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ANTIMICROBIAL ACTIVITY OF BLACK TEA WITH GINGER FORMULATIONS AND ITS CYTOTOXIC EFFECT—IN VITRO STUDY

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ABSTRACT:
Background: Black tea and ginger are well-known for their therapeutic and antimicrobial properties. This study aimed to evaluate the combined antimicrobial efficacy and cytotoxicity of black tea and ginger extract formulations in vitro..
Methods: Black tea-ginger extract combinations were prepared and tested for antimicrobial activity using agar well diffusion and cytotoxicity using brine shrimp lethality assay.
Results: The formulation exhibited dose-dependent antimicrobial activity against tested pathogens and showed minimal cytotoxicity at lower concentrations.
Conclusion: Black tea with ginger formulations demonstrated potential as a natural antimicrobial agent with acceptable cytotoxicity, encouraging further exploration in pharmaceutical and dental applications.

Keywords: Ginger, Black Tea, Antimicrobial, Cytotoxicity, Herbal Formulation, Brine Shrimp Assay

Introduction:

Ginger, botanically known as *Zingiber officinale*, has been recognized for its wide array of therapeutic applications in traditional and modern medicine. For centuries, it has played a crucial role in the management of inflammatory conditions, respiratory infections, nausea, gastrointestinal disturbances, and various systemic illnesses. Its bioactive constituents—most notably gingerols, shogaols, paradols, zingerone, and terpenoids—have demonstrated potent pharmacological actions including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Compounds such as gingerol and shogaol have been found to interfere with microbial cell wall synthesis, disrupt biofilms, and even modulate apoptotic pathways in malignant cells through the activation of tumor suppressor genes and inhibition of angiogenic signals such as VEGF. Additionally, terpenoids in ginger have been shown to induce apoptosis in cancerous cells by p53 activation, further underscoring its potential as a chemopreventive agent.

On the other hand, tea derived from *Camellia sinensis* is one of the most widely consumed beverages across the globe and is renowned not only for its stimulant and antioxidant properties but also for its considerable therapeutic potential. Black tea, specifically, is produced through full fermentation of

tea leaves and is distinct in its polyphenolic profile, being rich in theaflavins and thearubigins. These flavonoids exhibit various health-promoting effects, including protection against cardiovascular disease, regulation of blood glucose and lipid levels, and potential inhibition of tumor growth. Clinical studies suggest that regular consumption of black tea may aid in the prevention of conditions such as cancer, stroke, diabetes, hypercholesterolemia, and hypertension.

The bioactive compounds in black tea are broadly categorized into non-polymeric phenolics (NP) and polymeric tannins (PT). NP phenolics are readily absorbed in the gastrointestinal tract and exhibit direct oral bioactivity, while polymeric tannins contribute to stronger antioxidant and antimicrobial effects through their ability to bind to microbial membranes and inhibit enzymatic activity. These characteristics make black tea a highly valuable component in natural therapeutic formulations.

The alarming rise in multidrug-resistant pathogens has made it imperative to seek alternative antimicrobial strategies. The limitations of chemically synthesized antibiotics—such as adverse effects and resistance development—have driven global interest in phytotherapeutics. Natural plant extracts offer a safer, more sustainable avenue for antimicrobial agent development. Combining different plant-based extracts may yield synergistic interactions, enhancing their therapeutic efficacy while minimizing toxicity. This approach is now being adopted by many medical and dental institutions, which are actively researching and formulating novel herbal therapeutics. For instance, some institutions have successfully introduced herbal-based oral care products, such as toothpastes, mouthwashes, and topical gels derived from medicinal plants.

In particular, ginger and black tea stand out as two botanicals with overlapping and potentially synergistic antimicrobial and antioxidant profiles. Ethanol-based extracts of both plants have previously shown promising results against a variety of pathogens. A notable finding demonstrated that 10% ethanolic ginger extract exerted significant antimicrobial effects, further supporting its role in integrated herbal formulations.

Given the therapeutic promise of both *Zingiber officinale* and *Camellia sinensis*, and the global urgency for alternative antimicrobial agents, the present in vitro study was undertaken to evaluate the combined antimicrobial efficacy and cytotoxic safety of black tea and ginger extract formulations. By examining their performance in microbial inhibition assays and cytotoxicity screening using brine shrimp lethality testing, this study aims to validate the utility of such a formulation for further development in pharmaceutical and dental applications.

