



Research Article

Assessment of Phytoconstituents and Antimicrobial Activity of Tea Plant (*Camellia sinensis*)

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ABSTRACT

This study investigated the phytoconstituents and antimicrobial activity of *Camellia sinensis* leaves, with emphasis on their antioxidant potential. Ethanol extraction was carried out on dried leaf samples, followed by qualitative and quantitative phytochemical analyses using standard methods. Antimicrobial activity was evaluated using agar well diffusion and broth dilution techniques, while antioxidant activity was assessed using the DPPH radical scavenging assay. Qualitative analysis revealed the presence of alkaloids, carbohydrates, terpenoids, flavonoids, tannins, anthocyanins, phenols, and steroids, whereas saponins and glycosides were absent. Quantitatively, phenols (176.40 mg/mL) were most abundant, followed by tannins (42.28 mg/mL) and flavonoids (28.31 mg/mL). The extract exhibited concentration-dependent antimicrobial activity, with the highest inhibition observed against *Salmonella typhi* (51.7 mm) and *Enterococcus faecalis* (48.3 mm) at 100 mg/mL. MIC values ranged from 40–60 mg/mL. Synergistic studies showed enhanced antibacterial activity when the extract was combined with chloramphenicol discs, with notable effects against *Pseudomonas aeruginosa* and *S. typhi*, and induced antifungal activity against the fungal isolates tested. Antioxidant analysis showed moderate activity, with IC₅₀ values of 71.7–88.5 µg/mL compared with standard antioxidants. The study revealed that *Camellia sinensis* leaves are rich in bioactive phytochemicals, particularly polyphenols, and exhibit significant antimicrobial and moderate antioxidant properties, supporting their potential use in therapeutic applications.

Keywords: Antimicrobial Activity; Antioxidant Activity; Phytochemical Screening; Phytoconstituents; Tea plant

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INTRODUCTION

For centuries, natural products have been widely used not only in home remedies but also in over-the-counter medicines and as key ingredients in the production of pharmaceuticals, cosmeceuticals, and nutraceuticals around the world, in both developed and developing countries (Li, 2025; Wang, 2024). These natural products offer an affordable and effective way to treat many health issues and often work just as well as synthetic drugs. They tend to affect multiple body systems while causing fewer harmful side effects compared with synthetic medications (El-Saadony, 2025).

Tea, scientifically known as *Camellia sinensis* (L.) Kuntze or *Thea sinensis* F., of the family Theaceae, is a shrub or evergreen tree that can grow as tall as 16 metres (Horniman, 2024). Its leaves grow alternately, without stipules, and vary in shape from lance-shaped to obovate, reaching up to 30 cm in length and 2 to 5 cm in width. The leaves are initially covered with fine hairs but sometimes become smooth as they mature; their edges are serrated, and the tips are either sharp or tapering. Fresh tea leaves are typically harvested from late March or early April through to July each year (Chen *et al.*, 2023).

Tea is the second most popular beverage globally, after water. Approximately 78% of the tea consumed worldwide is black tea, with green tea accounting for roughly 20%. All types of tea, excluding herbal teas, are derived from the dried leaves of the *Camellia sinensis* plant, and the differences among them arise from the degree of oxidation the leaves undergo. Green tea is made from unoxidised leaves, making it one of the least processed types of tea (Wang, 2024). Today, around 3 billion people drink tea, making it one of the most widely consumed non-alcoholic beverages in the world (Wang *et al.*, 2019). Green tea, in particular, is rich in antioxidants and beneficial polyphenols (Wang, 2024). Historically, it has been used in traditional Chinese and Indian medicine to help control bleeding, heal wounds, aid digestion, promote heart and mental health, and regulate body temperature (El-Saadony, 2025). Research also suggests that green tea may positively affect a variety of health issues, from supporting weight loss and liver function to managing type 2 diabetes and potentially reducing the risk of Alzheimer's disease (El-Saadony, 2025).

Green tea makes up about 20% of all dried tea produced annually and is mainly consumed in Asian countries such as Japan (FAO, 2025), owing largely to its reputation for being relatively safe and more affordable than other beverages (FAO, 2025). Reflecting this trend, green tea production has been increasing steadily at an estimated rate of 6.4% per year. China, India, and Sri Lanka are the top three tea-producing countries, as highlighted during the 22nd Tea Session organised by the FAO Intergovernmental Group, while Kenya and Sri Lanka lead in exports (FAO, 2025).

