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Phytochemical content, antioxidant and antibacterial properties of *Typhonium flagelliforme* cultivated via soil and hydroponic methods

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Abstract

Background: *Typhonium flagelliforme* is a medicinal herb in the Araceae family, widely distributed in Southeast Asia. This study aimed to evaluate the effects of hydroponic and soil-based cultivation systems on the phytochemical content and bioactivity of *T. flagelliforme*.

Results: Using liquid chromatography-mass spectrometry (LC-MS) analysis, 9 and 16 phenolic compounds were identified in soil-grown and hydroponically grown plant extracts, respectively. Seven phenolic compounds were identified in both cultivation systems. Hydroponically grown *T. flagelliforme* exhibited a significantly higher total phenolic content (49.0 ± 3.30 mg gallic acid equivalent/100 g) and total flavonoid content (22.6 ± 3.45 mg catechin equivalent/100 g) compared to soil-grown plants. Hydroponic extracts demonstrated a lower half-maximal inhibitory concentration in radical scavenging (1.99 ± 0.19 mg/mL) and ferrous iron chelating (4.19 ± 0.08 mg/mL) assays than soil-grown extracts (2.37 ± 0.17 and 5.51 ± 0.07 mg/mL, respectively), indicating stronger antioxidant activity. The anti-radical power was also higher in hydroponically grown plants (0.50 ± 0.04) compared to soil-grown plants (0.42 ± 0.02). Hydroponically grown extracts showed superior antibacterial efficacy compared to soil-grown plants against *Bacillus subtilis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Conclusion: This study demonstrated that hydroponic cultivation enhanced the bioactive compound content and improved the plant's antioxidant and antibacterial properties.

KEYWORDS
antimicrobial, bioactive compound, medicinal plant, phytochemical, rodent tuber

INTRODUCTION

Typhonium flagelliforme, commonly known as the rodent tuber, is a medicinal herb belonging to the Araceae family and is widely distributed in regions such as China, Australia and Southeast Asian countries including Indonesia, Singapore and Malaysia.[1,2] Its widespread ethnopharmacological use among ethnic groups includes oral treatments for ailments such as asthma, diarrhoea and respiratory tract swelling.[3] The medicinal properties of *T. flagelliforme* are attributed to its diverse array of phytochemical compositions, including alkaloids, polyphenols, aliphatic compounds, amino acids and fatty acids. These compounds play crucial roles in mediating ecological competition, such as acting as defence mechanisms against herbivores or safeguarding against pathogen invasion.[3] It was also reported that *T. flagelliforme* exhibited

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a significant cytotoxicity effects on human lung carcinoma cells (NCI-H23), human mammary gland carcinoma cells (T-47D) and breast cancer cells (HS578T), indicating potential anticancer properties.[2,4,5]

The soil cultivation system, often referred to as conventional farming, is the most traditional and predominant growing system practiced in agriculture, where the use of natural soil is involved as the primary growing medium and the source of nutrients to support the plant's metabolic processes and growth.[6] This cultivation system offers numerous advantages, such as being cost-effective, fostering a diverse community of beneficial microflora and providing natural trace elements crucial for promoting plant health. However, the soil cultivation system is highly vulnerable to soil-borne pests and often necessitates the long-term use of synthetic fertilisers and pesticides, which can lead to the accumulation of chemical residues and soil acidification.[7] Another major drawback of the soil cultivation system is its high reliance on natural weather conditions, which are inherently unpredictable and uncontrollable. Therefore, the hydroponic cultivation system is an innovative growing system designed to address the limitations of traditional soil-based cultivation systems. This soil-less farming technique involves submerging the plant roots in a formulated nutrient-rich solution, allowing direct and continuous nutrient absorption, while using inert growing media like cocopeat to provide structural stability for the plants.[8,9] Generally, the nutrient solution is made up of nitrogen, phosphorous, calcium, magnesium and potassium as the major components, along with some trace elements, ensuring balanced plant nutrition.[10]

One of the significant advantages of this automated growing system is its capacity for high-density vertical farming in stacked layers with maximum space efficiency.[8,11] The controlled environment of a hydroponic system allows real-time monitoring and optimisation of environmental factors (abiotic) like temperature, humidity, light density and CO_2 concentration, significantly enhancing the plant growth rate.[12] Furthermore, this controlled growing environment has drastically reduced the risk of soil-borne pests and diseases, thereby controlling the reliance on pesticides and promoting a more sustainable and eco-friendly growing system.[11,13] To the authors' best knowledge, the phytochemical composition and bioactivity of hydroponically grown *T. flagelliforme* have not been reported previously. Therefore, the objective of this study was to compare the phenolic and flavonoid concentrations, antioxidant potential and antibacterial properties of *T. flagelliforme* cultivated in soil and hydroponic systems.

MATERIALS AND METHODS

Collection of plant samples

The plant samples were freshly harvested from the Agro-Biotechnology Institute, Selangor, Malaysia. For soil-cultivated plants, humus soil enriched with AB fertiliser was used as the growing medium and irrigation was carried out twice a day to maintain a consistent moisture level. Hydroponically cultivated plants were grown in cocopeat and immersed in a nutrient-rich solution with dissolved AB fertiliser. The temperature was maintained between 34°C and 36°C, while humidity levels were kept within a range of 60%–80% to prevent excessive dryness. Additionally, 70% shading was employed to shield the plants from direct exposure to excessive ultraviolet (UV) light.

The composition of AB fertiliser was follows: calcium, nitrate-N, ammonium-N, potassium, EDTA Fe, phosphate, nitrate-N, ammoniacal-N, potassium, magnesium, sulphate, boron, molybdate, EDTA Mn, EDTA Zn and EDTA Cu.

Preparation of sample extracts

The extraction process begins with the separation of leaves from the plant bodies, and the leaves were washed thoroughly to remove any adhering dirt or contaminants. The cleaned and dried leaves were then blended with ethyl acetate using a Waring blender. The blending was carried out at a solid-to-solvent ratio of 1:4 (m/v). The resultant mixture was then transferred to a clean container and incubated for 48 h to facilitate the extraction of bioactive compounds. After the incubation period, the plant extract was filtered using Whatman No. 1 filter paper to remove suspended solids. The filtered plant extracts were subsequently subjected to concentration using a rotary evaporator. The rotary evaporator was set to operate at a temperature of 50°C and a reduced pressure of 150 mbar. The dried plant extracts were then stored at a temperature of −20°C.

