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Research Article
Macroscopic and Microscopic Characterization, Phytochemical Profiling (HPLC-GC-MS) and Antioxidant Activity of Leaf and Stem Extracts of *Tinospora baenzigeri*

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Abstract

Background and Objective: *Tinospora baenzigeri* is used in traditional Thai medicine to reduce fever and restore general health. Limited information is available on the integration of anatomical characteristics with phytochemical and metabolite profiling across different plant tissues. This study aimed to investigate the macro and microscopic characteristics, phytochemicals, HPLC-GC-MS profiles and antioxidant activities of *T. baenzigeri*. **Materials and Methods:** Stems and leaves were extracted with ethanol, ethyl acetate and dichloromethane. Total phenolic and flavonoid contents were determined using colorimetric assays, while antioxidant activities were evaluated using DPPH and ABTS assays. HPLC analysis was conducted to identify major phenolic compounds and GC-MS analysis was used to determine the chemical constituents of the extracts. Triplicate assays were analyzed as Mean±SD using one-way ANOVA with Tukey's test ($p<0.05$) in SPSS. **Results:** Macro-microscopic observations showed diagnostic anatomical features including lenticellate stems, paired domatia on the abaxial leaf surface, anomocytic stomata and calcium oxalate crystals. The ethanolic stem extract showed the highest phenolic content (167.12 mg GAE/g extract) and the potent of antioxidant activity (DPPH 48.09%, ABTS 75.52%).The HPLC analysis identified catechin and rutin in the stem extract. GC-MS profiling revealed that fatty acid ethyl esters and hydrocarbons were the dominant compounds in both plant parts, particularly hexadecanoic acid ethyl ester, ethyl oleate and octadecanoic acid ethyl ester. **Conclusion:** These findings demonstrate tissue-specific differences in phytochemical composition and antioxidant activity between leaf and stem extracts of *T. baenzigeri* and contribute further evidence supporting its traditional medicinal use.

Key words: *Tinospora baenzigeri*, macro-microscopic characteristics, antioxidant activities, HPLC-GC-MS profiling, phytochemical analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tinospora baenzigeri Forman (Menispermaceae), commonly known in Thailand as "Chingcha chalee," is a climbing plant with a broad distribution and a long history of use in traditional herbal medicine. The stem has been used as an antipyretic remedy in a manner comparable to *T. crispa*, though the two species can be distinguished by the smooth surface of the stem and the presence of paired glands at the leaf base[1,2]. The bitter taste of the stem is characteristic and has been associated with its traditional application in the treatment of fever and malaria[3,4]. Phytochemical studies have identified a range of secondary metabolites in this species, including alkaloids, lignans, terpenoids and phenolic compounds, several of which have demonstrated pharmacological relevance[5].

Among these constituents, phenolic and flavonoid compounds have received particular attention for their antioxidant properties. The antioxidant potential of plant-derived compounds plays a key role in mitigating oxidative stress-related diseases, including cancer, cardiovascular disorders, neurodegenerative diseases and aging[6,7]. Antioxidants from medicinal plants neutralize free radicals and help maintain redox homeostasis. Phenolics, flavonoids, alkaloids and terpenoids act primarily through hydrogen atom transfer, single-electron transfer and metal-chelation mechanisms, thereby stabilizing reactive oxygen species (ROS). Increasing evidence supports their therapeutic relevance and value in functional food and phytomedicine development[8,9]. *Tinospora baenzigeri* is rich in phenolic acids, alkaloids, lignans and terpenoids that contribute to its strong antioxidant profile. Compounds from plants, including isoquinoline alkaloids, clerodane diterpenes (e.g., baenzigeride A, baenzigeroside A and B), lignans and phenolic derivatives, have demonstrated promising antioxidant properties in both *in vitro* and *in vivo* studies. These bioactivities support its traditional use in treating fever, inflammation, digestive disorders and as a general health tonic[10-12]. *Tinospora baenzigeri* is also incorporated into traditional formulations such as the "Saban remedy," used to enhance immunity and as a supportive treatment for cancer patients[13]. However, previous studies have focused predominantly on stem tissues and no HPLC or GC-MS chemical profiling data have been reported to date. Therefore, the present study aimed to investigate both the leaves and stems of *T. baenzigeri* through anatomical characterization, phytochemical screening, HPLC-GC-MS profiling and antioxidant activity assessment of extracts obtained using solvents of varying polarity.

