



***Typhonium trilobatum* (L.) Schott Extract: Phytochemical Profiling, Antioxidant, Anti Inflammatory, and Antimicrobial Activities**

Saikat Sen^{1,4}, Sakshar Saha¹, Shubham Paul¹, Srijan Panigrahi², Sreya Das³, Atanu Chatterjee⁴, Biplab Debnath⁵, and Ritu Khanra^{1*}

¹Department of Pharmaceutical Technology, JIS University, Kolkata 700109, India;

²Department of Pharmaceutical Technology, Jagannath Gupta Institute of Pharmacy, Kolkata-700137, India

³Department of Pharmaceutical Technology, Dr. Sudhir Chandra Sur Institute of Pharmaceutical Science and Technology, Dum Dum, West Bengal 700074

⁴Department of Pharmaceutical Technology, Bengal School of Technology, Chinsurah, Hooghly, West Bengal, India;

⁵Department of Pharmaceutical Technology, Bharat Technology, Uluberia, West Bengal, Pin- 711316.

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KEYWORDS

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ABSTRACT:

Typhonium trilobatum (L.) Schott, traditionally used for digestive disorders and wound healing, holds promising therapeutic potential, particularly its antioxidant and antimicrobial properties. The quantitative identification of lead bioactive compounds from *Typhonium trilobatum* extracts using various chromatographic techniques, along with a comprehensive evaluation of their antioxidant, anti-inflammatory, and antimicrobial activities—particularly involving membrane-active mechanisms—remains largely unexplored and inadequately characterized. This study investigates the phytochemical profile of the leaf extract of *Typhonium trilobatum*. Preliminary phytochemical screening confirmed the presence of flavonoids, polyphenols, tannins, glycosides, saponins, and steroids. Gas Chromatography- Mass Spectrometry (GC-MS) analysis identified a total of thirty four bioactive compounds. Further, Thin Layer Chromatography (TLC) indicated the presence of key phenolic and flavonoid compounds, including p-coumaric acid, chlorogenic acid, quercetin, apigenin, and caffeic acid. High-Performance Thin-Layer Chromatography (HPTLC) was subsequently employed for quantitative analysis, revealing significant concentrations of chlorogenic acid (195.58 ± 20.12 mg/g), caffeic acid (4.02 ± 0.11 mg/g), and kaempferol (2.62 ± 0.08 mg/g). Quantitative analysis indicated total phenolic content of 206.46 ± 0.21 mg/100 g and flavonoid content (quercetin) of 125.07 mg/100g. The plant extract exhibited significant DPPH radical scavenging activity as compared to ascorbic acid. The extract displayed significant antimicrobial activity by zone of inhibition process against gram-positive & gram-negative micro-organism.

Abbreviations

APL- Area per lipid CA - Chlorogenic acid

DPPH - 2,2-diphenyl-1-picrylhydrazyl GAE- Gallic acid equivalents

GC-MS - Gas chromatography-mass spectrometry HPTLC - High-performance thin-layer chromatography IC₅₀- Half maximal inhibitory concentration

TFC - Total flavonoid content

TLC - Thin-layer chromatography TPC - Total phenolic content

WHO - World Health Organization



Introduction

Medicinal plants have played a crucial role in traditional healthcare systems and continue to be important in modern drug discovery. They are rich in phytochemical - such as flavonoids, alkaloids, glycosides, and terpenoids - that exhibit diverse pharmacological properties [1]. Due to their complex genomes, plants produce structurally varied bioactive compounds, often more diverse than those in animals or microbes. According to the World Health Organization (WHO), over 80% of the global population depends on herbal medicines, and more than 21,000 medicinal plant species have been identified as potential therapeutic sources [2]. Many plant-derived compounds are directly used as drugs or serve as templates for synthetic pharmaceuticals [3,4]. Under-explored plants therefore represent promising candidates for discovering novel agents against infectious and different types of diseases.

Typhonium trilobatum (L.) Schott is a small to moderately sized perennial herb of the Araceae family, native to tropical Asia, the South Pacific, and Australia [5]. It is widely distributed across India, Bangladesh, China, Thailand, Vietnam, Malaysia, and Sri Lanka, and is locally known as Bengal arum, Ghatkanchu, or Ghatkol [6]. The plant is recognized by its three-lobed hastate leaves, subglobose tuber, long petioles, and a greenish-purple spathe [7]. Traditionally, various parts - including rhizome, root, and leaves - are used to treat ailments such as vomiting, asthma, gastric ulcers, sore throat, abscess, snakebite, diarrhea, dysentery, and menstrual disorders [8]. In some cultures, the leaves are consumed as vegetables for managing rheumatism and piles [9].