The chemical composition of green tea is complex, comprising polyphenols, alkaloids, proteins, minerals, vitamins, and amino acids. Beyond its rich chemistry, green tea is widely appreciated for its impressive health benefits, including anticancer properties (Radeva-Ilieva *et al.*, 2025), powerful antioxidant effects (Ratnani, 2022), cholesterol-lowering ability (Yang *et al.*, 2022), and antimicrobial actions (Radeva-Ilieva *et al.*, 2025). It has also been shown to help reduce body weight (Ratnani, 2022).

Despite this growing body of literature on the pharmacological properties of *Camellia sinensis*, there remains limited region-specific data on the phytochemical composition and antimicrobial efficacy of tea leaves sourced from local Nigerian markets, particularly against clinically relevant bacterial and fungal pathogens, and information on the synergistic potential of tea extracts with

conventional antibiotics remains scarce. This study therefore assessed the phytoconstituents, antimicrobial activity, and antioxidant potential of *Camellia sinensis* leaves obtained from Dutsin-Ma, Katsina State, with a view to providing scientific justification for their continued use as a source of natural bioactive compounds and to exploring their potential application in combination antimicrobial therapy.

MATERIALS AND METHODS

Plant Collection and Preparation

The tea leaves used in this experiment were obtained from Dutsin-Ma Market, Katsina State. The leaves were cut into pieces and ground into powder for extraction.

Preparation of Extracts (Ethanol Extraction)

Ten grams of powdered sample was extracted successively with 70% and 50% ethanol to produce crude extracts containing a wide range of active compounds. The extracts were prepared by macerating the plant material in the respective solvents on a shaker for 2 days. The extracts were filtered using Whatman No. 1 filter paper and dried under reduced pressure at a temperature below 45°C in a rotary evaporator to yield a dense residue (Azwanida, 2015).

Phytochemical Screening

Qualitative analysis

Qualitative phytochemical screening of the crude ethanol extract was carried out using standard colorimetric reagent tests to detect the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, carbohydrates, phenols, and steroids, following established protocols (Avato *et al.*, 2006; Preshant *et al.*, 2011). Briefly, alkaloids were tested using Dragendorff's reagent, with a red precipitate indicating a positive result; flavonoids were tested using lead ethanoate solution, with a buff-coloured precipitate indicating a positive result; tannins were tested using ferric chloride solution, with a blue-black or blue-green precipitate indicating a positive result; saponins were tested by the frothing method, with persistent froth indicating a positive result; terpenoids were tested using the Salkowski test (acetic anhydride and concentrated H₂SO₄), with a colour change from pink to violet indicating a positive result; glycosides were tested using Fehling's solution, with a brick-red precipitate indicating a positive result; carbohydrates were tested using Molisch's reagent (α -naphthol in ethanol, with concentrated H₂SO₄), with a purple ring at the interface indicating a positive result; phenols were

tested using 5% aqueous ferric chloride solution, with an intense blue or black colouration indicating a positive result; and steroids were tested using the Liebermann–Burchard test (chloroform, acetic anhydride, and concentrated sulfuric acid), with a red, green, bluish, or dark-pink colouration indicating a positive result.

Quantitative determination of phytochemicals

Estimation of total phenol

The total phenol content of the extracts was determined using the Folin–Ciocalteu method (Rossi & Singleton, 1965). Different concentrations of the samples were treated with 0.1 mL of Folin–Ciocalteu reagent and 2.5 mL of 0.2 N Na₂CO₃, then incubated at room temperature for 30 minutes. Absorbance was read at 760 nm using a spectrophotometer, with gallic acid as the reference standard. Results were expressed as milligrams of gallic acid equivalents per gram of fresh sample weight (mg GAE/g Fwt).

Estimation of total flavonoids

Total flavonoid content was determined using the aluminium chloride method (Chen *et al.*, 2016). A 0.3 mL aliquot of each plant extract was mixed with 0.15 mL of 0.5 M NaNO₃, 0.15 mL of 0.3 M AlCl₃·6H₂O, and 3.4 mL of 30% methanol. After 5 minutes, 1 mL of NaOH was added and the absorbance was read at 506 nm. Catechin was used as the standard, and total flavonoid content was expressed as milligrams of catechin equivalents per gram fresh weight.

