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RESEARCH ARTICLE

Qualitative Analysis of the Chemical Composition of *Cinnamomum tamala* leaves and Biological Activity

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Abstract: *Cinnamomum tamala* (Buch.-Ham.) Nees and Ebermaier. (Lauraceae) is a less explored medicinal plant in Uyghur medicine, with limited knowledge about its non-volatile compounds. We utilized chemical pre-screening in conjunction with UPLC-Q-Orbitrap-MS to analyze the phytochemical makeup of *C. tamala* leaves. Total polyphenols were measured using the Folin-Ciocalteu method, while total flavonoids were quantified through $NaNO_2$-Al $(NO_3)_3$ colorimetric analysis across four different commercial batches. In vitro assays for DPPH radical neutralization and nitrite elimination were additionally utilized to assess biological activity. Altogether, 153 substances were provisionally characterized, primarily including flavonoids, glycosides, phenolics, terpenoids, alkaloids, and carboxylic acids. The amounts of polyphenols and flavonoids varied significantly between batches, spanning from 5.00 to 14.28 mg/g for polyphenols and 44.41 to 71.57 mg/g for flavonoids. All samples demonstrated DPPH scavenging activity influenced by concentration and showed moderate nitrite elimination (56.91–62.55%). These results provide a foundation for understanding the chemical composition and biological activity of *C. tamala*, emphasizing the necessity of quality evaluation tailored to specific batches of this herbal material.

Keywords: *Cinnamomum tamala* Leaves, Chemical Composition, UPLC-Q-Orbitrap-MS, Total Polyphenols, Total Flavonoids, Antioxidant Activity

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Introduction

C. tamala, often referred to as Chai Gui Ye or Rou Gui Ye, is a prevalent herbal treatment in Uyghur traditional medicine and is officially listed in the Uyghur Medicinal Materials Standards of Xinjiang Uyghur Autonomous Region [1]. As per this guideline, the foliage is collected in the warmer months and fall, once mature, and then dried under sunlight for therapeutic purposes. Traditionally, it has been utilized for various ailments, including digestive and heart-related issues linked to cold-dampness conditions, as well as mental health concerns like anxiety and irregular heartbeat. Its recognized benefits include easing coldness, stimulating the stomach, reducing dampness, and enhancing cognitive function. Although it holds ethnopharmacological importance, the comprehensive analysis and assessment of the bioactivity of its non-volatile components have yet to be thoroughly investigated.

Phytochemically, Cinnamomum species are characterized by four major classes of constituents: volatile oils, polyphenols, flavonoids, and polysaccharides. Cinnamaldehyde dominates the essential oil fraction, accompanied by cinnamic acid and p-methoxycinnamaldehyde [2]. Among polyphenols, proanthocyanidins and catechins are considered the primary contributors to the antioxidant signature [3], while proanthocyanidin and rutin stand out as key flavonoids [4]. The polysaccharide fraction rich in mannose, galacturonic acid, and glucose has demonstrated hypoglycemic potential [5]. From a pharmacological perspective, a growing body of evidence has revealed multidimensional bioactivities of cinnamon, spanning anti-inflammatory [6], antitumor [7], antibacterial [8], glucose-lowering [9–11], antidepressant [12], and tyrosinase-inhibitory effects [13], underscoring its promise as a multifunctional medicinal resource.

The dried leaves of cinnamon exhibit notable regenerative capacity, extensive application scope, and abundant resources. Current phytochemical investigations of cinnamon leaves have been overwhelmingly oriented toward volatile oil profiling. Yet the practical yield of volatile oil remains modest typically 0.8–1.2% limiting its extraction efficiency [14, 15]. By contrast, the non-volatile fraction, particularly polyphenols and flavonoids, has received disproportionately little attention: quantitative data are sparse, bioactivity characterization is lacking, and the content activity relationship across different batches has not been established. Furthermore, the correlation between constituent content and in vitro activity has not been validated, and standardized methods for component determination are lacking. To elucidate the bioactive basis of *C. tamala* herbal material, this study systematically investigates its chemical composition, determines the total polyphenol and total flavonoid content in *C. tamala*, and preliminarily explores it's in vitro antioxidant capacity and nitrite scavenging ability.

A systematic chemical and biological characterization of the non-volatile constituents of *C. tamala* is therefore warranted not only to elucidate its pharmacologically active principles, but also to inform quality standardization and resource utilization strategies in the broader context of modernizing herbal medicine.

