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Pharmacognostical Profiling and GC-Ms Analysis of *Tinospora Cordifolia* Stems for Immunomodulatory Properties

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ABSTRACT

This paper is a pharmacognostical and chemical appraisal of *Tinospora cordifolia* stems with focus on their immunomodulatory activity. Authenticity and quality of the plant material were established by the use of pharmacognostical profiling such macroscopic and physicochemical analyses which indicated some features like greenish-brown in colour stems, fibrous fracture characteristic and good moisture (8.25%) and ash content equal to WHO recommended limits. Gas Chromatography Mass Spectrometry (GC-MS) revealed the characterization of tinosporaside (14.2%), berberine (12.5%), palmatine (10.8%), magnoflorine (9.4%), beta-sitosterol (8.6%), and stigmasterol (7.9%), to be the most significant bioactive components of ethanol extracts. An analysis of a Relative Contribution Index (RCI) identified tinosporaside (0.710) and berberine (0.625) as the core contributors to the potential activity of immunomodulation. These results provide a scientific foundation to the traditional use of T. cordifolia in immune-support formulations, a chemical road-map to quality control, and compounds of interest that may be considered priority to further bioactivity verification. The proposed integrated method helps with conceiving how to standardize herbal extracts and shape future pharmacological research.

Key Words:

Tinospora Cordifolia, Pharmacognostical Profiling, GC-MS Analysis, Immunomodulatory Activity, Relative Contribution Index

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1.INTRODUCTION

Traditional medicine systems have used medicinal plants as a source of bioactive agents with immense therapeutic values over the years [1.] Over the past decades, natural products became popular again because they produced more effective results with fewer side effects and prospects of designing new medications [2.] The types of these, immunomodulatory plants, are receiving increased interest in the potential to improve immune defence, control inflammatory responses and to work towards enhancing general body health. To correct the situation it is necessary to scientifically validate such plants requiring thorough pharmacognostical and phytochemical study of their safety, efficacy and quality control in the science of herbal medicine [3.]

Tinospora Cordifolia

1.1. Background Information

Tinospora Cordifolia (commonly known as Guduchi or Giloy) is a famous medicinal plant used in the systems of medicine Ayurveda, Siddha, and Unani, which are appreciated due to their multiple curative properties [4.] It is traditionally applied as an anti-fever, anti-diabetic, anti-jaundice, skin affliction and inflammation medication [5.] Specifically, its value as an immunomodulatory agent is of interest because it can be used to control and boost the immune response of the body [6.] The authenticity, purity, and quality of these herbal drugs are highly dependent on conducting pharmacognostical profiling of the plant materials by means of macroscopic, microscopic, and physicochemical characterization of plant materials. Combined with superior methods of analytical studies like Gas ChromatographyMass Spectrometry (GC-MS), scholars will be able to recognize and distinguish neuroactive substances responsible in pharmacological responses, hence, connecting the ancient wisdom with scientific appraisals [7.]

1.2. Statement of the Problem

On the one hand, this is due to popular use of *Tinospora Cordifolia* in folk medicine, on the other hand-a lack of thorough, scientifically substantiated data on its phytochemical composition, especially with regard to its stem components, which, in turn, are known to be the most powerful source of bioactive compounds. [8] Most of the studies that are available give attention on partial phytochemical screening or lack standardized pharmacognostical documentation [9.] More so, inadequate studies have been carried out in an attempt to specifically determine and quantify the chemical composition of immunomodulatory properties of GC-MS. Such knowledge gap restricts the standardisation of the plant in contemporary pharmacopoeias and its use in pharmaceutical formulations relating to immune health. [10.]

1.3. Objectives of the Study

The present study aims to:

1. Carry out elaborate pharmacognostical profiling of *Tinospora Cordifolia* stems comprising macroscopic, microscopic and physicochemical characterisation.

2. Determine the chemical constituents that make up the extracts of the stems through a GC-MS analysis.

3. Compare the established available phytoconstituents with the reported immunomodulatory effects so as to offer scientific support to its traditional uses.

2. METHODOLOGY

2.1. Description of Research Design

The current report used an experimental, laboratory based research design in which the pharmacognostical aspects and chemical constitution of stems of *Tinospora Cordifolia* was analyzed systematically and the results associated with the documented immunomodulatory effects. This was done in two stages i.e. (i) pharmacognostical profiling, (ii) phytochemical identification by GC-MS. The findings were matched to the literature concerning the immunomodulatory activity of reported in-vivo animal studies in the past.

