequent isolation and mechanistic studies [18].

Therefore, this study was designed with the following objectives: (i) to prepare a methanolic extract from the leaves of *T. cordifolia* (TCMLE), (ii) to perform a comprehensive GC-MS analysis to identify and quantify its major phytoconstituents, (iii) to evaluate its *in vitro* antioxidant potential via the DPPH radical scavenging assay, and (iv) to assess its cytotoxic efficacy against 2 human cancer cell lines, K562 (chronic myeloid leukemia) and HeLa (cervical adenocarcinoma). By integrating phytochemical profiling with biological screening, this research aims to provide a scientific foundation for the valorization of *T. cordifolia* leaves in the development of novel nutraceuticals, functional food ingredients, and potential chemotherapeutic leads.

Methodology

Plant (leaves) collection

Fresh leaves of *T. cordifolia* were collected in February 2024 from Warangal, Telangana, India. The collected leaf material was thoroughly washed with distilled water, surface-sanitized with ethanol, and subsequently shade-dried at ambient temperature under aseptic conditions. The dried leaves were then pulverized into a fine powder using a mechanical grinder [19].

Extract preparation

One hundred grams (100 g) of dried leaf powder was subjected to continuous Soxhlet extraction using 800 mL of methanol. The extraction was performed over a period of 48 h, maintaining the temperature below the boiling point of methanol. The resultant crude extract was then filtered through a 0.22 µm PVDF membrane filter. The filtration was concentrated to dryness under reduced pressure using a rotary evaporator. The dried methanolic extract was stored at 4 °C for subsequent use in all analyses [19].

GC-MS analysis

The phytochemical composition of the TCMLE was analyzed using a JEOL GCMATE II GC-MS. The GC was fitted with a capillary column (30 m length × 0.25 mm inner diameter, 0.25 µm film thickness) and helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was initiated at 40 °C and increased to 350 °C at a rate of 5 °C/min. A 1 µL sample was injected in split mode (split ratio 10:1). The MS operated in electron impact ionization mode with an ion source temperature of 200 °C and an ionization energy of 70 eV. Mass spectra were recorded across a scan range of 40 to 600 m/z. Compounds were tentatively identified by comparing the acquired mass spectra with those in the National Institute of Standards and Technology mass spectral library [19].

Antioxidative assay

The free radical scavenging (antioxidant) capacity of the extract was evaluated using the DPPH assay. This standard method measures a compound's ability to donate hydrogen atoms, reducing the stable purple DPPH radical to its yellow, non-radical form. The extent of this colorimetric change is directly proportional to the antioxidant potential. The assay was carried out in accordance with an established protocol [20]. No positive control (e.g., ascorbic acid) was included, as the study was designed to assess the extract's inherent dose-dependent activity rather than to benchmark against a standard.

Anticancer assay

The *in vitro* cytotoxic potential of TCMLE was evaluated against K562 and HeLa cell lines using the MTT assay. This

colorimetric method quantifies cell viability by measuring the reduction of the yellow tetrazolium salt to insoluble purple formazan crystals by mitochondrial dehydrogenases in living cells. The assay was carried out in accordance with an established protocol [20]. No positive control (e.g., cisplatin) was employed, as the primary objective was to evaluate the concentration-response relationship and cytotoxic efficacy of the extract itself.

Results and Discussion

GC-MS analysis

The GC-MS chromatogram of the TCMLE (Figure 1) shows a complex profile of phytoconstituents, consistent with the rich phytochemistry of *T. cordifolia* (Giloy/Guduchi), a revered medicinal plant in Ayurveda known for its diverse bioactive compounds [1, 2]. The methanolic leaf extract analyzed here reveals a chemical profile rich in alkaloids, fatty acids, esters, and aromatic compounds, which aligns with its known phytochemistry and bioactivities. Based on the GC-MS data (Figure 1 and table 1), TCMLE sample contains at least 37 distinct components, with a wide variety of chemical