Materials and Methods

Materials, Reagents, and Instruments

C. tamala originating from Guangxi (lot codes 250205, 250206, 20230202, and 250317). The rutin under investigation was provided by Shanghai Yuan Ye Biotechnology Co., Ltd., while gallic acid (2014052801, AR) originated from the Chengdu Kelong Chemical Reagent Plant. Analytical-grade Folin's reagent, sodium carbonate, sodium hydroxide, aluminum nitrate, and sodium nitrite were sourced from Shanghai Yuanye, Tianjin Beilian, Tianjin Damiao, Tianjin Shengao, and Tianjin Yongsheng, in that order. Additional substances that have been employed include KI, silicon dioxide, CCl3, among various alternatives. Every compound used was of high analytical grade quality. The experiment employed the ThermoVanquish UHPLC system, the Eppendorf 5430R refrigerated high-speed centrifuge, the Kunshan KQ5200DE ultrasonic washer, the ATERSHSST3 analytical column (100×2.1 mm, 1.8 μm), and the Shimadzu UV-2700 UV-Vis spectrophotometer.

Preliminary Chemical Composition Analysis of *C. tamala*

To evaluate the extraction performance systematically in solvents with varying polarities, 0.5 g of powdered *C. tamala* was subjected to ultrasonic extraction (85 W, 50°C, 30 minutes) using 20 mL of distilled water, pure ethanol, or petroleum ether. The obtained filtrates were labeled as the water-based, ethanol-based, and petroleum ether-based extracts, respectively. A 1 mL portion of *C. tamala* aqueous extract was subsequently utilized to test for the presence of glycosides, flavonoids, phenolic compounds, organic acids, polypeptides, amino acids, steroidal triterpenes, alkaloids, and anthraquinones. The identical method was utilized for the remaining three samples.

UPLC-Q-Orbitrap-MS

The resulting dataset comprises data from three aspects: retention time, m/z ratio, and peak intensity. The specimen was gradually defrosted at 4°C. Afterward, a precise measurement of 50–100 mg was taken and placed into a centrifuge tube. A 1 mL aliquot of the extraction solvent (composed of water, acetonitrile, and isopropanol in equal volumes, 1:1:1, v/v/v) was introduced, followed by vigorous vortex mixing for one minute. Subsequently, ultrasonic extraction at a low temperature was conducted for half an hour. The mixture was spun in a centrifuge (4°C, 12,000 rpm, 10 minutes), and the obtained supernatant was kept at -20°C for 1 hour to facilitate protein precipitation. Following a subsequent centrifugation step (4°C, 12,000 rpm, 10 minutes), the supernatant was subjected to vacuum drying and then dissolved in 200 μL of 50% (v/v) acetonitrile in water. The mixture was vortexed, subjected to centrifugation (4°C, 14,000 rpm, 15 minutes), and the resulting supernatant was placed into an autosampler vial for examination [16]. The analysis utilized a Waters HSS T3 column (100×2.1 mm, 1.8 μm), maintained at

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40°C. The dual-component mobile phase consisted of an aqueous solution with 0.1% formic acid (v/v) (A) and acetonitrile with 0.1% formic acid (B), pumped at a rate of 0.3 mL/min with a sample injection size of 2 µL. The gradient elution schedule proceeded as follows: from 0 to 1 minute, 0% B; from 1 to 12 minutes, a linear rise to 95% B; from 12 to 13 minutes, maintained at 95% B; at 13.1 minutes, reset to 0% B; and from 13.1 to 17 minutes, re-equilibrated at 0% B.High-precision mass spectrometry data were collected using a Thermo Fisher Q Exactive HFX system featuring an ESI source, functioning in a polarity-switching configuration. Essential source settings were adjusted as follows: sheath gas at 40 units; auxiliary gas at 10 units; spray voltage of +3000 V (positive mode) / −2800 V (negative mode); ion source operating at 350°C; capillary maintained at 320°C.Data collection for mass spectrometry utilized a full-scan approach alongside data-dependent secondary analysis (full-MS-DDMS^2), covering a detection window of 70–1050 m/z. This setup accommodates scans for both positive and negative ions. The mass spectrometer settings included an initial resolution of 70,000 and an additional resolution of 17,500.