Identification of phenolic compounds

Liquid chromatography-mass spectrometry (LC-MS) analysis was conducted to determine the phytochemicals in *T. flagelliforme*, according to the method of Lai et al.[2] with a slight modification. The phytochemical compounds were identified based on the mass-to-charge ratio (m/z) and retention time, as determined using the Agilent 1290 Infinity LC system coupled to an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with a dual electrospray ionisation source. The chromatographic separation was carried out using an Agilent Eclipse XDB-C18 narrow-bore column (150 mm × 2.1 mm, 3.5-µm particle size) at a column temperature of 25°C, with the autosampler maintained at 40°C. The mobile phase flow rate was set at 0.5 mL/min, using 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The injection volume was 1.0 µL. Gradient elution was performed initially at 95% solvent A and 5% solvent B for 5 min, followed by 100% solvent B for 20 min, and then held at 100% solvent B for another 5 min, with a total run time of 30 min. Mass spectrometry was performed in both positive and negative ion modes, covering an m/z range of 100–3200.

Total phenolic content (TPC)

The TPC was determined using the Folin–Ciocalteu method.[12] A total of 10 mg of the dried extract was accurately weighed and dissolved in

10 mL of 75% ethanol, and this mixture served as the sample solution for further analysis. The following reagents were added sequentially into a 0.3-mL sample solution to initiate the colorimetric reaction required for TPC quantification:

1. 1.2 mL of sodium carbonate (7.5% w/v)
2. 1.0 mL of Folin–Ciocalteu reagent (10 times diluted)

After the addition of the reagents, the sample is subjected to vortexing to ensure complete mixing, followed by 30 min of incubation at room temperature in a shadowed area. The intensity of the coloured complex was measured at 765 nm using a UV–visible (UV–Vis) spectrophotometer.

For the calibration curve, gallic acid standards were prepared at varying concentrations ranging from 0 to 150 μg/mL, following the steps described above. TPC was expressed as mg gallic acid equivalent/g fresh plant (mg GAE/g).

Total flavonoid content (TFC)

The TFC was determined using the aluminium chloride ($AlCl_3$) colorimetric assay.[13] Plant extracts (10 mg) were weighed and dissolved in 10 mL of 75% ethanol to create a homogeneous sample solution for analysis. The following reagents were added sequentially to 1.0 mL of sample solution to initiate the colorimetric reaction required for TFC quantification:

1. 0.2 mL of 10% $AlCl_3$
2. 0.2 mL of 1 M potassium acetate

After the addition of the reagents, the sample is subjected to vortexing to ensure complete mixing, followed by 30 min of incubation at room temperature in a shadowed area. The intensity of the coloured complex was measured at 420 nm using a UV–Vis spectrophotometer. For the calibration curve, catechin standards were prepared with varying concentrations ranging from 0 to 150 μg/mL, following the steps as described above. TFC was expressed as mg catechin equivalent/g fresh plant (mg GAE/g).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant potential was determined using the DPPH assay.[14] A total of 10 mg of plant extracts were weighed and dissolved in 10 mL of 75% ethanol to serve as the sample solution to assess the antioxidant capacity of the extracts. The DPPH solution (0.02 g/L) was prepared by dissolving 0.02 g of DPPH radicals in 1 L of ethanol. Then, 2.0 mL of DPPH solution was added to 1.0 mL of the plant extract solution. The mixture was vortexed thoroughly and incubated for 10 min at room temperature to allow colour change. This colour shifting can be detected at a wavelength of 515 nm using a UV–Vis spectrophotometer. The percentage of DPPH radical scavenging can be calculated by comparing the absorbance of the samples to a blank (DPPH solution without plant extracts), as shown below:

$$\frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control (DPPH solution without plant extracts) and A_s is the absorbance of the DPPH solution with plant extracts.

To determine the half-maximal inhibitory concentration (IC_{50}) of the plant extracts, varying concentrations of plant extracts ranging from 1 to 5 mg/mL were assessed. Each concentration was subjected to the same procedure as mentioned above. Additionally, the anti-radical power (ARP) can be derived from the IC_{50} value using the equation shown below:

$$\mathrm{ARP} = \frac{1}{\mathrm{IC}_{50}}$$

Ferrous iron chelating (FIC) activity

The antioxidant potential was also determined using the FIC assay, as described by Choo and Yong[14] with slight modifications. A total of 10 mg of plant extracts was weighed and dissolved in 10 mL of 75% ethanol to serve as the sample solution for assessing the FIC activity of the plant extracts. Subsequently, 1.0 mL of the sample solution was pipetted and mixed with the following reagents:

1. 1.0 mL of diluted iron sulphate ($FeSO_4$; 2 mM)
2. 1.0 mL of diluted ferrozine (5 mM)

$FeSO_4$ was added first, and the solution was allowed to rest to ensure a complete interaction between ferrous irons and the chelating agents present in the plant extract before ferrozine was added. The mixture was subsequently vortexed and allowed to incubate for 10 min at room temperature to facilitate the development of a coloured complex, which was then detected using a UV–Vis spectrophotometer at a wavelength of 620 nm. Concentrations of the plant extract ranging from 1 to 5 mg/mL were assessed. The percentage of FIC activity can be calculated by comparing the absorbance of the samples to a control ($FeSO_4$ solution without plant extracts), as shown below:

$$\frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control ($FeSO_4$ solution without plant extracts) and A_s is the absorbance of the $FeSO_4$ solution with plant extracts.

Antibacterial activity

The antibacterial activity was assessed using four Gram-positive bacteria strains, methicillin-resistant *Staphylococcus aureus* (MRSA;

American Type Culture Collection [ATCC] 33591), *S. aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 8188), *Enterococcus faecalis* (ATCC 29212) and four Gram-negative bacterial strains, *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Shigella flexneri* (ATCC 12022).

The antibacterial activities of *T. flagelliforme* were determined using a broth microdilution method, as described by Yong et al.[15] with slight modifications. The brain heart infusion (BHI) broth was prepared by weighing 3.7 g of BHI powder and dissolving it in 100 mL of distilled water. The broth was sterilised by autoclaving at 121°C for 15 min. Subsequently, the BHI broth was cooled to room temperature. The bacterial strains were obtained from the ATCC and inoculated into 100 mL of the sterilised BHI broth. The inoculated broth was incubated overnight at 37°C to allow the bacterial strains to proliferate.

The density of the bacterial suspension was compared to a McFarland standard of 0.5, which corresponds to an absorbance range of 0.08–0.10 at 625 nm. The standardised bacterial cultures were then subjected to further dilution in a 1:1000 ratio with fresh BHI broth. This antibacterial assay was performed in 96-well microtiter plates using two-fold serial dilutions, incubated at 37°C for 24 h to ensure sufficient time for bacterial growth in the presence of the plant extracts. In this assay, the positive control was bacterial suspension with chloramphenicol, while the negative control was bacterial suspension without plant extracts. The minimum inhibitory concentration (MIC) was indicated by a clear plate. Proper aseptic and sterilisation techniques were followed throughout the assay.

