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Comparative Antibacterial Efficacy and Phytochemical Characterization of Camellia Sinensis (Green Tea) Extracts against Clinically Important Bacterial Pathogens

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ABSTRACT: The increasing prevalence of antibacterial resistance has sped up the search for safe and effective natural alternatives to conventional antibiotics. Bioactive phytochemicals with antibacterial and antioxidant properties, such as polyphenols, flavonoids, and catechins, are abundant in green tea, or Camellia sinensis. The goal of the current study was to evaluate the phytochemical composition and in vitro antibacterial activity of green tea extracts in aqueous, methanolic, acetone, and hexane against clinically important bacterial pathogens, namely Escherichia coli, Pseudomonas spp., and Staphylococcus aureus. Qualitative phytochemical analysis revealed proteins, flavonoids, and tannins but not carbohydrates or saponins. Antibacterial activity at different extract concentrations was measured using the agar well diffusion technique. Acetone and methanolic extracts demonstrated comparatively greater antibacterial activity against all three bacterial strains, while hexane extract had the least inhibitory effect of the extracts under investigation. The aqueous extract also showed significant antibacterial activity, particularly against S. aureus and E. coli. The increased inhibitory activity with increasing extract concentration showed a concentration-dependent antimicrobial response. The reported antibacterial action is likely related to the presence of bioactive polyphenolic compounds that may prevent bacterial growth. These findings indicate that green tea extracts have strong antibacterial activity and may be effective natural antimicrobial agents against both Gram-positive and Gram-negative bacteria. To confirm their therapeutic potential, more research comprising the isolation of active compounds, the calculation of minimum inhibitory concentrations (MIC), toxicity assessment, and in vivo tests is advised.

1. INTRODUCTION

The rapid emergence and spread of antibiotic-resistant bacterial strains makes bacterial infectious diseases a major global public health concern. Multidrug-resistant (MDR) pathogens are becoming more common, which has significantly decreased the efficacy of traditional antibiotics. This has led to longer illnesses, higher healthcare costs, and higher death rates. Among the most common causes of respiratory, urinary tract, gastrointestinal, skin, and wound infections are pathogenic bacteria like Staphylococcus aureus, Pseudomonas species, and Escherichia coli. The widespread and often irresponsible use of antibiotics in human medicine, animal care, and agriculture has accelerated the development of bacterial resistance, underscoring the urgent need to develop safe, effective, and long-lasting alternatives to antibacterial medications. [1,2].

Medicinal plants have long been recognised as significant sources of bioactive compounds with potential therapeutic applications. Among the numerous secondary metabolites they generate are polyphenols, flavonoids, tannins, alkaloids, terpenoids, glycosides, coumarins, quinones, and saponins. Many of these substances have remarkable anti-inflammatory, antiviral, antibacterial, and antioxidant qualities.[3,4]. Due to their broad-spectrum biological actions and reduced potential to generate bacterial resistance, these naturally occurring phytochemicals have garnered significant interest as prospective substitutes for traditional antibiotics.

Green tea, or Camellia sinensis, is one of the medicinal plants that has garnered significant scientific interest due to its well-established health benefits and rich phytochemical composition. A common crop in tropical and subtropical regions is C. sinensis, a member of the Theaceae family. Based on the degree of leaf fermentation, tea is commercially classified into three groups: green tea (non-fermented), oolong tea (partially fermented), and black tea (completely fermented). Because green tea is less processed than other types of tea, it contains more physiologically active catechin [5].

The primary bioactive components of green tea are flavonoids, phenolic acids, and catechins such as epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC). These compounds have demonstrated potent antioxidant and antibacterial activity against a range of Gram-positive and Gram-negative bacteria. Numerous studies have shown that green tea polyphenols inhibit the growth of bacteria by weakening the integrity of cell membranes, interfering with essential metabolic processes, preventing biofilm formation, and reducing bacterial pathogenicity. These techniques make green tea a potentially natural source of antibacterial compounds with potential applications in the pharmaceutical, oral healthcare, and food preservation industries. [6,7].

Variations in extraction solvents significantly impact the recovery of bioactive compounds and, consequently, their biological activity, despite the fact that many studies have examined the antibacterial qualities of green tea. There is currently little data comparing the antibacterial effectiveness of extracts from organic and aqueous solvents against bacterial pathogens that are clinically significant. Additionally, determining the best extraction technique and optimizing the medicinal potential of green tea depend on an understanding of the connection between phytochemical composition and antibacterial activity.

In order to assess the phytochemical components of commercially available green tea (Camellia sinensis) and compare the in vitro antibacterial activity of aqueous, methanolic, acetone, and hexane extracts against three clinically significant bacterial pathogens—Escherichia coli, Pseudomonas spp., and Staphylococcus aureus—the current study was conducted.

The results of this study should add to the increasing amount of data that supports the use of green tea as a natural source of antibacterial compounds and offer a solid scientific foundation for its potential use in the creation of plant-based antibacterial formulations in the future.

The specific objectives of the present investigation were:

1. To prepare aqueous, methanolic, acetone, and hexane extracts of *Camellia sinensis* (green tea).
2. To perform qualitative phytochemical screening of the prepared extracts.
3. To isolate and identify selected bacterial cultures using morphological and biochemical characterization.
4. To evaluate the in vitro antibacterial activity of different green tea extracts against *Escherichia coli*, *Pseudomonas* spp., and *Staphylococcus aureus* using the agar well diffusion method.
5. To compare the antibacterial efficacy of different solvent extracts and determine the influence of extract concentration on bacterial growth inhibition.

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2. MATERIALS AND METHODS

2.1 Plant Material

Commercial green tea (Camellia sinensis L.) leaves were purchased as plant material from a local market in Dehradun, Uttarakhand, India (Organic India®, India). The information on the commercial product packaging served as the basis for the botanical identity. There was no independent herbarium authentication.. To avoid microbial contamination and the degradation of heat-sensitive phytochemicals, the tea leaves were shade-dried for three days at room temperature (25 ± 2°C) in a well-ventilated environment. Using an electric grinder, the dried leaves were ground into a fine powder, sieved to achieve a consistent particle size, and then kept in sterile, airtight containers until needed.

2.2 Preparation of Green Tea Extracts

The cold maceration method was used to create green tea extracts using aqueous, methanolic, acetone, and hexane solvents. In short, 100 mL of distilled water, methanol, acetone, or hexane were added to sterile 250 mL conical flasks along with 10 g of powdered green tea leaves. The flasks were placed on an orbital shaker for 48 hours at room temperature after being sealed with cotton plugs and aluminum foil to reduce solvent evaporation.