Determination of Total Polyphenol Content

The Folin-Ciocalteu spectrophotometric technique, as modified from [17], was utilized to measure the overall polyphenol concentration. Volumes ranging from 0 to 0.6 mL of gallic acid stock solution (0.104 mg/mL) were added to 10 mL amber volumetric flasks. Each flask was sequentially combined with 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% (w/v) sodium carbonate solution, topped up with purified water to the mark, and left to react at ambient temperature in the absence of light for 1 hour. The measurement of absorbance was taken at 760 nm using a reagent blank as the reference. Develop a calibration curve and perform experiments to assess accuracy, consistency, and durability. Determine the overall polyphenol concentration in *C. tamala* using the regression formula derived from the standard calibration curve.

Determination of Total Flavonoid Content

The total flavonoid content was measured through a colorimetric method utilizing sodium nitrite and aluminum nitrate reagents [18]. Rutin (0.10 mg/mL) was utilized as the standard reference. Slope segments (0–4.5 mL) were moved into 10 mL volumetric flasks and successively treated with 5% $NaNO_2$ (0.5 mL, allowed to react for 6 minutes), 10% $Al(NO_3)_3$ (0.6 mL, standing for 6 minutes), and 4% NaOH (4 mL), followed by dilution to volume using 60% ethanol. The measurement of absorbance was conducted at a wavelength of 510 nm [19]. The x-axis (X) indicated the rutin concentration (µg/mL), while the corresponding absorbance value was displayed on the y-axis (Y). A calibration curve was created, followed by experiments to assess accuracy, consistency, and robustness. Using the regression formula derived from the standard curve, the flavonoid concentration in each *C. tamala* sample was determined.

Determination of Antioxidant Capacity

The DPPH free radical neutralization test was conducted following the methodology described by [20, 21] with slight adjustments. In summary, serially diluted *C. tamala* total phenolic extract (with vitamin C as a reference) was combined with 0.1 mg/mL DPPH solution in a 96-well microplate and left to incubate in the absence of light for 30 minutes. The reduction in absorbance observed at 517 nm was tracked, and the antioxidant activity was calculated using the formula: Scavenging rate (%) = [(A1 − A2) / A1] × 100.

Determination of the Ability to Remove Nitrite

Nitrite scavenging capacity was assessed following the protocol of [22]. A reaction mixture containing 1.0 mL $NaNO_2$ (5 µg/mL), 0.25 mL sample extract, and 2.0 mL phosphate-citrate buffer (pH 3.0) was incubated at 37°C for 30 min. Subsequently, 1.0 mL each of 0.4% sulfanilic acid and 0.2% N-(1-naphthyl) ethylenediamine dihydrochloride were added. After color development at room temperature for 15 min, absorbance was read at 545 nm.

Results

Preliminary Chemical Composition of *C. tamala*

The preliminary phytochemical screening revealed solvent-dependent differences in the chemical profiles of *C. tamala* extracts (Tables 1-3). The aqueous extract displayed the broadest spectrum of constituents, testing positive for glycosides, flavonoids, phenolics, alkaloids, anthraquinones, steroids/triterpenes, and organic acids. The ethanolic extract shared glycosides, flavonoids, phenolics, organic acids, and steroids/triterpenes with the aqueous extract but lacked detectable alkaloids and anthraquinones. The petroleum ether extract similarly contained glycosides, flavonoids, phenolics, alkaloids, and organic acids, though anthraquinones were absent. The consistent detection of glycosides, flavonoids, and phenolics across all three solvents suggests these as the predominant secondary metabolite classes in *C. tamala*.

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Table 1: Preliminary test results of water extracts from *C. tamala*

Test solution	Test items	Test contents	Results	Positive reaction phenomenon
Water extract	Amino acids	Indigo carmine reagent	-	Blue or purple color
	Glycosides	Molish reaction	+++	Purple ring appears at the two interfaces.
	Flavonoids	Hydrochloric acid-magnesium powder test	++	Red or purplish red color
		0.2%aluminum chloride reaction	+	Yellow-green fluorescence
	Alkaloids	Iodine-potassium iodide test	+	Red-brown precipitate
	Saponins	Foam reaction	-	Foam appears after shaking and does not disappear within a short period of time.
	Phenols and tannins	1%ferric chloride test	++	Appears Blue-green
	Anthraquinones	1%magnesium acetate test	+	Appears red
	Sterols, triterpenoids	0.1%vanillin	+	Red-purple color
	Organic acids	PH test paper	＜7	Appears orange-red
		Bromophenol blue test	+	Yellow spots appear on a blue background

