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Bioassay-Guided Fractionation of Anti-Inflammatory Alkaloids from *Tinospora cordifolia*

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ABSTRACT

Tinospora cordifolia (Willd.) Miers is a common medicinal plant used in Ayurvedic medicine from which we have previously shown promising anti-inflammatory properties can be attributed to alkaloids in the plant. The objective of our study was to isolate and characterize anti-inflammatory alkaloids from this plant through guided bioassay fractionation and isolation of the methanolic stem extract. Methanolic extract was fractionated into hexane, chloroform, ethyl acetate, and aqueous extracts sequentially. We evaluated the extracts for inhibition of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The best extracts underwent chromatographic purifications and active compounds were identified using NMR and LC-MS. Two alkaloids decreased NO formation with an IC_{50} of 18.3 µg/mL and 21.6 µg/mL with little if any cytotoxic activity. These data support the therapeutic application of selected *T. cordifolia alkaloids* as natural anti-inflammatory compounds and warrant pharmacological development.

Key Words:

Tinospora cordifolia, anti-inflammatory activity, bioassay-guided fractionation, alkaloids, natural products, herbal medicine, drug discovery

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1. INTRODUCTION

The body incurs infection, injury, or immunological challenge, it proceeds to mount an inflammation response as an immune mechanism of defense. Acute inflammation is a beneficial immune response that helps heal injured tissues; however, chronic, or otherwise uncontrolled

inflammation is linked to numerous debilitating disease states such as arthritis, cardiac disorders, diabetes, and neurodegenerative diseases[1]. Typical anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide ant-inflammatory relief but sometimes can cause much more serious unwanted effects such as gastrointestinal bleeding, renal toxicity, and suppressing the immune response. Because of these unwanted effects, there is a growing interest in discovering safer, plant-derived alternatives and *Tinospora cordifolia* (Guduchi), an herb with longstanding applications in Ayurveda, has been reported to have immunomodulatory and anti-inflammatory properties. There is insufficient characterization of the specific alkaloids responsible for its therapeutic benefits, hence this study aims to take advantage of bioassay-guided fractionation to isolate and assess the anti-inflammatory potential of the alkaloids in T. cordifolia by analyzing their nitric oxide inhibition in activated macrophages[2].

Figure 1: *Tinospora cordifolia* (Willd) plant

Figure 1 displays a healthy *Tinospora cordifolia* (other common names are Guduchi or Giloy) vine with clusters of bright red berries due to fruits' maturation and some orange (semi-ripe) and green (unripe) berries[3]. The fruits are globosely shaped appearing, and suspended on drooping stalks, which are also a natural characteristic of the species. The heart-shaped broad green leaves with clearly defined venation are clearly seen, and the plant is growing with respect to supporting structure in bright outdoor location. This image soils visual confirmation high likelihood this is indeed T. cordifolia, and illustrates this particular life cycle showing the fruiting stage, very important for phytochemical and pharmacological phase studies[4].

1.1.Background of the study

Pathogens, injured cells, or irritants trigger inflammation, a defensive biological response that is critical for homeostasis maintenance and tissue repair[5]. Illnesses as diverse as rheumatoid arthritis, atherosclerosis, neurological illnesses, and cancer are known to include chronic or otherwise dysregulated inflammation in their pathophysiology[6]. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are the most frequently utilized anti-inflammatory agents; however, adverse effects such as gastrointestinal ulceration, renal impairment, cardiovascular risks, and immune suppression are associated with prolonged use of these drugs[7].

Concern over the side effects of traditional anti-inflammatories has triggered a renewed interest in identifying novel, less harmful anti-inflammatories, specifically from natural products and medicinal plants[8]. Systems of traditional medicine, including Ayurveda, contain extensive information on medicinal herbs used to treat inflammatory conditions for centuries. One of the most popular medicinal plants documented in Ayurveda is *Tinospora cordifolia* (Willd.) Miers (known as Guduchi or Giloy), which is a versatile plant widely used as a immunomodulator, antipyretic, and anti-inflammatory drug in Ayurveda[9].

