Journal of Chemical Health Risks

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JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

A Study on Phytochemical profiling and Bioactivities of *Tinospora cordifolia* against *Rhino- Cerebral Mucormycosis*

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(Received: 05 November 2025 Revised: 15 December 2025 Accepted: 23 January 2026)

KEYWORDS

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ABSTRACT:

Introduction: *Rhinocerebral mucormycosis* is a severe opportunistic fungal infection predominantly affecting immunocompromised individuals, particularly patients with uncontrolled diabetes mellitus and those recovering from COVID-19. The disease is most commonly caused by *Rhizopus arrhizus* and is associated with high morbidity and mortality. Conventional antifungal therapies, especially amphotericin B, though effective, are limited by severe nephrotoxicity and other adverse effects. This has necessitated the exploration of safer, plant-based bioactive compounds with antifungal and antioxidant potential. *Tinospora cordifolia*, a medicinal plant widely used in traditional medicine, is known for its immunomodulatory and therapeutic properties, making it a promising candidate for managing mucormycosis-related complications.

Objectives: The present study aims to: 1. Identify the secondary metabolites present in methanolic extracts of *Tinospora cordifolia* leaf and stem. 2. Characterize the bioactive compounds using Gas Chromatography–Mass Spectrometry (GC–MS). 3. Evaluate the antioxidant potential of the extracts using DPPH, nitric oxide scavenging, and lipid peroxidation assays. 4. Assess the antifungal efficacy of the extracts against *Rhizopus arrhizus.*

Methods: Methanolic extracts of *Tinospora cordifolia* leaf and stem were prepared and subjected to qualitative phytochemical screening to detect major secondary metabolites. The chemical constituents were characterized using GC–MS analysis. Antioxidant activity was evaluated through DPPH radical scavenging, nitric oxide scavenging, and lipid peroxidation inhibition assays. Antifungal activity against *Rhizopus arrhizus* was assessed using the agar diffusion method by measuring the zone of inhibition.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, and steroids in both leaf and stem extracts. GC–MS analysis identified several bioactive compounds, including guanosine, phytol, hexadecanoic acid, and neophytadiene. Antioxidant assays demonstrated significant free radical scavenging activity, with the leaf extract exhibiting superior antioxidant potential (IC_{50}: 41.2 µg/mL) compared to the stem extract. Antifungal evaluation showed notable inhibitory activity against *Rhizopus arrhizus*, with inhibition zones measuring up to 17 mm, indicating strong anti-mucormycotic efficacy.

Conclusions: The findings of this study suggest that *Tinospora cordifolia*, particularly its leaf extract, possesses potent antioxidant and antifungal properties. The presence of diverse bioactive secondary metabolites supports its potential as a natural and safer alternative for managing mucormycosis and associated oxidative stress-related complications. These results provide a scientific basis for further in-depth studies and development of plant-based antifungal therapeutics targeting mucormycosis..

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JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

1. Introduction

Global health concerns for immunocompromised patients are mostly affected by fungal infections. Opportunistic pathogens like *Rhizopus arrhizus* and other Mucorales species cause *Rhinocerebral mucormycosis* [1]. This rapidly progressing and often fatal infection predominantly affects patients with *Diabetes mellitus*, prolonged corticosteroid use, malignancies, or those recovering from COVID-19 [2]. In India, the burden of mucormycosis has escalated dramatically during the COVID-19 pandemic, with several cases linked to secondary fungal infections following immunosuppressive therapy [3].

The clinical management of mucormycosis relies primarily on antifungal drugs such as amphotericin B, often combined with surgical debridement. While effective in fungal clearance, amphotericin B is associated with nephrotoxicity, electrolyte imbalances, and systemic toxicity [4]. These limitations underscore the urgent need for safer and more affordable alternatives.

Medicinal plants provide a sustainable and eco-friendly source of therapeutic agents. Phytochemicals derived from plants are known for their antioxidant [5], antimicrobial, and immunomodulatory properties, making them valuable in developing adjunct or alternative therapies for infectious diseases [6]. *Tinospora cordifolia* (Guduchi), a widely used medicinal plant in Ayurveda, has been traditionally prescribed for fever, jaundice, diabetes, and immune disorders [7]. It is rich in bioactive compounds, including alkaloids, diterpenoid lactones, steroids, and phenolics [8], which contribute to its diverse pharmacological properties such as antidiabetic, neuroprotective, anticancer, and antimicrobial effects [9].