Modern studies have reported broad pharmacological activities for *T. trilobatum*, including antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antiulcer, and antidiarrheal, anticancer, antimalarial, neuropharmacological, wound-healing, and membrane-stabilizing effects [10]. Phytochemical investigations identified various bioactive constituents: leaves contain reducing sugars, alkaloids, glycosides, tannins, and saponins; rhizomes are rich in lectins; roots contain flavonoids, phenols, and carbohydrates [11]. The plant also provides micronutrients such as thiamine, niacin, carotene, folic acid, sterols, and β -sitosterol [12]. Despite these reports, mechanistic insights into its

pharmacological actions are lacking, and detailed chemical characterization using chromatographic techniques remains incomplete.

To address existing knowledge gaps, we conducted a comprehensive phytochemical analysis of *T. trilobatum* using multiple chromatographic techniques. Our methodology included preliminary phytochemical screening, thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), gas chromatography–mass spectrometry (GC–MS), and quantification of total phenolic (TPC) and flavonoid content (TFC). The extract was then evaluated for its antioxidant, anti-inflammatory, and antimicrobial activities *in vitro*. HPTLC identified chlorogenic acid as a principal phenolic compound. Note that CA exhibits broad-spectrum bactericidal activity against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Helicobacter pylori*, primarily by disrupting bacterial membranes and inducing leakage of intracellular contents [13,14].

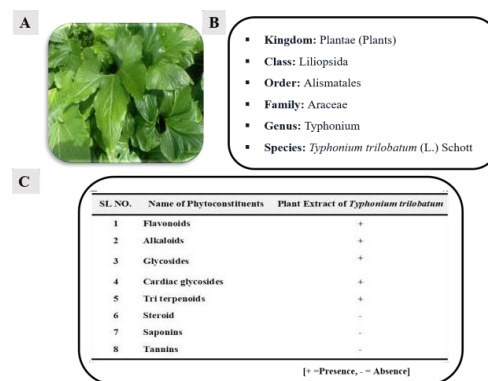


Figure 1. Plant Picture. Taxonomic Hierarchy of *Typhonium trilobatum*. Phytochemical Screening of Plant Extract of *T. trilobatum* L. (Schott) 1A: Picture of the *Typhonium trilobatum* (L.) Schott plant; 1B: Plant profile of *Typhonium trilobatum* (L.) Schott; 1C: Results of preliminary phytochemical screening

Materials and methods Phytochemical screening Plant material collection

The leaves of *Typhonium trilobatum* (L.) Schott was collected from the village areas of Hooghly district, West Bengal, India in July 2021. The plant was identified and authenticated by Central National Herbarium, Botanical Survey of India (Reference No. CNH/Tech II/2022/12), Howrah, West Bengal.



Preparation of plant extract

The leaves were shed dried and ground into coarse powder. Powdered material was taken in a clean, flat-bottomed container and soaked in methanol at room temperature for five days. Occasionally it was agitated for maximum wetting and extraction. The extract was filtered and evaporated using Rotary Evaporator.

Qualitative study of phytoconstituents

Preliminary Phytochemical group tests were carried out on crude extract in order to ascertain the presence of flavonoids, polyphenol, tannins, glycosides, saponins, steroid and other phytochemicals by employing standard procedure [15].

Gas chromatography-mass spectrometry (GC-MS)

The extract of *Typhonium trilobatum* (L.) Schott was subjected to GC-MS analysis for identification of volatile compounds. The GC-MS analysis was done in the Central University of Punjab using instrument Shimadzu QP 2010 Ultra GC -MS. Helium is used as carrier gas. The identification of components was based on NIST libraries as well as comparison of their retention time. The constituents were identified after comparison with those available in the computer library (NIST) attached to the GC-MS instrument and the results obtained have been tabulated.

Thin layer chromatography (TLC)

Chromatography is a separation technique for separating components. TLC was used to analyze the extract to identify the various components present in extract. It is based on the principle where molecules in mixture applied onto surface or into the solid surface, and fluid stationary phase is separating from each other while moving with the aid of a mobile phase. Using Chloroform: ethyl acetate: formic acid (5:4:1 v/v/v) as mobile phase separation was carried out. Either from visible spots or Ultraviolet (UV) and visible lights was used to see the separated constituents. Behavior of Separated compounds can be expressed as retardation factor (R_f value). R_f value can be used to assess the plates qualitatively.

High-performance thin-layer chromatography (HPTLC)

High Performance Thin Layer Chromatography gives

more sophisticated results as automation increase the resolution and accuracy. The method includes precise sample application, standard reproducible chromatogram software-controlled evaluation. Plant extracts (*Typhonium trilobatum*) and standards (1mg/μL) were applied to pre-coated HPTLC silica gel G F254 plates (Merck K GaA, Supelco, Germany) using Automatic TLC Sampler (ATS 4, CAMAG, Muttenz, Switzerland). The development of plate was carried out in a pre-saturated mobile phase of chloroform: ethyl acetate: formic acid (5:4:1 v/v/v) and toluene:methanol (9:1 v/v) using 100 μL HPTLC Syringe in a Twin Trough Chamber. The spot's migration was then observed under TLC-UV Cabinet 4 after drying. Evaluation of the chromatograms were done at 254 nm, in a TLC scanner 4 controlled through Vision CATS software.