To get rid of plant residues, the mixtures were filtered through Whatman No. 1 filter paper after extraction. In a water bath kept at 50–60°C, the filtrates were concentrated until the majority of the solvent had evaporated. Semi-solid residues were obtained by air-drying the concentrated extracts at room temperature. Until additional phytochemical and antibacterial analyses, the dried extracts were moved to sterile glass vials and kept at 4°C.

Figure 1. Dried leaves of Camellia sinensis.

Figure 2. Preparation of green tea extracts.

Fig. 3: Filtration of Green tea extracts

2.3 Preparation of Nutrient Agar Medium

In accordance with the manufacturer's instructions, the necessary amount of nutrient agar was dissolved in distilled water to create nutrient agar medium. Prior to sterilization, the pH was brought to 7.0. The medium was autoclaved for 15 minutes at 121°C and 15 psi of pressure. Following sterilization, the molten medium was aseptically transferred into sterile Petri dishes after being cooled to between 45 and 50°C. Before being used, the agar plates were allowed to solidify and kept in a refrigerator.

2.4 Isolation and Identification of Bacterial Isolates

Standard microbiological techniques were used to isolate and identify pure bacterial cultures. Colony morphology and Gram staining were used for initial identification; biochemical characterization came next. Tests for Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate Utilization, Catalase, Oxidase, Urease, Triple Sugar Iron (TSI), and carbohydrate fermentation were performed on the isolates. The bacterial isolates were identified as Escherichia coli, Pseudomonas spp., and Staphylococcus aureus based on their morphological and biochemical traits.

2.5 Gram Staining

To distinguish between Gram-positive and Gram-negative bacteria, Gram staining was performed in accordance with the standard microbiological protocol. Each bacterial culture was made into a thin smear, air-dried, and heat-fixed on a sterile glass slide. After staining the smear with crystal violet for one minute, Gram's iodine was applied for two minutes. After ethanol was used for decolorisation, the slides were counterstained with safranin for 30 to 60 seconds. The stained slides were examined under a compound light microscope with an oil immersion objective (100×) after being cleaned with distilled water and allowed to air dry.

2.6 Biochemical Characterization

Standard biochemical tests such as Indole production, Methyl Red, Voges-Proskauer, Citrate Utilisation, Catalase, Oxidase, Urease, Triple Sugar Iron (TSI), and carbohydrate fermentation assays were used to further characterise the bacterial isolates. The bacterial isolates' identities were verified by interpreting the biochemical reactions in accordance with accepted microbiological identification protocols.

2.7 Qualitative Phytochemical Screening

Qualitative phytochemical analysis of the prepared green tea extracts was performed to detect the presence of major bioactive constituents, including tannins, flavonoids, saponins, proteins, and carbohydrates. Standard phytochemical methods were employed for each analysis. The appearance of characteristic colour changes or precipitates was considered indicative of the presence of the corresponding phytochemical constituents.

2.8 Evaluation of Antibacterial Activity

The antibacterial activity of aqueous, methanolic, acetone, and hexane extracts of *Camellia sinensis* was evaluated using the agar well diffusion method. Fresh bacterial cultures (24 h old) were uniformly spread over the surface of sterile nutrient agar plates using a sterile L-shaped spreader.

Wells of approximately 7 mm diameter were aseptically prepared in the agar using a sterile cork borer. Different concentrations of each extract (0.02, 0.05, 0.10, and 0.50 g) were carefully dispensed into the respective wells. The inoculated plates were incubated at 37°C for 24 h. Following incubation, the antibacterial activity of each extract was determined by measuring the diameter of the clear inhibition zones (mm) surrounding each well using a calibrated ruler. Larger inhibition zones indicated stronger antibacterial activity.

2.9 Statistical Analysis

Tukey's multiple comparison test was used after one-way analysis of variance (ANOVA) for statistical analysis. At $p < 0.05$, differences were regarded as statistically significant.

Natural Resources for Human Health
eISSN: 2583-1194

3. RESULTS

3.1 Morphological and Biochemical Characterization of Bacterial Isolates

3.1.1 Gram Staining

Gram staining was performed to determine the cellular morphology and Gram reaction of the isolated bacterial strains. Samples 1 and 2 appeared as pink-colored rod-shaped bacteria after safranin staining, indicating that they were **Gram-negative bacilli**. In contrast, Sample 3 retained the crystal violet stain and appeared as purple-colored cocci, confirming its identity as a **Gram-positive coccus** (Fig. 4).

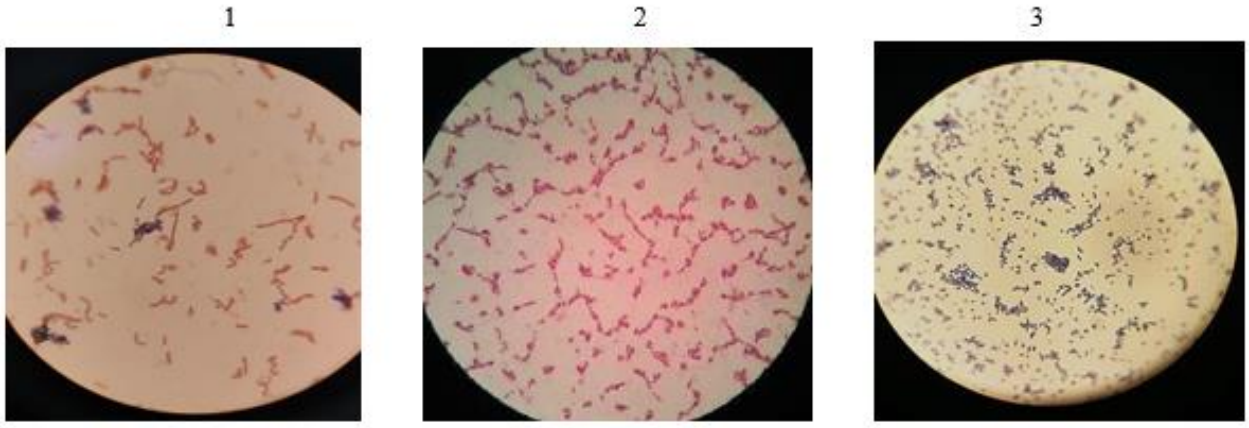

Figure 4. Gram staining results of the three bacterial isolates.

Sample 1 and 2 were stained pink with safranin and the bacteria were rod in shape and sample 3 was stained purple with crystal violet stain and it was coccus in shape.