Statistical analysis

Independent triplicates were prepared for all experiments and the results were reported as mean ± standard deviation ($n = 3$). All data analyses were performed in the Statistical Package for Social Sciences® (SPSS Statistics 27) software to determine the statistical significance at $p < 0.05$ using one-way analysis of variance, along with Tukey's test as the post hoc test.

RESULTS AND DISCUSSION

Identification of phenolic compounds

The identification of phenolic compounds in soil-grown and hydroponically grown *T. flagelliforme* was identified via LC-MS analysis. A total of 8 and 16 phenolic compounds were identified in soil-grown and hydroponically grown *T. flagelliforme* from both ionisation modes, respectively (Tables 1 and 2). Flavonoids, particularly isovitexin, apigenin, luteolin and kaempferol derivatives, were prominently identified in both soil-grown and hydroponically grown *T. flagelliforme*. The abundance of isovitexin and its derivatives was extensively identified in both growing conditions at *m/z* 564 $[M + H]^+$, *m/z* 432 $[M - H]^-$ and *m/z* 770 $[M - H]^-$ from positive and negative ionisation modes. The presence of these flavonoids was further confirmed by Farida et al.[16] from the ethyl acetate extract of *T. flagelliforme* via nuclear magnetic resonance spectroscopy. Isovitexin (apigenin-6-C-glucoside) has been extensively studied in the previous studies. The configuration of the o-dihydroxyl (catechol) structure located at the A ring enhances the free radical scavenging ability of the plant through resonance stabilisation and donation of hydrogen atom.[1,16] Furthermore, it has been found to demonstrate pro-apoptotic action, thereby hindering the proliferation of cancer cells. Meanwhile, apigenin-7-O-glucoside, a naturally occurring flavone (a subclass of flavonoid) with anti-inflammatory properties, was exclusively detected at *m/z* 433 $[M + H]^+$ in hydroponically grown samples. Apigenin has been shown to promote various anti-inflammatory pathways, including the p38/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/Akt pathways.[17,18] This flavonoid enhances the expression of several antioxidant enzymes such as glutathione synthase, catalase and superoxide dismutase, providing protection against oxidative stress.[19] Luteolin derivatives were also detected at *m/z* 595 $[M + H]^+$ and *m/z* 447 $[M - H]^-$ in both soil-grown and hydroponically grown plant extracts. Luteolin has been shown to be highly associated with anti-inflammatory activity, inhibiting several key inflammatory pathways, including cytokine expression, nuclear factor-κB signalling and Toll-like receptor 4 signalling, in immune cells such as mast cells at micromolar concentrations.[20,21] Kaempferol derivatives were also detected at *m/z* 709 $[M - H]^-$ in hydroponically grown extracts and this result is consistent with Septaningsih et al.[22] The pharmacokinetics of these polyphenolic compounds as antimicrobial agents have been extensively studied using in vitro and in vivo assays. Several mechanisms of action have been suggested in the previous studies, such as inhibiting bacterial DNA gyrase, efflux pumps (especially in Gram-negative bacteria like *E. coli* and *P. aeruginosa*), and disrupting the integrity of bacterial cell membranes, resulting in the seepage of cellular contents and the consequent loss of vital ions.[23,24] The presence of these phytochemicals suggests the potential of a hydroponic cultivation system to promote their synthesis.

The phenolic acids, such as 4-*p*-coumaroylquinic acid (hydroxycinnamate ester, where the coumaric acid is esterified with quinic acid), 16-feruloyloxypalmitat (ferulic acid moiety esterified to palmitic acid) and quinic acid, were detected in both soil-grown and hydroponically grown plants at *m/z* 337 $[M - H]^-$, *m/z* 447 $[M - H]^-$ and *m/z* 191 $[M - H]^-$, respectively. These phenolic acids may contribute to the antioxidant and antibacterial activities of *T. flagelliforme*. Coumaric and ferulic acids are two of the most common hydroxycinnamic acids in medicinal herbs.[25] Epidemiological studies have proven that they could effectively inhibit oxidative damage-induced diseases due to the reactivity of the phenol moiety.[25,26] The antimicrobial effectiveness of phenolic acids is significantly influenced by their chemical structure. Key factors include the length of the saturated carbon chains, the arrangement and quantity of substituents on the benzene ring, and the presence of ester groups. It is noteworthy that the antimicrobial activity tends to be lower in the phenolic acids themselves compared to their esters.[26] Moreover, the presence of quinic acid is in accordance with Septaningsih et al.[22] at the exact same *m/z* of 191.

TABLE 1 Identification of phenolic compounds in *Typhonium flagelliforme* at positive ionisation mode by LC-MS analysis.

Compound	Retention time (min)	Molecular weight (g/mol)	$[M + H]^+$	Peak area (%)
Soil-grown				
Isovitexin 2″-O-xyloside	8.33	564	565	0.525 ± 0.06
Hydroponically grown				
Luteolin 7-rhamnosyl(1- >6)galactoside	8.00	594	595	0.43 ± 0.06
Isovitexin 2″-O-xyloside	8.32	564	565	0.50 ± 0.01
3-*tert*-Butyl-5-methylcatechol	8.53	180	181	0.12 ± 0.03
Apigenin 7-O-glucoside	8.82	432	433	0.24 ± 0.04
8-*trans*-[2-(6-Benzoyloxy-4-hydroxy-2-methoxy-3-methylphenyl)ethenyl]-5-methoxyflavan-7-ol	13.84	538	539	0.68 ± 0.04

Abbreviation: LC-MS, liquid chromatography-mass spectrometry.

TABLE 2 Identification of phenolic compounds in *Typhonium flagelliforme* at negative ionisation mode by LC-MS analysis.