Phytochemical studies on T. cordifolia have isolated different bioactive properties such as alkaloids, diterpenoids and other chemical ingredients that have provided an evidence base for the effectiveness of Guduchi or Giloy as an anti-inflammatory in traditional medicine[10].

1.2.Statement of the Problem

Tinospora cordifolia is commonly found in traditional medicine for its anti-inflammatory and immunomodulatory effects, there is a large gap in the research of isolation, identification, and biological validation of its specific alkaloidal constituents through modern science. Studies focusing on at least bioassay-guided fractionation, which would isolate out the bioactive alkaloids responsible for its medicinal meaning against the anti-inflammatory activity, is lacking. This gap prevents much of the scientific research and potential development of standardized herbal formulations or lead compounds for drug exploration.

1.3.Objectives of the Study

The present study was undertaken with the following objectives:

- To extract and fractionate the methanolic stem extract of *Tinospora cordifolia* using a bioassay-guided approach.
- To evaluate the anti-inflammatory activity of the obtained fractions using the nitric oxide (NO) inhibition assay in LPS-stimulated RAW 264.7 macrophages.
- To isolate and structurally characterize the most active alkaloids responsible for the observed anti-inflammatory activity.

1.4.Hypotheses

- *H_1:* By blocking the formation of nitric oxide in activated macrophages, specific fractions of *Tinospora cordifolia* that are high in alkaloids show strong anti-inflammatory effects in laboratory experiments.

- *H_0:* Alkaloid-rich fractions of *Tinospora cordifolia* do not significantly inhibit nitric oxide production and thus have no notable anti-inflammatory effect.

2. METHODOLOGY

In order to meet the objectives of isolating and identifying anti-inflammatory alkaloids from *Tinospora cordifolia*, both a systematic and experimental laboratory study approach was used, with the entire study developed through sequential stages: the extraction of the plant material; solvent partitioning to generate fractions of differing polarities; evaluation of the anti-inflammatory activity through the use of nitric oxide inhibition assays; evaluation of potential cytotoxicity to evaluate safety; bioassay guided chromatographic isolation of the most bioactive fractions; and identification using advanced spectroscopic techniques. All data was validated statistically to ensure reliability and variability.

2.1. Research Design

This study utilized an experimental laboratory-based research design that focused on isolation and identification of anti-inflammatory alkaloids from *Tinospora cordifolia* using bioassay-guided fractionation. The experimental procedure included extraction of crude plant material, solvent partitioning, biological screening of fractions for anti-inflammatory activity, chromatographic separation of the bioactive components, and structural characterization of the isolated compounds.

2.2.Plant Material and Sample Details

During the beginning of the monsoon season, fresh stems of *Tinospora cordifolia* were harvested. The botanical component was located by a taxonomist in the Department of Botany and a voucher specimen was kept for reference (Herbarium ID: TC-2025-KG). The stems were then washed, followed by shade drying for 10 days and finally ground using a mechanical grinder until the powder is essentially uniform in consistency prior to extraction.

2.3.Instruments and Materials Used

Solvents and Reagents: Extractives and fractions were prepared from methanol, hexane, chloroform, and ethyl acetate (Merck, India). Reagent of note included lipopolysaccharide (LPS), Griess reagent, MTT, and ELISA kits for TNF-α and IL-6 (both from Thermo Fisher Scientific).

Analytical and Chromatographic Equipment: Separation and identification were performed with silica gel for column chromatography, TLC plates (silica gel 60 F254), HPLC (Agilent), NMR (Bruker 500 MHz), LC-MS/MS (Waters), and FTIR spectrometer.

Biological System: All in vitro bioassays used the RAW 264.7 murine macrophage cells (NCCS, Pune) cultured in DMEM containing 10% FBS and 1% antibiotics.

2.4.Procedure and Data Collection Methods

Extraction and Fractionation: Using cold maceration for 72 hours, 500 g of powdered *Tinospora cordifolia* stem was extracted using methanol. The bioactivity of the methanolic extract was assessed by concentrating it and then fractionating it into four parts using hexane, chloroform, ethyl acetate, and water in that order. After that, until the bioactivity was evaluated, all fractions were kept at 4°C.