MATERIALS AND METHODS

Plant material collection and authentication: Plants were collected from Khon Kaen Province during October 2024. The plants were taxonomically identified and voucher specimens were deposited at the Herbarium, Department of Biology, Faculty of Science, Mahasarakham University.

Extraction of phytochemicals: Powdered samples (50 g) of the leaf or stem were extracted with ethanol, ethyl acetate and dichloromethane using a Soxhlet apparatus for 4 hrs. The resulting mixtures were filtered through Whatman No. 1 filter paper and the filtrates were concentrated under reduced pressure using a rotary evaporator at $\leq$40°C. The dried residues were weighed to determine the extraction yield (% w/w) and stored at 4°C for subsequent analyses.

Microscopic characterization: Hand-cut transverse sections of leaves and stems of *T. baenzigeri* were mounted on glass slides and examined under a compound light microscope (ZEISS Primostar 3, Carl Zeiss, USA) at 4, 10 and 40× magnification. Surface characteristics were assessed from epidermal impressions. For each surface type, three to five sections were observed to obtain representative quantitative anatomical data.

Total phenolic content (TPC): Total phenolic content (TPC) was measured by the Folin-Ciocalteu colorimetric method with slight modifications as described[14]. An aliquot of 100 µL of appropriately diluted extract was combined with 500 µL of Folin-Ciocalteu reagent (1:10, v/v) and left to react for 5 min. Then, 400 µL of 7.5% Sodium Carbonate (Na_2CO_3) was added and the mixture was kept in the dark at room temperature for 30 min. Absorbance was recorded at 765 nm with a UV-Vis spectrophotometer. Gallic acid (0-200 µg/mL) served as the reference standard for the calibration curve and TPC values were reported as milligrams of gallic acid equivalent (GAE) per gram of dry extract.

Total flavonoid content (TFC): The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g). The procedure was adapted from the method described by Suriyaprom *et al.*[15] with minor modifications. Quercetin solutions ranging from 5 to 200 µg/mL were used to construct the standard calibration curve. For each sample, 500 µL of extract (1 mg/mL) was mixed with 1,500 µL of 95% ethanol and incubated at room temperature for 6 min.

Subsequently, 100 µL of 10% aluminum chloride solution was added, followed by a 5 min incubation. Then, 100 µL of 1 M potassium acetate was added and the total volume was adjusted to 5,000 µL with distilled water. The mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 415 nm in triplicate. The TFC was calculated from the quercetin standard curve and expressed as mg quercetin equivalent (QE) per gram of extract.

DPPH radical scavenging assay: Free radical scavenging activity was evaluated using the DPPH assay following a previously reported method with slight modifications[16]. DPPH solution (100 µM) was prepared in methanol. Each crude extract and ascorbic acid, used as a reference standard, was prepared as serial dilutions ranging from 62.5 to 500 µg/mL. Stock solutions of plant extracts were prepared in methanol at 1 mg/mL before dilution. In a 96-well microplate, 180 µL of the DPPH solution was combined with 20 µL of each test solution. Plates were kept in the dark at room temperature for 20 min before absorbance measurement at 517 nm with a microplate reader. Methanol without extract served as the blank control (Ac) and the absorbance of each sample mixture was recorded as (As). The DPPH radical scavenging activity (%) was then calculated according to the following (Eq. 1):

$$\text{Rradical scavenging (\%)} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100 \quad (1)$$

ABTS radical scavenging assay: The ABTS assay was performed following the method with slight modifications[16]. The $ABTS^{\cdot+}$ cation radical was generated by mixing a 7 mM $ABTS^{\cdot+}$ solution with 2.45 mM potassium persulfate and allowing the reaction to stand in the dark at room temperature for 12-16 hrs. Before analysis, the resulting $ABTS^{\cdot}$ solution was diluted with ethanol to obtain an absorbance of 0.700±0.02 at 734 nm. Serial dilutions of each crude extract and ascorbic acid (used as the standard) were prepared at concentrations of 1,000, 500, 250, 125 and 62.5 µg/mL. All the measurements were performed in triplicate, using methanol as the blank.

