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Research Article

Phytochemical Composition, GC-MS Profiling, Antibacterial and Antioxidant Activities of *Tarenna asiatica* Stem Extracts

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ABSTRACT

Tarenna asiatica is widely used in traditional medicine to treat a range of skin diseases. The current study focused on the stem extracts of the plant to evaluate its phytochemical composition, gas chromatography-mass spectroscopy (GC-MS) profile, and antibacterial and antioxidant efficacy. Alkaloids, flavonoids, steroids, terpenoids, and phenols were detected by qualitative phytochemical screening. The ethanol and chloroform extracts showed the highest phenol and flavonoid contents, respectively. The ethanol extract exhibited significant radical scavenging ability in the DPPH assay and reducing power assay. Antibacterial activity was assessed using the well diffusion method. The chloroform extract demonstrated significant antibacterial activity, exhibiting the highest zone of inhibition against *Escherichia coli* (17.67 ± 0.33 mm), while the methanolic extract indicated no activity. The chloroform extract had the lowest minimum inhibitory concentration (MIC) value against E. *coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, measuring a value of 0.156 mg/mL. In thin-layer chromatography (TLC) bioautography, inhibition zones for all tested pathogens were observed at Rf values of 0.16 and 0.37. GC-MS analysis revealed the presence of retusine and salvigenin as the chief flavonoid compounds in the chloroform extract. The results of the present study highlight the potential value of *T. asiatica* stem extracts as a source of antibacterial agents, especially the chloroform extract.

Introduction

Plants have significantly contributed to the evolution of human culture through their nutritional or therapeutic value. Medicinal plants, in particular, have consistently been a primary source of medicine across nearly all cultures and civilizations.[1] The World Health Organization (WHO) acknowledges that medicinal plants play a crucial role in healthcare, with approximately 80% of the population in developing countries relying primarily on traditional medicine, and about 85% of traditional medicine involves the use of plant extracts.[2] Plants are natural sources for phytochemicals that accumulate in the leaves, fruits, barks, stems, and roots of medicinal plants, which makes them an important source for therapeutic use in the treatment of various diseases.[3] Advances in phytochemical research have led to the identification of numerous bioactive chemicals from plants that are essential to nutraceuticals, cosmetics, and pharmaceuticals.

Tarenna asiatica belongs to the family Rubiaceae and is endemic to the southern part of India, Sri Lanka, and Malaysia.[4] Traditionally, it has been used to treat several human ailments, including wound healing, as an antidote for paralysis, eye infections, and skin diseases, and is also administered to stimulate suppuration.[5] Hence, *T. asiatica* holds an important position among medicinal plants due to its numerous phytotherapeutic properties. Generally, leaves are used as an antidote for paralysis, have wound-healing properties, and is a remedy for stomach problems.[6,7] Herbal remedies of *T. asiatica* fruit include treatment for eye infections, dermatological disorders, and stomach aches; fruit after smashing is placed on boils to induce suppuration.[8-10] Solvent

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extracts of leaves have shown good antibacterial,[8,11] antioxidant,[12] and anti-inflammatory properties.[13] Leaf extracts also have notable mosquitocidal activity against the *Aedes aegypti* and demonstrate substantial antiviral efficacy against the dengue virus.[11] Ethanol extract of *T. asiatica* fruit showed remarkable cytotoxic effects on the human breast cancer cell line.[14] A GC-MS investigation of the ethanolic leaf extract of *T. asiatica* showed the presence of nine compounds, namely benzaldehyde, glycerine, benzofuran-2,3-dihydro-, propane-1,3-diol 4-methyl-benzeneboronate, n-hexadecanoic acid, phytol, D-mannitol, propylene glycol monoleate, and squalene. [15] The acetone leaf extract revealed the presence of tetracontane, 2-methyltetracosane, and eicosane. [11]

Recently, there has been an increasing emphasis on the conservation of medicinal plants due to their usage in conventional as well as traditional practices, and some of them have a high demand for supply due to their large-scale utility in Commercial industries. However, some of the medicinal plants are scientifically validated for their therapeutic efficacy, while others are rooted in cultural beliefs and folklore. [16] Despite its Immense medicinal value, information with regard to *T. asiatica* biological efficacy against various ailments is lacking; hence, the present study focuses on the estimation of phytochemical constituents of *T. asiatica* stem solvent extracts and the evaluation of their antioxidant and antimicrobial properties.