+: Positive test; –: Negative test

Table 2: Preliminary test results of ethanol extract from *C. tamala*

Test solution	Test items	Test contents	Results	Positive reaction phenomenon
Ethanol extract	Flavonoids	Hydrochloric acid-magnesium powder test	++	Appears red or purplish red
		0.2%aluminum trichloride reaction	+	Has yellow-green fluorescence
	Phenols	1%ferric chloride reaction	+++	Appears greenish blue
	Sterols, triterpenoids	0.1%vanillin	+++	Appears reddish purple
	Alkaloids	Potassium bismuth iodide test	-	Orange-red precipitate
		Iodine-potassium iodide test	-	Reddish brown precipitate
	Glycosides	Molish reaction	+++	Purple ring appears at both interfaces.
	Anthraquinones	1%magnesium acetate test	-	Appears red
	Organic acids	PH test paper	＜7	Appears orange-red
		Bromophenol blue test	+	Yellow spots appear on a blue background

+: Positive test; –: Negative test

Table 3: Preliminary test results of petroleum ether extract from *C. tamala*

Test solution	Test items	Test contents	Results	Positive reaction phenomenon
Petroleum ether extract	Steroid, triterpenoid	0.1%vanillin	++	Appears reddish purple
	Glycoside	Molish reaction	+++	Purple ring appears at two interfaces.
	Flavonoid	Hydrochloric acid-magnesium powder test	+	Appears red or purplish red
	Saponin	0.2% aluminum trichloride reaction	+	Has yellow-green fluorescence
		Foam reaction	-	Foam appears after shaking and does not disappear for a short time
	Phenolic and tannin	1%ferric chloride test	+++	Strong positive, blue-black
	Alkaloid	Potassium bismuth iodide test	+	Orange-red precipitate
	Organic acid	PH test paper	＜7	Appears orange-red
		Bromophenol blue test	+	Yellow spots appear on a blue background

+: Positive test; –: Negative test

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UPLC-Q-Orbitrap-MS

The samples' comprehensive ion profiles (Fig. 1) were generated using UPLC-Q-Orbitrap-MS techniques. Mass spectrometry results were processed with Xcalibur 4.1 software to identify compounds exhibiting relative abundances over 0.1%. A total of 153 chemical constituents were discovered in *C. tamala*, accompanied by detailed data on the quantity of compounds and corresponding distribution illustrated through pie charts (Fig. 2). The 15 most abundant compounds were chosen, and detailed data regarding their chemical composition can be found in Table 4. This research prioritized the top 15 compounds with the highest relative abundance, as identified through UPLC-Q-Orbitrap-MS, for in-depth examination. The essential material foundation of *C. tamala* is formed by high-concentration compounds, which are closely linked to their biological activity. Due to their consistent and dependable qualitative outcomes, these substances offer vital insights into bioactivity determination. The biological importance and combined impacts of minor compounds require further detailed investigation, offering a promising direction for specialized future studies. The sample's chemical profile was analyzed utilizing both ESI+ and ESI− modes. In ESI mode, a variety of peaks corresponding to moderately polar compounds appeared within the 0–20 min range, highlighting significant signals from organic acids, flavone glycosides, and polyphenols. In ESI? mode, a group of prominent signals emerged between 26 and 28 minutes, suggesting that less polar flavonoid aglycones, terpenoids, and other substances demonstrated enhanced detection in positive ion mode. The synergistic functionality of both ionization techniques ensures thorough representation of the sample's chemical composition, delivering dependable chromatographic and mass spectrometric data to facilitate later analyses of component identification, measurement, and bioactivity assessment. These outcomes confirm the observations from initial chemical trials.