2.2. Participants/Sample Details

Fresh stems of *Tinospora Cordifolia* cultivated in verified herbal gardens, the were harvested as disease-free, mature plant material. The plant was taxonomically identified and authenticated by a known botanist and a voucher specimen taken by a botanist was placed in herbarium for future reference. The sampled materials were washed carefully, sun-dried and stored in a controlled laboratory environment to maintain the required phytoconstituent of the materials sampled**.**

2.3. Instruments and Materials Used

The instruments and materials used in the study included:

- Stereo zoom microscope anatomical studies and compound microscope
- Digital caliper to use macroscopic measurements
- Physicochemical tests Using analytical balance
- Soxhlet extraction 2. Apparatus
- GC-MS system (using proper column specifications) of chemical profiling
- Typical reagents and chemicals used in physicochemical and phytochemical examinations of these components (all are of analytical grades)

2.4. Procedure and Data Collection Methods

1. **Pharmacognostical Profiling**
    - Macroscopic Analysis: Morphological attributes were determined: color, odor, taste, texture of the surface, fracture appearance and size.
    - Microscopic Analysis: Transverse cross sections of stems are prepared, stained with appropriate reagents (e.g., safranin, fast green, etc.) and studied to look at

characteristic anatomical characters of that family of plants, including cortical tissues, vascular bundles, xylem fibers and parenchymatous cells.

- Physicochemical Parameters: Moisture, total ash, acid insoluble ash, water soluble ash and extractive values (water and alcohol) were analyzed based on WHO specifications.

2. Preparation of Extract

- Stem stock was dried and powdered and was Soxhlet extracted in ethanol as the first solvent. The extract was concentrated under lowered pressure by use of rotary evaporator and kept in air tight containers ready to be analyzed further.

3. GC-MS Analysis

- A GC-MS system was used to analyze the ethanol extract. The conditions were proper column type, carrier gas (Helium), the temperature of injection, temperature of the oven programming, and mass spectrometric detection in the EI mode.
- Identification of chromatographic peaks was done through retention time, mass spectral data and compared to the NIST library to identify the compounds.

4. Correlation with Immunomodulatory Properties

- Identified compounds were cross correlated with published literatures reporting immunomodulatory actions of in-vivo animal experiments. Direct animal tests were not involved in this study and this study only made inferences and correlations based on secondary data extracted on the existing experimental models.

2.5. Data Analysis Techniques

GC-MS data was examined with the help of specific chromatographic software, and detected substances were compared with spectral databases. Triplicated values of physicochemical values were calculated and the results were demonstrated in terms of averages with standard deviations. Correlation analysis based on literature was used to provide the association between phytoconstituents and the reported immunomodulatory effects.

3. RESULTS

The findings of the current research will include macroscopic description, physicochemical fingerprinting, and GC-MS-mediated phytochemical analysis of *Tinospora Cordifolia* stem material, and subsequent an examination of the relative addition of identified compounds to immunomodulatory actions.

3.1 Macroscopic Characteristics

Macroscopic analysis of *Tinospora Cordifolia* stems was performed to determine their organoleptic and morphological characters that are vital in authenticating the raw materials in pharmacognostic analysis. The stems were observed to be greenish-brown in colour, and when a

gentle odor inspired upon smelling a bit bitter and the taste distinctly bitter. It was smooth with clear nodes and also there was a fibrous nature of the fracture. Positive stem diameters were in the interval of 0.5 1.5 cm. These properties do not contradict with the reports as pharmacopoeials are used to identify *Tinospora Cordifolia*.

Table 1. Macroscopic characteristics of *Tinospora Cordifolia* stems

Parameter	Observation
Color	Greenish-brown
Odor	Slightly bitter
Taste	Bitter
Surface Texture	Smooth with nodes
Fracture	Fibrous
Size (cm)	0.5–1.5 diameter

The organoleptic and morphological characteristics of *Tinospora Cordifolia* stems agree with standardized monographs and the plant material is authentic. Its greenish-brown color and smooth nodal surface are fairly characteristic of this type of species, the notably bitter taste confirming its conventional medical role, in which bitter substances as alkaloids and glycosides are frequently involved. It has fibrous fracture that suggests a hard structure lignified that is typical to mature stems. Such macroscopic characteristics form the initial quality control prior to additional microscopic and physicochemical analysis of the material.