In-vitro bioassays

Total phenolic content (TPC)

Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method (16). All of extracts and Gallic acid (used as standard) were diluted by serial dilutions as (100 μg/mL, 200 μg/mL, 300 μg/mL, 400 μg/mL, 500 μg/mL.) then, on each test tube containing 1ml of diluted solution of sample and standard, following reagent solutions were added 5 ml folin-ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (7.5% sodium carbonate) of sodium carbonate. Samples were incubated at room temperature for 60 minutes and standard solution was incubated at room temperature for 30 minutes, after adding reagent mixtures. Absorbance of samples and standard were measured at 765 nm using UV-VIS spectrophotometer against blank, observation made in triplicate and mean value reported for the study. A typical blank solution contained the solvent used to dissolve the plant extract. The total content of phenol compounds in plant extracts in Gallic acid equivalents (GAE) was calculated using the following equation:

$$C = (c \times V)/m$$

Where, C = total content of phenol compounds, mg/gm plant extract, in GAE

c = the concentration of Gallic acid established from the calibration curve (mg/mL) V = the volume of extract in mL, m = the weight of crude plant extract in g.



Total flavonoid content (TFC)

Determination of total flavonoid content was carried out by colorimetric assay method and repeated three times to calculate the mean value based on the procedure of Nurul and Rabet [17]. Total flavonoid content was estimated from the linear equation ($Y=0.0105X-0.3686$) of a standard curve prepared with Quercetin. The amount of total flavonoid content in the extract was expressed in milligrams per gram of extract [18].

DPPH free radical scavenging activity

The 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method was used to evaluate the antioxidant property. Different concentrations of the plant extract of *Typhonium trilobatum* L. (Schott) were used to scavenge DPPH. The antioxidant activity of each sample was expressed in terms of IC₅₀. IC₅₀ was calculated from the graph after plotting inhibition percentage against extract concentration. DPPH assay was carried out after making some modifications in the standard protocol [19]. 3 mL of 0.1 mM DPPH solution was mixed with 1 ml of various concentrations (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL) of leaf extract. The mixture was incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. Methanol was used without any extract and with DPPH as control. The experiment was triplicate and three independent assays performed to calculate the mean value. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

Where, A_{control} is the absorbance of the control and A_{test} is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates ($n=3$), and average values were calculated.

Hydrogen peroxide scavenging activity

The scavenging activity of crude extract of *Typhonium trilobatum* L. (Schott) was determined by employing modified method [20]. Hydrogen peroxide solution (40 Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560 nm using UV spectrophotometer. 0.1 mg/mL of the extract was added to hydrogen peroxide solution and absorbance measured at 560 nm using UV

spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract was calculated respectively. The experiment was triplicate to calculate the mean.

Egg albumin assay Protein Denaturation Test

The principal objective behind the egg albumin denaturation assay is to determine whether agents or compounds can stop or hinder egg albumin from becoming denatured under particular circumstances. The egg albumin denaturation assay measures a drug or compound's capacity to prevent or lessen egg albumin denaturation to evaluate its anti-inflammatory effects [21]. The total 5 ml reaction mix consists of 0.2 mL of egg's albumin (fresh hen's egg), 2.8 ml of solution containing phosphate-buffered saline (pH 6.4), and 2 ml of different concentrations of crude extract. Therefore, the final result concentrations (100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL) were made as per the standard method [22].

An equal volume of distilled water was used as regulation. The mixtures were then incubated in an incubator at $37 \pm 2^\circ\text{C}$ for 15 min, and then heated for 5 min at 70°C . Using vehicles as blanks, their absorbance was assessed at 660 nm after cooling down. The standard drug used as a control in this experiment was diclofenac sodium at the final concentration range of 100–500 µg/mL and treated the same way for absorbance determination [23]. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times [A_{\text{bst}} / A_{\text{sc}} - 1] \text{ Where, } A_{\text{bst}} = \text{absorbance of test sample and } A_{\text{sc}} = \text{absorbance of control.}$$

The oil/drug concentration for 50% inhibition (IC₅₀) was determined from the dose–response curve by plotting percentage inhibition with respect to control against treatment concentration.

Antimicrobial activity

T. trilobatum extract was further assessed for antimicrobial activity against gram negative bacteria like *E. coli* (ATCC-8739) and gram-positive *Bacillus subtilis* (ATCC-6633) through disk diffusion method [24]. Freshly prepared cultures media, Mueller Hinton agar



were inoculated with different microbial strain by using sterile paper disk (0.6 mm diameter). Paper disk prepared with different concentration of *T. trilobatum* extract (300 µg/mL, 500 µg/mL, and 1 mg/mL). Incubation of the plates was done at 37°C for 24–48 h. Here Tetracycline used as standard drug, water and 4% ethanol solution was used as vehicle for the microbial test. Ethanolic solution (4%) used for the study, does not showing any antimicrobial effects.

2.7 Statistical analysis

The data were analyzed by one-way ANOVA followed by Dennett's t-test GraphPad prism and GraphPad In Stat Software. The data were expressed as mean ± SD. The significance was considered when $p < 0.05$ [25].