Materials And Methods

Collection of Plant Material

Young stem material of *T. asiatica* was collected from the Punajur region of Chamarajanagara district in Karnataka by taking help from a local expert. The plant was identified using local flora and verified through online databases. [17, 18] A herbarium specimen was prepared and deposited in the herbarium of the Department of Studies in Botany, University of Mysore, Manasagangotri, Mysuru, with Voucher No.: UOMBOT26TA004.The collected plant material (Fig. 1) was thoroughly rinsed with tap water and then with distilled water, shade-dried at room temperature, homogenized into a fine powder by using a blender, and stored in an airtight dry container for further use. [19]

Preparation of Extracts

The powdered plant material was subjected to the Soxhlet extraction method to separate phyto-constituents using different solvents, namely petroleum ether, chloroform, ethyl acetate, ethanol, and methanol, based on their polarity. [20] 50 g of powdered plant material was successively extracted with 250 mL of solvent. Following extraction, the recovered filtrates were concentrated and stored in a sterile container in a refrigerator at 4°C until further use. The percentage yield of the extracts that was obtained after evaporation was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder taken}} \times 100$$

Phytochemical Screening

The extracts were subjected to qualitative phytochemical screening to identify the presence of active phytochemical constituents using the standard methods. [19, 21, 22, 23] All tests were carried out in triplicate to ensure result reliability.

Total phenolic content

The Folin-Ciocalteu reagent method was followed to estimate total phenolic content with slight modification as described by Amir *et al*. (2011). [24] The Folin-Ciocalteu reagent (5 mL, 1:10 dilution) and aqueous sodium carbonate (4 mL, 1M) were mixed with an aliquot (0.5 mL of 1 mg/mL) of plant extract or gallic acid (standard). After 15 minutes of incubation at room temperature, the reaction mixtures' absorbance was measured at 765 nm. Gallic acid was used to develop a standard calibration curve (20–100 µg/mL) in methanol. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry crude extract (mgGAE/g DCE).

Total flavonoid content

The aluminum chloride colorimetric assay was used to estimate the total flavonoid content. [25] An aliquot (1 mL of 1 mg/mL) of extract or standard solutions of quercetin (20-100 µg/mL) was mixed with distilled water (4 mL). To the flask, sodium nitrite (0.3 mL, 5%) was added, followed by aluminum chloride (0.3 mL, 10%) after 5 minutes. Subsequently, sodium hydroxide (2 mL, 1 M) was added, and adjusting the volume to 10 mL using distilled water, the absorbance was measured at 510 nm. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry crude extract (mgQE/g DCE).

Antioxidant activities

DPPH(1, 1-Diphenyl-2-Picryl Hydrazyl) Assay

The DPPH assay was performed as described by Braca *et al*. (2001).[26] Plant samples were taken at different concentrations (20-100 µL) and made up to 100 µL using methanol. Subsequently, 3 mL of DPPH solution in methanol (0.004%) was added to the samples. Ascorbic acid was used as the reference standard. The negative control consisted of 100 µL of methanol combined with 3 mL of DPPH solution. The reaction mixtures were kept in the darkness for 30 minutes, and the absorbance was taken at 517 nm. The radical scavenging activity of the

test samples was quantified as IC_{50} values, which denoted the concentration required to scavenge 50% of DPPH free radicals.

Reducing power assay

The reductive ability of the extracts was determined based on the $Fe^{3+}$to Fe^{2+} reduction method described by Fejes *et al*. (200). [27] The extract (1 mL) was combined with phosphate buffer (2.5 mL, 0.2M, pH 6.6), and potassium ferricyanide (2.5 mL, 1%), then incubated at 50°C for 20 minutes. After adding trichloroacetic acid (2.5 mL, 10%), the mixture was subjected to centrifugation at 3000 rpm for 10 minutes. The supernatant (2.5 mL) was combined with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and absorbance was measured at 700 nm. Higher absorbance indicated better reducing power. Ascorbic acid served as the reference standard, whereas phosphate buffer was used as the blank solution.

