JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Analyzing the Efficacy of Different Formulations of *Camellia sinensis L.*

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Abstract: *Camellia sinensis L.*, also referred as Green tea is acclaimed as a therapeutic substance and has notable *in vitro* antimicrobial activity. The purpose of this investigation was to evaluate the antibacterial activity of green tea extracts in different formulations- ethanolic, concentrated, Dimethyl sulfoxide (DMSO), and aged (old) against two pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus*, through a disk diffusion assay using Mueller Hinton Agar plates. The inhibition zones were measured and compared as part of the antimicrobial activity assessment of each of the extracts. The inhibitory zone of the ethanolic green tea extract against *Escherichia coli* was the largest, followed by old and concentrated one, while there was no observable activity in the extract prepared in DMSO. Contrary to the results against *E. coli*, concentrated green tea extract exhibited highest antimicrobial measure against *Staphylococcus aureus*, whereas old and ethanolic extracts showed medium and equivocal activity respectively. Overall, these data imply that the activity of *Camellia sinensis* extracts is dependent on both the mode of extraction and the bacterial strain targeted.
Thus, the study determined to extract green tea phytochemicals in ethanol with antimicrobial activity appropriate against a Gram-negative bacterium like *E. coli*, and concentrated extract was appropriate against a Gram-positive bacterium like *S. aureus*. The aged extract also maintained substantial bioactivity, and demonstrates the antimicrobial phytochemicals have high persistence in green tea. This research highlights the potential use of green tea extracts as natural antimicrobial agents for pharmaceutical and food preservation applications. It also highlights the need to optimize extraction methods and storage conditions accordingly to potentialize the bioactive capacity.

Index Terms **- Green tea extract, Antimicrobial activity, Disc diffusion method, Zone of inhibition.**

I. INTRODUCTION

Treating bacterial infections caused by antibiotic-resistant pathogens remains a significant challenge. Consequences include prolonged hospital stays, increased mortality, higher antibiotic dosage requirements, and limited therapeutic options (Johnson et al., n.d.). Recent strategies to combat bacterial resistance include the development of next-generation antibiotics, combination therapies, natural and synthetic compounds, and novel drug delivery systems (Johnson et al., n.d.).
Green tea is derived from the leaves of *Camellia sinensis L.*, a plant native to Eastern Asia. In much of Asia, green tea is regarded as a healthy beverage and has traditionally been used in Chinese medicine for disease prevention (Abolfathi et al., 2012). Extracts from *Camellia sinensis* have garnered significant scientific interest for their potential as natural antimicrobials. Studies suggest these extracts possess a broad range of biological activities, including antiviral, antifungal, antioxidant, and antibacterial properties against both Gram-positive and Gram-negative bacteria, primarily due to their polyphenolic compounds (Pezeshki et al., n.d.).

Pharmacokinetic data support the safety and tolerance of 800 mg/day of epigallocatechin gallate (EGCG), a key catechin, in capsule form over a four-week period (Rahman et al., n.d.). Drinking three to five cups of green tea per day may yield approximately 250 mg of catechins, including EGCG (Rahman et al., n.d.). An antioxidant effects come from the ability of green tea to limit the number of free radicals by binding to reactive oxygen species (ROS). Anti-inflammatory effects may be a result of increased production of IL-10, an anti-inflammatory cytokine (Crespy & Williamson, as cited in Radji et al., 2013). Green tea is also linked to cardiovascular health benefits and has anti-inflammatory, antioxidative, antimutagenic, and anticarcinogenic properties. Epidemiological evidence suggests a potential role in reducing the prevalence of type II diabetes. In diabetic mice, green tea demonstrated antihyperglycemic effects (Abolfathi et al., 2012).

II. MATERIALS

Sr. No.	Types	Particulars
1.	Sample	Fresh green tea leaves
2.	Reagents	10.00 % Dimethyl sulfoxide (DMSO)
		Absolute Ethanol
3.	Medium used	Sterilized Mueller Hinton Agar
4.	Glassware	Sterile petri plates
		Sterile test tubes
		Sterile 1.0 ml, 10.0 ml pipettes
		Clean dry beakers
		Clean dry test tubes
		Clean dry pipettes of 1.0 ml and 10.0 ml
		Clean dry measuring cylinder
		Clean dry filtration funnel
		Clean dry glass rod stirrer
5.	Microorganisms Cheesbrough (2006)	*Escherichia coli* culture (18-24 hours old, adjusted to 0.1 Optical density at 530 nm)
		Staphylococcus aureus culture (18-24 hours old, adjusted to 0.1 Optical density at 530 nm)
6.	Miscellaneous	Muslin cloth
		Sterile Whatman filter paper discs
		Sterile cotton swabs
		Clean dry forceps
		Spatula
		Analytical weighing balance
		4 ℃ Refrigerator
		37 ℃ Incubator
		Magnetic stirrer
		Mortar and pestle
		Water bath
		Autoclave for steam/moist heat sterilization
		Distilled water