Estimation of carbohydrates

Carbohydrate content was determined using the Anthrone method (Hedge & Hofreiter, 1962). A 100 mg sample was weighed into a boiling tube and hydrolysed for three hours in a boiling water bath with 5 mL of 2.5 N HCl, then allowed to cool to room temperature. The hydrolysate was neutralised with solid Na₂CO₃ until effervescence ceased, made up to 100 mL, and centrifuged at 10,000 rpm for 15 minutes. Aliquots of 0.5 and 1 mL were taken for analysis, with the volume made up to 1 mL using distilled water. Glucose standards of 0, 0.2, 0.4, 0.6, 0.8, and 1 mL were prepared in the same way. Four millilitres of freshly prepared Anthrone reagent (200 mg anthrone in 100 mL of 95% ice-cold H₂SO₄) was then added to each tube, and the mixture was heated in a boiling water bath for 10 minutes, cooled rapidly, and the resulting dark green colour measured at 630 nm. A blank was prepared in the same manner, using distilled water in place of the sample.

Estimation of tannins

Tannin content was determined using the Folin–Denis method (Ci & Indira, 2016). One millilitre of sample extract was mixed with 5 mL of Folin–Denis reagent

and continuously shaken for three minutes. Ten millilitres of sodium carbonate solution was then added, and the mixture was left to stand for 2 hours before centrifugation to settle the residue. The supernatant was used to measure absorbance at 700 nm, with a blank prepared in the same way. A standard curve was constructed using serial dilutions of tannic acid (1:250, 1:50, 1:40, 1:25, and 1:10), and results were expressed as milligram tannic acid equivalents per gram fresh weight (mg TAE/g Fwt).

Estimation of anthocyanin

The pH-differential technique was used to quantify total anthocyanin pigments (Cheng & Breen, 1991), using KCl buffer (0.025 M, pH 1.0) and CH₃COONa buffer (0.4 M, pH 4.5). A 1 mL aliquot of extract was combined with 4 mL of each buffer and incubated at 28°C for 15 minutes to equilibrate. Deionised water served as the blank for absorbance measurements at 510 and 700 nm. Total anthocyanin content was expressed as milligrams of cyanidin-3-glucoside equivalents (CGE) per gram fresh weight, calculated as:

$$\text{Absorbance (A)} = (\text{A}_{520\text{nm}} - \text{A}_{700\text{nm}}) \text{ pH}1.0 - (\text{A}_{520\text{nm}} - \text{A}_{700\text{nm}}) \text{ pH}4.5$$

$$\text{Total anthocyanin (mg/g)} = (\text{A} \times \text{MW} \times \text{DF} \times 1000) / (\text{E} \times 1)$$

Where MW = molecular weight (449.2 g/mol for cyanidin-3-glucoside); DF = dilution factor; 1 = path length (1 cm); and E = molar extinction coefficient (26,900 L/mol/cm for cyanidin-3-glucoside).

Estimation of total alkaloids

Total alkaloid content was determined gravimetrically, following the principle described by Harborne (1998). A 5.0 g finely powdered, defatted sample was extracted with 100 mL of 10% acetic acid in ethanol for 4–6 hours with occasional stirring. The extract was filtered through Whatman No. 1 filter paper, and the filtrate concentrated on a water bath to approximately 25–30 mL. Concentrated ammonium hydroxide was added dropwise until the solution reached pH 9–10, causing alkaloids to precipitate. The precipitate was allowed to settle, filtered through pre-weighed filter paper, washed sequentially with 0.1% ammonium hydroxide, water, and dilute ethanol, and then dried to constant weight at 40–50°C. Total alkaloid content was expressed as a percentage of dry plant material (% w/w):

$$\% \text{ total alkaloids (w/w)} = (\text{weight of dried alkaloid residue (g)} \times 100) / \text{weight of dried sample (g)}$$

Estimation of total glycosides

Total glycoside content was determined using the phenol–sulfuric acid assay. Aliquots (0.5–1.0 mL) of the hydrolysate and glucose standards were mixed

with 1.0 mL of 5% phenol solution, followed by rapid addition of 5.0 mL concentrated sulfuric acid. The mixture was vortexed and allowed to develop colour for 10–20 minutes at room temperature, then incubated for a further 20–30 minutes at 25–30°C. Absorbance was measured at 490 nm against a reagent blank, and total glycosides were calculated as glucose equivalents from the calibration curve:

mg glucose equivalents/g dry sample = (concentration from calibration curve (µg/mL) × total volume of hydrolysate (mL)) / (sample weight (g) × 1000)