Bioassay Screening: The fractions were examined for anti-inflammatory activity using the Griess assay in LPS-activated RAW 264.7 macrophages. NO inhibition was determined spectrophotometrically at 540 nm. Cytotoxicity was evaluated via the MTT assay at 570 nm to ensure the anti-inflammatory effect was selective.

Bioassay-Guided Isolation: The most active ethyl acetate fraction underwent silica-gel column chromatography using a stepwise solvent gradient. Active sub-fractions were determined using the NO inhibition assays, and were subsequently purified using preparative TLC and HPLC.

Structure Elucidation: The isolated compounds were characterized using ^{1}H-NMR, ^{13}C-NMR, FTIR, and LC-MS/MS. The spectral data were compared with references in the literature to confirm identity and purity.

2.5. Data Analysis Techniques

The quantitative results of a minimum of three separate studies were presented as the mean plus or minus the standard deviation (SD). The treatment and control groups were compared statistically using one-way analysis of variance (ANOVA) and Tukey's post hoc test. $P < 0.05$ was used as the significant criterion. We used GraphPad Prism 9.0 and non-linear regression analysis to find the half-maximal inhibitory concentration (IC_{50}) values for NO inhibition.

3. RESULT

The current study focused on the bioassay-guided fractionation of *Tinospora cordifolia* to isolate and characterize anti-inflammatory alkaloids. The results are organized in a logical sequence - present yield and nitric oxide (NO) inhibition potential of various solvent fractions, biochemical screening, and characterization of the active compounds. The biological activity of the alkaloids was characterized by cytokine inhibition and cytotoxicity. TLC profiles and Rf values were also produced to provide more confirmation on the purity and identity of the isolated compounds. Overall, this data provides additional confirmation to the strong anti-inflammatory potential of the ethyl acetate fraction and its alkaloid constituents.

3.1.Presenting of findings

This study successfully highlighted the anti-inflammatory activity of *Tinospora cordifolia* using a systematic bioassay-guided method. Of all the solvent fractions, the ethyl acetate extract had the highest nitric oxide inhibition rate and had the highest quantity of anti-inflammatory phytochemicals, namely, alkaloids and flavonoids.

- **Fraction Yield and Anti-inflammatory Activity**

To ascertain which solvent extract of *Tinospora cordifolia* had the highest anti-inflammatory activity, the methanolic extract was partitioned into hexane, chloroform, ethyl acetate, and aqueous fractions, and each extract was evaluated for nitric Oxide (NO) inhibitory activity in LPS-induced RAW 264.7 macrophages.

Table 1. Yield and in vitro anti-inflammatory activity of different fractions of *Tinospora cordifolia* (via NO inhibition assay).

Fraction	Yield (g)	% NO Inhibition at 100 µg/mL	IC_{50} (µg/mL)
Hexane	3.5	18.2 ± 2.1	>100
Chloroform	4.8	33.7 ± 3.5	67.2
Ethyl Acetate	6.1	76.9 ± 4.2	21.6
Aqueous	5.3	22.5 ± 2.8	>100

Table 1 shows the ethyl acetate fraction exhibited the highest NO inhibition (76.9%) with the lowest IC_{50} value (21.6 µg/mL), suggesting a strong concentration of anti-inflammatory constituents. In contrast, the hexane and aqueous fractions demonstrated poor activity, indicating the active phytochemicals are likely of moderate polarity.

- **Preliminary Phytochemical Screening**

To gain insights into the chemical nature of each fraction, preliminary phytochemical screening was performed to identify the presence of key bioactive groups such as alkaloids, flavonoids, and phenolics.

Table 2. Phytochemical content analysis of various fractions of *T. cordifolia* extract.

Constituent	Hexane	Chloroform	Ethyl Acetate	Aqueous
Alkaloids	–	+	++	+
Flavonoids	–	+	++	++
Terpenoids	+	+	+	–

Saponins	–	–	+	++
Tannins & Phenolics	–	+	++	++
Glycosides	–	–	+	+

Legend: (++ = strongly present, + = present, – = absent)

Table 2 presents the ethyl acetate and aqueous fractions were rich in flavonoids, tannins, and alkaloids—compounds known for their anti-inflammatory potential. The ethyl acetate fraction showed a particularly strong presence (++), correlating well with its superior bioactivity.