Compound	Retention time (min)	Molecular weight (g/mol)	$[M - H]^-$	Peak area (%)
Soil-grown				
Catechin-4-ol 3-O-beta-D-glycopyranoside	0.68	468	467	0.585 ± 0.02
Isoorientin 2″-O-arabinoside	8.06	580	579	0.315 ± 0.01
Isovitexin	8.82	432	431	0.445 ± 0.05
Isovitexin 7-O-beta-[6‴-O-(*E*)-p-feruloyl]glucoside	8.89	770	769	0.885 ± 0.52
6-Hydroxyluteolin 5-rhamnoside	8.39	448	447	0.75 ± 0.04
Rhamnocitrin 3-(5‴-*p*-coumarylapiosyl)-(1- >2)-glucoside	9.05	741	739	0.905 ± 0.08
Desacetyl (7) khivorinic acid, methyl ester	15.04	537	535	0.38 ± 0.04
16-Feruloyloxypalmitate	19.92	449	447	0.602 ± 0.69
Hydroponically grown				
Quinic acid	0.68	192	191	0.77 ± 0.02
Catechin-4-ol 3-O-beta-D-glycopyranoside	0.69	468	467	0.595 ± 0.06
Robinetin 3-rutinoside	7.74	611	609	0.675 ± 0.04
Isoorientin 2″-O-arabinoside	8.07	581	579	0.50 ± 0.04
6-Hydroxyluteolin 5-rhamnoside	8.40	448	447	0.775 ± 0.02
4-*p*-Coumaroylquinic acid	8.55	338	337	0.23 ± 0.00
Isovitexin	8.83	432	431	1.71 ± 0.04
Isovitexin 7-O-beta-[6‴-O-(*E*)-*p*-feruloyl]glucoside	8.89	770	769	1.36 ± 0.06
Kaempferol 3-(2″-(*E*)-*p*-coumaroyl-alpha-L-arabinofuranoside)-7-rhamnoside	9.00	711	709	0.285 ± 0.02
Rhamnocitrin 3-(5‴-*p*-coumarylapiosyl)-(1- >2)-glucoside	9.05	741	739	1.955 ± 0.09
16-Feruloyloxypalmitate	19.7	449	447	0.36 ± 0.35

Abbreviation: LC-MS, liquid chromatography-mass spectrometry.

This type of phenolic acid has shown multifaceted pharmacological activities, including antibacterial, antiviral, antidiabetic and anticancer effects, with promising effectiveness. For example, quinic acid can exhibit antibacterial activity via the ribosomal modulation and disruption of aminoacyl-tRNA synthesis, along with changes in glycerophospholipid and fatty acid levels, which interfere with the oxidative phosphorylation and consequently affect membrane fluidity.[27,28] In terms of anticancer activity, quinic acid has been found to promote cellular apoptosis and enhanced endothelial permeability while inhibiting invasive behaviours via the downregulation of activator protein 1 and specific MAPK signalling pathways.[29] Catechin derivatives were detected at *m/z* 467 $[M - H]^-$ in both soil-grown and hydroponically grown plant extracts. Catechin is a well-documented polyphenolic compound with a general configuration of flavon-3-ol with an additional benzene ring.[30] It has been shown to exhibit remarkable antioxidant properties, primarily due to the catechol structure in the phenolic

B ring.[30,31] Unlike other flavonoid compounds, the absence of a 1,4-pyron moiety in catechin enables efficient electron delocalisation across the rings with relatively lower bond dissociation enthalpy.[30]

Phenolic compounds that were solely observed in soil-grown *T. flagelliforme* are likely a result of the environmental stressors associated with soil cultivation. In soil, plants tend to face more challenges, such as fluctuating nutrient availability, water scarcity due to competition and increased exposure to microbial activity.[32,33] These stressors often lead to stomatal closure with reduced carbon dioxide absorption in the Calvin cycle. As a result, the plant synthesises more secondary metabolites, such as phenolic compounds, as an auxiliary system to dissipate excess energy.[32] Conversely, hydroponic cultivation systems with precisely controlled environmental factors have supported the metabolic activity and biosynthesis of secondary metabolites.[32] Hence, the results suggest that the phenolic compounds observed solely in hydroponically grown plant extracts may be associated with nutrient abundance and favourable growing conditions rather than adaptive responses to stress factors in the soil cultivation system. The controlled nature of hydroponic cultivation systems havepotentially provoked the production of certain phenolics while suppressing the production of stress-induced phenolic compounds.[34] Both cultivation systems influenced the phenolic profile in different ways: soil-grown plants tend to produce phenolic compounds in response to environmental stress, such as 6-hydroxyluteolin 5-rhamnoside and desacetyl (7) khivorinic acid, methyl ester (Table 2). Hydroponically grown plants synthesise phenolic compounds as a result of optimal nutrient conditions and favourable growth parameters, such as luteolin 7-rhamnosyl (1- >6)galactoside, apigenin 7-O-glucoside, quinic acid and kaempferol derivatives (Tables 1 and 2).

TPC and TFC

Assessing the total phenolic and flavonoid content of *T. flagelliforme* is crucial in this study because these compounds are known to contribute to the plant's antioxidant properties, which involve neutralising reactive oxygen species (ROS) and preventing oxidative damage in biological systems. Phenolic compounds are a class of secondary metabolites synthesised in plant bodies that can be further classified into four main categories: (1) flavonoids, (2) stilbenes, (3) lignins and (4) phenolic acids.[35,36] In the preliminary study, plant extracts obtained with absolute ethyl acetate showed the highest TPC values compared to those obtained with other solvents, as shown in the Appendix Table A1. As a result, absolute ethyl acetate was selected as the extractant for this study. It was found that hydroponically grown *T. flagelliforme* exhibited a significantly higher TPC than soil-grown plants (Table 3). The TPC values observed for the soil-grown extracts were slightly lower than those reported in previous literature. For example, Mohan et al.[82] reported TPC values for *T. flagelliforme* ranging from 2.20 ± 0.25 mg GAE/g to 5.21 ± 0.82 mg GAE/g, depending on the extraction solvent. Their study noted the highest TPC in dichloromethane extracts, followed by hexane, ethyl acetate and methanol. Several factors may explain this discrepancy, including soil conditions, geographic factors, plant maturity stage and differences in extraction methods.

TABLE 3 TPC and TFC for soil-grown and hydroponically grown *Typhonium flagelliforme*.

Cultivation method	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	Ratio
Soil	16.7 ± 0.57[a]	6.75 ± 2.38[a]	0.41
Hydroponic	49.0 ± 3.30[b]	22.6 ± 3.45[b]	0.46

Note: [ab]Different superscript letters within a column indicate significant difference at $p < 0.05$.
Abbreviations: CE, catechin equivalent; GAE, gallic acid equivalent; TFC, total flavonoid content; TPC, total phenolic content.