Results

Phytochemical screening

Typhonium trilobatum L. (Schott), a medicinal plant traditionally used in Southeast Asia, is valued for its antimicrobial and anti-inflammatory properties [5]. To identify the bioactive constituents potentially responsible for these effects, a phytochemical test was conducted as a comprehensive phytochemical analysis of plant extract. This included qualitative screening for secondary metabolites, gas chromatography–mass spectrometry (GC-MS) for the identification of volatile compounds, determination of total phenolic content (TPC) and total flavonoid content (TFC), and phytochemical profiling and quantification using thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC).

Presence of Phytoconstituents from major classes

The objective of the preliminary phytochemical

screening was to assess the presence of key secondary metabolites in the crude extract of *Typhonium trilobatum* L. (Schott). The analysis revealed the presence of several major classes of phytoconstituents, including flavonoids, alkaloids, glycosides, cardiac glycosides, and triterpenoids (Table 1). These findings indicate that the extract exhibits a chemically diverse phytochemical composition.

Identification of major volatile compounds by GC-MS analysis

To chemically profile the volatile components of *Typhonium trilobatum* L. (Schott), the extract was analyzed using GC-MS. The analysis identified thirty four distinct phytochemical constituents. The retention times, molecular formulas, peak area percentages, and peak heights of these phytochemicals are presented in Figure 2 and Table 1. Among them, the compounds with high peak area percentages were marked as major constituents 1-Nonadecene, Behenyl Alcohol, 1-Heptacosanol etc. Beyond volatile compounds, the next experiment investigated the abundance and types of antioxidant-related phytochemicals.

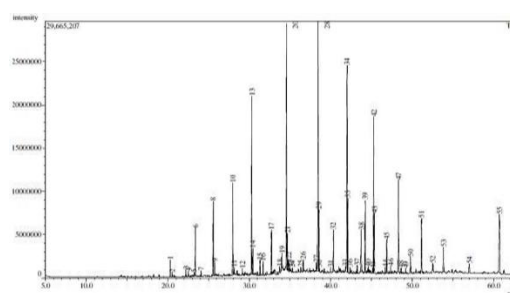


Figure 2. GC-MS Chromatogram of extract of *T. trilobatum* L. (Schott).

Table 1. Phytochemical constituents identified in the leaf extract of *Typhonium trilobatum* (L.) Schott by GC-MS.

SL No.	Name	Molecular Formula	Retention Time	Area %	Height
1.	Behenic Alcohol	C ₂₂ H ₄₆ O	38.454	12.54	28895659
2.	1-Nonadecene	C ₁₉ H ₃₈	34.58	12.05	28421086
3.	1-Heptacosanol	C ₂₇ H ₅₆ O	42.001	10.19	2388681
4.	Dococyl heptafluorobutyrate	C ₂₆ H ₄₅ F ₇ O ₂	48.302	5.19	10672767
5.	Tris(2,4-di-tert-butylphenyl) phosphate	C ₄₂ H ₆₃ O ₄ P	60.685	4.59	6723739
6.	Hexadecyl Acrylate	C ₁₉ H ₃₆ O ₂	27.97	3.37	10692395



7.	Bis-(2-ethyl hexyl)-phthalate	C ₂₄ H ₃₈ O ₄	44.223	3.13	8342044
8.	1-Heptadecene	C ₁₇ H ₃₆	25.583	2.75	8590686
9.	Tetracosylpentafluoropropionate	C ₂₇ H ₄₉ F ₅ O ₂	51.132	2.69	6138230
10.	Tetracosane	C ₂₄ H ₅₀	42.074	2.45	8613364
11.	Docosane	C ₂₂ H ₄₆	38.535	2.17	7137850
12.	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9	C ₁₇ H ₂₄ O ₃	32.725	2.09	5102127
13.	Heneicosane	C ₂₁ H ₄₄	45.334	2.06	6920019
14.	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	23.388	1.91	5565756
15.	Tetratetracontane	C ₄₄ H ₉₀	43.65	1.72	4870366
16.	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	33.997	1.06	1739154
17.	1-Pentadecene	C ₁₅ H ₃₀	20.305	0.65	1953721
18.	2-Pentadecanone, 6,10,14-trimethyl	C ₁₈ H ₃₆ O	31.321	0.57	1711120
19.	Butylated Hydroxytoluene	C ₁₅ H ₂₄ O	34.83	0.53	1400438
20.	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl	C ₁₆ H ₂₂ O ₄	31.697	0.52	1536750
21.	Octacosylpentafluoropropionate	C ₃₁ H ₅₇ F ₅ O ₂	56.983	0.48	964324
22.	Hexadecanoic acid, butyl ester	C ₂₀ H ₄₀ O ₂	38.224	0.32	980239
23.	Cyclononasiloxane, octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	43.2	0.26	831194
24.	Hexatriacontane	C ₃₆ H ₇₄	52.512	0.24	766323
25.	2,5-di-tert-Butyl-1,4-benzoquinone	C ₁₄ H ₂₀ O ₂	22.211	0.23	713931
26.	Di-3,7-dimethyl-1-octyl phthalate	C ₂₈ H ₄₆ O ₄	33.701	0.20	688967
27.	1-Dodecanol	C ₁₂ H ₂₆ O	22.527	0.20	585257
28.	3-Methylbutyl hexadecanoate	C ₂₁ H ₄₂ O ₂	45.125	0.18	510399
29.	Eicosane	C ₂₀ H ₄₂	24.09	0.18	630339
30.	Octadecanoic acid, butyl ester	C ₂₂ H ₄₄ O ₂	41.799	0.18	535412
31.	Tetradecane	C ₁₄ H ₂₈	20.534	0.17	523383
32.	Propanoic acid, 3-mercapto-, dodecyl ester	C ₁₅ H ₃₀ O ₂ S	34.965	0.15	509830
33.	Oxirane, hexadecyl	C ₁₈ H ₃₆ O	35.295	0.12	428594
34.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15	C ₁₆ H ₅₀ O ₇ Si ₈	38.608	0.09	448857