3.1.2 Indole Test

The indole production test was performed to evaluate the ability of the isolates to hydrolyze tryptophan into indole using the enzyme tryptophanase. Formation of a cherry-red ring after the addition of Kovac's reagent indicated a positive reaction.

Sample 1 showed a positive indole reaction, whereas Samples 2 and 3 did not produce a red ring, indicating negative results (Fig. 5).

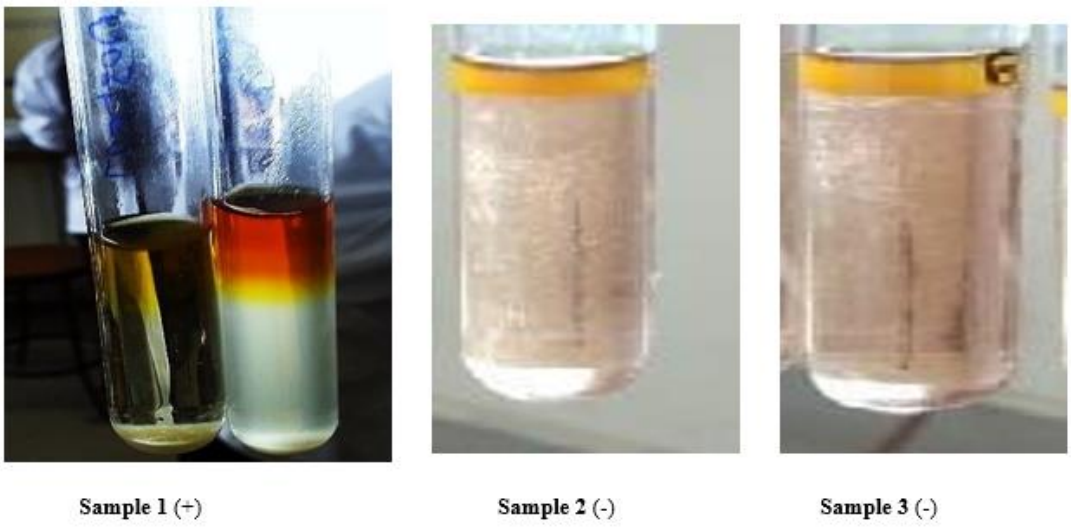

Figure 5. Indole test results of the bacterial isolates.

3.1.3 Methyl Red Test

The Methyl Red (MR) test was carried out to determine the ability of bacterial isolates to produce stable acidic end-products from glucose fermentation.

Samples 1 and 3 exhibited a distinct red coloration, indicating positive MR reactions, whereas Sample 2 remained yellow, confirming a negative result (Fig. 6).

Fig.6.1: Sample 1 (+)

Fig.6.2: Sample 2 (-)

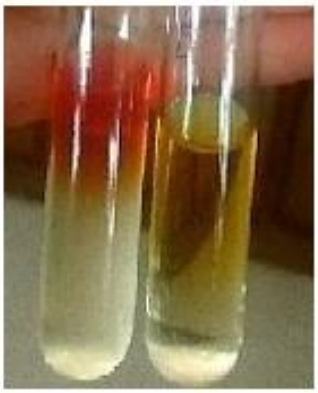

Fig.6.3: Sample 3 (+)

Figure 6. Methyl Red test results.

3.1.4 Voges–Proskauer Test

The Voges–Proskauer (VP) test was used to detect acetoin production during glucose fermentation.

Only Sample 3 produced a characteristic red coloration after the addition of VP reagents, indicating a positive reaction. Samples 1 and 2 remained unchanged and were therefore considered VP negative (Fig. 7).

Fig.7.1: Sample 1 (-)

Fig.7.2: Sample 2 (-)

Fig.7.3: Sample 3 (+)

Figure 7 Voges–Proskauer test results.

3.1.5 Citrate Utilization Test

The citrate utilization test assessed the ability of bacterial isolates to utilize citrate as the sole carbon source.

Samples 2 and 3 successfully utilized citrate, resulting in a color change of Simmons citrate agar from green to blue. Sample 1 showed no color change and was therefore considered citrate negative (Fig. 8).

Fig.8.1: Sample 1 (-)

Fig.8.2: Sample 2 (+)

Fig.8.3: Sample 3 (+)

Figure 8. Citrate utilization test.

3.1.6 Catalase Test

Catalase activity was determined by observing oxygen bubble formation following the addition of hydrogen peroxide.

Samples 2 and 3 produced immediate effervescence, indicating positive catalase activity, whereas Sample 1 did not produce bubbles and was considered catalase negative (Fig. 9).

Fig.9.1: Catalase (-)

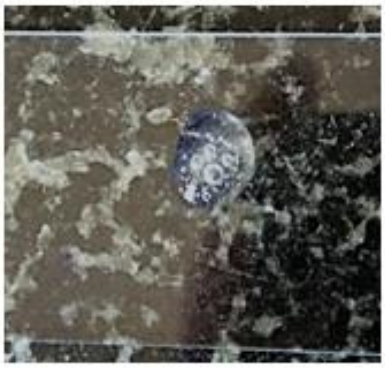

Fig.9.2: Catalase (+)

Fig.9.3: Catalase (+)

Figure 9. Catalase test results.

3.1.7 Oxidase Test

The oxidase test was performed to determine the presence of cytochrome c oxidase enzyme.

Sample 2 produced a dark purple coloration within 30 seconds and was considered oxidase positive. Samples 1 and 3 showed no color change and were therefore oxidase negative (Fig. 10).

Natural Resources for Human Health
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Fig.10.1: Sample 1 (-)

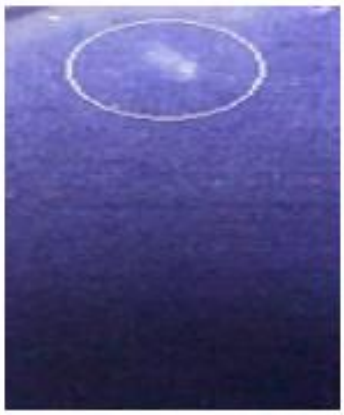

Fig.10.2: Sample 2 (+)

Fig.10.3: Sample 3 (-)

3.1.8 Urease Test

The urease test was carried out to evaluate the ability of bacterial isolates to hydrolyze urea into ammonia.

Only Sample 3 showed a pink coloration indicating urease activity, whereas Samples 1 and 2 remained unchanged and were recorded as urease negative (Fig. 11).

Fig.11.1: Sample 1 Urease (-)

Fig.11.2: Sample 2 Urease (-)

Fig.11.3: Sample 3 Urease (+)

Figure 11. Urease test

3.1.9 Triple Sugar Iron (TSI) Agar Test

The Triple Sugar Iron (TSI) agar test was performed to evaluate carbohydrate fermentation patterns and hydrogen sulfide production. The observed reactions assisted in confirming the biochemical characteristics of the isolates (Fig. 12).