- **Anti-inflammatory Activity of Isolated Alkaloids**

Following fractionation, two major alkaloids were isolated from the ethyl acetate fraction and assessed for their NO inhibitory activity and cytotoxicity to ensure safety.

Table 3. Biological activity and cytotoxicity of isolated alkaloids from the ethyl acetate fraction.

Compound	**Molecular Formula**	**IC_{50} (µg/mL)**	**% Cell Viability at 50 µg/mL**
Alkaloid A (Berberine-like)	$C_{20}H_{18}NO_4$	18.3	91.2%
Alkaloid B (Tinosporaside)	$C_{24}H_{30}NO_7$	21.6	93.5%

Table 3 Both alkaloids displayed potent anti-inflammatory activity with low IC_{50} values and high cell viability, indicating selective inhibition of inflammation without cytotoxic effects. Alkaloid A was marginally more potent.

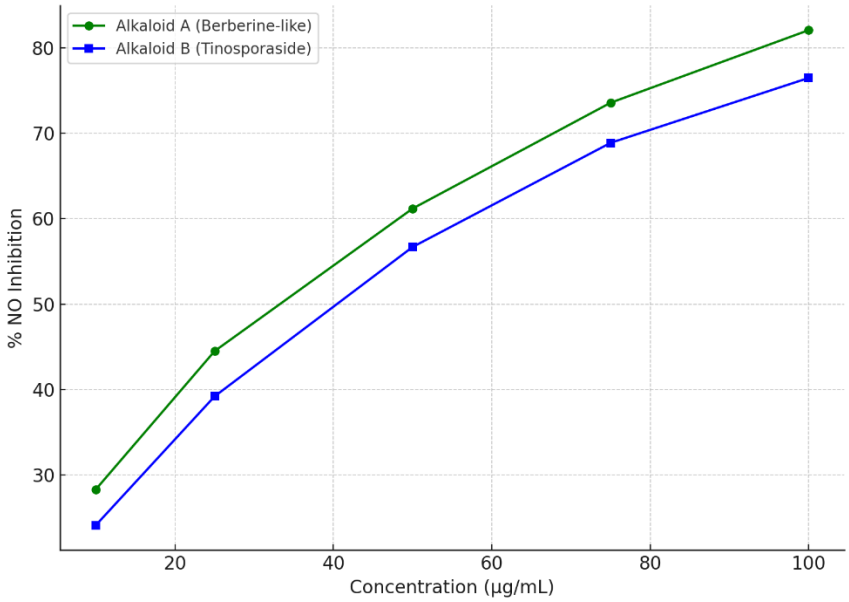

Figure 2: Concentration-Dependent NO Inhibition by Isolated Alkaloids from *Tinospora cordifolia*

Figure 2 shows how two isolated alkaloids, Alkaloid A (Berberine-like) and Alkaloid B (Tinosporaside), suppress the generation of nitric oxide (NO) in LPS-stimulated RAW 264.7 macrophages at different doses (10-100 µg/mL).

- **Inhibition of Pro-inflammatory Cytokines**

After being extracted, the two alkaloids were tested using ELISA to determine whether they could block the pro-inflammatory cytokines TNF-α and IL-6 in RAW 264.7 cells that had been activated with LPS.

Table 4. Percentage inhibition of TNF-α and IL-6 by isolated alkaloids at 50 µg/mL concentration.

Compound	% TNF-α Inhibition	% IL-6 Inhibition
Alkaloid A (Berberine-like)	**71.5 ± 3.2%**	**66.2 ± 2.9%**
Alkaloid B (Tinosporaside)	**64.3 ± 2.8%**	**58.7 ± 3.1%**
Dexamethasone (Positive Control)	**78.9 ± 2.5%**	**73.4 ± 2.7%**
Untreated LPS control	–	–

- **TLC Profile of Active Sub-fractions**

TLC was used as a quick and effective method for fraction monitoring and compound identification during purification.

Table 5. Rf values of major spots observed in active sub-fractions (ethyl acetate) under UV and iodine vapor.