Recent studies suggest that significant reduction in oxidation leads to oxidative stress and improper function of immune system play an important role in the infection of mucormycosis [10]. Given the potent antioxidant and immunomodulatory activities of *T. cordifolia*, it holds promise as a natural therapeutic option for combating mucormycosis and associated neurological complications.

2. Objectives

The present study objective is to investigate the phytochemical constituents, antioxidant and antifungal bioactivity from leaf and stem methanolic extracts of *Tinospora cordifolia*. Specifically, this work focuses on phytochemical screening, GC–MS analysis, antioxidant assays (DPPH, nitric oxide scavenging, lipid peroxidation), and antifungal evaluation against *Rhizopus arrhizus*. By elucidating the bioactivities of *T. cordifolia*, this study seeks to provide scientific evidence supporting its potential role as a sustainable plant-based intervention against mucormycosis.

3. Methods

Sample preparation:

Tinospora cordifolia (leaf and stem parts) were identified and collected from SRM college campus, Kattankulathur, Chengalpattu district, Tamil Nadu. The samples were washed, shade-dried, and powdered. Methanolic extracts were prepared and kept in shaker for 24hrs and extracted the samples using Soxhlet extraction (1:5 ratio) for 36 h, concentrated with a rotary evaporator, and stored at 4 °C (Fig.1).

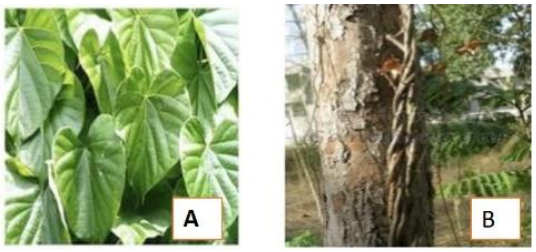

Fig.1: Collection of *T.cardifolia* from A -leaf and B - stem

Phytochemical Screening:

Qualitative tests were performed for alkaloids, carbohydrates, glycosides, saponins, proteins, phenols, flavonoids, terpenoids, and steroids using standard methods [11].

Gas chromatography – Mass Spectrometry:

Methanolic extracts were analyzed using GC–MS (NRC, SRM Institute of Science and Technology). The compounds present in the sample were screened and identified based on retention time on the graph and mass spectral database [12].

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Antioxidant assay:

Sample preparation:

The extracts were prepared by dissolving in methanol as a stock solution. From this, different concentrations 10, 20, 40, 80, and 160 µg/mL were prepared with standard ascorbic acid in triplicate manner and percentage of inhibition was calculated using the formula:

% of Inhibition = Control – sample/Control * 100

DPPH radical scavenging assay:

To 0.1mM of DPPH + methanol solution equal volume of extract were mixed as per the concentration level and incubated in dark for 30 minutes in room temperature with standard ascorbic acid and absorbance was measured at 517 nm [13].

Nitric Oxide Scavenging assay:

The test sample was mixed with an equal volume of sodium nitroprusside solution and incubate at 25°C for 150minutes under light. After incubation, Griess reagent was added and observed for pink chromatophore at 546 nm with ascorbic acid as standard [14].

Lipid peroxidation:

Different concentrations of the test samples + egg yolk homogenate (10% w/v) in phosphate buffer (0.1 M, pH 7.4) + ferric chloride solution. The reaction was incubated for 30 minutes at 37 °C, and trichloroacetic acid (15%) was added to inhibit the reaction, followed by thiobarbituric acid (0.67%), and boiled in a water bath for 15 minutes, forming the complex. The samples after cooling were centrifuged, and the supernatant was measured at 532 nm with ascorbic acid as standard [15].

Anti-fungal Activity:

The agar well diffusion method was used against *Rhizopus arrhizus*. Extracts at concentrations of 125–1000 µg/mL were tested, with fluconazole as the standard drug [16].

4. Results

Phytochemical composition:

Leaf and stem extracts showed the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins and phenols. Carbohydrates and proteins were observed efficiently in the stem extract compared to the leaf extract, while steroids were abundant only in leaves and absent in stem extract (Table 1, Fig.2).