Detection of phenolic compounds by TLC analysis

To identify specific phytoconstituents, the extract of *Typhonium trilobatum* L. (Schott) was subjected to TLC analysis. Twelve known phytochemical standards were used for comparison, including chlorogenic acid, vanillic acid, p-coumaric acid, 4-hydroxybenzoic acid, trans-cinnamic acid, caffeic acid, gallic acid, naringenin, apigenin, quercetin, rutin, and myricetin. Visualization under ultraviolet light at 254 nm and 366 nm revealed 5 major phytoconstituents – p-coumaric acid, chlorogenic acid, quercetin, apigenin and caffeic acid (Figure 3).

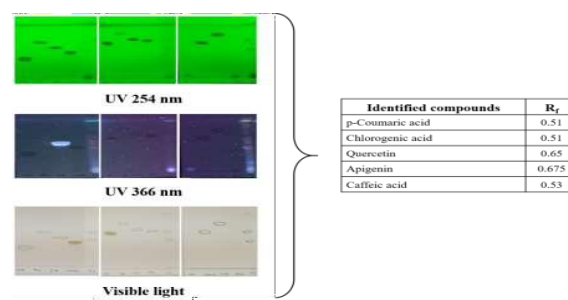


Figure 3A.Thin Layer Chromatogram of extract of *Typhonium trilobatum* at 254nm, 366nm and visible light: **3B:** List of identified compounds in *T.*



trilobatum extract with their Retention Quantification of key phenolic compounds by HPTLC analysis

To further identify and quantify key phenolic compounds, the extract of *Typhonium trilobatum* L. (Schott) was analyzed using HPTLC. Out of 12 standard compounds, chlorogenic acid, caffeic acid, and

kaempferol were positively identified and quantified in the said extract. Concentrations were determined as $195.58 \pm$

20.12 mg/g for chlorogenic acid, 4.02 ± 0.11 mg/g for caffeic acid, and 2.62 ± 0.08 mg/g for kaempferol in Figure 4.

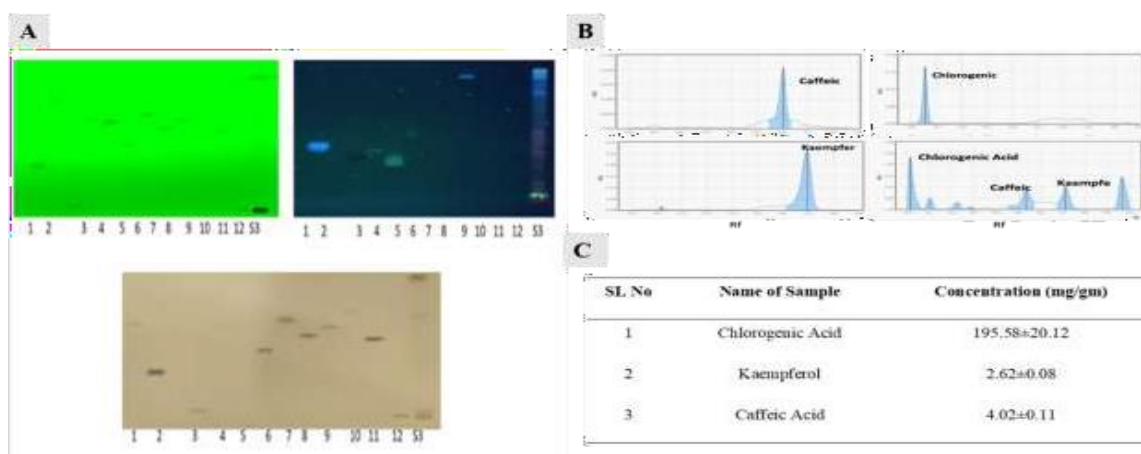


Figure 4A: HPTLC plate view at UV 254, UV 366 and white light of *Typhonium trilobatum* extract and standard, **4B:** HPTLC chromatogram of Chlorogenic Acid, Kaempferol, Caffeic Acid and extract of *Typhonium trilobatum* at UV 254 nm, **4C:** Quantitation results expressed as average concentration of the different compounds (mg/g) of *Typhonium trilobatum* extract

In-vitro activities

To evaluate the pharmacological potential of *Typhonium trilobatum* L. (Schott), a series of *in vitro* assays were performed targeting antioxidant, anti-inflammatory, and antimicrobial activities. The extract was tested for its ability to scavenge free radicals, reduce oxidative stress, inhibit inflammation, and suppress bacterial growth.