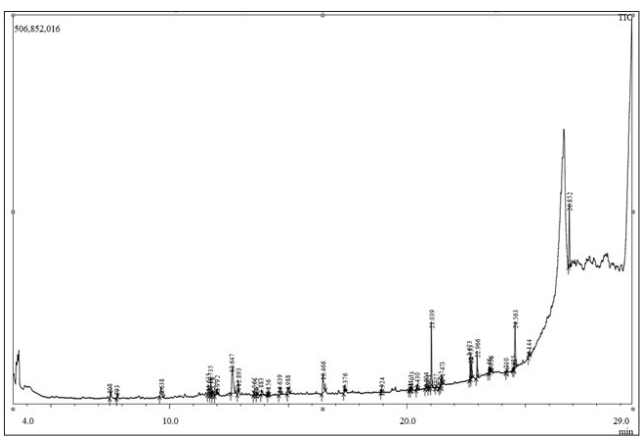

Figure 1: GC-MS spectra of TCMLE.

classes present, indicating significant complexity. The major components are dominated by esters, aromatic compounds, and nitrogen-containing heterocycles.

The most striking feature of the chromatogram is the high abundance of 2 nitrogen-containing heterocyclic compounds. 9-methoxycanthin-6-one (peak 37, 12.79%): This is a β-carboline alkaloid, a class well-documented in *T. cordifolia*. These compounds are renowned for their wide-ranging pharmacological effects, including antioxidant, anti-inflammatory, immunomodulatory, and anticancer activities. The presence of this as a major constituent is a key fingerprint for *T. cordifolia* and is a strong candidate for contributing to its therapeutic reputation. 6H-indolo[3,2,1-de][1,5] naphthyridine (peak 35, 10.46%): This is a complex polycyclic alkaloid structurally related to the canthin-6-one alkaloids. Its significant concentration further underscores the extract's richness in this important alkaloid class.

Other notable phytoconstituents include benzofuran, 2,3-dihydro (peak 8, 12.32%): A simple benzofuran derivative. Benzofuran derivatives have been reported to possess antimicrobial, antioxidant, and anti-inflammatory properties, and may contribute to the overall bioactivity profile of the extract. Benzofuran motifs are present in various natural products and can exhibit biological activity. Fatty acid methyl esters: Peaks 24 (methyl myristate), 28 (methyl linoleate), and 30 (methyl stearate) are prominent. These are likely natural components of leaf waxes and oils, but they can also be artifacts formed during the methanolic extraction/analysis (transesterification of lipids). They may contribute to the extract's permeability-enhancing or emollient properties. Phenolic derivatives: Compounds like 2-methoxy-4-vinylphenol (peak 12, a volatile phenol with antioxidant potential) and phenol, 3,5-bis(1,1-dimethylethyl) (peak 16, a butylated hydroxytoluene-like compound) are present. These contribute to the antioxidant capacity of the extract. Benzeneacetaldehyde (peak 3, 3.82%): An aromatic aldehyde that contributes to the extract's aroma and may have mild bioactive properties.

The high abundance of methyl esters of fatty acids (peaks 24, 28, and 30) strongly suggests that transesterification occurred during the methanolic extraction or in the hot GC injector, converting the plant's triglycerides and free fatty acids into their methyl ester forms. This is a common phenomenon and does not necessarily mean the esters were originally present. Certain compounds are almost certainly laboratory contaminants and not genuine plant metabolites. Dibutyl phthalate (peak 26) and bis(2-methylpropyl) ester of phthalic acid (peak 21) are ubiquitous plasticizers leaching from laboratory plasticware. Their presence is a common artifact in GC-MS analyses and was therefore excluded from subsequent correlations with the observed bioactivities.

This GC-MS profile of the TCMLE provides a strong chemical rationale for its traditional use. The extract is dominated by alkaloids, particularly the β-carboline type (9-methoxycanthin-6-one), which are prime candidates for its documented immunomodulatory and antioxidant effects. The supporting cast of phenolic compounds, aromatic aldehydes, and fatty acid derivatives adds to the complex mixture that likely acts synergistically (the 'entourage effect') to produce the plant's full therapeutic benefits.