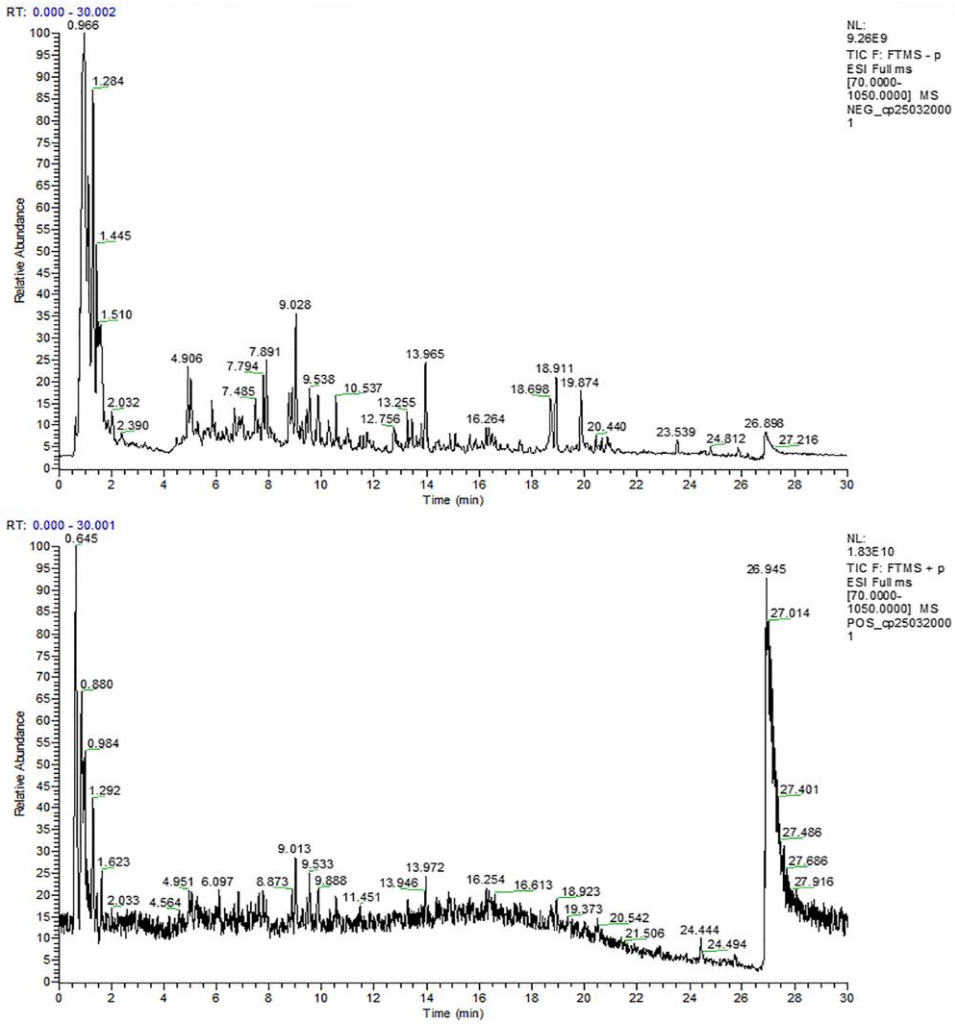

Fig. 1: Total ion current diagram of *C. tamala* in negative and positive ion modes

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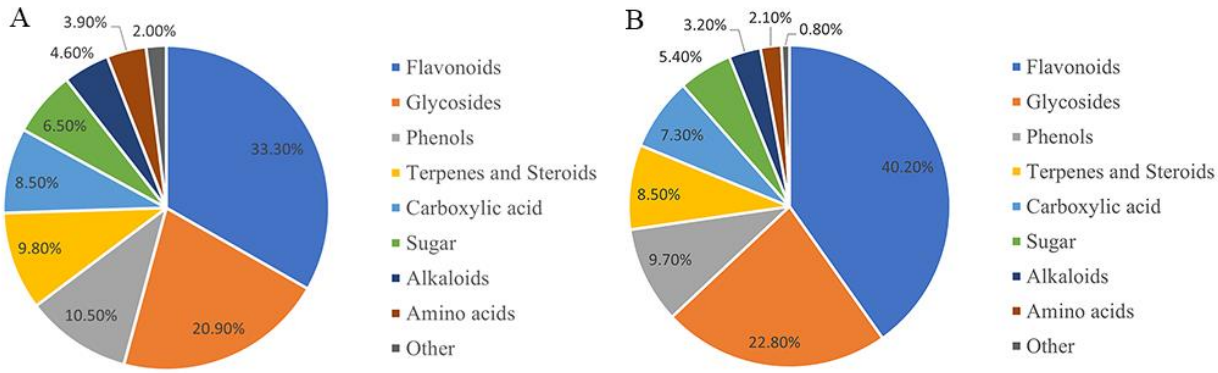

Fig. 2: (A) Pie chart showing the chemical composition of *C. tamala*; (B) Pie chart showing the relative content of *C. tamala*