Table 1. Phytochemical analysis of *Tinospora cordifolia* extracts

Phytochemical	Leaf Extract	Stem Extract
Alkaloids	+++	+++
Flavonoids	+++	+++
Phenols	+++	+++
Glycosides	+++	+++
Saponins	+++	+++
Proteins	+	+++
Carbohydrates	+	+++
Steroids	+++	-

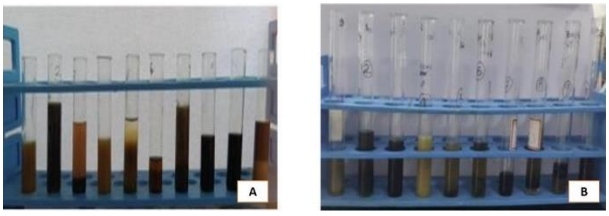

Fig.2: Phytochemical analysis of *Tinospora cordifolia* A- leaf and B- stem extracts

Gas Chromatography and Mass spectral (GC-MS) analysis:

The characterization profiling of identified compounds showed the presence of bioactive properties. Leaf extracts revealed phytol, neophytadiene, guanosine, and hexadecanoic acid derivatives (Fig. 3, Table 2), while stem extracts showed glyceraldehyde, guanosine, and 3-deoxy-d-mannoic lactone (Fig. 4, Table 3).

Table 2: Major peaks, name of derivatives and its application obtained from the leaf extract of *T. cardifolia.*

1	9.204	2,3-dihydro-3,5-dihydroxy-6-methyl-4h- pyran-4-one	Bioactive flavonoid

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

2	13.57	Guanosine	Nucleosideantioxidant
3	15.5499	Inositol, 1-deoxy-	Neuroprotective role
4	18.363	Neophytadiene	Antimicrobial
5	19.66	n-hexadecanoic acid	Antioxidant
6	21.232	Phytol	Strong antioxidant
7	24.843	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antifungal

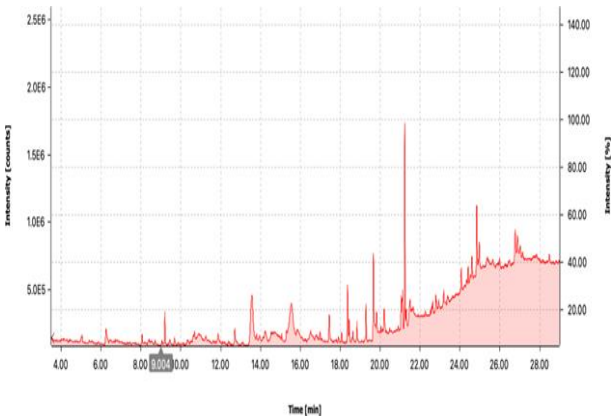

Fig.3: GC - MS Profile of the *T.cordifolia* Leaf.

Table 3: Major peaks, name of derivatives and application obtained from the stem extract of *T. cardifolia*

Peak	**Retention time**	**Name**	**Application**
1	3.989	Glyceraldehyde	Metabolic intermediate
2	9.201	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Oxidation reduction potential
3	13.623	Guanosine	Nucleoside, antioxidant
4	15.513	3-Deoxy-d-mannoic lactone	Antimicrobial
5.	24.842	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antifungal

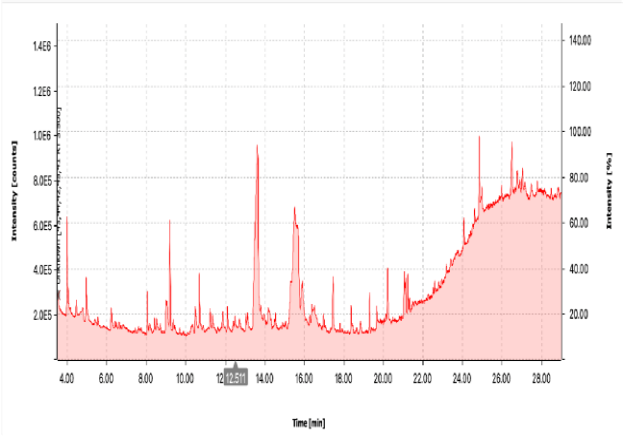

Fig. 4: *GC - MS Profile of the T.cordifolia stem*

Antioxidant activity:

DPPH radical scavenging activity:

Ascorbic acid, the DPPH standard, demonstrated a maximum (60.48%) free radical scavenging activity at 100 g/mL for the leaf extract (60.48%) and stem extract (67.55%). The scavenging activity of the methanolic stem extract was highest (73.31%) at 160 µg/mL (Table 4, Fig. 5).The IC50 value of the leaf extract was 41.286 µg/ml compared to 44.996 µg/ml of the stem extract which shows that leaf extract potentially has a slightly better radical scavenging activity than the stem.