When comparing with other plants, the TPC of hydroponic *T. flagelliforme* (48.96 ± 3.30 mg GAE/100 g) has surpassed common cruciferous vegetables such as cauliflower (19.1 ± 3.42 mg GAE/100 g) and Brussels sprouts (37.7 ± 4.51 mg GAE/100 g), as reported by Singh et al.[37] Similarly, it was found that hydroponically grown *T. flagelliforme* contained a significantly higher TFC than soil-grown plants. However, there is no existing literature reporting on the TFC of *T. flagelliforme*. The TFC of hydroponically grown *T. flagelliforme* (22.62 ± 3.45 mg CE/100 g) was higher than that of common vegetables such as onion (17 ± 2.16 mg CE/100 g), tomato (11 ± 0.94 mg CE/100 g) and garlic (12 ± 1.69 mg CE/100 g), as reported by Saeed et al.[38] The ratio of TFC to TPC was slightly higher for hydroponically grown plants (0.46) than that for soil-grown plants (0.41). Furthermore, the TPC and TFC values were found to be higher than those of some common medicinal herbs, such as *Saraca asoca*, *Cassia fistula*, *Caesalpinia sappan* and *Acacia sinuta*.[39]

This result is also in accordance with Abeysinghe et al.,[40] where hydroponically grown medicinal herb *Acmella oleracea* from *the Asteraceae* family was found to demonstrate significantly higher TPC and TFC. In the study of Agarwal et al.[41] it was confirmed that the type of cultivation system significantly affects the phytochemical composition of lettuce, and the TFC of hydroponically grown plant extract (0.12 ± 0.003 g/100 g) was significantly higher than that of soil-grown plants (0.07 ± 0.01 g/100 g). However, in this study, hydroponically grown lettuce has demonstrated a lower TPC than soil-grown plants. Contradictory results were also found in the study of Dass et al.,[42] where both TPC and TFC were significantly lower in hydroponically grown medicinal herb *Plectranthus amboinicus* than in soil-grown plants. These discrepancies between the findings of each study may be attributed to variations in experimental design, including differences in environmental parameters such as light intensity and humidity levels, as well as the use of different extraction methods with varying types and concentrations of solvents being used as extractants. Moreover, it is noteworthy that the phytochemical response to the cultivation system may be species-specific, which is influenced by the plant physiology.

While phenolic and flavonoid content has been extensively studied in soil-grown *T. flagelliforme*, there is no published data available for hydroponically grown variants. This study has addressed this

TABLE 4 IC_{50} and anti-radical power for soil-grown and hydroponically grown *Typhonium flagelliforme*.

Method	IC_{50} (mg/mL)		
	DPPH	FIC	ARP
Soil	2.37 ± 0.17[a]	5.51 ± 0.07[a]	0.42 ± 0.02[a]
Hydroponic	1.99 ± 0.19[b]	4.19 ± 0.08[b]	0.50 ± 0.04[b]

Note: [ab]Different superscript letters within a column indicate significant difference at $p < 0.05$.
Abbreviations: ARP, anti-radical power; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; FIC, Ferrous iron chelating; IC_{50}, half-maximal inhibitory concentration.

research gap by conducting the first quantitative assessment of TPC and TFC in hydroponically cultivated *T. flagelliforme* and comparing it with the traditional soil-grown system. The observed variations in TPC and TFC between hydroponically grown and soil-grown plants could be attributed to several factors. Hydroponic cultivation systems offer more controlled and consistent nutrient delivery, which may enhance the production of secondary metabolites such as phenolics and flavonoids in plants. Moreover, Sambo et al.[43] highlighted that hydroponic cultivation systems enable maximum absorption of both macro- and micronutrients, which are readily available in the nutrient solution. This statement is supported by Kumar et al.,[44] who found that most hydroponically grown plants have higher bioactive content, especially phenols and flavonoids in mint crops. In contrast, soil-grown counterparts might experience more environmental stress from both abiotic and biotic factors, such as salinity, temperature fluctuations and plant pathogens, potentially leading to higher or lower phytochemical concentrations depending on the plant's adaptive responses.[45] The potential lack of beneficial soil microbes in hydroponic cultivation systems could explain the contradictory results in Dass et al.,[42] as the absence of these microbes might limit the natural processes (nutrient cycling and plant metabolism) that enhance nutrient uptake and stimulate the production of secondary metabolites. Moreover, the discrepancies between the findings of each study may also be attributed to variations in the experimental design, including differences in environmental parameters such as light intensity and humidity levels, as well as the use of different extraction methods with varying types and concentrations of solvents being used as extractants.

DPPH radical scavenging activity

In this study, the antioxidant potential of *T. flagelliforme* was evaluated by assessing DPPH radical scavenging activity and FIC activity. The findings showed that the IC_{50} of hydroponically grown extracts was significantly lower ($p < 0.05$) than that of soil-grown extracts (Table 4). This suggests that the hydroponic samples have a higher antioxidant potential. As a result, plants grown in hydroponic systems had significantly higher ARP (0.50 ± 0.04) than plants grown in soil (0.42 ± 0.02). These significant differences in IC_{50} and ARP between the cultivation methods may be attributed to the higher TPC observed in hydroponic plants. The IC_{50} values of soil-grown extracts reported in this study were relatively higher than those found by Farida et al.,[16] who reported IC_{50} values as low as 0.056 ± 0.003 mg/mL for ethyl acetate fractions of *T. flagelliforme*. This discrepancy may be attributed to differences in extraction methods, solvent polarity, cultivation conditions, plant maturity and storage conditions, which can affect the concentration and composition of antioxidant compounds in plant extracts. Meanwhile, there is no existing literature reporting on the DPPH scavenging capacity of hydroponically grown *T. flagelliforme*.

In terms of comparative antioxidant efficacy, the IC_{50} of hydroponically grown *T. flagelliforme* reported in this study is lower than that of several commonly consumed vegetable extracts, such as carrot, cucumber and turnip, as reported by Djenidi et al.[46] This highlights the potential of hydroponically grown *T. flagelliforme* as a potent source of natural antioxidants with comparable efficacy in antioxidant activity.

These findings are consistent with previous studies that have shown higher antioxidant potential in hydroponically grown plants. This is in accordance with the study by Surendran et al.,[47] which reported that a stronger scavenging effect was observed in hydroponically grown *Mentha spicata* (a medicinal herb from the *Lamiaceae* family) than in soil-grown samples across ABTS, DPPH and nitric oxide assays. Similarly, Negrao et al.[48] reported that lettuce grown in a hydroponic cultivation system exhibited a higher content of bioactive compounds and greater antioxidant capacity compared to soil-grown lettuce. These results suggest that hydroponic cultivation may create an optimal environment for the synthesis of phenolic compounds, enhancing the plant's overall antioxidant properties.

The increase in antioxidant activity in hydroponically grown plants may be attributed to higher TPC, which is in accordance with the studies of Polumackanycz et al.[49] and Muflihah et al.[50] Both studies have suggested that the antioxidant properties of plant extracts are strongly correlated with the TPC values. Additionally, Wang et al.[51] reported a strong and significant correlation between TPC and DPPH after Pearson correlation analysis (PCA), as shown in the PCA score plot and PCA loading plot.