Estimation of total saponins

Total saponin content was determined gravimetrically. A 5.0 g dried, powdered sample was extracted with 50 mL of 20% aqueous ethanol by refluxing for 4 hours (Azwanida, 2015). The extract was filtered, and the residue re-extracted twice with 50 mL of 20% ethanol. The combined extracts were concentrated under reduced pressure to approximately 40 mL, mixed with an equal volume of diethyl ether to remove lipids, and the aqueous layer collected. This fraction was mixed with 60 mL of n-butanol, washed twice with 10 mL of 5% sodium chloride solution, and the n-butanol extract evaporated to dryness in a pre-weighed dish at 40°C. Total saponin content was expressed as a percentage of dry plant material (% w/w):

% total saponins = (weight of dried saponin residue (g) × 100) / weight of dried sample (g)

Estimation of total steroids

Total steroid content was estimated using the Liebermann–Burchard colorimetric method (Abubakar & Haque, 2020). A 1.0 mL aliquot of hydrolysed extract was mixed with 2 mL chloroform, followed by 2 mL acetic anhydride and 2 mL concentrated sulfuric acid, and incubated at room temperature for 30 minutes for colour development. Absorbance was measured at 620 nm against a reagent blank, and a standard curve was prepared using cholesterol standards (0, 50, 100, 150, 200, and 250 µg/mL). Total steroids were expressed as milligram cholesterol equivalents per gram dry sample:

mg steroid equivalent/g dry sample = (concentration from calibration curve (µg/mL) × total extract volume (mL) × 1000) / sample weight (g)

Estimation of total terpenoids

Total terpenoids were estimated gravimetrically (Abubakar & Haque, 2020). A 10 g powdered sample was extracted with 100 mL of 95% ethanol by maceration for 24 hours. The extract was filtered and concentrated under reduced pressure to about 20

mL, then washed with 20 mL petroleum ether to remove chlorophyll and other impurities. Terpenoids were precipitated by adding 50 mL of diethyl ether and filtered through pre-weighed filter paper, and the precipitate dried at 40–50°C to constant weight. Total terpenoid content was calculated as a percentage of dry plant material (% w/w):

% total terpenoids = (weight of dried terpenoid residue (g) × 100) / weight of dry sample (g)

Preparation of Different Concentrations of the Extract

A stock solution was prepared by dissolving 6 g of each crude ethanolic and aqueous extract in 10 mL of 5% DMSO, from which working concentrations of 40, 60, and 100 mg/mL were obtained by appropriate dilution.

Antimicrobial Assay

Test organisms

The pathogens used in this study were obtained from General Hospital, Dutsin-Ma. Bacterial strains used were *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, while the fungal strains used were *Fusarium* sp. and *Candida albicans*. Bacterial strains were maintained by weekly subculturing in nutrient broth, and fungal strains in Sabouraud dextrose broth.

Antibacterial and antifungal activity of the extract

The antibacterial and antifungal activities of the leaf extracts were evaluated using the agar well diffusion and paper disc diffusion methods. In the agar well diffusion test, 0.2 mL of a 24-hour broth culture of each bacterial or fungal test organism was aseptically spread on the surface of sterile Mueller–Hinton agar plates using a sterile L-rod. Three wells (about 6 mm in diameter) were aseptically cut into each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between wells and the edge of the plate. A fixed volume (0.1 mL) of the leaf extract was introduced into each well, with a control well at the centre containing the extracting solvent alone.

In the paper disc diffusion test, sterile paper discs were soaked in the leaf extract for 2 hours, after which 0.2 mL of the test bacterial suspension was spread on the surface of Mueller–Hinton agar plates and the discs placed at different positions on the plate. Plates were incubated at 37°C for 24 hours for bacteria and at room temperature for 2–4 days for fungi. All experiments were performed in duplicate (Hudzicki, 2009).

Synergistic activity of tea extract and antibiotics

Antibiotic discs were soaked in the leaf extract for 30 minutes before use. Bacterial and fungal cultures

were swabbed onto their respective Mueller–Hinton agar and Sabouraud dextrose agar plates. A chloramphenicol disc was used as the standard antibiotic. The soaked disc was placed on the inoculated plate, incubated, and the resulting zones of inhibition were compared with those produced by the antibiotic disc alone to assess synergistic activity (Archana & Jayanthi, 2011).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the crude extract was determined using the broth dilution method (Wiegand *et al.*, 2008). Five millilitres of sterile nutrient broth was dispensed into sterile test tubes, and each tube inoculated with a loopful of the test bacterial strain. The leaf extract was then added to each tube in increasing volumes (60, 80, and 100 μ L). The tubes were gently shaken to ensure proper mixing and incubated at 37°C for 24 hours. A control tube without the test organism was included.