3.2 Physicochemical Parameters

The *Tinospora Cordifolia* stems were evaluated in physicochemical parameters in accordance with the WHO quality control guidelines on herbal raw material. The evaluated parameters were moisture content, ash values and extractive values, which reflect the vital measures of purity, quality and presence of bioactive component. Each has been measured in three replicates and the results presented as the mean value ± standard deviation (SD). According to WHO requirements, physicochemical parameters will give crucial information about the quality, purity, and the possible phytochemical constituents of T. cordifolia stems.

Table 2. Physicochemical parameters of *Tinospora Cordifolia* stems

Parameter	Value (Mean ± SD)
Moisture Content (%)	8.25 ± 0.12
Total Ash (%)	6.48 ± 0.09
Acid-Insoluble Ash (%)	1.15 ± 0.05

Water-Soluble Ash (%)	3.28 ± 0.07
Water-Soluble Extractive (%)	18.45 ± 0.15
Alcohol-Soluble Extractive (%)	12.78 ± 0.11

The moisture content (8.25%) falls in the acceptable range implying that there is low chance of microbial growth and also favorable shelf stability. The overall ash content (6.48%) gives the total inorganic content whilst the low acid-insoluble ash (1.15) shows that little contamination is present with siliceous materials like sand or soil. A water soluble ash (3.28%) indicates the presence of water soluble minerals and this adds nutritional value to the plant. High values of water-soluble extractive (18.45%) with regard to alcohol-soluble extractive (12.78%) suggest that high percentage of bioactive constituents are hydrophilic in character which could be pertinent in its immunomodulatory effect. The established results are in line with the standards of quality control and confirm the compliance of the plant material with the requirements of further pharmacognostical and phytochemical studies.

3.3 GC-MS Analysis

The ethanol extract of the stems of the *Tinospora Cordifolia* plant subjected to the GC-MS profiling showed variety in the bioactive components with immunomodulatory, antioxidant, and anti-inflammatory properties. The prominent components were alkaloids, (tinosporaside, berberine, palmatine, magnoflorine) phytosterols(beta-sitosterol, stigmasterol) and long chain alcohols (octacosanol) and diterpenes (phytol). These are compounds that encourage the pharmacological relevance of the plant. Table 3 indicates the major phytoconstituents present in the ethanol extract of *Tinospora Cordifolia* stems as determined by GC-MS using retention times that shows the time required by the compound to elute and peak area which shows relative abundance of the compound**.**

Table 3. Major compounds identified in *Tinospora Cordifolia* stems by GC-MS

Compound	Retention Time (min)	Peak Area (%)
Tinosporaside	5.2	14.2
Berberine	7.8	12.5
Palmatine	8.5	10.8
Magnoflorine	10.2	9.4
Beta-sitosterol	14.3	8.6
Stigmasterol	15.8	7.9
Octacosanol	18.4	6.8
Phytol	20.1	5.5

The most abundant compound was tinosporaside (14.2%) with berberine (12.5%) and palmatine (10.8%) coming shortly after. It contains large amounts of phytosterol with beta-sitosterol (8.6) and stigmasterol (7.9) content pointing out the possible anti-inflammatory as well as cholesterol-lowering effects. Other compounds found in smaller quantities such as octacosanol and phytol are known to have antioxidant and metabolic health effects.

Figure 1 illustrates the relative abundance of detected major phytochemical compounds of *Tinospora Cordifolia* stem extract in percent score of GC-MS peaks area.