The percentage inhibition of $ABTS^{\cdot+}$ radical scavenging activity was calculated using the following (Eq. 1).

High-Performance Liquid Chromatography (HPLC) analysis: Phenolic compounds in ethanol extracts of the leaf and stem of *T. baenzigeri* were analyzed using HPLC following a modified method[17]. Separation was performed on an InertSustain® C18 column (4.6×250 mm, 5 µm; GL Sciences, Japan) with a guard column. The column temperature was maintained at 38°C and the flow rate was set at 1.0 mL/min. Samples were prepared by dissolving crude extracts in 50% methanol (1 mg/mL) and phenolic standards (catechin, gallic acid, rutin and quercetin) were prepared in methanol at concentrations of 10-150 µg/mL. A volume of 20 µL of each sample and standard was injected using an autosampler. The mobile phase consisted of water:1% acetic acid: methanol: Acetonitrile (40:15:45, v/v/v/v) and detection was carried out at 260, 280, 340 nm using a UV detector. Phenolic compounds were identified by comparing retention times with authentic standards and quantified using external standard calibration curves.

Gas Chromatography-Mass spectrometry (GC-MS) analysis: The leaf and stem extracts were analyzed by a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) Model 8890 gas chromatograph equipped with a mass selective detector (MS). Data were processed using Agilent MassHunter Qualitative Analysis software version 12.0. Separation was achieved on a fused silica capillary column HP-5 (5% phenyl methyl siloxane; 30×0.25 mm i.d., film thickness 0.25 µm) following the method[18]. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was initially held at 70°C for 3 min and then increased at a rate of 5°C/min to 280°C and maintained at this temperature for 10 min. The transfer line temperature was set at 280°C. Mass spectra were recorded in full scan mode over the m/z range 50-550 amu. Compounds were identified by comparing their retention times and mass spectra with reference data from the Wiley23/NIST23 (National Institute of Standards and Technology) Mass Spectral Library.

Statistical analysis: All the assays were performed in triplicate, with data expressed as Mean±Standard Deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test ($p<0.05$) was conducted using statistical software (SPSS).

RESULTS AND DISCUSSION

Macroscopic features of *Tinospora baenzigeri*: *Tinospora baenzigeri* Forman is a robust woody climber reaching up to 15 m in height and is entirely glabrous throughout. The stems are cylindrical, pale green to greyish when young and become progressively thicker with age, attaining up to 6 cm in diameter. Mature stems bear scattered, finely pustulate lenticels and a bitter milky sap exudes when the stem is cut. Long, slender, filiform aerial roots commonly arise from both nodes and internodes Fig. 1(a-e). The leaves are simple,

Fig. 1(a-e): Macroscopic features of *T. baenzigeri*: (a) Leaves, (b) Stem, (c) Stem showing lenticels, (d) Leaf petioles swollen and geniculate at the base and (e) Leaf with a hollow domatia in the basal nerve axils

alternate and broadly ovate to sub-orbicular, with a thin papery (papyraceous) texture. The leaf blade has an acuminate apex and a cordate base. Leaf and stem morphology are shown in Fig. 1(a-c), including the lenticellate surface of mature stems and the petiole structure. The petiole is 4-10 cm long, swollen and geniculate at the base (Fig. 1d). Venation is palmate, with 5-7 primary nerves radiating from the petiole insertion. Paired domatia are present on the lower leaf surface and represent a defining characteristic of this species (Fig. 1e).