DPPH radical scavenging activity

As oxidative stress contributes to the onset and progression of various chronic disorders, assessing the antioxidant activity of *Typhonium trilobatum* L. (Schott) is essential to understand its therapeutic potential. The antioxidant capacity of the extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [5,38], with ascorbic acid serving as the reference standard. The extract exhibited concentration-dependent radical scavenging activity, yielding an IC_{50} value of 9.0 ± 0.1 μ g/mL, which was markedly lower than that of ascorbic acid (28.5 ± 0.35 μ g/mL) (Figure 5A). These results suggest that the extract has strong antioxidant potential and can

effectively scavenge free radicals at relatively low concentrations.

Hydrogen peroxide scavenging activity

To further assess the antioxidant efficacy of *Typhonium trilobatum* L. (Schott), its ability to neutralize hydrogen peroxide was examined using a standard method with ascorbic acid as the positive control. Although the extract demonstrated moderate scavenging activity, it was less potent than ascorbic acid. The IC_{50} value of the extract was determined to be 60.10 ± 0.12 μ g/mL, compared to 18.520 ± 0.22 μ g/mL for ascorbic acid (Figure 5B). This indicates that while the extract contains constituents capable of reducing oxidative stress, its hydrogen peroxide scavenging efficiency is relatively lower than that of the pure standard antioxidant [39].

High phenolic and flavonoid content detected in the extract

To evaluate the abundance of antioxidant-related



phytochemicals, the TPC and TFC of the extract of *Typhonium trilobatum* L. (Schott) were quantitatively determined. Gallic acid and quercetin were used as reference standards for phenolics and flavonoids, respectively. The TPC was measured as 206.46 ± 0.21 mg/100 g gallic acid equivalents, while the TFC was found to be 125.07 mg/100 g quercetin equivalents (Figure 5D). These results indicate that the extract is a rich source of polyphenolic and flavonoid compounds.

Protein Denaturation Test

Inflammation is a key factor in many chronic diseases [42], and plants with anti-inflammatory potential, such

as *Typhonium trilobatum* L. (Schott), are of considerable therapeutic interest due to their traditional use in reducing swelling and pain. To evaluate this potential, the protein denaturation effects of the extract was compared with the standard non-steroidal anti-inflammatory drug diclofenac sodium [43]. The assay revealed that the extract inhibited inflammatory responses with an IC_{50} value of 410.97 ± 0.26 μ g/mL, while diclofenac sodium exhibited a slightly lower IC_{50} of 300.980 ± 0.52 μ g/ml (Figure 5C). Although the

extract was marginally more potent than the standard drug, its notable inhibitory activity supports its traditional use in managing inflammatory conditions.