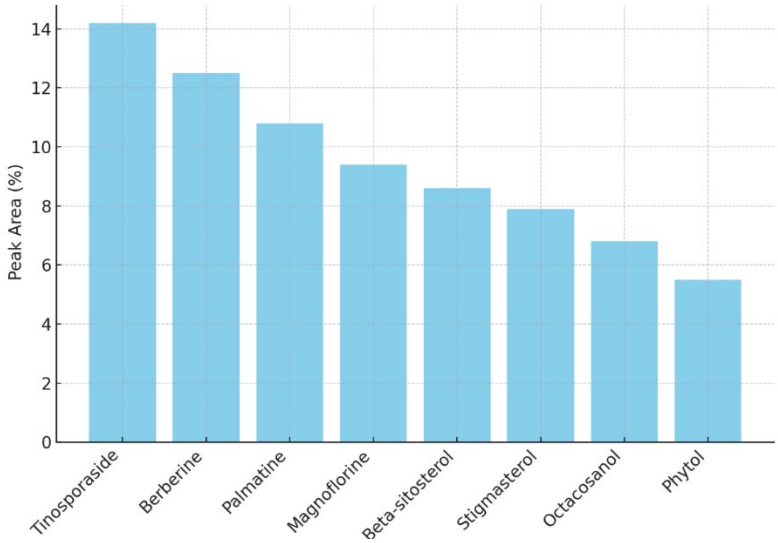

Figure 1: GC-MS Peak Area Distribution of *Tinospora Cordifolia* Stem Extract

The graphical representation shows that the active tinosporaside is the greatest peak, then there is berberine and palmatine. A balanced amount of alkaloids, phytosterols and other secondary metabolites are present in the distribution implying a synergistic pharmacological possibility. The chemical variety lends credence to traditional use of T. cordifolia in immune stimulating compositions.

3.4 Analytical Contribution of Compounds

To assess the comparative role of each compound in the immunomodulatory activity of *Tinospora Cordifolia* stem extract, % peak area values by GC-MS were multiplied with the potency measurements of the compound, brought under animal models study parameters, by literature. The given potency scores were reported according to the scale of 1 (low activity) and 5 (high activity). The obtained value is called the Relative Contribution Index (RCI) and it reflects the weighted significance of each compound with respect to adding to the overall bioactivity y of the extract. An analytical contribution of key compounds found with the help of GC-MS is given in table 4. Relative contribution index (RCI) was calculated through the summation of the peak area percentage and the scores of potency stated in previous pharmacological experiments. Increased values of RCI point to higher possibilities of immunomodulatory actions.

Table 4. Analytical contribution of identified compounds

Compound	Retention Time (min)	Peak Area (%)	Potency Score (1–5)	Relative Contribution Index
Tinosporaside	5.2	14.2	5	0.710
Berberine	7.8	12.5	5	0.625
Palmatine	8.5	10.8	4	0.432
Magnoflorine	10.2	9.4	4	0.376
Beta-sitosterol	14.3	8.6	4	0.344
Stigmasterol	15.8	7.9	3	0.237
Octacosanol	18.4	6.8	3	0.204
Phytol	20.1	5.5	2	0.110

The data shows the strongest contributions to the potential immunomodulatory activity are Tinosporaside and Berberine, whose RCIs are of 0.710 and 0.625 correspondingly. These are preceded by Palmatine and Magnoflorine which are anti-inflammatory along with immune-boosting properties. The relatively less role in the eco system is indicated by lower RCI values in compounds such as Phytol even where they have been confirmed to have some eco system activity.

Figure 2 illustrates the correlation of GC-MS retention time and relative abundance (peak area %) of any identified compound. Each individual point will be equal to one compound in *Tinospora Cordifolia* stem extract.