Natural Resources for Human Health
eISSN: 2583-1194

Fig.12.1: Sample 1 (-)

Fig.12.2: Sample 2 (-)

Fig.12.3: Sample 2 (-)

Figure 12. Triple Sugar Iron (TSI) agar test.

4. Biochemical Identification of Bacterial Isolates

The biochemical characteristics of the three bacterial isolates are summarized in Table 1. Based on the combined results of Gram staining and biochemical analyses, Sample 1 was identified as Escherichia coli, Sample 2 as Pseudomonas spp., and Sample 3 as Staphylococcus aureus.

Table 1. Biochemical characteristics of the bacterial isolates.

S.No.	Name of Test	Sample 1	Sample 2	Sample 3
1	Gram staining	Negative	Negative	Positive
2	Indole	Positive	Negative	Negative
3	Methyl red	Positive	Positive	Negative
4	Voges prausker	Negative	Negative	Positive
5	Citrate utilization	Negative	Positive	Positive
6	Catalase test	Negative	Positive	Positive
7	Urease test	Negative	Negative	Negative
8	Oxidase tests	Negative	Positive	Negative

Identification of isolated microorganisms

From the above biochemical methods, the bacteria that were isolated are identified as: sample 1 *Escherichia coli*, sample 2 as Pseudomonas sp., and sample 3 as *Staphylococcus aureus* (Table 1).

4.1 Qualitative Phytochemical Screening of Green Tea Extract

Qualitative phytochemical analysis of the green tea extract revealed the presence of several biologically active compounds (Fig. 13). Tannins, flavonoids, and proteins (Ninhydrin test) showed positive reactions, whereas saponins and carbohydrates (Molisch test) were absent.

The presence of tannins and flavonoids suggests that these phytochemicals may contribute significantly to the antibacterial activity of green tea extracts.

4.2 Antibacterial Activity of Green Tea Extracts

The antibacterial activity of aqueous, methanol, acetone, and hexane extracts of green tea was evaluated against Escherichia coli, Pseudomonas spp., and Staphylococcus aureus using the agar well diffusion method. Antibacterial efficacy was assessed by measuring the diameter of the inhibition zone (mm) at different extract concentrations.

4.3 Acetone Extract

Among all solvent extracts, the acetone extract exhibited strong antibacterial activity against all three bacterial species (Table 3). The highest inhibition zones were recorded at 0.5 g concentration, measuring 20.3 mm against *E. coli*, 19.2 mm against *Pseudomonas spp.*, and 21.3 mm against *S. aureus*. The inhibitory effect increased with increasing extract concentration.

4.4 Hexane Extract

The hexane extract demonstrated comparatively weak antibacterial activity (Table 4). Little or no inhibition was observed at lower concentrations. At the highest concentration (0.5 g), inhibition zones of 2.1 mm, 2.3 mm, and 6.5 mm were observed against *E. coli*, *Pseudomonas spp.*, and *S. aureus*, respectively.

4.5 Aqueous Extract

The aqueous extract exhibited considerable antibacterial activity, particularly against *Staphylococcus aureus* and *Escherichia coli* (Table 5). The maximum inhibition zone of 22.6 mm was observed against *S. aureus* at 0.5 g concentration, while *E. coli* showed an inhibition zone of **20.3 mm*. Limited antibacterial activity was observed against *Pseudomonas spp.*, with inhibition becoming evident only at higher concentrations.

4.6 Methanol Extract

The methanol extract also demonstrated strong antibacterial activity against all tested organisms (Table 6). At 0.5 g concentration, inhibition zones reached 18.0 mm for *E. coli*, 15.0 mm for *Pseudomonas spp.*, and 20.6 mm for *S. aureus*. Similar to the acetone extract, antibacterial activity increased with extract concentration.

4.7 Comparative Analysis

Among the four solvent extracts, acetone and methanol extracts exhibited the highest antibacterial activity, followed by the aqueous extract, whereas the hexane extract showed the weakest inhibitory effect. Staphylococcus aureus was the most susceptible bacterial strain, exhibiting the largest inhibition zones across all effective extracts. The antibacterial activity was concentration-dependent, with the greatest inhibition consistently observed at 0.5 g extract concentration.

Overall, the findings demonstrate that green tea extracts possess significant antibacterial potential against both Gram-positive and Gram-negative bacteria, with solvent polarity playing an important role in the extraction of bioactive antibacterial compounds.

Fig.13.1: Tannins

Fig.13.2: Saponins

Fig.13.3: Flavonoids

Fig.13.4: Proteins

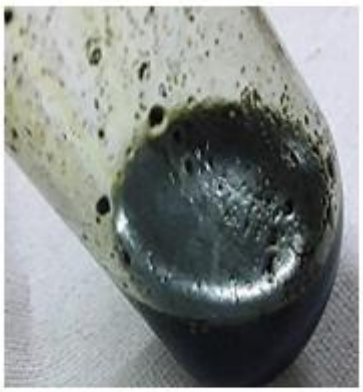

Fig.13.5: Carbohydrates

Figure 13. Qualitative phytochemical screening.

Saponin and Molisch test showed negative result, while Tannin, Flavonoids and ninhydrin test showed positive result (Table 2).

Table 2: Qualitative Phytochemical Tests

S. No.	Phytochemical Tests	Results
1	Saponin	Negative
	No foam is observed	
2	Tannin	Positive
	Brownish black color is observed	
3	Flavonoids	Positive
	Appearance of white colored layer on top is observed	
4	Proteins (ninhydrin test) Yellow color is observed	Positive
5	Carbohydrates (Molisch test)	Negative
	No color change is observed	

RESULTS OF ANTIBACTERIAL ACTIVITY OF EXTRACTS (FIGS 14.1–16.4 AND TABLES 3 – 6)

Fig.14 Pseudomonas sp.