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JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

Table 4: DPPH Assay of *T. cordifolia* leaf and stem extract

S. No	Concentration	Leaf				Stem			
		Test sample	Control	% of Inhibition	IC_{50} value µg/mL	Test sample	Control	% of Inhibition	IC_{50} value µg/mL
1.	Control	0.267	-	-		0.356	-	-	
2.	Positive control Ascorbic acid (100 µg/mL)	0.105	0.165	60.48	41.268	0.115	0.240	67.55	44.996
3	10	0.187	0.080	25.46		0.296	0.06	16.85	
4	20	0.162	0.105	38.2		0.260	0.09	26.82	
5	40	0.153	0.113	42.5		0.217	0.13	38.9	
6	80	0.141	0.125	47		0.12	0.23	69.1	
7	160	0.125	0.142	53.18		0.103	0.25	73.31	

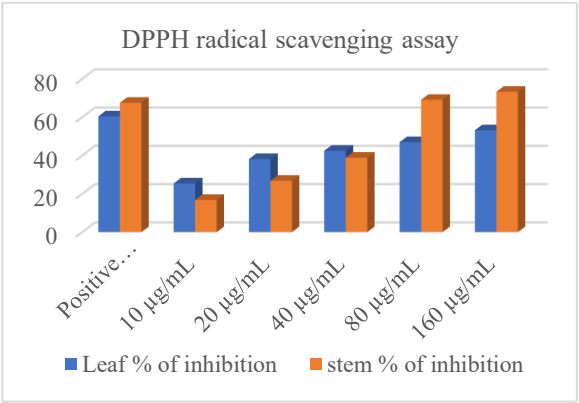

Fig.5: DPPH radical scavenging assay of leaf and stem extract of *T. cordiofolia* L.

Nitrous oxide scavenging assay:

In leaf extract Ascorbic acid, the standard, demonstrated a maximum (76.85%) activity at 100 µg/mL. The methanolic leaf extract had the maximum scavenging activity at 20 g/mL whereas the stem extract had the maximum scavenging activity at 160 g/mL (Table 5, Fig. 6). The IC_{50} value of leaf extract was 62.884µg/ml compared to 67.584 µg/ml of the stem extract which shows that leaf extract is slightly better radical scavenger than the stem.

Table 5: Nitrous oxide scavenging assay of *T. cordifolia* leaf and stem extract

S. No	Concentration	Leaf				Stem			
		Test sample	Control	% of Inhibition	IC_{50} value (µg/mL)	Test sample	Control	% of Inhibition	IC_{50} value (µg/mL)
1	Control	0.27	-	-		0.37	-	-	
2	Positive control Ascorbic acid (100	0.06255	0.2075	76.85		0.06255	0.3075	83.1	

Journal of Chemical Health Risks
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JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

	µg/mL)								
3	**10 µg/mL**	0.0415	0.2285	84.62	62.884	0.263	0.107	28.91	67.546
4	**20 µg/mL**	0.04555	0.2245	83.14		0.112	0.258	69.72	
5	**40 µg/mL**	0.00675	0.2025	75		0.088	0.282	76.21	
6	**80 µg/mL**	0.11455	0.1555	57.59		0.071	0.299	80.8	
7	**160 µg/mL**	0.2332	0.038	14.07		0.063	0.307	82.08	

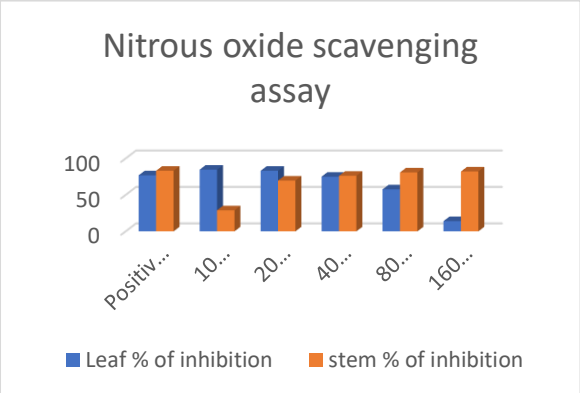

Fig.6: Nitrous oxide scavenging assay of leaf and stem extract of *T. cordiofolia* L.