Microscopic features of *T. baenzigeri*: Microscopic examination of the leaf and stem of *T. baenzigeri* showed anatomical features consistent with the family Menispermaceae (Fig. 2). In stem cross-sections (Fig. 2a), a thin epidermis was observed, followed by 3-5 layers of angular collenchyma. The cortex consisted of chlorenchymatous parenchyma containing scattered calcium oxalate crystals. Vascular bundles were arranged in a continuous ring, with phloem on the outer side and xylem vessels positioned internally in radial groups. Calcium oxalate crystals were also present in the leaf tissue (Fig. 2b), mainly in cubic form. The leaf epidermis (Fig. 2c) was uniseriate, composed of polygonal cells with straight to slightly wavy anticlinal walls and bore anomocytic stomata typical of the family. The mesophyll (Fig. 2d) consisted of loosely arranged parenchyma with distinct intercellular spaces and numerous chloroplasts. Lenticels were observed on the outer periderm as ruptured zones of loosely arranged suberized cells, suggesting a role in gas exchange.

Extraction yield and phytochemical constituents: Extraction yields varied depending on the type of solvent and plant part (Table 1). In the leaf samples, ethanol produced the highest extraction yield (11.832%), followed by ethyl acetate (4.245%) and dichloromethane (3.503%). A similar trend was observed in stem samples, where ethanol also gave the highest yield (5.737%), with ethyl acetate (2.262%) and dichloromethane

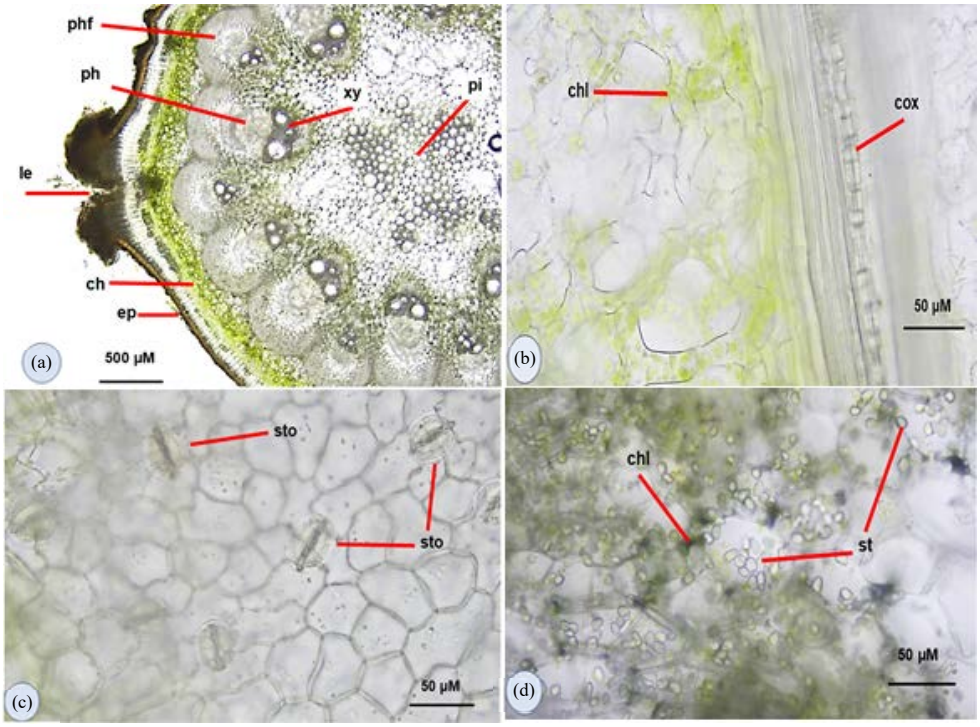

Fig. 2(a-d): Microscopic features of *T. baenzigeri*, (a) Transverse section of stem showing epidermis (ep), chlorenchyma (ch), phloem (ph), xylem (xy) and pith (pi), (b) Lenticel on the stem; showing chloroplast, calcium oxalate cubic crystals, (c) Abaxial leaf epidermis showing anomocytic stomata and (d) Leaf mesophyll parenchyma, showing starch(st), chloroplast (chl)

Ep: Epidermis, chl: Chloroplast, ch: Chlorenchyma, co: Collenchyma, ph: Phloem, xy: Xylem, pi: Pith, sto: Stomata, cox: Calcium oxalate cubic crystal and le: Lenticel, st: Starch

Table 1: Extraction yields of leaf and stem extracts of *T. baenzigeri* using different solvents

	Ethanol		Dichloromethane		Ethyl acetate	
Plant part	Plant (gram)	Yield (%)	Plant (g)	Yield (%)	Plant (g)	Yield (%)
Leaf	5.916	11.832	1.639	3.503	2.123	4.245
Stem	2.869	5.737	0.829	1.656	1.133	2.262

(1.656%) yielding lower amounts. The leaf extracts exhibited higher extraction yields than the stem extracts across all the solvents. These extracts were subsequently used for phytochemical and antioxidant analyses.