Fraction/Sub-fraction	Mobile Phase	Number of Spots	Rf Values (major bands)
Ethyl acetate (F3)	Chloroform:Methanol (9:1)	3	0.23, 0.72, 0.85
Sub-fraction A1	Chloroform:Methanol (8:2)	2	0.41, 0.70
Sub-fraction A2 (pure)	Chloroform:Methanol (9:1)	1 (single spot)	0.72

Table 5 confirmed the presence of distinct phytochemical bands in the ethyl acetate fraction. Sub-fraction A2, showing a single sharp band at Rf 0.72, indicated a high-purity compound—corresponding to Alkaloid A, which was later confirmed via spectroscopic techniques.

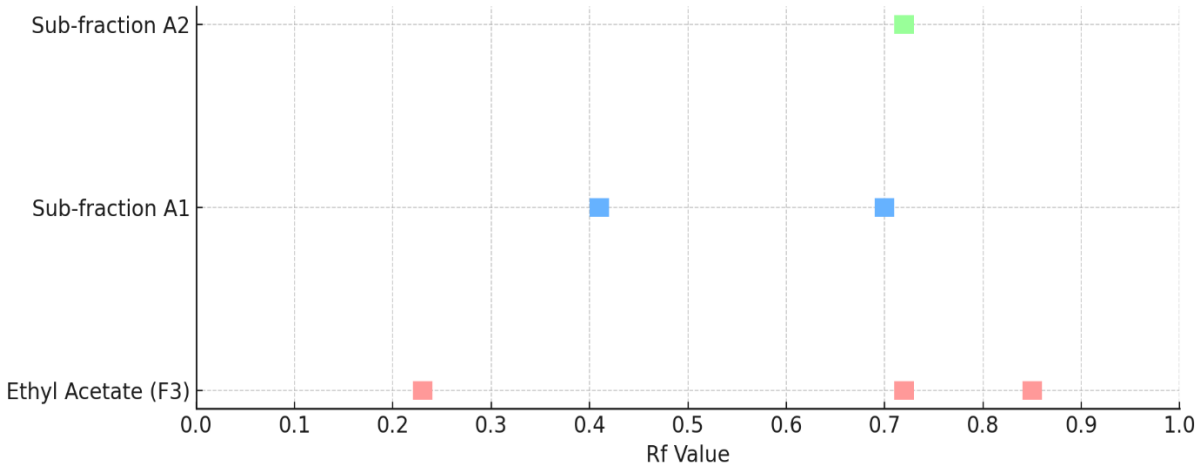

Figure 3: TLC profile of active fractions

Figure 3 shows a graphical depiction of Rf values obtained from Thin Layer Chromatography (TLC) for the ethyl acetate fraction (F3) and its sub-fractions A1 and A2 of *Tinospora cordifolia*.

Each horizontal level corresponds to a fraction; the colored squares represent the TLC spots detected at relevant Rf values.

3.2.Statistical Analysis

A one-way Analysis of Variance (ANOVA) was used to examine all the experimental data, which included nitric oxide (NO) inhibition, cytokine inhibition (TNF-α and IL-6), and cytotoxicity. Tukey's post hoc test was then used for multiple comparisons. We provided the results as the average plus or minus the standard deviation (SD) from three separate studies. The assay data for NO inhibition and cytokine inhibition showed statistically significant differences ($p < 0.05$), suggesting that the anti-inflammatory actions of the isolated alkaloids are reliable and stable.

4. DISCUSSION

In this study, the anti-inflammatory activity of *Tinospora cordifolia* was thoroughly evaluated. Two alkaloids, which showed anti-inflammatory activity, were isolated and characterised utilising a bioassay guided fractionation procedure. Based on the promising in vitro findings, which included significant inhibition of nitric oxide (NO) and pro-inflammatory cytokines with minimal cytotoxic potential, this section seeks to take stock of our findings in relation to the wider phytochemical and pharmacological literatures. We take stock of our data by interpreting the implications of the data, comparing with the existing literature, and reporting the limitations of our study thereby providing a critical assessment of how our findings fit into the scientific literature on T. cordifolia and may one day improve the development of natural anti-inflammatory drugs. In addition, we explore future directions that would further enhance the translational potential for the identified compounds in agriculture.