Lipid peroxidation assay:

The positive control (L.Ascorbic acid 100µg/ml) demonstrated an inhibition rate of 78.62% for both leaf and stem. Leaf extract showed highest percentage of inhibition (56.17) at 160µg/mL and stem extract showed the highest percentage of inhibition (73.81) at 160µg/mL (Table 6, Fig 7). The IC50 value of the leaf was 34.284 µg/mL and the stem was 49.806 µg/mL. So, it can be concluded that leaf extract is better than the stem extract in lipid peroxidation inhibition activity.

Table 6: Lipid peroxidation assay of *T. cordifolia* leaf and stem extract

S.No	Concentration (µg/mL)	Leaf				Stem			
		Test sample	Control	% of Inhibition	IC_{50} value (µg/mL)	Test sample	Control	% of Inhibition	IC_{50} value (µg/mL)
1.	**Control**	0.737	-	-		0.737	-	-	
2.	**Positive control Ascorbic acid (100)**	0.157	0.579	78.62	34.28	0.157	0.579	78.62	49.80
3	**10**	0.6033	0.134	18.18		0.63	0.107	14.51	
4	**20**	0.5445	0.191	25.98		0.482	0.254	34.53	
5	**40**	0.502	0.235	31.88		0.366	0.370	50.27	

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JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

6	**80**	0.448	0.289	39.21		0.193	0.559	65.47	
7	**160**	0.323	0.414	56.17		0.177	0.554	68.05	

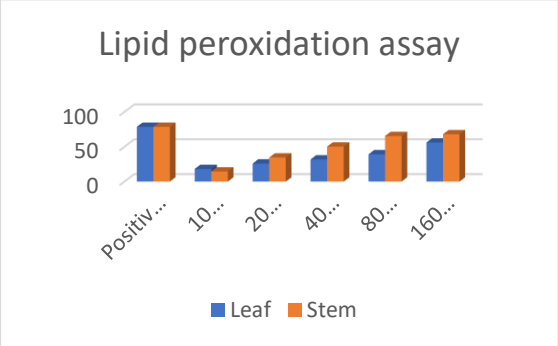

Fig.7: Lipid peroxidation assay of extract (leaf and stem) of *T. cordiofolia* L.

Thus, both extracts of *T. cordiofolia* exhibited strong free radical scavenging activity. Leaf extract had slightly better IC_{50} values (41.2 µg/mL in DPPH, 62.8 µg/mL in NO scavenging, 34.2 µg/mL in lipid peroxidation) compared to stem (44.9 µg/mL in DPPH, 67.54 µg/mL in NO scavenging, 49.80 µg/mL in lipid peroxidation).

Antifungal Activity:

Tinospora cordifolia was tested for its antimicrobial effectiveness against *Rhizopus arrhizus* and the results were presented in Table 7. Figure 8 A and B display photographs of the inhibitory zones created by both extracts of *T. cordifolia*, respectively. From the results obtained it was inferred that leaf extract showed maximum zone of inhibition (14mm) at 1000 µg/mL concentration and stem extract showed maximum zone of inhibition (17mm) at 1000 µg/mL in comparison to the standard drug (fluconazole).

Table 7: Antifungal activity measuring zone of inhibition in methanolic extract of *T. cordifolia* of leaf and stem extract.

S.No	**Concentration** (µg/mL)	**Leaf extract (mm)**	**Stem extract (mm)**
1	125	-	-
2	250	10.6	9.3
3	500	14.2	12.4
4	1000	17.8	14.5
5	Standard drug (Fluconazole)	19.3	16.4

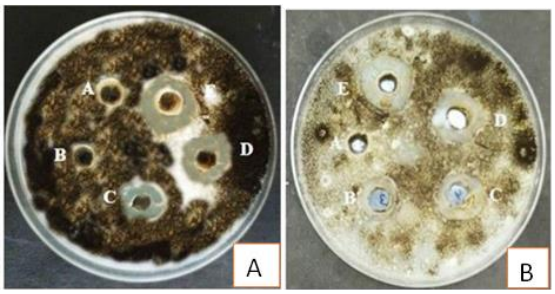

Fig.8: Antifungal activity of *T.cordifolia* (A) leaf (B) stem (E – standard, D- 1000 µg/mL, C- 500 µg/mL, B - 250 µg/mL, A- 125 µg/mL.