Antioxidant Assay by DPPH Radical Scavenging Assay

Antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, based on the reduction of the stable DPPH radical upon accepting a hydrogen atom from an antioxidant compound. Radical scavenging activity of the extract against stable DPPH• was determined spectrophotometrically (Kedare *et al.*, 2011), with colour changes (from deep violet to light yellow) measured at 515 nm using a UV/visible spectrophotometer (Ultrospec 3000, Pharmacia Biotech).

Briefly, each dry extract (0.025 g) was dissolved in 10 mL of hot water, and a freshly prepared solution of DPPH• in ethanol (6×10^{-5} M) was used for all measurements. An aliquot of 77 μ L of the extract solution was added to 3 mL of the ethanolic DPPH solution in a 1 cm path-length disposable microcuvette and mixed thoroughly, giving final extract-to-DPPH mass ratios of approximately 3:1, 1.5:1, and 0.75:1. Samples were kept in the dark for 15 minutes at room temperature before absorbance was measured. A blank containing the same amount of ethanol and DPPH solution was prepared and measured daily, and all experiments were carried out in triplicate. Radical scavenging activity was calculated as:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} is the absorbance of DPPH alone, and A_{sample} is the absorbance in the presence of the test compound.

Data Analysis

Data obtained were analysed using SPSS version 23. Results were presented as mean $\pm$ standard error, and comparisons were made using one-way ANOVA with a significance level set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The qualitative profile of the ethanol extract of *Camellia sinensis* leaves (Table 1) provides a visual overview of the secondary metabolite classes present or absent, based on classical qualitative phytochemical tests: alkaloids (yellow precipitate), carbohydrates (brick-red precipitate), terpenoids (violet colour), flavonoids (yellow-brown colour), tannins (blue-green precipitate), anthocyanins (green hue), phenols (blue colour), and steroids (green-bluish colour). Saponins and glycosides produced no diagnostic colour change and were recorded as absent under the test conditions. These colorimetric responses are consistent with standard spot and reagent tests, indicating that ethanol selectively extracted several polar to mid-polarity phytochemical groups commonly associated with antioxidant and bioactive properties.

The presence of these secondary metabolites in the ethanol extract of *C. sinensis* leaves aligns with previously documented phytochemical profiles of the species, which is recognised for its diverse bioactive constituents that contribute to its therapeutic properties (Aziz *et al.*, 2021; Zhao *et al.*, 2023). The detection of alkaloids, indicated by a yellow precipitate, suggests potential pharmacological activities such as antimicrobial and anticancer effects (Harborne, 1998). Carbohydrates, indicated by a brick-red precipitate, may contribute to plant structural integrity and defence (Harborne, 1998). Terpenoids, confirmed by a violet colour, are widely reported for their anti-inflammatory and antioxidant activities, corroborating studies highlighting their abundance in tea leaves (Aziz *et al.*, 2021). Flavonoids, indicated by a yellow-brown colour, represent a significant class of polyphenols known for strong antioxidant and free-radical scavenging properties (Kumar & Pandey, 2013). Tannins, indicated by a blue-green precipitate, are phenolic compounds with known antioxidative and antimicrobial activities (Aziz *et al.*, 2021), while anthocyanins, indicated by a green hue, act as natural antioxidants that protect cells against oxidative damage (Kumar & Pandey, 2013).

The absence of diagnostic colour changes for saponins and glycosides suggests that these compound classes were either not efficiently

extracted by ethanol under the test conditions or are present only in negligible amounts, as ethanol typically favours the extraction of polar to mid-polar compounds such as phenolics and alkaloids but may exclude highly polar glycosides or saponins (Aziz *et al.*, 2021; Harborne, 1998). This finding is consistent with the reports of Cho *et al.* (2023), Al-Naimi *et al.* (2024), and Singh and Yadav (2025), who similarly reported the absence of saponins and glycosides in *Camellia sinensis* leaf extracts.