Antibacterial activity

The antibacterial activity of the extracts was tested against *Staphylococcus aureus* (MTCC 740), *Escherichia coli* (MTCC 7410), *Bacillus subtilis* (MTCC 121^T), and *Salmonella typhi* (MTCC 733).

Well-diffusion method

The antibacterial assay of solvent extracts was evaluated using the well-diffusion method.[28] Using 50 µL of the inoculum, a uniform bacterial lawn was obtained on nutrient agar plates. Wells were prepared using a cork borer measuring 10 mm in diameter. The wells were loaded with 100 µL of plant extract (100 mg/mL in DMSO), solvent (DMSO, negative control), or streptomycin (1 mg/mL, positive control) in aseptic condition. The inoculated plates were incubated at 37°C for 24 hours, and then the plates were examined for zones of inhibition. The diameter of the zone was measured in millimeters (mm) and compared to the positive control (streptomycin).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using a 96-well microtiter plate (300 µL capacity per well). The solvent extracts were prepared at a concentration of 10 mg/mL and 0.1 mg/mL for the plant extracts and reference antibiotic (streptomycin), respectively. 100 µL of Muller-Hinton broth was dispensed into each well. A 100 µL volume of each test sample was introduced into the initial well of the plate. Serial dilution was prepared to attain concentration ranges of 5.0-0.039 mg/mL for extracts and 0.05-0.00039 mg/mL for the standard reference antibiotic. To each well, 20 µL of bacterial inoculum was then added. The first and second wells functioned as sterile (broth only) and negative controls (broth and inoculum), respectively. Plates were wrapped and incubated at 37°C for 24 hours. MIC was confirmed by adding 20 µL of 2,3,5-triphenyl tetrazolium chloride (TTC) at 2 mg/mL to the incubated culture. Viable bacteria reduce TTC from colorless to pink. The lowest concentration well without pink coloration was considered as the MIC value. [29,30]

Thin layer chromatography (TLC) bioautography

The TLC bioautography agar overlay method was employed to evaluate the biological activity of separated compounds. About 50 µL of chloroform extract (10 mg/mL) was loaded onto a TLC plate developed using a solvent system consisting of hexane:chloroform:methanol (7:2:1, v/v/v). The developed chromatogram was air-dried, and clear bands were identified, and their retention factor (Rf) was determined. The TLC plate was placed in a sterile petri plate, and overlaid with 15 mL of Muller- Hinton agar combined with 20 µL of bacterial suspension (1.5×10^8 CFU/mL) and TTC (5 mg/mL), and then allowed to solidify. The plates were initially kept at 4°C for 2 hours for effective and rapid diffusion of the bioactive compound and then incubated at 37°C for 24 hours. A zone of inhibition was observed with the pink background.[31]

Gas chromatography- mass spectroscopy (GC-MS) analysis

The chloroform extract was subjected to GC–MS analysis using a Shimadzu Nexis GC-2030 system equipped with an AOC-30/20i autosampler. An SH-I-5Sil MS capillary column (30m length, 0.25 mm inner diameter, and 0.25µm film thickness) was used for separation. The mass spectra of the compounds were identified using GC-MS Solutions software and compared with data libraries from NIST 20 (National Institute of Standards and Technology, Washington, DC, USA) and Wiley8.

Statistical Analysis

All experiments were performed in triplicate. Data were analysed using one-way analysis of variance (ANOVA).

Results And Discussion

Yield percentage (%)

The percentage yield obtained from of *T. asiatica* stem through Soxhlet extraction varied between the solvents (Table 1). Extraction with ethanol showed highest yield (6.48%), followed by chloroform (5.36%), petroleum ether (3.06%), methanol (2.22%) and ethyl acetate (1.34%).

Table 1: Percentage yield (w/w) of *T. asiatica* stem extracts

Solvent extracts	*Yield of extraction (%)*
Petroleum ether	3.06
Chloroform	5.36
Ethyl acetate	1.34
Ethanol	6.48
Methanol	2.22

The results indicated that extract yield is influenced by the choice of solvent with ethanol being the good solvent with high yield.