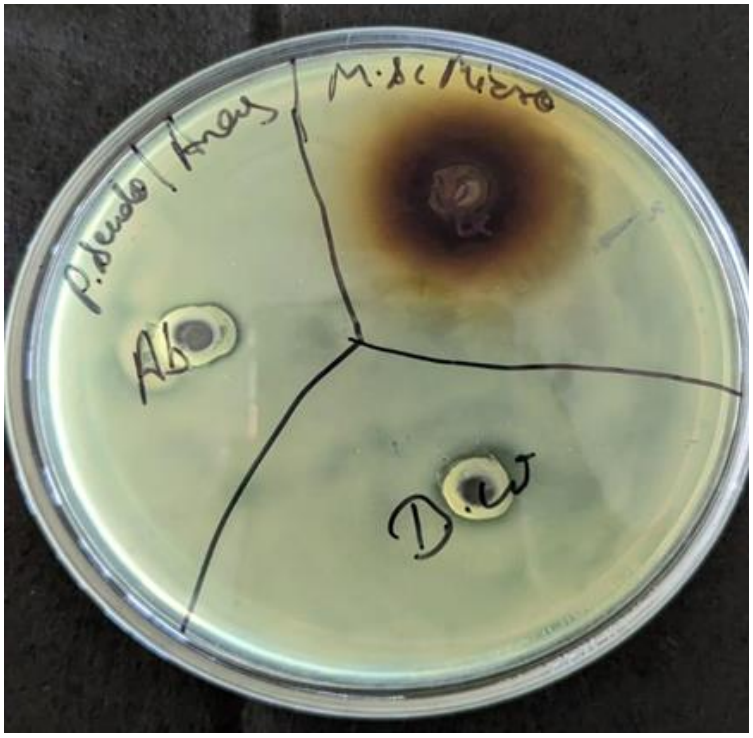

Fig. 14.1: Aqueous extract (green tea)

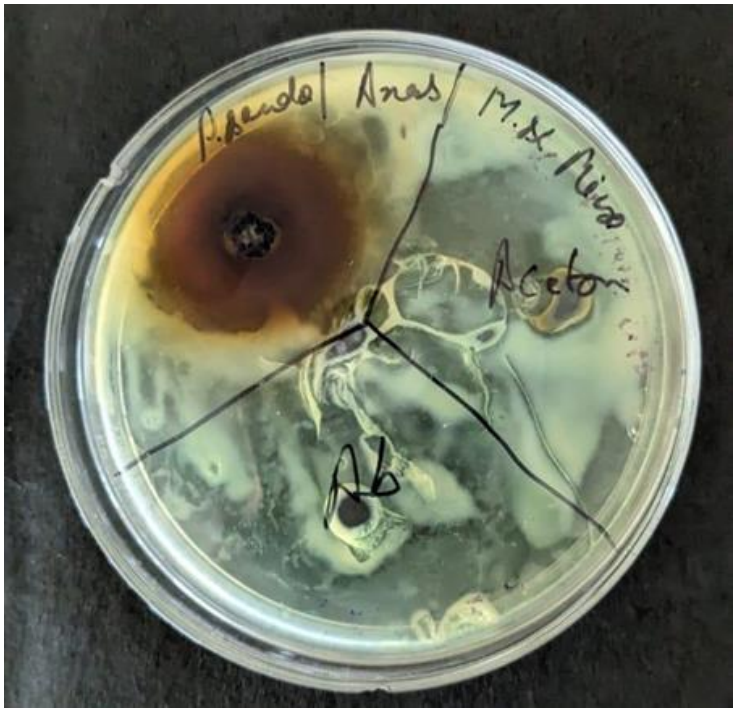

Fig. 14.2: Acetone extract (green tea)

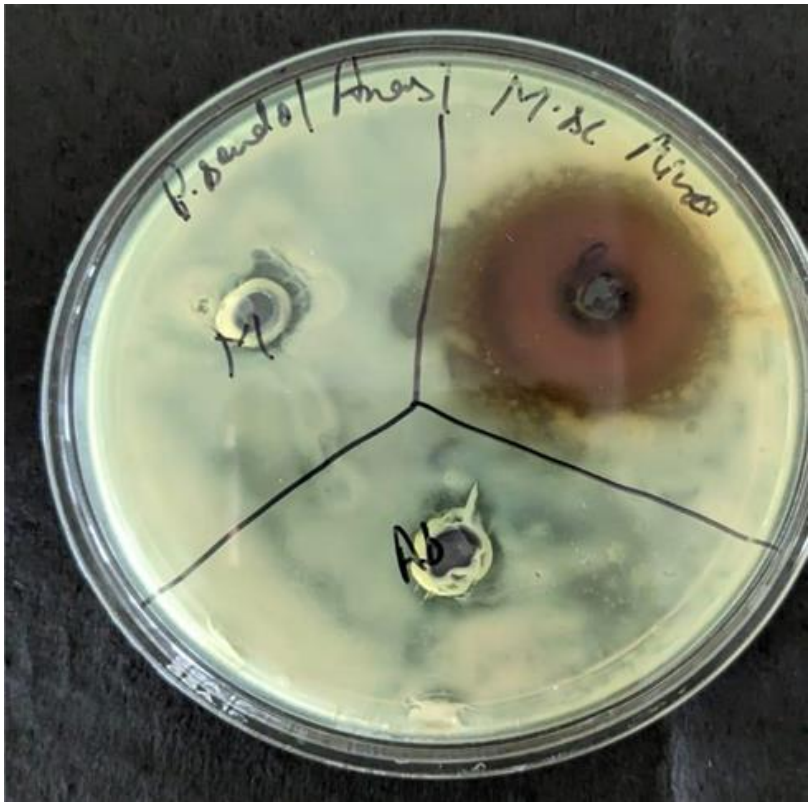

Fig. 14.3: Methanol extract (green tea)

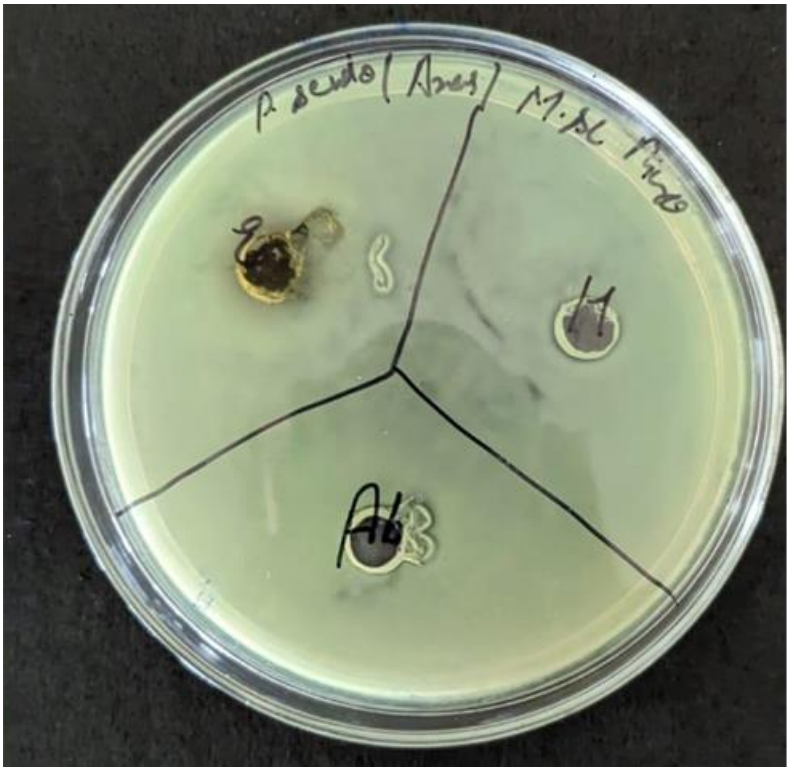

Fig. 14.4: Hexane extract (green tea)