Phenolic compounds, including flavonoids (dominant groups), tannins, phenolic acids and anthocyanins, are natural antioxidants. They have a C15 skeleton (C6–C3–C6) and hydroxyl groups (-OH) with a reduction potential that allows them to effectively stabilise ROS and prevent autoxidation.[52,53] The resonance effect from the aromatic ring structure of phenolic compounds stabilises the phenoxyl radical that forms when an electron is donated.[54] This is achieved by the delocalisation of unpaired electrons within the conjugated double bonds (π-system) of the aromatic ring. This electron delocalisation reduces the reactivity of the phenoxyl radical (lower energy state). Moreover, the abundance of phenolic compounds enhances the antioxidant activity by quenching singlet oxygen, which readily reacts with lipid molecules and causes consequent cellular damage.[55] The donation of electrons intercepts singlet oxygen and reverts it to the ground state (less reactive triplet oxygen), thereby reducing the oxidative stress.[56] In the study of Zeb,[57] the antioxidant potential of

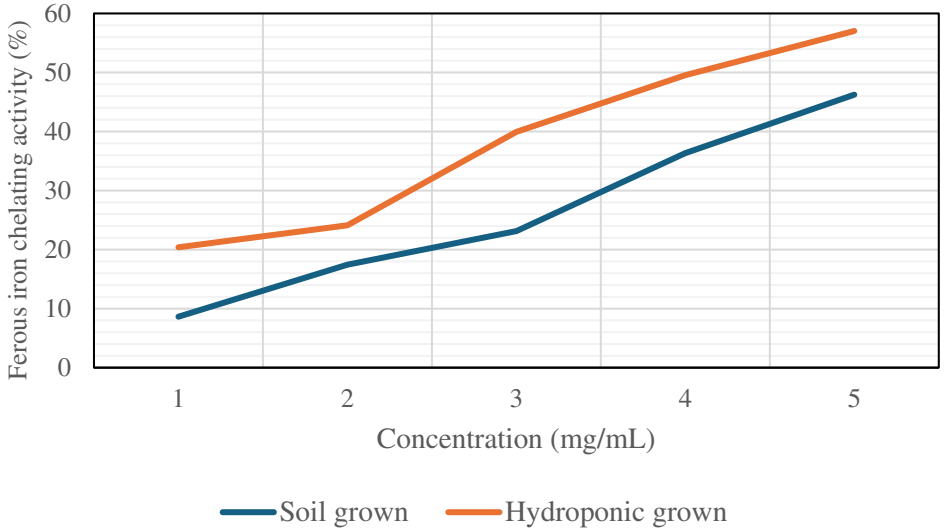


FIGURE 1 Ferrous iron chelating activity of soil-grown and hydroponically grown *Typhonium flagelliforme*.

phenolic compounds can be attributed to other mechanisms, such as sequential proton loss electron transfer, where phenolic compounds initially lose a proton to a free radical, forming a phenoxide anion that donates an electron consequently, resulting in the formation of a stable molecule. It has been reported that this phenomenon is commonly observed in flavonoid compounds like epicatechin and catechin, with the hydroxyl group at the 4-site from the B-ring being the preferential site for hydrogen donation.[57,58]

FIC activity

For both soil-grown and hydroponically grown *T. flagelliforme*, FIC activity increased with increasing plant extract concentration (Figure 1). A linear relationship was observed between the FIC activity (%) and concentration of both plant extracts. The hydroponically grown *T. flagelliforme* consistently showed higher FIC activity compared to soil-grown plants at all tested concentrations (Table 5). Previous studies have not investigated the FIC activity of *T. flagelliforme*. The hydroponically grown *T. flagelliforme* was found to exhibit a similar FIC activity to that of other medicinal herbs from botanical families such as *Lamiaceae*, *Apiaceae* and *Asteraceae*.[59] This similarity suggests that *T. flagelliforme* may share common bioactive compounds or structural characteristics with these other medicinal plants, such as higher levels of flavonoids and other polyphenols.

This enhanced activity can be attributed to the higher flavonoid content in hydroponically cultivated plants, as shown in other studies.[60,61] The hydroponically grown *T. flagelliforme* also exhibits similar FIC activity to that of medicinal herbs from botanical families such as *Lamiaceae*, *Apiaceae* and *Asteraceae*, as indicated in the study of Ulewicz-Magulska et al.[59] This similarity suggests that *T. flagelliforme* may share common bioactive compounds or structural characteristics, such as higher levels of flavonoids and other polyphenols, compared to soil-grown *T. flagelliforme*. Increasing in vitro studies agree that

TABLE 5 Ferrous iron chelating activity of soil-grown and hydroponically grown *Typhonium flagelliforme*.

	Ferrous iron chelating activity (%)	
Concentration (mg/mL)	Soil-grown	Hydroponic grown
1	8.68 ± 0.92^{a}	20.4 ± 3.86^{b}
2	17.5 ± 1.02^{a}	24.2 ± 3.14^{b}
3	23.2 ± 2.94^{a}	40.0 ± 1.88^{b}
4	36.4 ± 2.92^{a}	49.6 ± 5.99^{b}
5	46.3 ± 2.02^{a}	57.1 ± 1.02^{b}

Note: ^{ab}Different superscript letters within a row indicate significant difference at $p < 0.05$.

flavonoids are potent metal chelators due to the presence of metal-binding sites (carbonyl and hydroxyl moieties) and the ortho-dihydroxyl (catecholate) structure with metal-chelating ability.[62] Flavonoids like myricetin and quercetin, with strong affinity for ferrous iron, actively prevent ferrous iron from catalysing Fenton reactions (redox cycling processes) that involve the decomposition of hydrogen peroxide or lipid peroxides, which contribute to oxidative stress exacerbation by generating ROS.[62] Ultimately, this redox cycling process results in lipid peroxidation, disrupting cellular homeostasis (metal-induced hemolysis) and causing DNA damage.[61,63]

Flavonoids like quercetin with three metal-binding sites (α-hydroxy-carbonyl, β-hydroxy-carbonyl or catechol) are potent metal chelators, especially for pro-oxidant metal ions like copper ions and ferrous iron.[64–66] Additionally, other phenolic compounds such as propyl gallate, gallic acid, caffeic acid and pyrogallol also exhibit metal-chelating ability due to the enhanced stability of Fe^{3+} interactions

TABLE 6 MIC values of soil-grown and hydroponically grown *Typhonium flagelliforme* and positive control, CAM.