The quantitative phytochemical profile (Table 2) shows that the ethanol extract of *Camellia sinensis* leaves was heavily enriched in phenolic constituents: total phenols (176.40 mg/mL) were the most abundant, followed by tannins (42.28 mg/mL) and flavonoids (28.31 mg/mL). Lesser amounts of carbohydrates (21.33 mg/mL), steroids (14.35 mg/mL), terpenoids (12.19 mg/mL), alkaloids (8.25 mg/mL), and anthocyanins (4.93 mg/mL) were also detected. This composition indicates a polyphenol-rich extract, as phenols, tannins, and flavonoids are well known for their antioxidant activities.

Phenols, detected by a blue colour reaction, are major contributors to the antioxidant potential of *C. sinensis* extracts (Zhao *et al.*, 2023), while steroids, identified by a green-bluish colour, possess anti-

inflammatory and regulatory bioactivities that further support the pharmacological scope of the extract (Harborne, 1998). The presence of these phytochemical constituents—flavonoids, tannins, alkaloids, steroids, and terpenoids—in the ethanol extract of *Camellia sinensis* leaves is consistent with the findings of Awad *et al.* (2021), Onohuean *et al.* (2022), and Zhao *et al.* (2023), who reported that such compounds in *Camellia sinensis* extracts underpin its antioxidant and antimicrobial activities.

The ethanol extract of *C. sinensis* leaves produced clear, concentration-dependent antimicrobial effects against all tested organisms, with mean zones of inhibition increasing progressively from 40 to 100 mg/mL for each isolate. At the highest tested concentration (100 mg/mL), *Salmonella typhi* (51.7 mm) and *Enterococcus faecalis* (48.3 mm) showed the largest inhibition zones, comparable to or exceeding the positive control values recorded for those organisms, whereas *Pseudomonas aeruginosa* (29.4 mm) and *Fusarium* sp. (23.2 mm) were the least susceptible. *Candida albicans* and *Escherichia coli* displayed intermediate sensitivity (38.1 mm and 42.5 mm at 100 mg/mL, respectively). The negative control (distilled water) showed no activity, confirming the extract as the source of inhibition (Table 3).

Table 1. Qualitative Analysis of Phytochemical Constituents of the Ethanol Extract of *Camellia sinensis* Leaves

S/N	Compounds	Colour Observed	Inference
1	Alkaloids	Yellow ppt	Present
2	Carbohydrates	Brick-red ppt	Present
3	Saponins	No colour change	Absent
4	Terpenoids	Violet colour	Present
5	Flavonoids	Yellow-brown colour	Present
6	Tannins	Blue-green ppt	Present
7	Glycosides	No colour change	Absent
8	Anthocyanins	Green hue	Present
9	Phenols	Blue colour	Present
10	Steroids	Green-bluish colour	Present

Table 2. Quantitative Analysis of Phytochemical Constituents of the Ethanol Extract of *Camellia sinensis* Leaves

Tea Compound	Content (mg/mL)
Alkaloids	8.25
Carbohydrates	21.33
Anthocyanin	4.93
Terpenoids	12.19
Flavonoids	28.31
Tannins	42.28
Phenols	176.40
Steroids	14.35

Table 3 Antimicrobial Activity (Mean Zone of Inhibition, mm) of the Ethanol Extract of *Camellia sinensis* Leaves against Test Pathogens

Test Pathogen	100 mg/mL	60 mg/mL	40 mg/mL	Positive Control	Negative Control
<i>E. coli</i>	42.5 ^b	25.1 ^c	11.3 ^d	51.33 ^a	0.00 ^e
<i>E. faecalis</i>	48.3 ^{ab}	31.3 ^{ac}	18.4 ^{ad}	49.2 ^b	0.00 ^e
<i>S. typhi</i>	51.7 ^a	37.2 ^{cd}	18.3 ^{ad}	45.2 ^{ab}	0.00 ^e
<i>P. aeruginosa</i>	29.4 ^d	19.1 ^c	8.3 ^d	51.2 ^a	0.00 ^e
<i>Fusarium</i> sp.	23.2 ^d	13.7 ^b	5.2 ^a	40.7 ^c	0.00 ^e
<i>C. albicans</i>	38.1 ^c	29.1 ^d	20.5 ^b	44.9 ^c	0.00 ^e

Means followed by different superscripts in the same row are significantly different ($P < 0.05$). Positive control = Ciprofloxacin (20 mg/mL) for bacteria and Fluconazole for fungi; Negative control = distilled water.