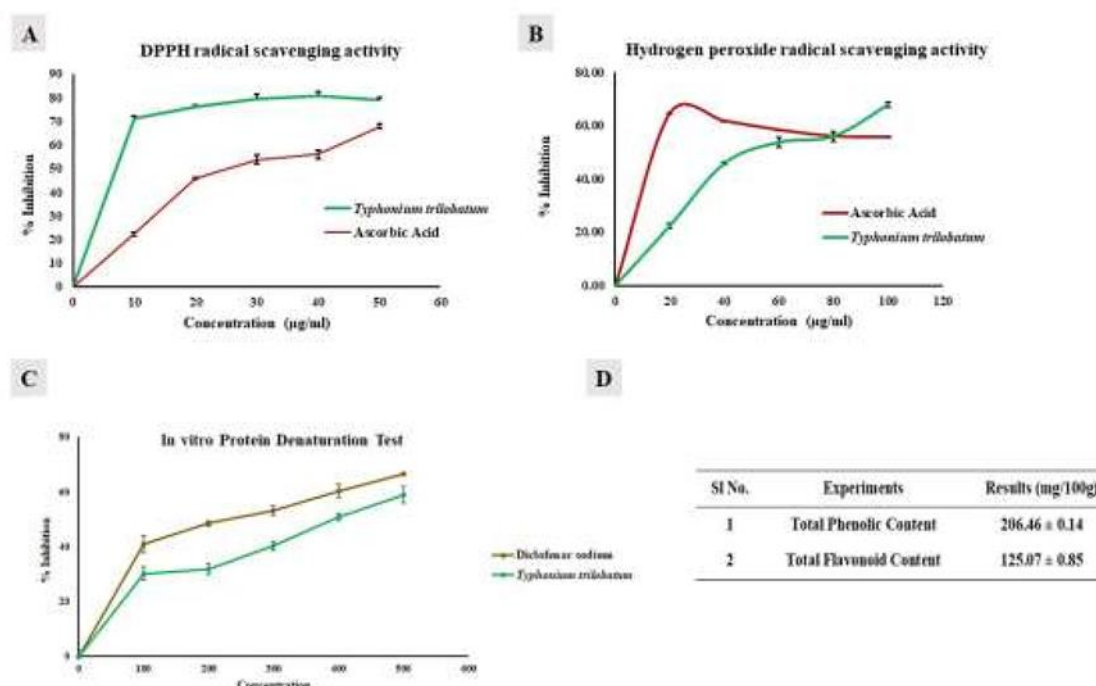


Figure 5: *In vitro* activities of the leaf extract of *Typhonium trilobatum* (L.) Schott. 5A: DPPH radical scavenging activity, 5B: H_2O_2 radical scavenging activity, 5C: anti-inflammatory activity, and 5D: total phenolic and flavonoid contents.

Antimicrobial activity

The rise of antibiotic resistance has intensified the search for plant-derived antimicrobials [44], and *Typhonium trilobatum* L. (Schott) has long been used in traditional medicine for treating infections. To evaluate its antimicrobial potential, the extract was tested against Gram-positive (*Bacillus subtilis*, ATCC 6633) and Gram-negative (*Escherichia coli*, ATCC 8739) bacterial strains using the disk diffusion method [45]. At the

concentration of 300 mg/mL, the extract produced a zone of inhibition measuring 1.2 ± 0.01 cm against *E. coli* and 0.9 ± 0.05 cm against *B. subtilis* (Figure 6A-B). The inhibitory effect increased with concentration dependent manner (Figure 6C). These results suggest that the extract possesses concentration-dependent antimicrobial activity.

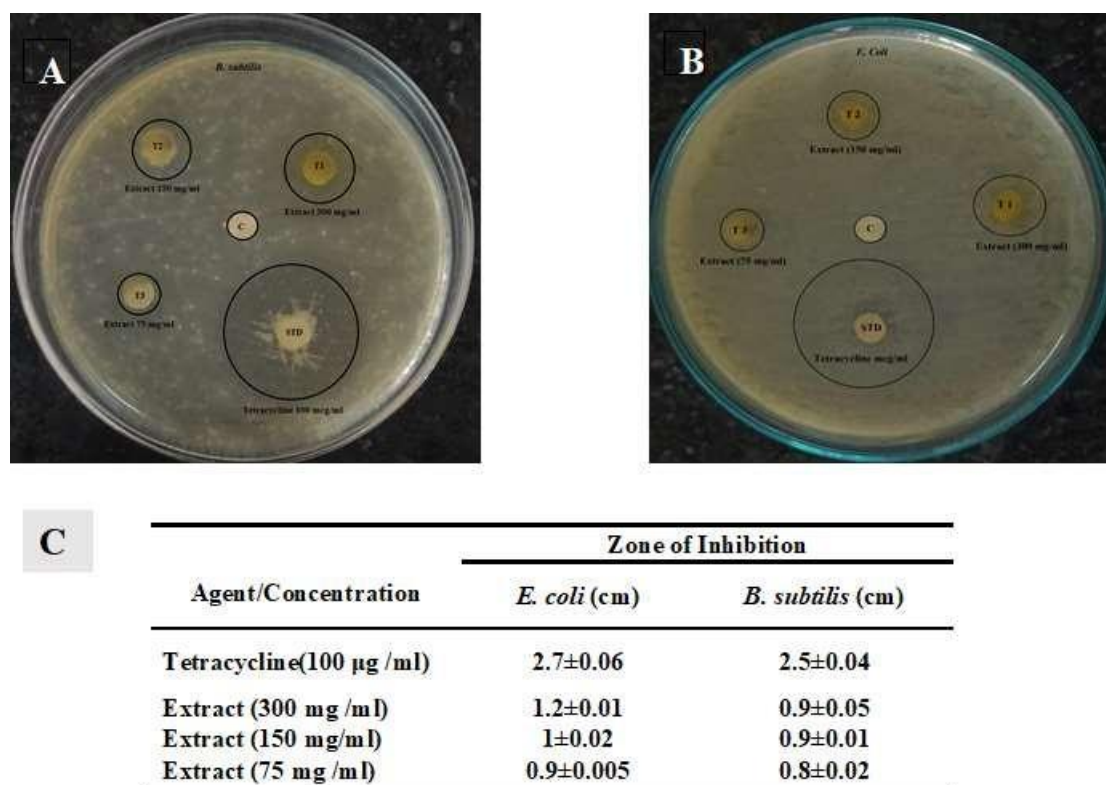


Figure 6A.Evaluation of Anti-microbial activity of *TyphoniumTrilobatum*(L) Schottextract against gram-positive *Bacillus subtilis* (ATCC 6633). **6B:** Evaluation of Anti-microbial activity of *Typhonium Trilobatum*(L) Schottextract against gram-negative *Escherichia coli* (ATCC 8739) a **6C:** Observation table to evaluate zone of inhibition.