Phytochemical Screening

Phytochemical analysis of *T. asiatica* stem extracts revealed the presence of major phyto-constituents, including alkaloids, glycosides, flavonoids, steroids, terpenoids, and phenols (Table 2). Petroleum ether, chloroform, and ethyl acetate extracts revealed the presence of alkaloids. Glycosides were observed in petroleum ether and ethyl acetate extracts. Carbohydrates tested positive in chloroform and ethanol extracts. Polar solvents showed the presence of tannins, whereas chloroform, ethanol, and methanol extracts showed the presence of flavonoids and phenols. Steroids were solely present in the chloroform extract. Terpenoids were reported in all extracts except methanol. The present results corroborate previous reports on leaf extracts from *T. asiatica*, where ethanol extract of leaves was reported to have flavonoids, glycosides, tannins, steroids, and triterpenoids, whereas in methanol extract alkaloids, flavonoids, phenols, saponins, and tannins were reported. Flavonoids and alkaloids were similarly reported in the chloroform extract. [4, 11, 15, 32]

Fig. 1: *Tarenna asiatica* (L.) Kuntze ex K. Schum

Total phenolic content

The phenolic content of extracts varied from 29.43 ± 0.33 mg GAE/g DCE to 42.44 ± 0.16 mg GAE/g DCE(Table 3). The ethanol extract possessed the highest phenolic concentration (42.44 ± 0.16 mg GAE/g DCE) among the

Table 2: Qualitative phytochemical analysis of *T. asiatica* stem extracts.

Phytochemicals	*Phytochemical tests*	*Petroleum ether*	*Chloroform*	*Ethyl acetate*	*Ethanol*	*Methanol*
Alkaloids	Dragendorff's test	+	+	+	-	-
	Hager's test	+	+	+	-	-
	Wagner's test	+	+	+	-	-
Glycosides	Keller-Killiani's test	+	-	+	-	-
	Sulfuric acid test	+	-	+	-	-
	Modified Borntrager's test	+	-	+	-	-
Carbohydrates	Fehling's test	-	+	-	+	-
	Benedict's test	-	+	-	+	-
Tannin	Gelatin test	-	-	-	+	+
	Braymer's test	-	-	-	+	+
Flavonoids	Alkaline reagent test	-	+	-	+	+
	Ferric chloride test	-	+	-	+	+
Steroids	Libermann-Burchard's test	-	+	-	-	-
Saponin	Foam test	-	-	-	-	-
Terpenoids	Salkowski's test	+	+	+	+	-
Phenols	Ferric chloride test	-	+	-	+	+

Note: '+' indicates the presence of phytochemicals. '-' indicates the absence of phytochemicals.

Table 3: Total phenolic and total flavonoid content of *T. asiatica* stem extracts.

Solvent extracts	*TPC (mg GAE/g DCE)*	*TFC (mg QE/g DCE)*
Petroleum ether	-	-
Chloroform	35.81 ± 0.16	79.83 ± 2.20
Ethyl acetate	-	-
Ethanol	42.44 ± 0.16	69.00 ± 1.44
Methanol	29.43 ± 0.33	34.00 ± 2.88

Each value represents the mean ± SEM; (n=3); Note: '-' Not determined

extracts, followed by the chloroform extract (35.81 ± 0.16 mg GAE/g DCE).The findings of the present study were compared with previous reports, in which the acetone extract of *T. asiatica* leaves showed the highest phenolic content (57.21 g GAE/100 g), followed by acetone (48.26 g GAE/100 g)and methanol (43.98 g GAE/100 g) extracts of bark. [33]

Total flavonoid content

The chloroform, ethanol, and methanol extracts that showed the presence of flavonoids during phytochemical screening were further subjected to total flavonoid content estimation (Table 3). Among the examined solvent extracts, the chloroform extract exhibits the highest (79.83 ± 2.20 mg QE/g DCE) total flavonoid content, followed by the ethanol extract (69.00 ± 1.44 mg QE/g DCE). The lowest value was recorded in methanol extract (34.00 ± 2.88 mg QE/g DCE). In earlier reports, the acetone extract of *T. asiatica* leaves showed a high flavonoid content (619.67 mg RE/g), followed by acetone extracts of bark (561.00 mg RE/g) and flowers (497.00 mg RE/g). [33]