Combining a 60 mg/mL ethanol extract of *C. sinensis* leaves with a chloramphenicol disc increased the zones of inhibition for all tested bacterial pathogens compared with chloramphenicol alone, with values increasing from 14.1 mm to 19.6 mm for *E. coli*, 21.5 mm to 30.3 mm for *E. faecalis*, 11.4 mm to 21.1 mm for *S. typhi*, and 8.3 mm to 20.8 mm for *P. aeruginosa*. This indicates enhanced antibacterial activity in the presence of the extract. The largest potentiation was observed against *Pseudomonas aeruginosa* (an increase of approximately 12.5 mm) and *Salmonella typhi* (an increase of approximately 9.7 mm), suggesting a particularly strong adjuvant effect of the tea extract against these organisms.

Chloramphenicol alone showed no activity against the tested fungi (*Fusarium* and *Candida albicans*); however, combining the antibiotic disc with the extract produced clear inhibition zones (12.3 mm and 23.5 mm, respectively), indicating that the tea extract confers antifungal activity when applied together with the antibiotic disc. This synergistic enhancement may be attributed to the polyphenolic constituents of the extract, particularly tannins and flavonoids, which are reported to disrupt microbial cell membranes and potentiate the action of co-administered antimicrobial agents (Fahmi *et al.*, 2022; Onohuean *et al.*, 2022).

The broth dilution MIC results (Table 5) showed that the ethanol extract of *C. sinensis* leaves exerted measurable antimicrobial activity against all tested organisms. A concentration of 40 mg/mL was sufficient to inhibit *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*, and *Candida albicans*, whereas a higher concentration (60 mg/mL) was required to inhibit *Pseudomonas aeruginosa* and *Fusarium* spp. This pattern is consistent with the findings of Archana and Jayanthi (2011) and reflects the known antimicrobial potential of *C. sinensis* ethanol extracts, which exhibit bacteriostatic and fungistatic properties largely attributable to bioactive compounds such as polyphenols, catechins, and

flavonoids. These compounds are reported to disrupt microbial cell membranes, inhibit essential enzymes, and induce oxidative stress in pathogens, leading to growth inhibition (Fahmi *et al.*, 2022; Onohuean *et al.*, 2022). The comparatively lower MIC values observed against *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*, and *Candida albicans*, relative to *Pseudomonas aeruginosa* and *Fusarium* spp., may be attributed to differences in the structural and physiological characteristics of these organisms, including cell wall composition and efflux pump activity, which can confer greater resistance in *Pseudomonas* and *Fusarium* (Fahmi *et al.*, 2022).

The DPPH assay showed that the ethanol extract of *C. sinensis* leaves exhibited moderate free-radical scavenging activity, with IC₅₀ values ranging from 71.7 µg/mL at the lowest tested concentration to 88.5 µg/mL at the highest, whereas the positive controls, ascorbic acid and gallic acid, gave considerably lower IC₅₀ values of 13.7 µg/mL and 19.2 µg/mL, respectively (Figure 1).

This moderate antioxidant activity is consistent with the extract's phytochemical composition, which is dominated by phenolic compounds, tannins, and flavonoids—constituents widely recognised for their free-radical scavenging capacity through hydrogen-donation and metal-chelation mechanisms (Zhao *et al.*, 2023). The comparatively higher IC₅₀ values relative to the standard antioxidants suggest that, while the crude extract possesses appreciable radical-scavenging potential, its activity is lower than that of purified reference compounds, likely due to the presence of non-antioxidant constituents that dilute the effective polyphenol concentration in the crude extract (Radeva-Ilieva, 2025). Similar moderate DPPH scavenging activity has been reported for *Camellia sinensis* leaf extracts by Aziz *et al.* (2021) and Onohuean *et al.* (2022), who attributed the antioxidant capacity primarily to catechins and other polyphenolic compounds. These findings support the potential of *C. sinensis* as a natural source of

antioxidant compounds for use in nutraceutical and pharmaceutical formulations.

Table 4. Synergistic Antimicrobial Activity (Mean Zone of Inhibition, mm) of the Ethanol Extract of *Camellia sinensis* Leaves with Chloramphenicol Disc against Test Pathogens

Test Pathogen	Chloramphenicol Disc Alone	Tea Extract (60 mg/mL) + Chloramphenicol Disc
<i>E. coli</i>	14.1 ^b	19.6 ^{ac}
<i>E. faecalis</i>	21.5 ^a	30.3 ^a
<i>S. typhi</i>	11.4 ^c	21.1 ^b
<i>P. aeruginosa</i>	8.3 ^{ac}	20.8 ^{bc}
<i>Fusarium</i> sp.	–	12.3 ^d
<i>C. albicans</i>	–	23.5 ^{ab}

Means followed by different superscripts in the same row are significantly different (P<0.05).