III. METHODOLOGY

A) Formulation of Green tea extracts: 0.75 grams of fresh green tea leaves were weighed and grounded using a mortar and pestle with 2.0 ml of 1:4 ethanol solution (1:4 ethanol to distilled water) and stirred thoroughly. The resultant ethanolic green tea extract was divided into four parts **(Fig. 1)** as follows:

Tube 1: Concentrated ethanolic green tea extract (Ethanolic extract subjected to boiling water bath for 10 minutes).
Tube 2: Ethanolic green tea extract (used directly without modifications).
Tube 3: Ethanolic green tea extract incubated at 4 ℃ for 03 days (72 hours) referred as old/aged extract.
Tube 4: Ethanolic green tea extract + 0.5 ml of 10 % DMSO.

B) Preparation of Culture Plates: Sterile Mueller-Hinton agar (MHA) plates were prepared (HiMedia Laboratories, n.d.) and swabbed with standardized bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus*. This medium is recommended by clinical guidelines due to its consistent composition and optimal diffusion properties, which support accurate measurement of antimicrobial activity using the disk diffusion method (Dewees et al., 1970). To assess the antimicrobial potential of prepared green tea extracts, the agar disk diffusion method was employed, adhering to standardized protocols for assessing antibacterial activity. (European Committee on Antimicrobial Susceptibility Testing [EUCAST], n.d.)

C) Application of Green Tea Extracts: Sterile Whatman filter paper discs were immersed in the prepared extracts of ethanolic, DMSO treated, concentrated and aged green tea samples. Aseptically, using sterile forceps, the green tea extracts loaded Whatman filter paper discs were placed on the microbial culture inoculated Mueller Hinton agar surfaces. Sufficient space was kept between the extracts impregnated discs to restrict coinciding of the inhibition zones (Nyuykonge et al., 2021).

D) Pre-diffusion: The plates were then refrigerated at 4℃ for 15 minutes to facilitate the diffusion of the extracts into the agar medium prior to incubation, for boosting the accuracy in recording the inhibition zone (Pongtharangkul & Demirci, 2004).

E) Incubation: Maintaining aerobic conditions, the culture plates were incubated for 24 hours at 37℃. This incubation period allows for optimal bacterial growth and interaction with the antimicrobial agents present in the green tea extracts.

F) Measurement of Inhibition Zones: After 24 hours incubation at 37℃, the diameters of the clear zones surrounding each disc were measured in millimeters. These zones indicate the extent of bacterial growth inhibition and were compared across the different green tea preparations. This methodology aligns with established practices in antimicrobial research, where green tea extracts have demonstrated inhibitory effects against both gram-positive and gram-negative organisms (Badger et al., 2019).

IV. RESULTS

Antibiotic susceptibility testing by agar disc diffusion implicated that all 04 extracts of *Camellia sinensis L.* (Green tea); ethanolic, DMSO treated, concentrated and aged extracts exhibited different sized zones of clearance/inhibition **(Table 1 and 2)** against *Staphylococcus aureus* and *Escherichia coli* after 24 hours of incubation at 37 ℃. For *Staphylococcus aureus*, the heat concentrated green tea extract marks the highest zone of inhibition (20 mm), while the old green tea extract and ethanolic extract

showcased the lesser value of inhibition zone which were 14 mm and 10 mm respectively **(Fig. 2)**. The sequence of the zone of inhibition/clearance is as follows: Concentrated > Old > Ethanol > DMSO treated **(Table 1)**.

Ethanolic extract demonstrated the largest zone of inhibition of 20 mm, followed by aged (14 mm) and concentrated (10 mm) for *Escherichia coli* **(Fig. 3)**. The sequence of the zone of inhibition/clearance is as follows: Ethanol > Old > Concentrated > DMSO treated **(Table 2)**. DMSO treated extracts exhibited least size of zone of inhibition against both the organisms.

V. DISCUSSION

The current research explored the antimicrobial properties of *Camellia sinensis L.* (Green tea) extracts against two clinically relevant bacterial isolates, *Escherichia coli* and *Staphylococcus aureus*, using various forms of extracts: ethanolic, heat concentrated, aged (stored for 72 hours), and DMSO treated. The zones of inhibition outlined that the ethanolic green tea extract was the most effective against *Escherichia coli*, and the highest inhibitory action was recorded with the concentrated green tea extract against *Staphylococcus aureus*. Findings of the study indicate the importance of extraction strategies and storage conditions in engineering plant-based antimicrobials.