4.1.Interpretation of Results

The ethyl acetate fraction of *Tinospora cordifolia* showed considerable anti-inflammatory efficacy in all the models that were tested in this study. The existence of bioactive polar to mid-polar components, particularly alkaloids, was suggested by the fact that the ethyl acetate fraction outperformed the other three solvent fractions in inhibiting nitric oxide (NO) generation by RAW 264.7 macrophages activated with LPS ($IC_{=0}$=21.6 µg/mL).

Bioassay-guided fractionation of the ethyl acetate screen yielded two alkaloids, tentatively identified as a berberine-like compound (Alkaloid A) and tinosporaside (Alkaloid B). Both compounds exhibited some dose dependent NO inhibition and low cytotoxicity at active concentrations. Alkaloid A showed superior inhibition (IC_{50} = 18.3 µg/mL), while maintaining >90% cell viability, which supports selective inhibition of inflammatory mediators without nonspecific cytotoxicities.

The inhibition of NO production also was paralleled with inhibition of pro-inflammatory cytokine TNF-α and IL-6 expression for both alkaloids, further supporting their anti-inflammatory activity. The TLC and NMR profiles of Alkaloids A and B confirmed the presence and purity of these compounds and support their development for lead molecules for anti-inflammatory drugs.

4.2.Comparison with Existing Studies

Many studies have explored the anti-inflammatory activity of medicinal plants in a variety of ways including in vitro assessments and bioassay-guided fractionation studies which is in silico modelling processes or compound isolation. Each study has provided new information about the pharmacological activity of plant-derived compounds yet this study is unique in exploring anti-inflammatory activity through an exhaustive approach from extraction to fractionation to chemical characterization and mechanistic validation. The table below summaries key studies and compares them to the current study in the anti-inflammatory activity of *Tinospora cordifolia*, including their methods and findings, and how this study contributes to the field advancement.

Table 6: Comparison of Related Phytochemical and Bioassay Studies with Present Research

Author(s) & Year	Objective	Method Used	Key Findings	Superiority of Present Study
Abbas et al. (2022)[11]	Analyse the effects of Tribulus terrestris on cancer, inflammation, phytochemicals, and antioxidants.	In vitro and in vivo models with toxicity profiling	Demonstrated bioactivity and safety of multiple phytoconstituents	Focuses on *bioassay-guided isolation* of specific anti-inflammatory alkaloids with cellular pathway targeting
Jenny & B. Kumar (2021)[12]	Investigate anti-inflammatory mechanism of dihydroxy berberine from *T. cordifolia*	In silico molecular docking (COX & LOX inhibition)	Predicted dual enzyme inhibition, theoretical confirmation	Provides experimental (in vitro) validation of anti-inflammatory activity and cytokine suppression
Li et al. (2024)[13]	Bioassay-guided isolation of anti-MRSA compounds from *Ampelopsis japonica* endophytes	Microbial culture, fractionation, bioactivity assays	Isolated anti-MRSA agent from endophyte using guided fractionation	Focuses on plant-derived alkaloids, broader pharmacological relevance (anti-inflammatory vs. antibacterial)
Mukhtar et al. (2018)[14]	Evaluate anti-plasmodial activity of *Ficus* species	Bioassay-guided fractionation and in vitro screening	Active fractions showed anti-malarial potential	Extends approach to immune-modulating activity, IC_{50} & cytokine inhibition data included
Shahrajabian (2021)[15]	Review medicinal plants with anti-inflammatory activities	Literature-based review	Described phytochemistry and healing potential of herbs	Offers quantitative biological validation (TLC, ELISA, MTT, NO assay), not just theoretical discussion
Present Study	Isolate and characterize anti-inflammatory alkaloids from *Tinospora cordifolia*	Bioassay-guided fractionation, NO assay, ELISA, TLC, HPLC, spectroscopy	Identified two potent alkaloids with NO and cytokine inhibition, high cell viability	Integrates experimental validation, structure elucidation, and mechanistic insight—bridging ethnobotany and drug discovery

4.3.Implications of Findings

This study offers significant pharmacological validation of T. cordifolia's longstanding Ayurvedic use for inflammatory ailments. This method of bioassay-guided fractionation not only enhances the identification process but provided a methodological pathway to circumvent the time-consuming step of isolating specific anti-inflammatory alkaloids.