Table 4: UPLC-Q-Orbitrap-MS data information

Rank	Metabolite	Formula	m/z	Retention time(min)	Mode	Adducts	STJ(%)[a]	Compound Classification
1	2-amino-4-(4-hydroxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile	$C_{19}H_{12}N_2O_4$	723.1721	16.3706	-	2M+Hac-H	9.65	flavonoid
2	Narcissoside	$C_{28}H_{32}O_{16}$	623.1616	9.5383	-	M-H, M+Cl	7.39	glycoside
3	3,10-Dihydroxy-5,11-dielmenthadiene-4,9-dione	$C_{20}H_{28}O_4$	333.2054	9.0126	+	M+H, M+ACN+H, M+H-H2O, M+H-2H2O	3.73	terpenoids
4	Citric acid	$C_6H_8O_7$	191.0196	1.5215	-	M-H2O-H, M-H	3.52	carboxylic acids
5	13-Oxo-9E,11E-octadecadienoic acid	$C_{18}H_{30}O_3$	293.2119	18.9108	-	M-H, M+Cl, 2M-H	3.10	carboxylic acids
6	Quercetin 3,7-dirhamnoside	$C_{27}H_{30}O_{15}$	595.1653	9.4490	+	M+H, M+Na, M+H+Na	2.51	glycoside
7	(+)-Puerol B 2"-O-glucoside	$C_{24}H_{26}O_{10}$	439.1383	16.6132	+	M+H-2H2O	2.33	glycoside
8	afzelin	$C_{21}H_{20}O_{10}$	431.0980	10.5368	-	M-H, 2M-H, M+Cl	2.15	glycoside
9	(+)-Catechin hydrate		289.0716	7.8908	-	M-H, M+Cl, 2M-H	1.99	flavonoid
10	2"-O-E-p-Coumaroylafzelin	$C_{30}H_{26}O_{12}$	577.1349	13.7834	-	M-H, M+Cl	1.93	glycoside
11	Catechin	$C_{15}H_{14}O_6$	291.0859	7.8899	+	M+H-H2O, M+H	1.87	flavonoid
12	2'-FUCOSYLLACTOSE	$C_{18}H_{32}O_{15}$	533.1720	0.9802	-	M+FA-H	1.76	glycoside
13	Phenethyl rutinoside	$C_{20}H_{30}O_{10}$	475.1818	7.8091	-	M+FA-H	1.75	glycoside
14	1-Heptanol	$C_7H_{16}O$	274.2736	14.8111	+	2M+ACN+H	1.74	Other
15	Paederosidic acid	$C_{18}H_{24}O_{12}S$	463.0883	9.1816	-	M-H, 2M-H	1.67	glycoside

+: Positive ion; –: negative ion

STJ (%): Relative percentage of each compound, calculated as the ratio of its peak area to the total peak area of all identified compounds in the total ion chromatogram

Total Polyphenol Content

The gallic acid calibration curve exhibited strong linearity over the range of 1.04–6.24 μg/mL, described by the equation Y = 0.1095X + 0.0411 (R^2 = 0.9996). Method validation parameters were within acceptable limits: precision (RSD, 1.08%), repeatability (RSD, 1.55%), and stability (RSD, 0.87%). Spike-recovery experiments yielded a mean recovery of 101.0% (RSD = 1.77%), confirming satisfactory accuracy and precision for quantitative purposes (Table 5).

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Table 5: Experimental results of total polyphenol content determination in *C. tamala* (n = 4, $\bar{X}$±SD)

Consignment	Total polyphenol content(mg/g)	RSD (%)
250205	5.00±0.026	0.51
250206	9.89±0.051	0.52
20230202	14.22±0.231	1.62
250317	14.28±0.186	1.30

Total Flavonoid Content

The regression equation is Y = 0.0152X + 0.0012, with R^2 = 0.9994, indicating a significant linear relationship for rutin standards within the range of 20–45 μg/ml. The relative standard deviation (RSD) for precision experiments was 1.12%, for repeatability experiments was 1.26%, and for stability experiments was 1.94%. The results of the total flavonoid content determination for *C. tamala* (Table 6) showed an average recovery rate of 101.0% in the spiked recovery experiment, with a relative standard deviation of 1.93%.