Antioxidant activities

DPPH Assay

The stem extracts of *T. asiatica* exhibited notable radical scavenging activity in the DPPH assay, which is evident from the IC_{50} values. Among the tested extracts, the ethanol extract showed the highest radical scavenging activity, with IC_{50} values of 57.74 ± 0.52 µg/mL, followed by chloroform (83.27 ± 0.66 µg/mL), methanol (173.88 ± 0.85 µg/mL), ethyl acetate (178.45 ± 0.89 µg/mL), and petroleum ether (212.18 ± 1.13 µg/mL) extracts. However, when compared with the standard ascorbic acid results, which showed IC_{50}=22.91± 0.76 µg/mL, all extracts were not on par with the standard (Fig. 2). The radical scavenging activity was in agreement with earlier reports where ethanolic leaf extract possessed a lower IC_{50} value (26.6 µg/mL) and acetone extract of leaves had an IC_{50} value of 20.38 µg/mL. [4,33]

Reducing Power Assay

The reducing power of both plant extracts and the standard reference increased gradually with rising concentrations

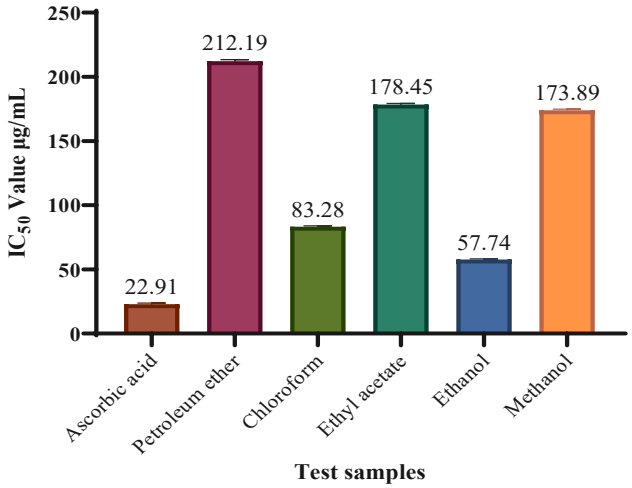

Fig. 2: IC_{50} value of *T. asiatica* stem extracts by DPPH assay Values are expressed as mean ± SD (n=3). Statistical significance was determined using one-way ANOVA, and differences were considered significant at $p < 0.0001$ compared to ascorbic acid.

(Fig. 3). Among the tested extracts, the ethanol extract exhibited the highest absorbance value (0.73 ± 0.00), whereas the ethyl acetate extract showed the lowest value (0.05 ± 0.00). The results were compared to the reducing power of the standard ascorbic acid (2.36 ± 0.06). These findings corroborate the earlier reports where fruits of *T. asiatica* showed excellent reducing power values in ethanol and chloroform extract, [34] and methanol leaf extract also showed reductive capabilities. [35]

Antibacterial activity

Well diffusion method

The antibacterial activity of *T. asiatica* stem extracts was determined by the agar well diffusion method. All solvent

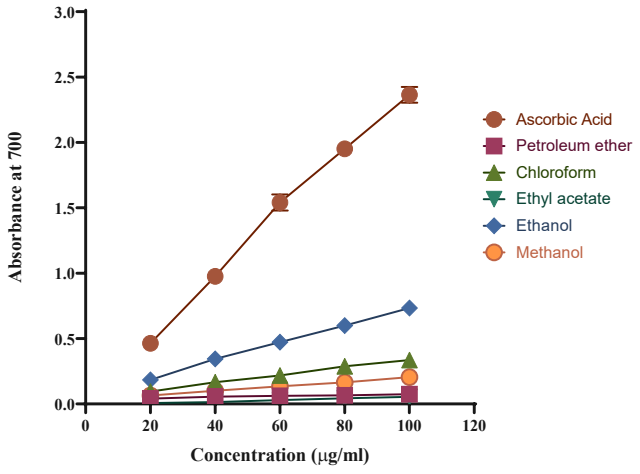

Fig. 3: Antioxidant activity of *T. asiatica* stem extracts by reducing power assay Values in figure 3 are expressed as mean ± SD (n= 3). Statistical significance was analysed by one-way ANOVA. $p < 0.0001$ compared with ascorbic acid.