Bacteria	ATCC no.	MIC (mg/mL)		
		Soil-grown	Hydroponic grown	CAM
MRSA	33,591	>25	>25	0.015
Staphylococcus aureus	29,213	12.5	12.5	0.005
Bacillus subtilis	8188	3.13	1.56	0.020
Enterococcus faecalis	29,212	12.5	6.25	0.015
Pseudomonas aeruginosa	10,145	3.13	1.56	0.005
Escherichia coli	25,922	12.5	3.13	0.020
Salmonella typhimurium	14,028	6.25	6.25	0.015
Shigella flexneri	12,022	6.25	3.13	0.015

Abbreviations: ATCC, American Type Culture Collection; CAM, chloramphenicol; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.

with the oxygen ligands of phenol groups, which have strong electron-donating properties that stabilise the Fe^{3+} state.[67] Moreover, the hydroponically grown plant extracts (4.19 ± 0.08 mg/mL) have demonstrated comparable FIC activities in comparison with the other common medicinal plants from Turkey, such as the aqueous fractions of *Hedysarum varium*, *Onobrychis hypargyrea* and *Vicia truncatula*.[68]

Antibacterial activity

In the case of Gram-positive bacteria, both plant extracts showed MIC values greater than 25 mg/mL against MRSA (ATCC 33591), indicating limited inhibitory activity (Table 6). For *S. aureus* (ATCC 29213), a similar MIC value of 12.5 mg/mL was determined for both soil- and hydroponically grown plant extracts. For *B. subtilis* (ATCC 8188), hydroponically grown extracts exhibited a significantly lower MIC value of 1.56 mg/mL compared to 3.13 mg/mL for soil-grown extracts. A similar trend was observed for *E. faecalis* (ATCC 29212), where hydroponically grown extracts had an MIC of 6.25 mg/mL, whereas soil-grown extracts required 12.50 mg/mL to inhibit bacterial growth.

For Gram-negative bacteria, hydroponically cultivated plant extracts showed an MIC of 1.56 mg/mL against *P. aeruginosa* (ATCC 10145), which was more effective than the 3.13 mg/mL observed for soil-grown extracts. The largest discrepancy was observed in *E. coli* (ATCC 25922), where hydroponically grown plant extracts showed a significantly lower MIC value of 3.13 mg/mL compared to 12.5 mg/mL for soil-grown samples. For bacterial strains such as *S. typhimurium* (ATCC 14028) and *S. flexneri* (ATCC 12022), hydroponically grown extracts showed slightly stronger antibacterial activity, with MIC values of 6.25 and 3.13 mg/mL, respectively. In contrast, soil-grown plant extracts showed a similar MIC value of 6.25 mg/mL for both bacterial strains. Overall, hydroponically grown plant extracts exhibited stronger antibacterial activity against most tested strains compared to soil-grown plant extracts. This finding is in alignment with Farida et al.,[16] which found that *T. flagelliforme* has inhibitory activity against both Gram-positive and Gram-negative bacteria. For the positive control, chloramphenicol displayed complete inhibition against both Gram-positive and Gram-negative bacteria. Meanwhile, turbidity was observed for all bacteria tested, indicating bacterial growth.

The results indicate that hydroponic cultivation improved the potency of plant extracts against most of the tested bacteria. This corresponded to the higher TPC and TFC values, as shown in Table 3. The enhanced antibacterial activity in hydroponically grown plants was also observed in the study by Tadevosyan et al.,[69] in which hydroponically grown *Moringa oleifera* displayed superior performance in inhibiting both Gram-positive and Gram-negative bacteria. Additionally, the hydroponically grown extracts have demonstrated stronger antibacterial activity against Gram-negative bacteria than Gram-positive bacteria. These results align with studies by Tadevosyan et al.[69] and Juan et al.,[70] suggesting that the thinner peptidoglycan layer in Gram-negative bacteria as a contributing factor to their susceptibility. This particularly high susceptibility in these specific strains may be due to their increased sensitivity to certain phytochemicals found in *T. flagelliforme* (Tables 1 and 2), which are more abundant in hydroponically grown extracts, as revealed in the studies by Coppo and Marchese[71] and Ergüden.[72] Coppo and Marchese[71] have stated that phenolic acids like gallic acid and ethyl gallate have demonstrated enhanced antibacterial activity against Gram-negative bacteria, especially respiratory pathogens such as *P. aeruginosa*, *S. aureus* and *E. faecalis*, which were assessed in this study. This is in accordance with Alibi et al.[73] and Lobiuc et al.,[74] where a wide range of bacteria showed highly susceptibility to phenolic acids and chlorogenic acid, which cause drastic changes in the extracellular pH (hyper-acidification) due to their intrinsic chemical properties. Phenolic terpenes and terpenoids are reported to inhibit both Gram-positive and Gram-negative bacteria through ion leakage (interruption of ion homeostasis) and disruption of structural integrity (membrane permeabilisation), respectively.[72,75,76] The hydrophobic skeleton of terpenoids facilitates easier penetration through the lipid bilayer composed of phospholipids. It was found that flavonoids like quercetin could affect DNA/RNA synthesis by inhibiting the enzymatic activity of DNA gyrase and protein kinase in bacteria.[77] Moreover, it was

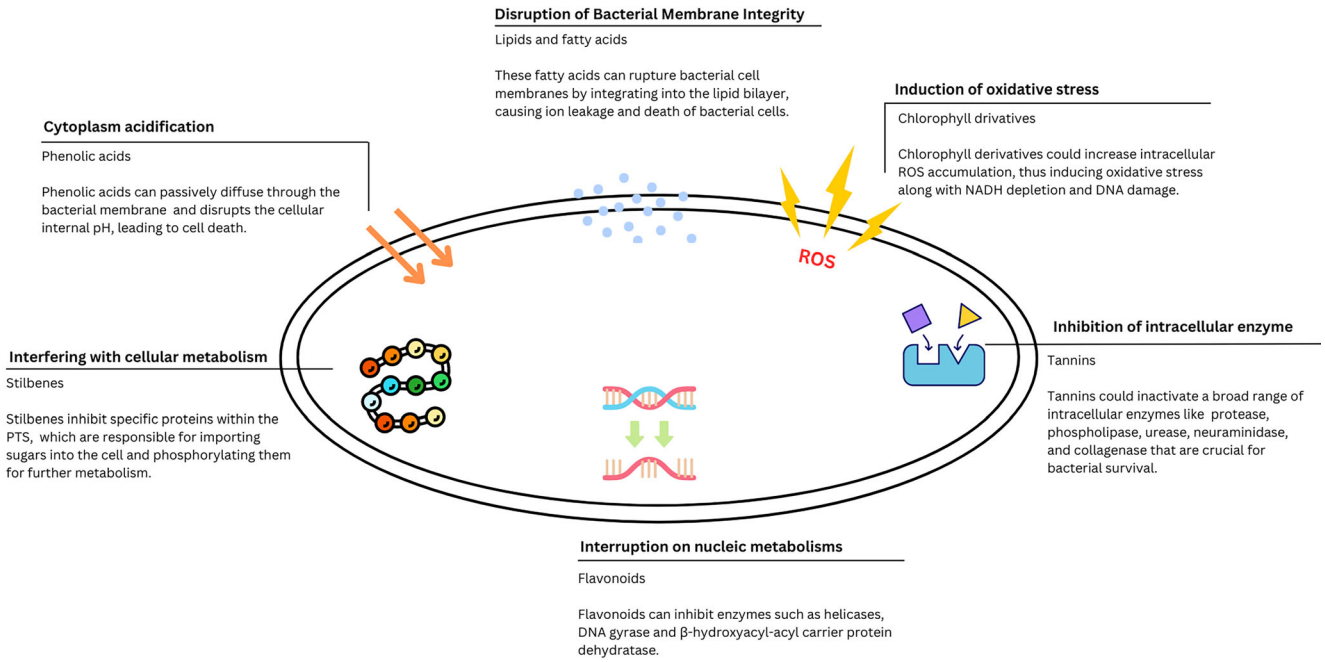


FIGURE 2 A summary of the possible modes of action for phytochemicals present in *Typhonium flagelliforme*.