Table 6: Experimental results of total flavonoid content determination in *C. tamala* (n = 4, $\bar{X}$±SD)

Consignment	Total flavonoid content (mg/g)	RSD (%)
250205	45.41±0.233	0.51
250206	73.32±0.436	0.60
20230202	72.17±0.711	0.99
250317	71.57±0.355	0.49

Antioxidant Capacity

All four batches of cinnamon extract exhibited dose-dependent DPPH radical scavenging activity, with scavenging rates increasing from 12.0–48.0% at 0 mg/mL to 60.0–86.0% at 1.4 mg/mL. The mean scavenging rate across all concentrations was 60.07±12.89%, with a 95% confidence interval of (52.15%, 67.99%). A one-sample t-test confirmed the extract's scavenging rate was significantly higher than zero (t = 21.15, df = 27, P<0.001). Univariate ANOVA revealed significant differences in DPPH scavenging capacity among batches (F = 18.62, df = 3,24, P<0.001). LSD post-hoc tests indicated: Batch 20230202 exhibited the highest scavenging activity, significantly superior to Batches 250205 and 250317 (both P<0.01); Batch 250206 showed no significant difference from Batch 20230202 (P>0.05), but was significantly higher than Batches 250205 and 250317 (both P<0.05). Compared to the positive control group (vitamin C, average scavenging rate: 96.43%), the extract exhibited significantly lower DPPH scavenging capacity (t = 10.83, df = 12, P<0.001) (Fig. 3).

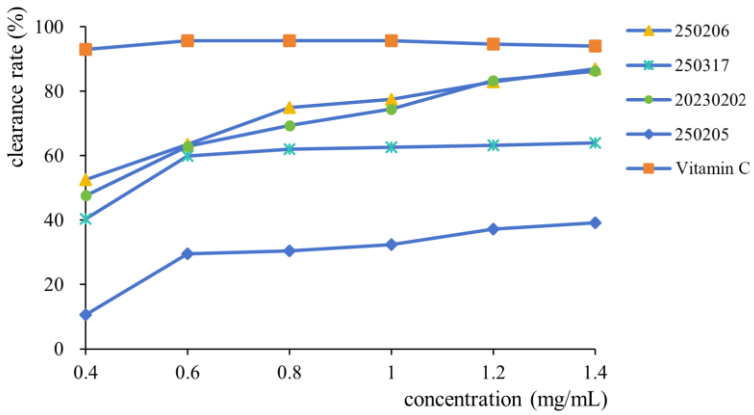

Fig. 3: Results of DPPH radical scavenging

Nitrite Removal Effect

This study evaluated the ability of *C. tamala* extract to remove nitrite through a nitrite removal experiment. The color of the solution with *C. tamala* extract was lighter than that of the solution without the extract. The nitrite removal rates differed

among the four batches of C. tamala extract. The clearance rates were 56.91%, 59.82%, 60.55%, and 62.55%, respectively, with a mean of 59.96% and a standard deviation of 2.34%. The results indicate that the nitrite clearance rate of the *C. tamala* extract was significantly higher than zero ($t = 51.32$, $p<0.001$), with a 95% confidence interval of (56.24%, 63.68%). The experimental results showed that *C. tamala* extract has a certain ability to remove nitrite (Table 7).

Table 7: Experimental results of nitrite removal capacity

Consignment	Nitrite clearance (%)
250205	56.91
250206	59.82
20230202	60.55
250317	62.55

Discussion

This study offers a two-fold analysis of *C. tamala*, combining chemical composition evaluation with laboratory-based bioactivity testing. A significant discovery highlights the considerable variation between batches: the total polyphenol levels varied by almost three times (5.00–14.28 mg/g), while the total flavonoid levels showed a difference of approximately 1.6 times (44.41–71.57 mg/g) among the four analyzed batches. The significant differences observed are probably indicative of how harvest timing and localized environmental conditions impact the buildup of secondary metabolites.