Discussions:

Rhinocerebral mucormycosis is a type of invasive fungus caused by mucoracea family, a class of Phycomycetes (Zygomycetes). It causes infections of the nose, skin, lungs, digestive system, brain, and sinuses. People with Diabetes mellitus are more susceptible to the disease as they are immunocompromised. The disease is primarily caused by predominantly caused by Rhizopus arrhizus. Rhizopus species are unique in their capacity to use chelators as a siderophore to ingest iron for better growth [17]. Iron chelation and aluminium overload, which were most frequent in hemodialysis patients, were treated with deferoxamine. The disease is characterized by aggressive tissue invasion, oxidative stress, and vascular damage.

Tinospora cordifolia L. belongs to the Menispermaceae family used in traditional medicine for the management of infectious diseases, inflammatory conditions, and immune-related disorders, indicating its potential relevance in combating opportunistic fungal infections [18]. It is characterized by oxidative stress, iron-dependent fungal proliferation, and compromised host immunity. The multifunctional bioactive profile of T. cordifolia provides a strong scientific rationale for its selection as a potential supportive antifungal agent.

Journal of Chemical Health Risks
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JCHR (2026) 16(1), 1131-1139 | ISSN:2251-6727

The extracts of *T. cordifolia have* diverse secondary metabolites, and each metabolite indicates the plant as a potential bioactive material. The presence of compounds proves that plant extracts are rich in antioxidant, antifungal, and immunomodulatory activities. The presence of Phenols and flavonoids indicates their role in scavenging free radicals and mitigating oxidative stress, whereas the presence of alkaloids and saponins interacts with the integrity of the fungal cell membrane and its metabolic processes [19]. The glycoside and steroids have anti-inflammatory and protective effects. Primary metabolite carbohydrate and protein, enhance the biological interactions and possess the extract stability. Thus, the Rhizopus-mediated infections, such as phytochemical metabolites, help to inhibit the growth of fungal infection and reduce oxidative tissue damage, which are key pathological features of rhino-cerebral mucormycosis.

GC–MS profiling of *Tinospora cordifolia* leaf and stem extracts has shown a prominent profile of bioactive constituents that contribute to antioxidant and antifungal activities [20]. The leaf extract showed the presence of lipophilic compounds that interact with microbial membranes and inhibit the growth of Rhizopus arrhizus through destabilization of the membrane. The stem extract has polar and redox-active compounds to scavenge the free radicals and control oxidative stress. Thus, the fungal proliferation suppresses the oxidative tissue injury and shows the potential action of T. cordifolia against Rhizopus arrhizus associated rhino-cerebral mucormycosis.

The antioxidant activity indicates that the plant extract can neutralize the free radicals and rhino-cerebral mucormycosis plays a prominent role in tissue necrosis and vascular damage [21]. Therefore the antioxidant potential contribute in inhibiting the oxidative injury and prevent the cell integrity during fungal activity. Secondary metabolites such as terpenoids, alkaloids and phenolic compounds interfere with fungal cell wall, membrane permeability, and enzymatic action to inhibit the growth of fungus in immunocompromised patients [22]. Thus, the antioxidant and antifungal activities have a dual action, protecting the body from oxidative damage and fungal infection. The multi-targeted effects of plant-based extracts have a synergistic effect on fungal growth and mitigate oxidative stress.

Conclusion:

The methanolic extracts of *Tinospora cordifolia* exhibit strong antioxidant and antifungal properties, with significant inhibitory effects against *Rhizopus arrhizus*. Leaf extracts were more effective in antioxidant assays, while stem extracts showed superior antifungal activity. The study highlights *T. cordifolia* as a promising candidate for developing plant-based therapeutic activity.

Acknowledgement:
The authors thank the Nanotechnology Research Centre (NRC), SRM Institute of Science and Technology, Tamil Nadu, for GC–MS analysis support.

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Journal of Chemical Health Risks

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