GC-MS analysis of leaf and stem extracts: Gas chromatography-mass spectrometry (GC-MS) analysis of the ethanolic extracts showed that long-chain fatty acid ethyl esters and saturated hydrocarbons were the main chemical constituents in both leaf and stem samples of *T. baenzigeri*. The chromatograms of the leaf and stem extracts are presented in Fig. 3a-b, respectively. In the leaf extract (Fig. 3a), the major peaks were assigned to hexadecanoic acid ethyl ester, ethyl oleate and octadecanoic acid ethyl ester, together with smaller contributions from hexadecane, octadecane and isopropyl myristate. The stem extract (Fig. 3b) showed a similar pattern, although linoleic acid ethyl ester and heptadecanoic acid ethyl ester were more clearly detected, along with hexadecanoic acid ethyl ester as a dominant component. GC-MS analysis of *T. baenzigeri* identified several bioactive lipid-derived compounds that may help explain the traditional medicinal uses of this species. Ethyl oleate, detected in both leaf and stem extracts, has been reported to possess antioxidant and antibacterial activities[19], which is consistent with the radical-scavenging activity observed in this study. Hexadecanoic acid ethyl ester, the major constituent in both plant parts, is similarly associated with antioxidant and antimicrobial properties[19], aligning with the high total phenolic content and the DPPH and ABTS scavenging capacities, particularly in the stem extract. Linoleic acid ethyl ester, detected predominantly in the stem extract, has been reported to exhibit antimicrobial, hepatoprotective and hypocholesterolemic activities[20,21].