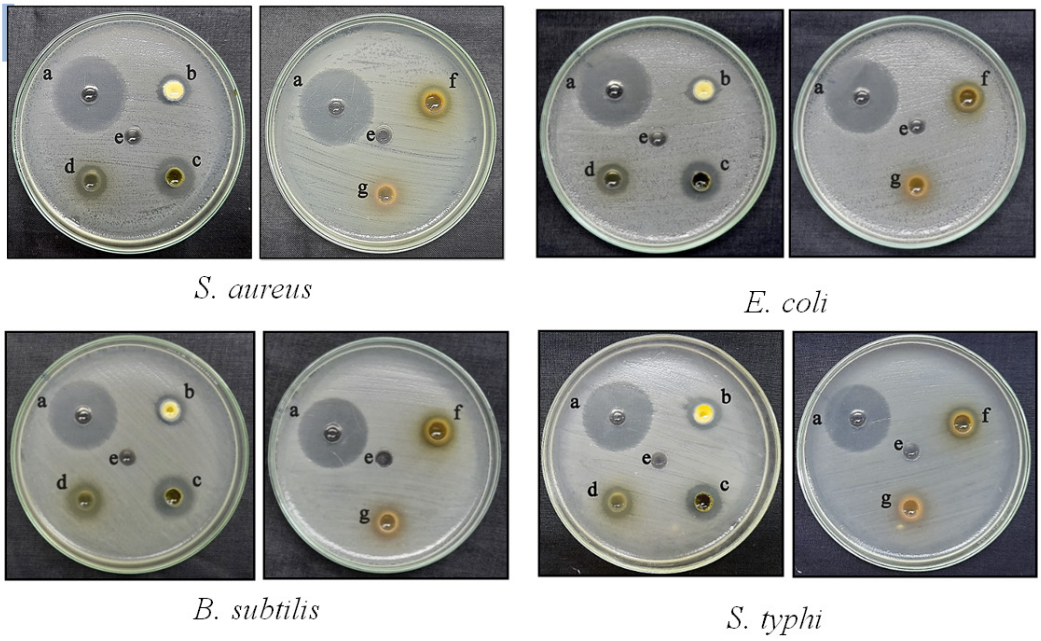

Fig. 4: Antibacterial activity of *T. asiatica* stem extracts
Note: a-Standard (Streptomycin); b-Petroleum ether; c-Chloroform; d-Ethyl acetate; e- DMSO; f-Ethanol; g-Methanol

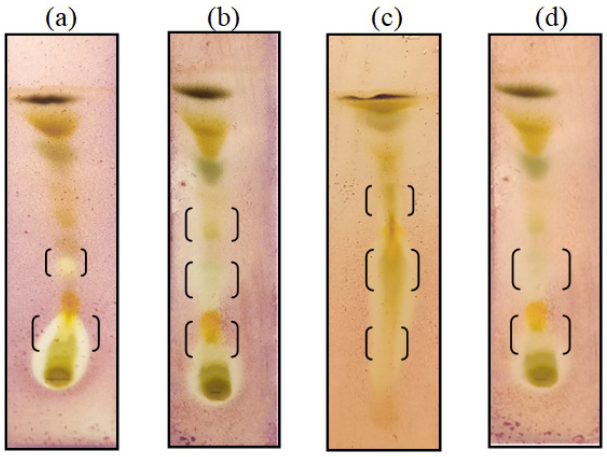

Fig. 5: TLC-bioautography of *T. asiatica* chloroform extract
(a) *S. aureus;* (b) *B. subtilis*; (c) *E. coli;* (d) *S. typhi*

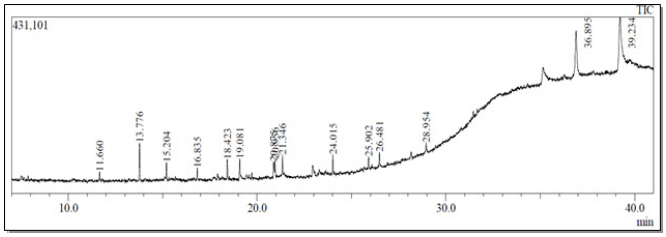

Fig. 6: GC-MS Chromatogram of *T. asiatica* chloroform extract

extracts displayed antibacterial activity against the tested pathogens, except for the methanol extract (Table 4). Among the extracts, the chloroform extract demonstrated the most substantial antibacterial activity with the highest zone of inhibition towards *E. coli* (17.67 ± 0.33 mm), followed by *S. typhi* (17.33 ± 0.33 mm), *S. aureus* (17.00 ± 0.00 mm), and *B. subtilis* (16.33 ± 0.33 mm). In contrast, the methanol extract showed no inhibition (Fig. 4).