reported that flavonoids could bind to the GyrB subunit of *E. coli* DNA gyrase, reducing ATPase activity and increasing inner membrane permeability.[78,79] Furthermore, flavonoids disrupt bacterial energy metabolism by interacting with the respiratory electron transport chain, thereby affecting oxygen consumption.[78] For phenolic compounds like tannins, which have been tested positive in *T. flagelliforme* in the previous studies, they were also found to demonstrate antibacterial activity through inhibition of enzymes that are responsible for cell wall synthesis and oxidative phosphorylation.[79] Meanwhile, another phenolic compound, such as stilbenes, affects the bacterial energy metabolism by inhibiting the enzymatic activity involved in the phosphotransferase system.[23] This is a common bacterial mechanism for transporting and phosphorylating sugars (such as glucose, mannose and sorbitol) for ATP production. The mode of action for phytochemicals present in *T. flagelliforme* has been summarised in Figure 2.

In comparison to the results shown in Bussmann et al.[80] and Sharma et al.,[81] the hydroponically cultivated plant extracts exhibited a significantly stronger antibacterial activity than soil-grown counterparts, particularly against *E. coli* (a human pathogenic bacterium), surpassing most of the medicinal plants used in Northern Peru and Mexico. Nevertheless, there is no existing literature reporting on the MIC values of *T. flagelliforme*. Given its enhanced antimicrobial activity, it is suggested to test hydroponically grown *T. flagelliforme* against a broader spectrum of bacteria, including multidrug-resistant strains like vancomycin-resistant *Enterococci* (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE) and *Mycobacterium tuberculosis* (MDR-TB).

Both soil-grown and hydroponically grown extracts showed limited efficacy against MRSA (>25 mg/mL), indicating that neither cultivation method enhanced the antimicrobial activity of the extracts against MRSA, which is consistent with the study by Syam Mohan et al.[82] The limited inhibitory effect against MRSA may be due to several reasons, such as the (1) presence of altered penicillin-binding proteins (PBP2a) encoded by methicillin-resistant genetic component (*mecA) genes*, (2) production of the penicillinase enzyme, (3) abundance of long-chain fatty acyl peptidoglycan and (4) presence of efflux pumps that work as a defence mechanism for MRSA.[83–85] Despite the results from this study, it is still believed that phytochemicals in herbal plants could effectively inhibit MRSA by interfering with efflux pumps, altering active sites, enhancing plasma membrane permeability and modifying bacterial enzymes, as stated by Shoaib et al.[86] Since neither cultivation method has produced an effective inhibition at the concentrations tested, it has highlighted the need for either higher concentrations or different extraction methods that might improve yield or efficacy against MRSA in future studies. The consistency in MIC values for *S. aureus* (12.5 mg/mL) and *S. typhimurium* (6.25 mg/mL) across both cultivation methods suggests that the specific antibacterial compounds that are responsible for inhibiting these bacteria are perhaps naturally less susceptible to changes in phytochemical profiles caused by differences in cultivation methods.

CONCLUSIONS

This study has demonstrated that hydroponic cultivation significantly enhanced the phytochemical content, antioxidant capacity and antibacterial efficacy of *T. flagelliforme* compared with conventional soil-based cultivation. Hydroponically grown *T. flagelliforme* exhibited a significantly higher TPC (49.0 ± 3.30 mg GAE/100 g), higher TFC (22.6 ± 3.45 mg CE/100 g), lower IC_{50} in radical scavenging activity (1.99 ± 0.19 mg/mL), lower FIC activity (4.19 ± 0.08 mg/mL) and

higher ARP (0.50 ± 0.04) compared to those (16.7 ± 0.57 mg GAE/100 g, 6.75 ± 2.38 mg CE/100 g, 2.37 ± 0.17 mg/mL, 5.51 ± 0.07 mg/mL and 0.42 ± 0.02, respectively) of soil-grown plants. In LC-MS analysis, 9 and 16 phenolic compounds were identified in soil-grown and hydroponically grown plant extracts, respectively. The seven phenolic compounds, including isovitexin 2″-O-xyloside, catechin-4-ol 3-O-beta-D-glycopyranoside, isoorientin 2″-O-arabinoside, isovitexin, isovitexin 7-O-beta-[6‴-O-(*E*)-*p*-feruloyl]glucoside, 6-hydroxyluteolin 5-rhamnoside and 16-feruloyloxypalmitate, were identified in both cultivation systems. Hydroponically grown plants exhibited promising levels of total phenolic and flavonoid contents, which correlated with stronger DPPH radical scavenging and FIC activities. Additionally, hydroponic extracts showed greater antibacterial potency against both Gram-positive and Gram-negative bacteria than soil-grown extracts, particularly *B. subtilis* (1.56 mg/mL vs. 3.13 mg/mL), *P. aeruginosa* (1.56 mg/mL vs. 3.13 mg/mL), *E. coli* (6.25 mg/mL vs. 12.5 mg/mL) and *S. flexneri* (3.13 mg/mL vs. 6.25 mg/mL). These findings have underscored the potential of hydroponically cultivated *T. flagelliforme* as a potent and novel source of natural antioxidants and antimicrobial agents, supporting its use in functional food and pharmaceutical applications. Given that most previous studies were in vitro, it is recommended to conduct in vivo studies to validate the efficacy of *T. flagelliforme*. While this study has shown promising in vitro results, the safety of *T. flagelliforme* extracts in vivo remains unknown. Hence, establishing a safety profile, including cytotoxicity studies and evaluations of the long-term effects in humans, is crucial before considering any in vivo studies.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A

TABLE A1 TPC of soil-grown *Typhonium flagelliforme* using various solvents.

Solvent	TPC (mg GAE/g)
70% ethanol	0.52 ± 0.05[a]
100% ethanol	3.36 ± 0.49[b]
70% ethyl acetate	0.51 ± 0.05[c]
100% ethyl acetate	3.86 ± 0.35[da]

Abbreviations: GAE, gallic acid equivalent; TPC, total phenolic content.