1. Escherichia coli

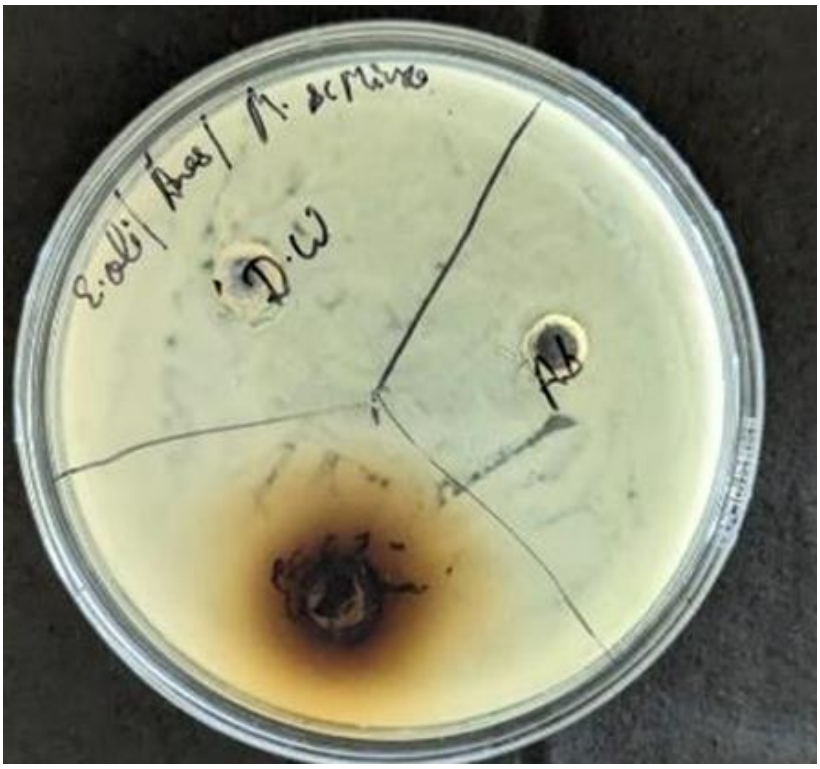

Fig. 15.1: Aqueous extract (green tea)

Fig. 15.2: Acetone extract (green tea)

Fig. 15.3: Methanol extract (green tea)

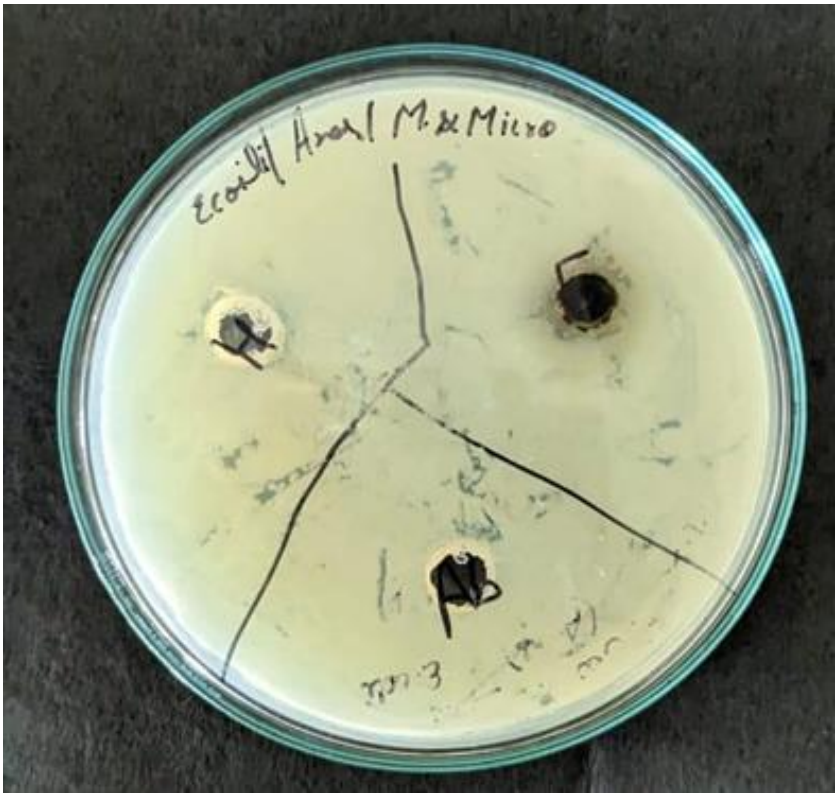

Fig. 15.4: Hexane extract (green tea)