Antioxidative assay

Figure 2 presents the results of a DPPH radical scavenging assay for a TCMLE. The DPPH assay is a standard, quick,

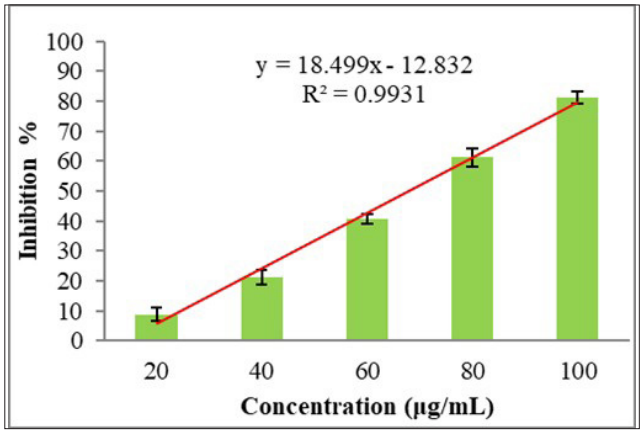

Figure 2: Antioxidative (DPPH radical scavenging) assay results.

Table 1: GC-MS analysis and relative quantification of phytochemical constituents in TCMLE.

Peak#	Retention time	Area	Area%	Height	Height%	Ampere-hour	Name
1	7.508	7869486	0.61	4675439	0.79	1.68	Butyrolactone
2	7.793	8249793	0.64	4864104	0.82	1.70	6-oxa-bicyclo[3.1.0]hexan-3-one
3	9.638	49200170	3.82	12454162	2.09	3.95	Benzeneacetaldehyde
4	11.635	28135436	2.19	13100676	2.20	2.15	Acetic acid, phenylmethyl ester
5	11.735	45897106	3.57	23673621	3.98	1.94	3,5,5-trimethylhexyl acetate
6	11.829	16997756	1.32	6664419	1.12	2.55	Benzeneacetic acid, methyl ester
7	11.992	18244703	1.42	7560911	1.27	2.41	Naphthalene
8	12.647	158602827	12.32	35745178	6.01	4.44	Benzofuran, 2,3-dihydro-
9	12.893	27927243	2.17	16996190	2.86	1.64	Benzene, 1,3-bis(1,1-dimethylethyl)-
10	13.560	7979275	0.62	5119474	0.86	1.56	ortho tert-butyl cyclohexyl acetate
11	13.675	4354858	0.34	2946023	0.50	1.48	Indole
12	13.883	7818819	0.61	5968141	1.00	1.31	2-methoxy-4-vinylphenol
13	14.156	6605953	0.51	4581497	0.77	1.44	4-tert-butylcyclohexyl acetate
14	14.639	22448738	1.74	10500628	1.77	2.14	4-tert-butylcyclohexyl acetate
15	14.988	22551739	1.75	8676603	1.46	2.60	DL-proline, 5-oxo-, methyl ester
16	16.466	84679665	6.58	25374415	4.27	3.34	Phenol, 3,5-bis(1,1-dimethylethyl)-
17	17.376	15735490	1.22	8939387	1.50	1.76	Syn-tricyclo[5.1.0.0(2,4)]oct-5-ene, 3
18	18.924	6222461	0.48	4087281	0.69	1.52	Methyl tetradecanoate
19	20.111	4906148	0.38	3086918	0.52	1.59	Neophytadiene
20	20.193	8108577	0.63	4816417	0.81	1.68	2-pentadecanone, 6,10,14-trimethyl-
21	20.430	12325803	0.96	7104531	1.19	1.73	1,2-benzenedicarboxylic acid, bis(2-methyl-propyl) ester
22	20.804	10256302	0.80	5200833	0.87	1.97	Hexadecanenitrile
23	20.921	4212010	0.33	2095275	0.35	2.01	Phthalic acid, butyl undecyl ester
24	21.039	156114599	12.13	85753695	14.42	1.82	Tetradecanoic acid, 12-methyl-, methyl ester
25	21.227	2187161	0.17	1572520	0.26	1.39	Benzoic acid, 2-benzoyl-, methyl ester
26	21.395	4914253	0.38	2996903	0.50	1.64	Dibutyl phthalate
27	21.473	32453484	2.52	13699323	2.30	2.37	n-hexadecanoic acid
28	22.673	59903267	4.65	35123977	5.91	1.71	9,12-octadecadienoic acid, methyl ester
29	22.733	48342209	3.76	28237312	4.75	1.71	9,12-octadecadienoyl chloride, (Z,Z)-
30	22.966	60522931	4.70	35172282	5.91	1.72	Methyl stearate
31	23.466	14396972	1.12	7800050	1.31	1.85	2,6-dimethoxybenzoyl chloride
32	23.536	10765574	0.84	5855112	0.98	1.84	Tetradecanamide
33	24.210	2801744	0.22	1700241	0.29	1.65	N,N-dimethyldecanamide
34	24.485	4037355	0.31	2227168	0.37	1.81	Dimethylaminoethyl palmitate
35	24.563	134633390	10.46	60762044	10.22	2.22	6H-indolo[3,2,1-de][1,5]naphthyridin
36	25.144	12183157	0.95	6457130	1.09	1.89	4,8,12,16-tetramethylheptadecan-4-olide
37	26.852	164594819	12.79	83128670	13.98	1.98	9-methoxycanthin-6-one