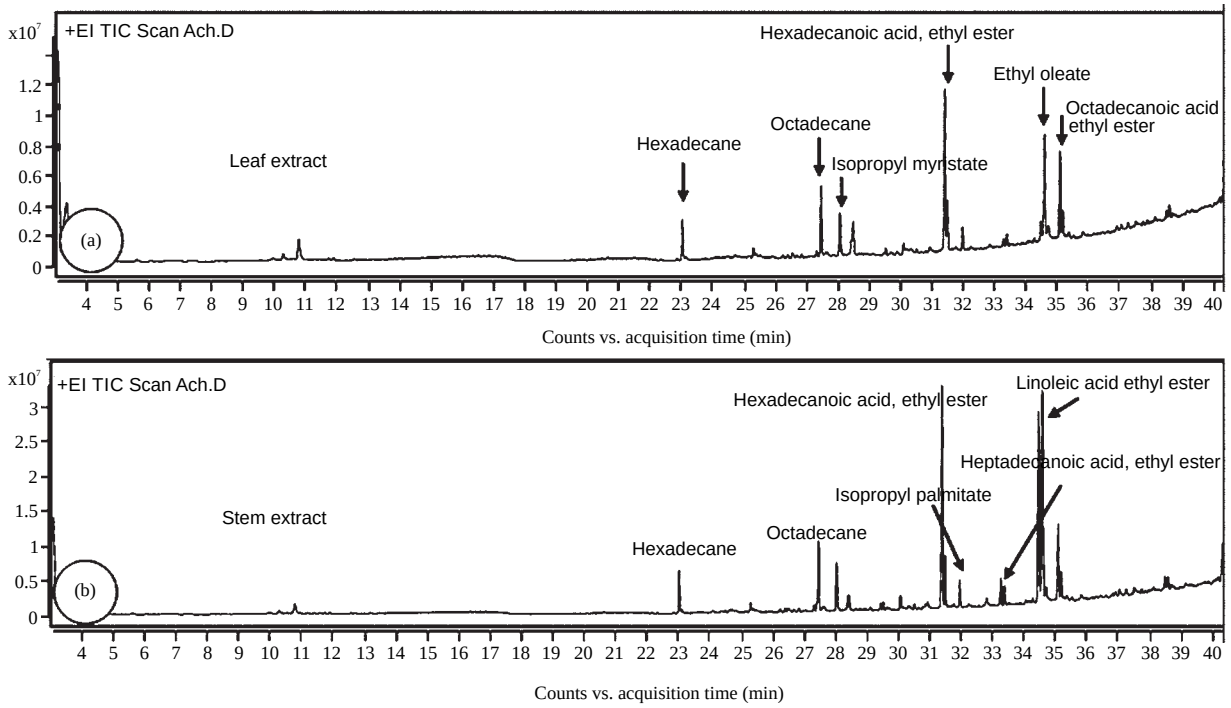

Fig. 3(a-b): GC-MS chromatograms of ethanol extracts of *Tinospora baenzigeri*, (a) Leaf extract showing major compounds including hexadecane, octadecane, isopropyl myristate, hexadecanoic acid ethyl ester, ethyl oleate and octadecanoic acid ethyl ester and (b) Stem extract showing predominant compounds including hexadecane, octadecane, isopropyl palmitate, hexadecanoic acid ethyl ester, linoleic acid ethyl ester and heptadecanoic acid ethyl ester
X-axis: Acquisition time (min) and Y-axis: Intensity ($\times 10^7$)

The distribution of chemical constituents observed in *T. baenzigeri* is consistent with reports from other medicinal plants showing tissue-specific variation in lipid-derived compounds. For example, *Allium nigrum* exhibits organ-dependent differences in hydrocarbon composition, while hexadecanoic acid and related esters are consistently present across tissues[22]. A similar pattern was evident in *T. baenzigeri*, where n-hexadecane, heptadecane, octadecane, indicating that these saturated hydrocarbons constitute part of a conserved structural lipid matrix within the species.

HPLC analysis: The HPLC analysis of the ethanol extracts of *T. baenzigeri* revealed the presence of several phenolic compounds when compared with authentic standards (Fig. 4a). The chromatogram of the stem extract (Fig. 4b) showed the presence of catechin and rutin. Catechin was the dominant compound with a concentration of 190.55 µg/mL, while rutin was present at a lower concentration of 11.17 µg/mL. In contrast, the leaf extract chromatogram (Fig. 4c) exhibited only minor phenolic peaks, including gallic acid and rutin. Gallic acid and quercetin were not detected in the stem extract under the experimental conditions. The presence of catechin and rutin in the stem extract may contribute to the antioxidant potential of *T. baenzigeri*. Catechin is a well-known flavan-3-ol widely reported for its strong antioxidant and free radical scavenging activities, while rutin is a flavonoid glycoside associated with anti-inflammatory, antimicrobial and cardioprotective effects[23,24]. The relatively high concentration of catechin observed in the stem extract suggests that this compound may play a major role in the antioxidant activity detected in the present study. Similar phenolic profiles have been reported in other medicinal plants, where catechin and rutin are key contributors to antioxidant capacity[25,26].

Phytochemical constituents and antioxidant activities: The total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of the leaf and stem extracts are presented in Table 2. Among all the extracts, the stem ethanolic extract exhibited the highest TPC (167.120 mg GAE/g extract) and the strongest antioxidant activity in both DPPH (48.092%) and ABTS (75.517%) assays. By contrast, the leaf ethyl acetate extract contained higher TFC (89.198 mg QE/g extract) but showed lower radical scavenging activity (DPPH: 5.507%; ABTS:24.202%).