Discussion

Phytochemical investigation of *Typhonium trilobatum* (L.) Schott has previously been limited to basic qualitative assessments, lacking detailed characterization and quantification of individual bioactive constituents [55]. While earlier studies reported the presence of general classes of secondary metabolites [56], a systematic and comprehensive chemical profiling remained largely unexplored. In this study, we addressed this gap through a multi-tiered chromatographic approach, including preliminary phytochemical screening, GC-MS, TLC, HPTLC, and quantification of TPC and TFC. Preliminary screening revealed the presence of flavonoids, alkaloids, glycosides, cardiac glycosides, and triterpenoids, indicating chemical diversity. Similar profiles are reported in methanolic and ethanolic extracts [57]. GC-MS of *T. trilobatum* identified major volatiles including 1-nonadecene, behenyl alcohol, and 1-heptacosanol, which have not been previously documented [58]. TLC confirmed the presence of *p*-coumaric acid, chlorogenic

acid (CA), quercetin, apigenin, and caffeic acid. The extract was found to be a rich source of polyphenols and flavonoids, as reflected by high TPC and TFC values, suggesting strong antioxidant potential. These findings align with established phenolic/flavonoid profiles linked to antioxidant activity [58]. Most notably, HPTLC identified CA as a principal phenolic compound in the extract, highlighting a key chemical marker that may contribute to the plant's pharmacological activities and was previously underreported in the literature.

CA is a polyphenolic compound composed of a caffeic acid moiety esterified with quinic acid [12]. Its structure contains multiple hydroxyl groups, which contribute to its strong antioxidant properties by scavenging free radicals and chelating metal ions [59]. CA is widely recognized for its diverse pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and neuroprotective effects [60, 61]. The identification of CA as a principal phenolic compound in *T. trilobatum* through HPTLC aligns with its known bioactivity and supports its relevance to the



plant's therapeutic potential. In our *in vitro* assays, the extract exhibited potent antioxidant activity, moderate anti-inflammatory effects, and concentration-dependent antimicrobial action - all of which are consistent with the reported pharmacodynamics of CA. The high TPC and TFC further suggest that CA, along with other phenolic and flavonoid constituents, may act synergistically to mediate the observed pharmacological effects.

The antibacterial activity of polyphenols has been widely attributed to their ability to interact with and disrupt microbial membranes [62–64]. CA, a major phenolic compound identified in *T. trilobatum*, has demonstrated potent bactericidal effects against both Gram-positive and Gram-negative bacteria primarily through membrane disruption leading to leakage of intracellular components and cell death [60, 64, 65].

This study provides important insight into the pharmacological potential of *T. trilobatum* (L.) Schott extract, but several limitations should be noted. The *in vitro* antioxidant, anti-inflammatory, and antimicrobial assays were conducted using the crude extract, which contains a complex mixture of phytochemicals. Cytotoxicity and selectivity toward bacterial versus mammalian membranes were also not assessed. Future studies involving bioactivity-guided fractionation, broader pharmacological screening, and more physiologically relevant membrane models are needed to fully understand the therapeutic potential of *T. trilobatum* (L.) Schott and its individual constituents.

Conclusions

This study offers an integrated chromatographic and mechanistic understanding of the pharmacological potential of *Typhonium trilobatum* (L.) Schott. A chemically diverse extract rich in flavonoids, phenolics, and other bioactive constituents was characterized through traditional comparative comprehensive chromatographic analyses - including preliminary phytochemical screening, gas chromatography–mass spectrometry, thin-layer chromatography, high-performance thin-layer chromatography, and quantification of total phenolic and flavonoid content. Chlorogenic acid was identified as a principal phenolic compound and was closely associated with the extract's strong antioxidant, moderate anti-inflammatory, and concentration-dependent antimicrobial activities.

Molecular dynamics simulations showed that chlorogenic acid actively engages with phospholipid membranes, favoring negatively charged bacterial membranes, and perturbs their structure by altering lateral packing, thickness, and hydrophobic core organization. These mechanistic insights extend the understanding of plant-derived phenolics as membrane-active agents and position *Typhonium trilobatum* (L.) Schott was a valuable candidate for advancing natural product-based antimicrobial strategies.

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Author contributions

S S: practical performance, draft preparation, figure preparation, collection, and resources; S P: practical performance; S S: draft preparation, figure preparation; A C and B D: primary editing, supervising the work, conception, and design; S P: secondary editing, formal analysis, data curation; R K: editing, collection, supervising the whole work and sorting of data.

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Declarations competing interests

The authors declare no competing interest.

Highlights

1. Phytochemical Profiling: TLC, HPTLC, GC–MS
2. Identification of Chlorogenic Acid as lead Compound
3. Strong Antioxidant and Anti-inflammatory Activities
4. Antimicrobial Efficacy
5. Mechanistic Insight via Molecular Dynamics Simulation

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