and widely used method to evaluate the *in vitro* antioxidant capacity of plant extracts by measuring their ability to donate hydrogen atoms or electrons to neutralize the stable, purple-colored DPPH free radical, resulting in a color change to yellow [15].

The data demonstrates a strong, clear dose-dependent relationship between the concentration of TCMLE and its free radical scavenging activity. At the lowest tested concentration (20 µg/mL), the extract shows only 8.75% inhibition, indicating a minimal effect. However, the activity increases dramatically with concentration, at 40 µg/mL, inhibition more than doubles to 21.23%. At 60 µg/mL, it nearly doubles again to 40.84%, representing significant midpoint activity. At 80 µg/mL, a substantial leap to 61.31% inhibition is observed. Finally, at the highest tested concentration (100 µg/mL), the extract achieves 81.20% inhibition of the DPPH radical.

This classic sigmoidal dose-response pattern confirms that TCMLE contains potent antioxidant compounds, and their efficacy is directly proportional to their concentration in the assay mixture. The relatively low standard deviations (ranging from 1.61 to 3.10) across all concentrations indicate good reproducibility and reliability of the experimental data.

This robust antioxidant activity can be directly correlated with the rich phytochemical profile identified in the GC-MS analysis of the same extract. The activity is primarily attributed to (i) major alkaloids, the high concentrations of 9-methoxycanthin-6-one and related alkaloids (β-carbolines) are strong candidates [21]. Alkaloids from *T. cordifolia* are well-documented for their radical scavenging potential. (ii) phenolic compounds, the presence of 2-methoxy-4-vinylphenol and other phenolic derivatives contributes significantly [22]. Phenolics are renowned antioxidants due to their hydroxyl groups, which readily donate hydrogen to stabilize free radicals. The overall activity is likely the result of synergistic interactions among the complex mixture of alkaloids, phenolics, and other minor constituents present in the extract, rather than the action of a single compound.

In summary, the DPPH assay confirms that the TCMLE possesses significant *in vitro* antioxidant activity, which increases in a concentration-dependent manner. This provides a scientific basis for its traditional use in conditions linked to oxidative stress, such as inflammation, aging, and metabolic disorders. The activity is a direct consequence of its rich and diverse phytochemistry, particularly its alkaloid and phenolic content.

Anticancer assay

The results show concentration-dependent cytotoxic effect of TCMLE on K562 leukemia cells (Figure 3). At a concentration of 25 µg/mL, the extract already shows 12.48% inhibition/cell death, indicating a bioactive effect even at low doses. The effect escalates steadily: at 50 µg/mL, inhibition rises to 21.84%. At 75 µg/mL, a more substantial effect is observed at 37.32%. The activity crosses the critical threshold at 100 µg/mL, with 52.95% inhibition. At 150 µg/mL, a strong effect of 72.40% inhibition is achieved. Most strikingly, at the highest tested concentration of 200 µg/mL, the extract induces 95.77% inhibition, demonstrating near-complete cytotoxicity against this cancer cell line. The low standard deviations across all concentrations indicate highly reproducible and reliable results.