Table 5. Minimum Inhibitory Concentration (MIC) of the Ethanol Extract of *Camellia sinensis* Leaves against Test Pathogens

<i>E. coli</i>	<i>E. faecalis</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>Fusarium</i> sp.	<i>C. albicans</i>
40	40	40	60	60	40

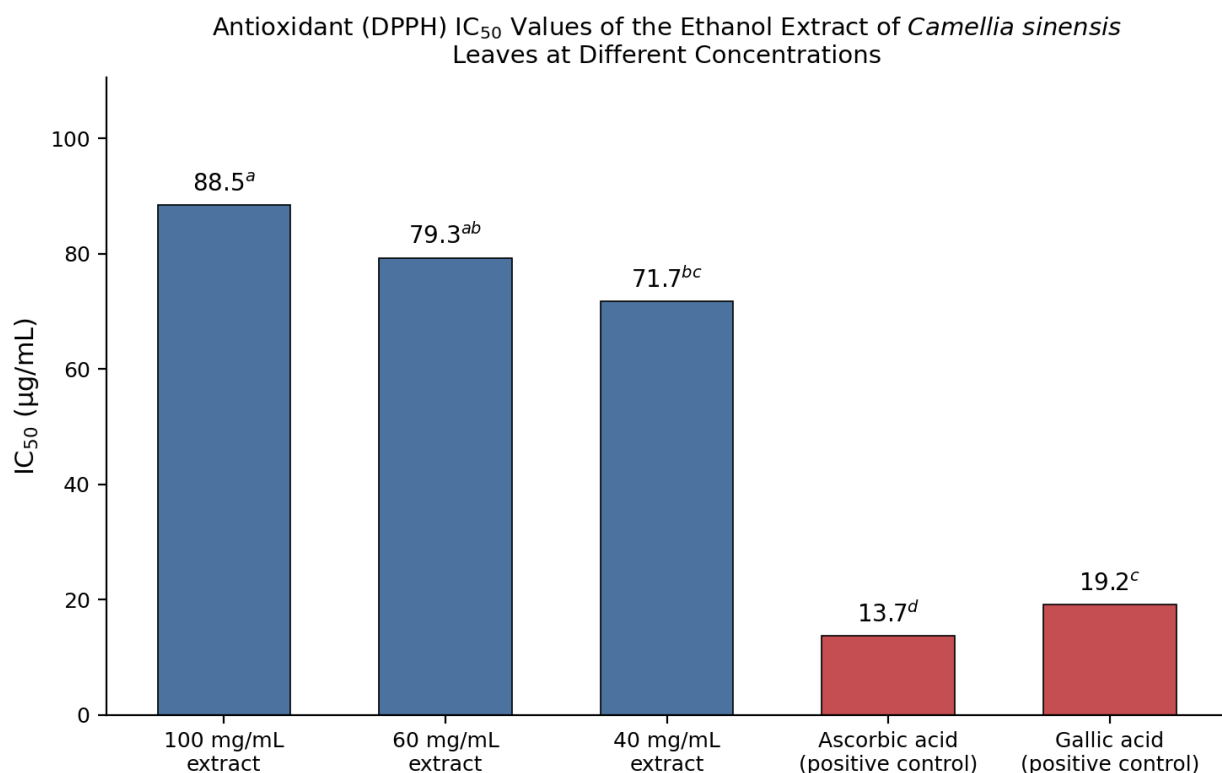


Figure 1: Antioxidant (DPPH) IC₅₀ Values of the Ethanol Extract of *Camellia sinensis* Leaves at Different Concentrations

Means followed by different superscripts within the same column are significantly different (P<0.05).

CONCLUSION

The study confirms that the ethanolic extracts of *C. sinensis* leaves are valuable sources of bioactive phytochemicals, predominantly phenols, tannins, and flavonoids, which contribute to its notable antimicrobial and antioxidant activities. The extract exhibited strong inhibition against several pathogenic

bacteria and fungi, either alone or synergistically with chloramphenicol, indicating its potential as a natural antimicrobial agent. Its moderate antioxidant capacity further supports its role in scavenging free radicals, contributing to overall health benefits. These findings highlight the potential of tea leaves for

application in the development of natural antimicrobial and antioxidant formulations.

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