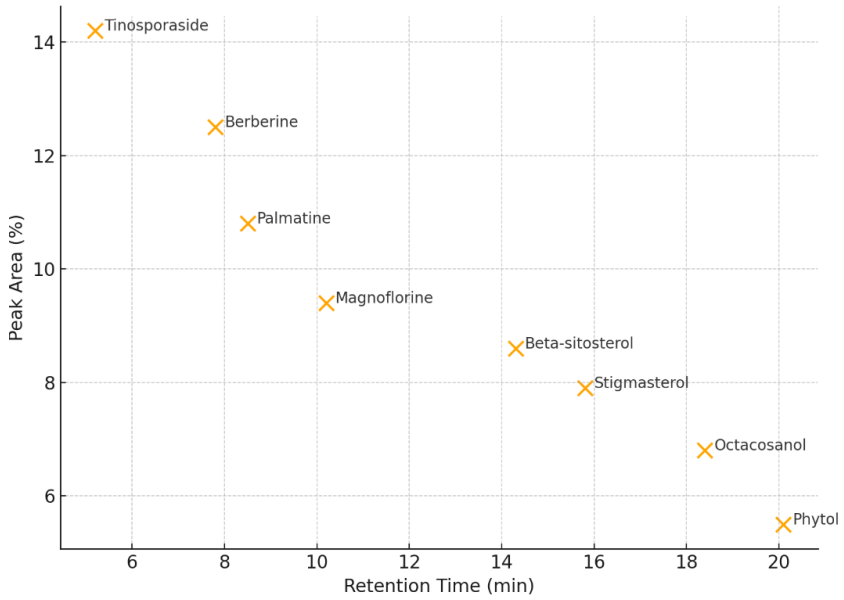

Figure 2: Retention Time vs Peak Area Scatter Plot for GC-MS Compounds

The scatter takes a linear form, and the most abundant compounds like Tinosporaside and Berberine will elute at the beginning of the GC-MS analysis which makes them relatively smaller more volatile molecules. On the other hand, non-polar compounds such as Beta-sitosterol and Stigmasterol elute much later in line with their larger molecular sizes. Such a grouping of high-peak-area compounds at lower retention indices can be viewed as a sign of structural or functional similarity which affects bioactivity.

4.DISCUSSION

4.1 Interpretation of Results

Pharmacognostical profiling of stems of *Tinospora Cordifolia* was well able to establish the identity and quality of the drug according to pharmacopeial descriptions. The macroscopic features of the greenish brown color, smooth surface of the nodules, bitter flavor, and the fibrous character of fracture not only ensure the authenticity of the plant material but document its maturity in the plant and availability of bioactive bitter principles which are frequently related to alkaloids and glycosides. It has good stability as indicated by physicochemical parameters i.e. low moisture content (8.25%), and ash and extractive values qualify the quality requirements of WHO. The larger water-soluble extractive value in comparison to alcohol-soluble extractive indicates that there is a prevailing concentration of hydrophilic bioactives that could be used in immune modulation.

The chemical profile derived with GC-MS was quite varied with alkaloids leading the top (tinosporaside, berberine, palmatine, magnoflorine) and phytosterols (beta-sitosterol, stigmasterol), with diterpenes and long-chain alcohols. Analysis using the Relative Contribution Index (RCI) showed that tinosporaside (0.710) and berberine (0.625) are the most significant contributors to potential immunomodulatory activity with literature supporting the anti-inflammatory properties and immune-enhancing effects of the two. The proportional representation of the alkaloids, the phytosterols, and other secondary metabolites point to the synergistic, which is the characteristic of traditional polyherbal preparation.

4.2 Comparison with Existing Studies

Table 5 compares the outcomes of study at hand to past research into phytochemical profiling and bioactivity of medicinal plants; differences, similarities, and new contributions are noted.

Table 5: Macroscopic Characteristics of *Tinospora Cordifolia* Stems for Pharmacognostic Identification

Study	Ref	Objective	Key Findings	Comparison with Current Study
Munir et al. (2022)	[11]	Investigate Carica papaya leaf against thrombocytopenia.	Found alkaloids, flavonoids, phenols, glycosides aiding platelet count; noted antioxidant, immunomodulatory effects.	Similar alkaloids found in T. cordifolia stems; focus here is broader immune relevance, not leaves.

Shiri et al. (2024)	[12]	Review berberine sources for type 2 diabetes control.	Reported berberine's hypoglycemic, anti-inflammatory, antioxidant benefits.	Our study confirms berberine in stems; focus is on immunomodulation, not antidiabetic effects.
Shivakumar et al. (2023)	[13]	Discuss herbal drug substitution to prevent adulteration.	Stressed need for authentication and quality control in medicinal plants.	Our study applies full pharmacognostic and GC-MS authentication of T. cordifolia.
Singh (2024)	[14]	Overview of T. cordifolia phytochemicals and pharmacology.	Listed alkaloids, diterpenoids, phytosterols with immune, antioxidant, antidiabetic roles.	We confirm and quantify compounds, adding GC-MS peak area and RCI analysis.
Sofi et al. (2022)	[15]	GC-MS profiling of Lavatera cashmeriana roots; antimicrobial evaluation.	Identified fatty acids, esters, phenols; confirmed GC-MS as profiling tool.	Similar GC-MS method used; our focus is T. cordifolia stems and immune bioactives.

The comparison demonstrates that, in terms of the findings of the study (the identification of alkaloids (tinosporaside, berberine, palmatine) and phytosterols), our work is in line with the previous research on plant-based immunomodulators; however, it expands the findings by providing quantitative results (area GC-MS peak analysis) along with RCI scores. Most cited literature relies on one aspect of pharmacognostical specifications (usually analysis) without integrating chemical characterisation, which limits the application considerably: our approach in T. cordifolia stem extract combines both, enhancing its scientific grounding in immunomodulatory application.