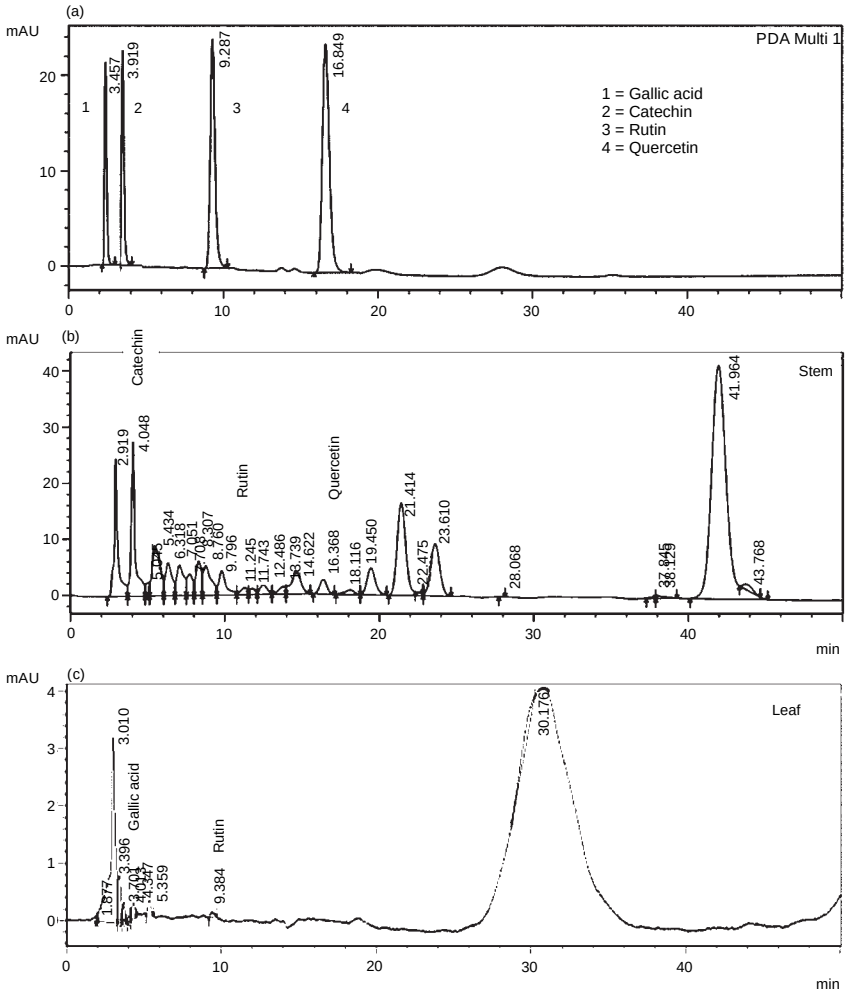

Fig. 4(a-c): HPLC chromatograms of phenolic standards and extracts of *T. baenzigeri*, (a) HPLC chromatogram of phenolic standards: (1) Gallic acid, (2) Catechin, (3) Rutin and (4) Quercetin, (b) HPLC chromatogram of the stem ethanol extract showing the presence of catechin and rutin and (c) HPLC chromatogram of the leaf ethanol extract showing minor phenolic peaks including gallic acid and rutin

Table 2: Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (%) of the extracted plant parts

Plant part	Solvent	DPPH inhibition (%)	TPC (mg GAE/g extract)	ABTS inhibition (%)	TFC (mg QE/g extract)
Leaf					
	Ethyl acetate	5.507±1.718^{e}	53.596±1.302^{e}	24.202±0.819^{e}	89.198±0.694^{a}
	Dichloromethane	2.399±0.526^{f}	46.195±0.745^{f}	12.586±0.717^{f}	74.916±0.667^{b}
	Ethanol	14.722±0.526^{d}	78.364±0.231^{d}	27.740±0.912^{d}	28.060±1.155^{d}
Stem					
	Ethyl acetate	19.466±0.619^{c}	100.767±0.302^{c}	61.396±0.339^{c}	41.080±0.639^{c}
	Dichloromethane	22.519±1.183^{b}	120.804±0.681^{b}	64.371±2.796^{b}	19.319±0.206^{e}
	Ethanol	48.092±1.546^{a}	167.120±1.144^{a}	75.517±0.799^{a}	7.290±0.156^{f}

Results are presented as Mean±SD of triplicate measurements. Mean values in the same row with different superscript letters are significantly different ($p<0.05$). DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, TPC: Total phenolic content, GAE: Gallic acid equivalent, ABTS: 2,2 -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity, TFC: Total flavonoid content and QE: Quercetin equivalent

For both plant parts, the extracts obtained with dichloromethane and ethyl acetate exhibited comparatively lower TPC, TFC and antioxidant activity than the ethanolic extracts. The results indicated that ethanol was the most efficient solvent for extracting antioxidant-related compounds in *T. baenzigeri* and that stem tissues contained higher phenolic constituents, contributing to stronger antioxidant potential than the leaf extracts.