Table 4: Antibacterial activities of T. *asiatica* stem extracts by well diffusion assay

Pathogens	*Zone of inhibition in mm*						
	Positive control (Streptomycin)	*Petroleum ether*	*Chloroform*	*Ethyl acetate*	*Ethanol*	*Methanol*	*Negative control (DMSO)*
S. aureus	30.00 ± 0.00^{a}	13.33 ± 0.00^{c}	17.00 ± 0.00^{b}	12.66 ± 0.33^{cd}	12.33 ± 0.33^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
B. subtilis	28.33 ± 0.33^{a}	13.33 ± 0.33^{c}	16.33 ± 0.33^{b}	12.33 ± 0.33^{d}	11.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}
E. coli	29.66 ± 0.33^{a}	13.00 ± 0.00^{c}	17.66 ± 0.33^{b}	13.33 ± 0.33^{c}	12.33 ± 0.33^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
S. typhi	30.00 ± 0.00^{a}	12.33 ± 0.33^{d}	17.33 ± 0.33^{b}	14.00 ± 0.00^{c}	12.33 ± 0.33^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

Each values represent mean ± SEM (n = 3). Based on Tukey's B test, means followed by the same letter(s) within the same row are not significantly different at $p < 0.05$.

Table 5: MIC of *T. asiatica* stem extracts by micro-dilution method (in mg/mL)

Pathogens	*Positive control (Streptomycin)*	*Petroleum ether*	*Chloroform*	*Ethyl acetate*	*Ethanol*	*Methanol*	*Negative control (DMSO)*
S. aureus	0.003	0.313	0.156	2.500	2.500	0.00	0.00
B. subtilis	0.003	0.313	0.156	2.500	2.500	0.00	0.00
E. coli	0.003	0.625	0.156	2.500	2.500	0.00	0.00
S. typhi	0.006	0.625	0.313	2.500	2.500	0.00	0.00

Values presented were average of triplicate experiments.

Table 6: GC-MS profile of *T. asiatica* chloroform extract

Peak No.	*Retention time*	*Area of %*	*Compound name*	*Molecular formula*	*Biological application*
1	11.660	1.40	1-Tetradecene	$C_{14}H_{28}$	-
2	13.776	6.65	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	Antibacterial, antiviral, antifungal, antioxidant, cytotoxic[37]
3	15.204	2.71	Tetradecyl trifluoroacetate	$C_{16}H_{29}F_3O_2$	-
4	16.835	2.10	Hexadecyl acrylate	$C_{19}H_{36}O_2$	-
5	18.423	3.73	1-Nonadecene	$C_{19}H_{38}$	Antibacterial [38]
6	19.081	3.52	Neophytadiene	$C_{20}H_{38}$	Antimicrobial, antipyretic, analgesic, anti-infla mmatory, antioxidant[39]
7	20.875	4.76	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Anti-infla mmatory, antioxidant, antibacterial[11,40,41]
8	20.956	4.12	(-)-Cembrene A	$C_{20}H_{32}$	-
9	21.346	3.97	Octacosanol	$C_{28}H_{58}O$	Anti-Parkinsonian effect, lipid metabolism regulation, reduce fatigue, cardiovascular and liver protection, anti-infla mmatory[42,43]
10	24.015	3.74	Docosyl trifluoroacetate	$C_{24}H_{45}F_3O_2$	-
11	25.902	2.35	3,4',5,6'-tetra-tert-butylbiphenyl-2,3'-diol	$C_{28}H_{42}O_2$	-
12	26.481	2.80	Heneicosyl trifluoroacetate	$C_{23}H_{43}F_3O_2$	-
13	28.954	1.97	1-Hexacosanol	$C_{26}H_{54}O$	Antibacterial, antitumor[44,45]
14	36.895	22.44	Salvigenin	$C_{18}H_{16}O_6$	Antioxidant, anti-infla mmatory, cytotoxic, anti-tumor activities[46,47,48]
15	39.234	33.74	Retusine	$C_{19}H_{18}O_7$	Antibacterial, anti-infla mmatory[49,50]