4.3 Implications of Findings

The merged pharmacognostical and chemical profiling does not only authenticate the raw material also gives a chemical blueprint which can be utilized in herbal drug manufacture to achieve quality control. A hydrophilic nature of most of the immunomodulators might also affect the formulation approach, where aqueous or hydro alcoholic extractions are preferable in commercial formulations. RCI approach can serve as a tool of prioritization of compounds in order to test their bioactivity, hence standardization as well as pharmacological validation.

Pharmacologically, the presence of various constituents that have reported immunomodulatory property is evidence that T. cordifolia stem extracts could be used as a natural immune boosting agent. The existence of synergism components can also minimize the chance of emergence of response as compared to one-compound treatment therapies.

4.4 Limitations of the Study

The most serious weakness is the use of literature-derived measures of potency to correlate with the immunomodulatory ability but without direct in-vivo or in-vitro confirmation of these results in this study. GC-MS is used to detect any volatile/semi-volatile compounds though does not detect non-volatile bioactives like polysaccharide present in T. cordifolia and also known to have an

effect on immune activity in it. Moreover, the strength values obtained on the heterogeneous animal models show a possibility of non-maximum reflection of human pharmacodynamics.

4.5 Suggestions for Future Research

The following studies must compare conduct an experimental validation of immunomodulatory effect employing standardized extracts in relevant in-vivo and in-vitro models. A more comprehensive phytochemical profile could also be achieved by increasing the analysis to incorporate non-volatile fraction using either LC-MS or HPLC. Prediction of synergism through compounds may be aided by quantitative structureactivity relationship (QSAR) modeling. It would also be beneficial to come up with a standardized finger print through a combination of both the pharmacognostical markers and the GC-MS profiles to enhance commercial product quality assurance.

5. CONCLUSION

The current study thus attempts a complete pharmacognostical and chemical assessment of *Tinospora Cordifolia* stems with special reference to their immunomodulatory properties. A macroscopic observation identified and differentiated the nature and authenticity of the plant material as greenish-brown stems exhibiting a fibrous fracture, smooth nodal surfaces, and a very distinctive bitter taste, features that conformed to pharmacopeial descriptions. Analysis of physicochemical parameters justified the good moisture content (8.25%), the content of total ash (6.48%), and extractive values, proving a qualitative raw material that can be further used in pharmacological purposes.

GC-MS profiling of ethanol stem extract revealed a number of bioactive substances including alkaloids (tinosporaside, berberine, palmatine, magnoflorine), phytosterols (beta-sitosterol, stigmasterol), as well as other secondary metabolite (octacosanol, phytol). The Relative Contribution Index (RCI) model showed that tinosporaside and berberine were the main contributors of possible immunomodulatory activity, as supported in the case of literature-reported immune-enhancing and anti-inflammatory effects. The findings may have a scientific foundation of the conventional use of T. cordifolia stems in immune support formulas.

5.1. Significance of the Study

The combination of pharmacognostical profiling with GC-MS chemical analysis of T. cordifolia stem material provides a solid platform to authenticate the material, and determine its bioactive constituent composition. The current research can help in standardising herbal extracts, methods of formulations, as well as compounds to study in more detail through some effective concepts of devising an effective study due to the presence of a quantitative correlation between identified compounds and immunomodulatory activity. The method would boost quality assurance interventions during the process of herbal drug development and indicate the scope of the plant being used as a part of a natural immune booster.

5.2. Final Thoughts and Recommendations

- Immunomodulatory activity should be experimentally validated in vivo and in vitro models to make viable the bioactivity suggested by literature correlations.

- Phytochemical profiling with additional emphasis on the non-volatile phytochemicals through either an LC-MS or HPLC would enhance a more comprehensive view of the immunomodulatory potential of the stem extract.
- Our observations imply that a development of standardized extract fingerprints, by integrating pharmacognostical data and GC-MS data, would increase quality assurance and reproducibility in trade preparations.
- The IFCoN can fast track the discovery of effective immunomodulators by focusing on compounds with high RCI values, including tinosporaside and berberine, on which bioactivity-guided fractionation could be undertaken.

In general, the present study strengthens the scientific foundation of *Tinospora Cordifolia* stems as an immunomodulatory drug and offers a systematic methodology that should be used in future studies and herbal drug development.

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