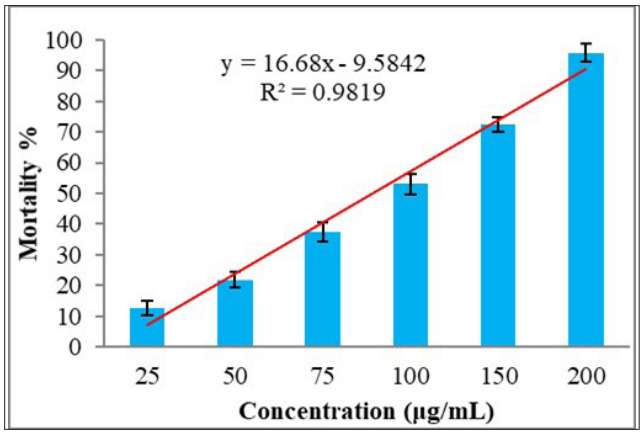

Figure 3: Anticancer assay results against K562 cancer cell line.

The activity against HeLa cells is remarkably strong, initiating at a substantial level even at the lowest concentration (Figure 4). At 25 µg/mL, the extract induces 21.24% inhibition/cell death, which is notably higher than the 12.48% observed at the same concentration for K562 cells. This suggests HeLa cells may be more sensitive to the extract's components. The dose-response is steep and effective: at 50 µg/mL, inhibition rises sharply to 36.81%. At 100 µg/mL, a very strong effect of

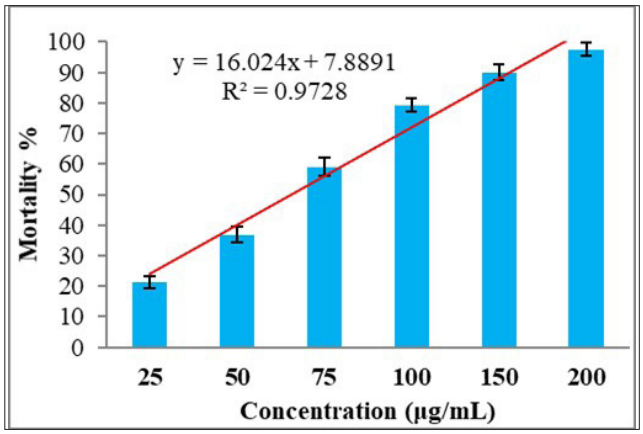

Figure 4: Anticancer assay results against HeLa cancer cell line.

79.24% inhibition is achieved. Near-complete cytotoxicity is observed at 150 µg/mL (89.93%) and 200 µg/mL (97.51%). The consistently low standard deviations confirm the reliability and reproducibility of these potent effects.

This potent anticancer activity can be directly linked to the specific phytochemicals identified in the GC-MS analysis. Primary role of alkaloids, the major constituents, 9-methoxycanthin-6-one and 6H-indolo[3,2,1-de][1,5] naphthyridine, are β-carboline and related alkaloids. This class of compounds is extensively documented for pro-apoptotic, anti-proliferative, and chemopreventive activities against various cancer cell lines [21]. They often work by intercalating into DNA, inhibiting topoisomerases, inducing cell cycle arrest, and triggering mitochondrial apoptosis pathways. Contributing bioactive: Other components like 2-methoxy-4-vinylphenol (a phenolic with known anticancer properties) and various esters may contribute to the overall cytotoxic effect through synergistic actions, potentially enhancing permeability or targeting multiple pathways simultaneously [22]. Potential pro-oxidant mechanism: the extract's strong antioxidant activity (shown in the DPPH assay) can, in the complex cellular environment of cancer cells, manifest as a pro-oxidant effect. This can exacerbate oxidative stress within cancer cells, which already operate under high basal reactive oxygen species levels, pushing them beyond a survivable threshold and inducing apoptosis.

In summary, the complex mixture in TCMLE likely attacks multiple cellular pathways simultaneously-a common advantage of plant extracts. This could include anti-mitotic effects, inhibition of signal transduction (e.g., NF-κB, often active in HeLa), and interference with metabolic adaptations,

making it harder for cancer cells to develop resistance.