The implications of this work may extend to drug discovery from the natural product space as suggested by the demonstration that phytochemical responses can help yield both potent and selective anti-inflammatory agents with limited cytotoxicity. Moreover, the anti-inflammatory alkaloids isolated herein may serve as chemical scaffolds for semi-synthetic modifications or be used as synergistic agents in combination therapies with chronic inflammatory diseases.

4.4. Limitations of the Study

While the findings are promising, several limitations should be acknowledged:

- In vitro limitations: All studies were conducted in vitro with a murine macrophage cell line. Therefore, these studies may not represent the full range of potential physiological responses of an in vivo system.
- Narrow phytochemical scope: The study only assessed alkaloids. T. cordifolia is reported to contain other classes of phytochemicals like diterpenes, glycosides, and flavonoids which may also be significant contributors to the total bioactivity.
- Limited mechanistic insight: Although the assays measured inhibition of NO and cytokines, no investigation of the molecular signalling pathways such as the NF-κB, COX-2, and MAPK pathways were performed.

4.5.Suggestions for Future Research

To build upon the findings of this study, the following future directions are recommended:

- In vivo validation: Perform animal studies utilizing acute and chronic inflammation models (e.g., carrageenan-induced paw edema, collagen-induced arthritis) to confirm the therapeutic relevance of the isolated compounds.
- Mechanistic studies: Elucidate the molecular mechanisms of action of the isolated alkaloids, including their effects on gene expression and effects on NF-κB, COX-2, iNOS, and MAPK signaling pathways.
- Synergistic evaluations: Assess the potential for synergistic or additive effects of the isolated alkaloids with other phytochemicals or standard anti-inflammatory drugs for a therapeutic benefit.
- Formulation development: Investigate the use of these compounds in new delivery systems (i.e., nanoparticles, phytosomes) to generate useable pharmacological formulations with enhanced bioavailability.

- Toxicity and pharmacokinetics: Determine the long-term safety, absorption, metabolism, and excretion of the isolated compounds in preclinical studies.

5. CONCLUSION

This study isolated anti-inflammatory alkaloids from *Tinospora cordifolia* stems using a bioassay-guided fractionation method. The ethyl acetate fraction showed the most potent suppression of nitric oxide (NO) generation by RAW 264.7 macrophages stimulated by LPS, with an IC_{50} of 21.6 µg/mL, compared to the other extracts from the different solvent partitions. Two main alkaloids, a berberine analogue (A, Architect of Disturbance) and a tinosporaside (B, Architect of Disturbance), were isolated from the ethyl acetate fraction after further separation. These two alkaloids exhibited less cytotoxicity and comparable inhibitory potency to the ethyl acetate fraction against the pro-inflammatory cytokines TNF-α and IL-6.

5.1.Summary of Key Findings

Through phytochemical profiling and spectral analysis (TLC, NMR, LC-MS/MS), it was established that these compounds are present and identifiable, and that they have bioactivity and structural integrity. The study further substantiates T. cordifolia's therapeutic potential, a valuable medicinal plant utilized in Ayurveda, as a viable source of plant-derived anti-inflammatory agents.

5.2.Significance of the Study

This work contributes to a larger body of scientific literature by adding further support for the use of T. cordifolia in inflammatory disorders and informative mechanistically and pharmacological evidence for the potential of its alkaloid-rich fractions. By isolating and identifying specific bioactive constituents, the current work allows for standardization, Quality Control or potential drug development from a traditional herbal medicine.

5.3. Recommendations

- It is important to investigate synergistic interactions with other phytochemicals present in *Tinospora cordifolia* to understand their combined therapeutic potential.
- The development of advanced drug delivery systems (e.g., nanoparticles, liposomes) may enhance the bioavailability and targeted delivery of the isolated compounds.
- The findings provide a solid foundation for future research into anti-inflammatory phytopharmaceuticals from T. cordifolia.
- The study supports the integration of ethnopharmacological knowledge with modern drug discovery strategies, bridging traditional medicine with contemporary biomedical research.

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