2. ***Staphylococcus aureus***

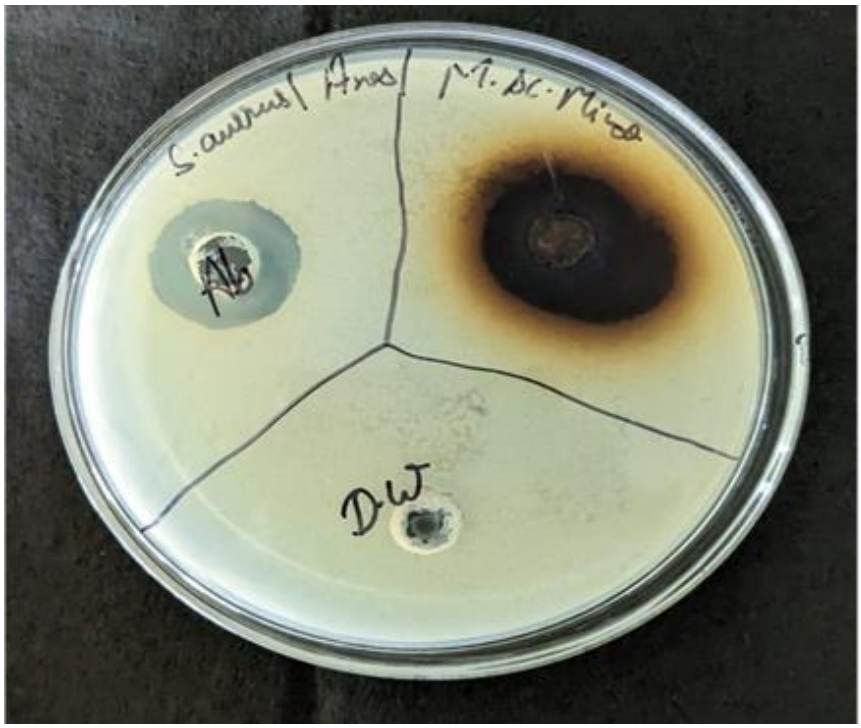

Fig. 16.1: Aqueous extract of green tea

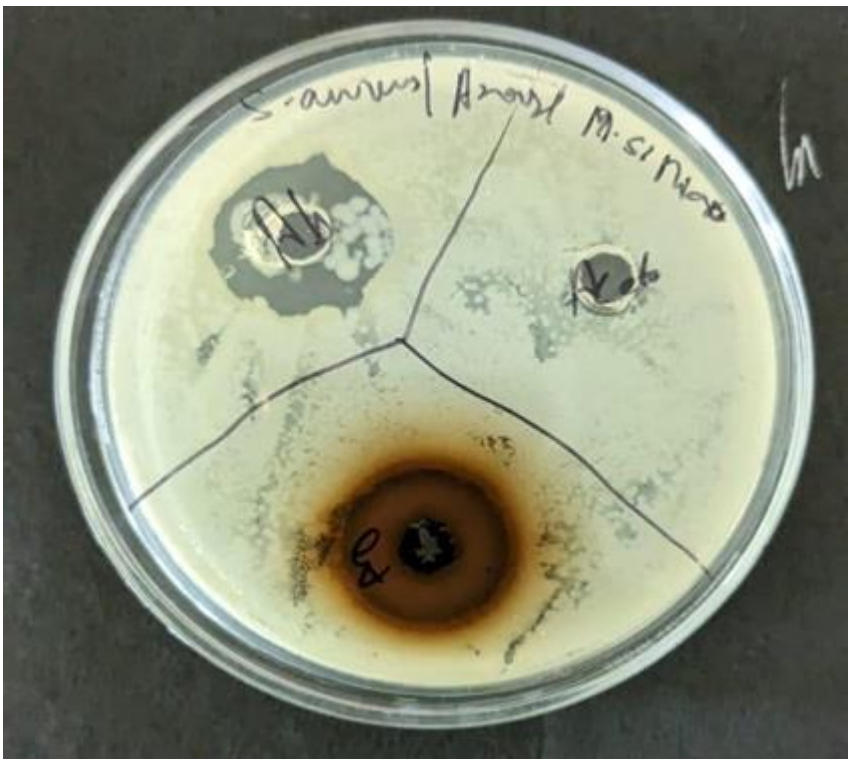

Fig. 16.2: Acetone extract of green tea

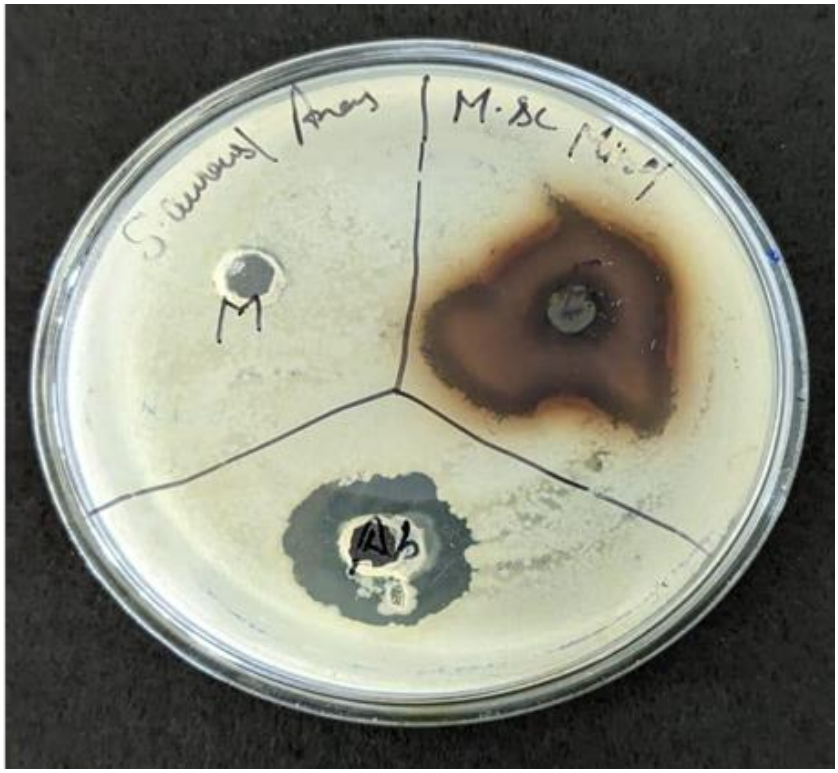

Fig. 16.3: Methanol extract of green tea

Fig. 16.4: Hexane extract of green tea

Table 3: Acetone Green Tea Extract

S.No.	Test organism	Zone of inhibition (in mm) on different concentrations			
		0.02g	0.05g	0.1g	0.5g
1.	*E. coli*	-	6.6	13.6	20.3
2.	*Pseudomonas*	8.2	11.7	14	19.2
3.	*S. aureus*	7.5	10	15.4	21.3

Table 4: Hexane Green Tea Extract

S.No.	Test organism	Zone of inhibition(in mm) on different concentrations			
		0.02g	0.05g	0.1g	0.5g
1.	*E. coli*	-	-	-	2.1
2.	*Pseudomonas*	-	-	-	2.3
3.	*S. aureus*	-	-	1.9	6.5

Table 5: Aqueous Green Tea Extract

S.No.	Test organism	Zone of inhibition(in mm) on different concentrations			
		0.02g	0.05g	0.1g	0.5g
1.	*E. coli*	7.67	10.16	13.6	20.3
2.	*Pseudomonas*	-	-	8	10
3.	*S. aureus*	11	12	19	22.6

Table 6: Methanol Green Tea Extract

S.No	Test organism	Zone of inhibition(in mm) on different concentrations			
		0.02g	0.05g	0.1g	0.5g
1.	*E. coli*	-	-	9	18
2.	*Pseudomonas*	-	10.33	12.3	15
3.	*S. aureus*	10	15	18.6	20.6

5. DISCUSSION

The current study shows that green tea, or Camellia sinensis, has significant antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli and Pseudomonas spp.) bacteria. The antibacterial efficacy of the various solvent extracts differed, suggesting that the extraction solvent is essential for separating the bioactive phytochemicals that inhibit bacterial growth. The antibacterial activity generally rose as the extract concentration increased, indicating a concentration-dependent inhibitory effect.