The UPLC-Q-Orbitrap-MS analysis unveiled a profile abundant in polyphenols and flavonoids, consistent with the known phytochemical characteristics of Lauraceae family plants [23]. However, the substantial disparity in total polyphenols (5.00–14.28 mg/g) and the 1.6-fold difference in flavonoids (44.41–71.57 mg/g) observed across just four batches is remarkable. The observed variability between batches aligns with the established responsiveness of phenylpropanoid and flavonoid production pathways to subtle environmental factors factors such as elevation, soil properties, and harvest schedules have all been reported to influence phenolic content in Lauraceae species [24, 25]. Significantly, these variations are not just theoretical interference; they resulted in observable distinctions in biological activity, as elaborated further. The generation of these compounds is also associated with the plants' defensive strategies against environmental challenges. This research identified batch 250317 as having the greatest overall polyphenol concentration and achieving DPPH radical scavenging equilibrium within the 0.4 to 1.4 mg/mL range, unequivocally establishing polyphenols as the key antioxidant component in *C. tamala*. While batch 20230202 showed a marginally reduced total polyphenol concentration (14.22 mg/g) compared to batch 250317 (14.28 mg/g), it demonstrated the strongest DPPH radical scavenging efficacy across all four batches. The following explanations are proposed to address this occurrence. Batch 20230202 exhibited a substantial flavonoid concentration (72.17 mg/g), suggesting the potential for cooperative antioxidant interactions between flavonoids and polyphenols. Additionally, the makeup of polyphenols can vary between batches for example, the levels of key active components like catechins and proanthocyanidins might fluctuate, and simply measuring total polyphenol content does not reflect these compositional variations. Moreover, various elements like terpenoids and steroids contributed to 22.8% of the relative composition observed in the UPLC-MS analysis (Fig. 2B) and could potentially enhance antioxidant effects through supplementary mechanisms. Collectively, these results indicate that the antioxidant potential of C. tamala arises not merely from its overall polyphenol concentration, but from the combined effects of various active compounds working in harmony. In contrast to the closely related species Cinnamomum camphora, *C. tamala* exhibited elevated concentrations of flavanol glycosides, which may be integral to its distinctive biological properties.

The *C. tamala* extract demonstrated a significant dose-responsive ability to neutralize DPPH radicals, with sample 20230202 reaching an 86% neutralization rate at 1.4 mg/mL. This action aligns with the antioxidant capacity earlier documented for *C. tamala* stem bark and its essential oil, effectively validating its remarkable ability to counteract oxidative stress. In four separate trials, *C. tamala* exhibited nitrite scavenging rates between 56.91% and 62.55%, aligning with the efficacy associated with natural polyphenols [26]. While somewhat less effective compared to pure catechins and prickly ash polyphenols, it outperformed the majority of typical plant extracts, highlighting its promise as a natural agent for nitrite removal.

This research presents some constraints: Various considerations require recognition. Firstly, translating in vitro DPPH and nitrite scavenging results to in vivo antioxidant effectiveness is challenging; validation through studies in cellular or animal models is necessary prior to making therapeutic assertions. Secondly, while mass spectrometry currently provides extensive

coverage for identification, it remains at a preliminary stage advancing towards focused extraction, purification, and structural analysis via NMR for the key active or prevalent components would be a reasonable progression. Third, the use of a four-batch approach, though adequate for showcasing variability, limits the capacity for comprehensive statistical analysis of the interaction between genotype, environment, and bioactivity. Broadening the scope of sampling to include diverse geographic regions and various harvest periods would significantly enhance the applicability of these findings.

Conclusion

This research enhances the scientific knowledge of the chemical makeup and biological properties of the herbal remedy, offering valuable insights for assessing bioactivity and advancing the pharmaceutical development of C. tamala. This study presents a comprehensive analysis of the stable chemical composition of *C. tamala* leaves, utilizing advanced mass spectrometry techniques along with specific spectrophotometric measurements and laboratory-based bioassays. Key discoveries highlight the provisional identification of 153 compounds, including flavonoids, glycosides, phenolics, terpenoids, carboxylic acids, and alkaloids; variability in total polyphenol levels (ranging from 5.00 to 14.28 mg/g, a 2.9-fold difference) and total flavonoid concentrations (spanning 44.41–71.57 mg/g, a 1.6-fold gap) across four commercial samples. These findings enhance understanding of the chemical makeup and bioactivity of *C. tamala* from Yunnan and Guangxi, serving as a resource for investigating the non-volatile components within Cinnamomum species. Additionally, concentration-dependent DPPH scavenging efficacy was observed across all samples, with batch 20230202 exhibiting the highest activity, achieving 86% inhibition at 1.4 mg/mL. Furthermore, stable nitrite-scavenging performance (56.91%–62.55%) points to the potential of *C. tamala* as a promising natural nitrite scavenging agent. This research provides foundational chemical and functional insights into *C. tamala*, highlighting the importance of assessing batch-specific quality in the pharmaceutical advancement of this largely untapped medicinal plant.

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Author's Contributions

Qianwen Gong: Writing original draft, review and editing, methodology, conceptualization.
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Linyang Wang: Review and edited, conceptualization.
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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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