The antioxidant activity observed in the stem extracts of *T. baenzigeri* in this study is consistent with earlier reports. Previous work by Saravanan *et al.*[27] found that stem-derived extracts of this species showed free radical scavenging capacity, attributed to phenolic, flavonoid and related compound classes. This pattern is reflected in the present findings, where higher phenolic content in the stem extract corresponded with greater DPPH and ABTS activity compared with the leaf extract. Although the crude extract showed lower antioxidant activity than standard reference antioxidants, the consistency across studies supports the traditional use of *T. baenzigeri* as a tonic herb. Taken together, the findings suggest that the stem of *T. baenzigeri* may serve as a useful natural source of antioxidant compounds with potential applications in functional food or phytopharmaceutical contexts. The study also confirmed that ethanol was a more effective solvent than others for extracting phenolic compounds and that the stem generally contained higher phenolic content and antioxidant activity than the leaf. The results confirmed that *T. baenzigeri* was rich in phenolic and fatty acid-based compounds, which contributed to its antioxidant properties. Ethanol was the most effective solvent for extracting phenolic compounds, while the stem contained higher levels of phenolics and antioxidant activity than the leaf.

The macroscopic and microscopic characteristics of *T. baenzigeri* observed in this study provide important structural evidence supporting its phytochemical accumulation and biological activities. The presence of lenticellate stems, paired hollow domatia, well-developed epidermal tissues and chlorenchymatous mesophyll with abundant chloroplasts, together with calcium oxalate cubic crystals, is consistent with the diagnostic anatomical features of the family Menispermaceae[28]. These anatomical adaptations are known to facilitate active photosynthesis, secondary metabolite biosynthesis and tissue protection, thereby supporting the medicinal potential of the species.

The GC-MS analysis of both leaf and stem extracts of *T. baenzigeri* showed that fatty acid ethyl esters and saturated hydrocarbons were the predominant compound classes. The main constituents identified were hexadecanoic acid ethyl ester, ethyl oleate, octadecanoic acid ethyl ester and linoleic acid ethyl ester. Linoleic acid ethyl ester was detected only in the stem extract, which may be relevant to the traditional use of *T. baenzigeri* in preparations such as the Saban remedy, where it is used for detoxification, fever reduction and metabolic support. These structural features are associated with mechanical protection, gas exchange and resistance to microbial entry, which may contribute to plant defense and the stability of secondary metabolites. The HPLC analysis identified catechin and rutin in the extracts, consistent with the results from total phenolic content and antioxidant assays. The antioxidant activity of the stem extract, which was higher than that of the leaf extract, appears to reflect its greater phenolic content. The strong antioxidant activity observed in the stem extract is likely associated with its higher phenolic content, which is consistent with previous studies reporting that phenolics and flavonoids are major contributors to antioxidant activity[29].

CONCLUSION

Tinospora baenzigeri contains bioactive phytochemicals with measurable antioxidant activity, supporting its traditional medicinal use. The stem extract demonstrated higher total phenolic content and stronger radical scavenging activity against both DPPH and ABTS, while the leaf extract contained higher flavonoid content. HPLC analysis identified catechin and rutin as the principal phenolic compounds in the stem extract, which likely account for its antioxidant capacity. Anatomical examination revealed characteristic features including lenticels, paired domatia and calcium oxalate crystals, which may play roles in metabolite accumulation and tissue protection. These results support the traditional medicinal use of *T. baenzigeri* and provide useful information for future studies on its phytochemical composition and biological activities.

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