Green tea is well known for its high polyphenolic composition, especially catechins like epigallocatechin gallate (EGCG), which are responsible for its strong antimicrobial, antioxidant, and anti-inflammatory activities (Rahman et al., n.d.). Ethanol extraction, being a solvent-effective process, would most likely have boosted the solubility and bioavailability of the phytochemicals and hence increased their effectiveness against Gram-negative bacteria like *E. coli*. In contrast, the concentrated extract with potentially higher levels of active constituents as a result of lower content of solvent showed better activity against *S. aureus*, a Gram-positive pathogen.

The difference in the efficacy of the extracts further identifies the role of extract aging. The aged extract, while less potent than fresh ethanol or concentrated extracts, remained resistant to antimicrobial activity, indicating a level of bioactive stability in green tea over time. But the chronically poor performance of the DMSO treated extract assures that the solvent by itself had no antibacterial activity. Overall, these findings corroborate the promise of green tea extracts as antimicrobial agents of natural origin, with real-world relevance in food safety, alternative therapy, and pharmaceuticals. Refining extraction and storage procedures can further maximize their effectiveness. With the increasing alarm of antibiotic resistance, green tea-based products might provide a cost-effective, safe, and health-enhancing alternative to treat bacterial infections. Quantitative phytoconstituent profiling and mechanistic studies should be included in further research to confirm these results.

VI. CONCLUSION

Based on the data from the present study, it can be concluded that the antibacterial efficacy of *Camellia sinensis L.* (Green tea) extracts is highly dependent on extraction method, and the handling and storage of the extracts. The ethanolic extract demonstrated the largest zone of inhibition against *Escherichia coli* (a Gram-negative bacterium), suggesting that the ethanol solvent can increase the extraction of active phytochemicals such as catechins and flavonoids, many of which are known for their antimicrobial efficacy (Rutter et al., n.d.). In contrast, the concentrated extract showed stronger antimicrobial activity against *Staphylococcus aureus* (a Gram-positive bacterium), suggesting that higher concentrations of green tea bioactive compounds may be more useful against some microbial cell wall structures.

The moderate antimicrobial activity of the stale extract also supports that green tea does have some antibacterial efficacy over time, although lower than active compounds that are freshly prepared. The solvent DMSO was found to show little to no inhibition confirming that the antimicrobial activity was mainly due to the phytoconstituents in green tea and not the solvents. These results collectively emphasize the necessity of correct extraction techniques and storage procedures to realize the optimum therapeutic activity of plant antimicrobials. The wide-spectrum antimicrobial activity of *Camellia sinensis L.*, combined with its well-known antioxidant, anti-inflammatory, and health-benefiting activities, especially in light of rising antibiotic resistance, indicates a very strong potential as a natural and innocuous alternative to chemical antimicrobial compounds.

Future studies should aim to identify and quantify the possible active compounds responsible for these effects and investigate their mechanisms of action across various microbial strains. Utilization of such natural antimicrobials in pharmaceutical and food preservation applications represents an avenue for sustainable and health-related innovation.

Figures and Tables

Fig. 1. Concentrated, ethanolic, old, DMSO treated green tea extracts (Left to right)

Fig. 2. Zones of inhibition against *Staphylococcus aureus*

Fig. 3. Zones of inhibition against *Escherichia coli*

(D: DMSO treated, E: Ethanolic, O: 72 hours old ethanolic, C: Heat concentrated green tea extracts)

Table 1 Zones of inhibition of different green tea extracts against *Staphylococcus aureus*

Sr. No.	Type of Green tea extract	Zone of inhibition in mm
1.	Heat concentrated	20
2.	Ethanolic	10
3.	72 hours old	14
4.	DMSO treated	3.0

Table 2 Zones of inhibition of different green tea extracts against *Escherichia coli*

Sr. No.	Type of Green tea extract	Zone of inhibition in mm
1.	Heat concentrated	10
2.	Ethanolic	20
3.	72 hours old	14
4.	DMSO treated	5.0

VII. Acknowledgment

I would like to express my sincere gratitude to my guide, **Ms. Dnyanada Sadanand Ghadi**, for her constant guidance, support, and valuable insights throughout the research work. I extend my heartfelt thanks to our respected Director, **Mrs. Jyoti Gaitonde**, and I/C Principal, **Dr. Mahendra Kanojia**, for providing the necessary facilities and a supportive environment to carry out this project. I am also grateful to **Mrs. Shweta Vijay Khopde**, Department Coordinator, for her encouragement and coordination, and to **Dr. Priyanka Pushkaraj Vartak**, for her valuable suggestions and academic support. Special thanks to **Mr. Vighnesh Borsutkar**, Lab Assistant, and **Mr. Satyadeep Kadam**, Lab Attendant, for their technical assistance and cooperation during the experimental work. This research would not have been possible without their collective support and encouragement.

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