Conclusion

Based on comprehensive phytochemical and biological analyses, the TCMLE demonstrates significant potential for therapeutic development and application in functional foods. The GC-MS profiling revealed a rich chemical matrix dominated by bioactive alkaloids, particularly 9-methoxycanthin-6-one and related β-carbolines, alongside phenolic compounds and fatty acid derivatives, which collectively underpin the observed bioactivities. The extract exhibited strong, dose-dependent antioxidant capacity in the DPPH assay and potent cytotoxic effects against both K562 (leukemia) and HeLa (cervical carcinoma) cell lines, with notable efficacy against the latter. These findings scientifically validate the traditional use of *T. cordifolia* and position it as a promising source of multifunctional phytochemicals. The identified compounds, especially the major alkaloids, warrant further investigation as lead molecules for drug development, while the overall extract, pending further safety studies, holds immediate promise for incorporation into nutraceuticals and functional foods aimed at oxidative stress management and chemoprevention.

Future Prospects

Based on the promising bioactivity profile of *T. cordifolia* leaf extract, a primary prospect involves the application of food nanotechnology to overcome the inherent challenges of bioactive delivery. The major alkaloids, such as 9-methoxycanthin-6-one, identified as key therapeutic agents, often suffer from poor solubility, stability, and bioavailability. Future work should focus on developing nanoencapsulation systems-using liposomes, biopolymer nanoparticles, or nanoemulsions-to protect these sensitive phytochemicals, enhance their absorption, and enable their controlled release in functional food matrices. Concurrently, the extract's potent antioxidant and antimicrobial properties, validated in this study, make it an excellent candidate for developing active and intelligent packaging materials. Incorporating the extract or its isolated fractions into biocomposite films could create next-generation packaging that actively extends shelf-life by scavenging oxygen and inhibiting microbial growth, while nanosensors derived from its components could potentially detect food spoilage indicators, aligning with the focus on food safety, packaging, and shelf-life extension.

Further research should also delve into the food application and safety toxicology of the characterized extract to bridge the gap between laboratory bioactivity and commercial food product development. Systematic studies are needed to incorporate the standardized extract into model food systems (e.g., beverages, spreads, or supplements) to evaluate its impact on organoleptic properties, stability during processing, and synergistic effects with other food constituents. Crucially, comprehensive toxicological assessments, including *in vitro* genotoxicity assays and *in vivo* sub-chronic oral toxicity studies in relevant models, are essential to establish its safety for human consumption as a food ingredient. Finally, scaling up the green synthesis of nanoparticles using the extract as a reducing and capping agent presents an opportunity for creating nanofiltration membranes or nanosensors for the detection of contaminants like heavy metals or pesticides in food products. This multidisciplinary approach, integrating phytochemistry, nanotechnology, and food engineering, can transform *T. cordifolia* from a traditional remedy into a validated, high-value ingredient for nutraceuticals and innovative functional foods, directly contributing to the journal's mission of advancing food quality, safety, and production.

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Conflict of Interest

None.

References

1. Gupta A, Gupta P, Bajpai G. 2024. *Tinospora cordifolia* (Giloy): an insight on the multifarious pharmacological paradigms of a most promising medicinal ayurvedic herb. *Heliyon* 10(4): 1-18. https://doi.org/10.1016/j.heliyon.2024.e26125
2. Chaudhary A, Das R, Mehta K, Mehta DK. 2024. Indian herb *Tinospora cordifolia* and *Tinospora* species: phytochemical and therapeutic application. *Heliyon* 10(10): 1-23. https://doi.org/10.1016/j.heliyon.2024.e31229
3. Arunachalam K, Yang X, San TT. 2022. *Tinospora cordifolia* (Willd.) Miers: protection mechanisms and strategies against oxidative stress-related diseases. *J Ethnopharmacol* 283: 114540. https://doi.org/10.1016/j.jep.2021.114540
4. Payyappallimana U, Ravikumar K, Venkatasubramanian P. 2023. Can Guduchi (*Tinospora cordifolia*), a well-known ayurvedic hepato-protectant cause liver damage? *J Ayurveda Integr Med* 14(1): 100658. https://doi.org/10.1016/j.jaim.2022.100658
5. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, et al. 2025. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits-a comprehensive review. *Front Immunol* 16: 1-37. https://doi.org/10.3389/fimmu.2025.1491777
6. Aware CB, Patil DN, Suryawanshi SS, Mali PR, Rane MR, et al. 2022. Natural bioactive products as promising therapeutics: a review of natural product-based drug development. *South African J Bot* 151: 512-528. https://doi.org/10.1016/j.sajb.2022.05.028
7. Anjum V, Bagale U, Kadi A, Potoroko I, Sonawane SH, et al. 2023. Unveiling various facades of *Tinospora cordifolia* stem in food: medicinal and nutraceutical aspects. *Molecules* 28(20): 7073. https://doi.org/10.3390/molecules28207073
8. Siddiqui S, Jadaun S, Anusha R, Kumar N, Ashraf SA, et al. 2025. Medicinal importance of *Tinospora cordifolia* (Willd.) Miers and its possible use in food industry for value addition. *Discov Food* 5(1): 1-17. https://doi.org/10.1007/s44187-025-00652-8
9. Alum EU, Manjula VS, Uti DE, Echegu DA, Ugwu OPC, et al. 2025. Metabolomics-driven standardization of herbal medicine: advances, applications, and sustainability considerations. *Nat Prod Commun* 20(8): 1-16. https://doi.org/10.1177/1934578X251367650
10. Mondello L, Cordero C, Janssen HG, Synovec RE, Zoccali M, et al. 2025. Comprehensive two-dimensional gas chromatography–mass spectrometry. *Nat Rev Methods Prim* 5(1): 7. https://doi.org/10.1038/s43586-024-00379-3

11. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, et al. 2023. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Front Chem* 11: 1-24. https://doi.org/10.3389/fchem.2023.1158198

12. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, et al. 2023. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch Toxicol* 97(10): 2499-2574. https://doi.org/10.1007/s00204-023-03562-9

13. Dixit V, Joseph Kamal SW, Bajrang Chole P, Dayal D, Chaubey KK, et al. 2023. Functional foods: exploring the health benefits of bioactive compounds from plant and animal sources. *J Food Qual* 2023(1): 5546753. https://doi.org/10.1155/2023/5546753

14. Rahaman MM, Hossain R, Herrera-Bravo J, Islam MT, Atolani O, et al. 2023. Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: an update. *Food Sci Nutr* 11(4): 1657-1670. https://doi.org/10.1002/fsn3.3217

15. Gulcin İ, Alwasel SH. 2023. DPPH radical scavenging assay. *Processes* 11(8): 2248. https://doi.org/10.3390/pr11082248

16. Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, et al. 2021. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules* 26(4): 1109. https://doi.org/10.3390/molecules26041109

17. Gavanji S, Bakhtari A, Famurewa AC, Othman EM. 2023. Cytotoxic activity of herbal medicines as assessed *in vitro*: a review. *Chem Biodivers* 20(2): e202201098. https://doi.org/10.1002/cbdv.202201098

18. Mazumder K, Aktar A, Roy P, Biswas B, Hossain ME, et al. 2022. A review on mechanistic insight of plant derived anticancer bioactive phytocompounds and their structure activity relationship. *Molecules* 27(9): 3036. https://doi.org/10.3390/molecules27093036

19. Reddy MJ, Sampathkumar Y, Godishala V. 2025. Exploring the bioactive phytochemicals of *Tinospora cordifolia* via gas chromatography-mass spectrometry: implications for drug development and functional foods. *J Food Chem Nanotechnol* 11(2): 52-58. https://doi.org/10.17756/jfcn.2025-202

20. Kurra H, Velidandi A, Sarvepalli M, Pabbathi NPP, Godishala V. 2025. Aqueous *Cymbopogon citratus* extract mediated silver nanoparticles: part I. Influence of synthesis parameters, characterization, and biomedical studies. *Nanomaterials* 15(5): 328. https://doi.org/10.3390/nano15050328

21. Yunos NM, Amin NDM, Jauri MH, Ling SK, Hassan NH, et al. 2022. The *in vitro* anti-cancer activities and mechanisms of action of 9-methoxycanthin-6-one from *Eurycoma longifolia* in selected cancer cell lines. *Molecules* 27(3): 585. https://doi.org/10.3390/molecules27030585

22. Rubab M, Chelliah R, Saravanakumar K, Barathikannan K, Wei S, et al. 2020. Bioactive potential of 2-methoxy-4-vinylphenol and benzofuran from *Brassica oleracea* L. var. capitate f, rubra (red cabbage) on oxidative and microbiological stability of beef meat. *Foods* 9(5): 568. https://doi.org/10.3390/foods9050568