The presence of bioactive phytochemicals like flavonoids, tannins, and polyphenolic catechins—especially epigallocatechin gallate (EGCG), which is known to disrupt bacterial cell membranes, inhibit essential bacterial enzymes, interfere with nucleic acid synthesis, and suppress bacterial biofilm formation—is primarily responsible for the observed antibacterial activity. Tannins, flavonoids, and proteins were found in the current study's qualitative phytochemical screening, but saponins and carbohydrates were not. These results lend credence to the theory that green tea extracts' antibacterial activity is primarily attributed to polyphenolic compounds.

Staphylococcus aureus showed the greatest susceptibility to green tea extracts among the tested bacterial isolates, especially at higher concentrations. The acetone (21.3 mm) and methanol (20.6 mm) extracts were closely followed by the aqueous extract, which generated the largest inhibition zone (22.6 mm at 0.5 g). While Pseudomonas species showed relatively lower sensitivity, particularly to aqueous and hexane extracts, Escherichia coli also showed significant susceptibility. Pseudomonas species' highly impermeable outer membrane, multidrug efflux pumps, and intrinsic resistance mechanisms that lessen the penetration and efficacy of antibacterial compounds may all contribute to their comparatively lower susceptibility.

The antibacterial efficacy was significantly impacted by the extraction solvent. All three of the bacterial isolates were strongly inhibited by acetone and methanol extracts, suggesting that these solvents effectively extracted the phenolic compounds and flavonoids that have antibacterial properties. The hexane extract, on the other hand, exhibited very little activity, indicating that polar compounds rather than non-polar molecules are the main antibacterial components of green tea. Additionally, the aqueous extract showed strong antibacterial activity, especially against S. aureus and E. coli, suggesting that water-soluble phytochemicals play a major role in green tea's antibacterial potential.

The results of this study are in line with earlier findings regarding Camellia sinensis's antibacterial qualities. The antibacterial potential of tea polyphenols in oral health applications was highlighted by Rathinavelu et al. (2019), who showed that green tea extracts significantly inhibited the growth of Streptococcus mutans. Similar to the concentration-dependent inhibition seen in this study, Amit Kumar et al. (2012) reported that the antibacterial activity of green tea depends on both the extraction solvent and extract concentration.

Camellia sinensis has strong antibacterial activity against bacterial pathogens that are resistant to drugs, according to Farooqui et al. (2015), who also suggested that it could be used either on its own or in conjunction with other medicinal plants. The strong inhibitory activity of the aqueous extract seen in the current study is corroborated by Roy et al. (2018), who found that aqueous green tea extracts successfully inhibited the growth of Escherichia coli. Additionally, Camellia sinensis leaf extracts were found to have strong antibacterial activity against Escherichia coli and Staphylococcus aureus by Tariq et al. (2013), which supports the results of this investigation.

The synergistic interaction of several phytochemicals rather than the action of a single compound may also be linked to the antibacterial activity found in this study. Tannins precipitate membrane proteins and inhibit microbial enzymes, which together increase the inhibition of bacterial growth, while flavonoids and catechins can increase the permeability of bacterial membranes. Green tea extracts' broad-spectrum antibacterial activity could be explained by these synergistic mechanisms.

While the current study validates the antimicrobial properties of green tea extracts, it is important to recognise some limitations. The agar well diffusion method was the only technique used to measure antibacterial activity, and only three bacterial species were examined. To confirm the therapeutic potential and safety of the extracts, additional research should include MIC and MBC determination, quantitative phytochemical profiling, toxicity assessment, time-kill kinetics, biofilm inhibition assays, and in vivo evaluation. The antibacterial mechanisms and therapeutic potential of phytochemicals derived from green tea would be better understood thanks to these analyses.

Overall, the current research shows that Camellia sinensis is a potentially useful natural source of antimicrobial substances. Its potential use as a natural antibacterial agent in pharmaceutical formulations, food preservation, oral healthcare products, and

as a supplement to traditional antibiotics for treating bacterial infections is supported by its strong inhibitory activity against both Gram-positive and Gram-negative bacteria.

To further determine the therapeutic potential and safety of Camellia sinensis extracts, additional research should include MIC and MBC determination, quantitative phytochemical profiling, toxicity assessment, time-kill kinetics, biofilm inhibition studies, and in vivo evaluation. Clarifying the compounds causing the observed antibacterial activity would also be aided by the identification and quantification of individual bioactive compounds using suitable analytical techniques.

6. CONCLUSION

The current study showed that leaf extracts from Camellia sinensis have antibacterial activity against S. aureus, E. coli, and Pseudomonas spp., with activity varying depending on concentration and extraction solvent. While the aqueous extract demonstrated especially strong inhibition against S. aureus and E. coli, the acetone and methanol extracts generally demonstrated strong antibacterial activity. The activity of the hexane extract was relatively low. Tannins, flavonoids, and proteins were found through qualitative phytochemical screening, indicating that these components might be involved in the observed antibacterial effects. However, before any therapeutic application can be established, more research involving MIC/MBC determination, quantitative phytochemical characterization, toxicity assessment, and in vivo validation is needed.

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Author contributions

PREETI HANDA KAKKAR[1]: Conceptualization, formulation of the research objectives, research supervision, interpretation of results, manuscript writing, critical review and editing, and final approval of the manuscript.

CHANDRIKA BHATT[2]: Methodology, experimental design, field/laboratory experimentation, data collection, and analysis of experimental observations.

PRERANA BADONI[3]: Laboratory experimentation, sample processing, data collection, laboratory analysis, and documentation of experimental results.

SHEFALEE SINGH[4]: Field experimentation, field observations, sample collection, maintenance of experimental records, and data compilation.

DHYAL SINGH[5]: Data curation, statistical analysis, interpretation of results, preparation of tables and figures, and validation of data.

SHRUTI SAXENA[6]: Literature review, collection of relevant references, data interpretation, and drafting of the manuscript.

PRIYANKA KUMARI[7]: Manuscript preparation, literature compilation, preparation of tables/figures, editing, and critical review of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

The datasets generated or analysed during the current study are available from the corresponding author upon reasonable request.

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