Similarly, as mentioned in previous studies, leaf extract of *T. asiatica* showed moderate activity against *S. aureus* and *P. aeruginosa* with a clear zone of inhibition of 11 mm [36], and acetone extract of the leaf also showed a favorable antibacterial property. [32]

MIC

The chloroform extract demonstrated the lowest MIC value (0.156 mg/mL) against *S. aureus*, *B. subtilis,* and *E. coli*, and 0.313 mg/mL for *S. typhi*, followed by petroleum ether with an MIC value of 0.313 mg/mL against *S. aureus*, and *B. subtilis,* and a value of 0.625 mg/mL against *E. coli*, and *S. typhi.* The lowest value was observed in ethyl acetate and ethanol extract, whereas the methanol extract showed no inhibitory effect against the tested pathogens (Table 5). The result exhibits that even at the lowest concentration chloroform extract was efficient in inhibiting the bacterial growth and showed a significant difference.

TLC bioautography

The chloroform extract was subjected to estimate antibacterial activity by the TLC bioautography method. TLC separation of the extract showed a total of 7 bands with the Rf values of 0.90, 0.85, 0.77, 0.53, 0.37, 0.24, and 0.16. The developed TLC plates were dried and subsequently used for bioautographic analysis. Zones of inhibition were observed at specific Rf values (Fig. 5). At Rf values of 0.37, 0.24, and 0.16, inhibition zones were

observed against *B. subtilis* and *E. coli*, whereas inhibition zones were observed at Rf values of 0.16 and 0.37 against *S. aureus* and *S. typhi*. The observed inhibition zones indicate that the antibacterial constituents are associated with compounds present at these specific Rf values.

GC–MS analysis

The chloroform extract, which confirmed prominent antibacterial activity along with low MIC values, was therefore selected for GC-MS analysis. The GC-MS profile revealed the presence of 15 compounds belonging to phenolic, flavonoid, and terpenoid classes (Fig. 6 and Table 6). The major constituents identified were retusine (33.74%) and salvigenin (22.44%), collectively accounting for 56.18% of total extract composition. The minor constituent contains1-hexacosanol (1.97%) and 1-tetradecene (1.40%). Several identified compounds, including 2,4-di-tert-butylphenol, 1-nonadecene, 1-hexacosanol, and retusine, have been previously reported to exhibit biological activities. The antibacterial activity of the chloroform extract may result from the individual or synergistic interactions of multiple phyto-constituents present in the extract. Earlier GC-MS investigations on *T. asiatica* have reported the presence of tetracontane, 2-methyltetracosane, and eicosane in acetone leaf extract and n-hexadecanoic acid in ethanol leaf extract.[11,15]

Conclusion

The present study provides scientific insight into the phytochemical composition and evaluates the measurable free radical scavenging and antibacterial potential of stem extracts of *T. asiatica*, a medicinal plant traditionally used in the Punajur region of Chamarajanagara district, Karnataka, India. Among tested solvents, chloroform extract exhibited comparatively stronger antibacterial activity, while ethanol extract showed strong antioxidant potential. The enhanced antibacterial activity of the chloroform extract may be due to the presence of moderately non-polar bioactive compounds, like flavonoids and terpenoid derivatives identified through GC–MS analysis, as well as possible synergistic interactions between the phytochemicals.

The present study is limited to *in-vitro* evaluation of antioxidant and antibacterial activities. Further experimental validation is required to confirm the pharmacological relevance of the individual bioactive compounds. These findings provide a scientific basis for the traditional use of *T. asiatica* and warrant further investigation into its potential therapeutic applications. Therefore, additional research for structural characterization of active constituents, and *in-vivo* pharmacological validation is necessary to validate therapeutic relevance *T. asiatica.*

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