Materials And Methods

The present in vitro study was conducted in the Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai, following institutional ethical approval from the Institutional Review Board (IRB), Saveetha Institute of Medical and Technical Sciences (SIMATS). All protocols adhered to standardized laboratory biosafety and plant extract handling regulations.

Collection and Preparation of Plant Materials

Dried black tea leaves (*Camellia sinensis*) and fresh ginger rhizomes (*Zingiber officinale*) were obtained from local certified organic suppliers in Chennai. The raw materials were thoroughly cleaned, shade-dried, and pulverized using a mechanical grinder to obtain a uniform powder.

Extraction Process

The powdered plant material was subjected to Soxhlet extraction using 70% ethanol as solvent. Approximately 25 g of black tea and 25 g of ginger powder were each extracted separately for 6 hours. The extracts were filtered and concentrated using a rotary evaporator at 45°C under reduced pressure to obtain viscous crude extracts. These were stored in airtight amber bottles at 4°C until further use.

Formulation Preparation

Equal volumes of black tea and ginger extracts were mixed to form the combined formulation. Serial dilutions were performed using sterile distilled water to prepare working concentrations of 5 µL, 10 µL, 20 µL, 40 µL, and 80 µL for antimicrobial and cytotoxic analysis.

Antimicrobial Activity Assessment

The antimicrobial activity was evaluated by agar well diffusion method. Standard bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were used as test organisms. Mueller-Hinton agar plates were inoculated with 100 µL of each microbial suspension adjusted to 0.5 McFarland turbidity. Wells of 6 mm diameter were punched into the agar, and 50 µL of each concentration of the formulation was loaded into respective wells. Plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters using a digital caliper.

Cytotoxicity – Brine Shrimp Lethality Assay

The brine shrimp (*Artemia salina*) lethality assay was performed to assess cytotoxicity. Brine shrimp eggs were incubated in artificial seawater (38 g sea salt/L distilled water) and allowed to hatch over 48 hours under constant aeration and light. Ten nauplii were transferred into test tubes containing 5 mL of seawater and various extract concentrations (5–80 µL). A control tube containing only seawater and ethanol (vehicle) was also maintained. After 24 and 48 hours, the number of surviving nauplii was counted. Cytotoxicity was expressed as the percentage of mortality

Results

Antimicrobial Activity

The black tea and ginger extract formulation demonstrated a dose-dependent inhibitory effect on all tested microbial strains. At concentrations of 40 µL and 80 µL, the formulation produced clear and measurable zones of inhibition against *Staphylococcus aureus* and *Escherichia coli*, indicating significant antibacterial activity. For *Candida albicans*, antifungal activity was moderate but still appreciable, especially at the 80 µL concentration.

Inhibition zones were recorded as follows (mean ± SD in mm):

Microorganism	20 µL	40 µL	80 µL
S. aureus	8.4 ± 0.5 mm	11.2 ± 0.4 mm	14.3 ± 0.6 mm
E. coli	7.6 ± 0.4 mm	10.5 ± 0.7 mm	13.1 ± 0.5 mm
C. albicans	6.2 ± 0.3 mm	8.8 ± 0.6 mm	11.0 ± 0.4 mm

Lower concentrations (5 µL and 10 µL) did not produce measurable inhibition zones, indicating that the minimum inhibitory concentration (MIC) lies above 10 µL under the experimental conditions used.

These results indicate that both gram-positive and gram-negative bacteria, as well as fungal strains, were susceptible to the phytochemical activity of the black tea–ginger formulation, although the efficacy was more pronounced in bacterial species.

Cytotoxicity Assay

The brine shrimp lethality assay provided preliminary insights into the cytotoxic potential of the test formulation. At concentrations of 5 µL and 10 µL, shrimp survival remained at 100% over both 24-hour and 48-hour exposure periods. At 20 µL, 80% survival was recorded by Day 2, and at 40 µL, survival dropped to 60%. Interestingly, the 80 µL group showed only a mild decrease in viability (80% survival), suggesting that toxicity may plateau beyond a certain concentration.

Mortality rates (%) across groups at 48 hours:

Concentration	Mortality (%)
5 µL	0%
10 µL	0%
20 µL	20%
40 µL	40%
80 µL	20%
Control	70%

The control group (ethanol vehicle) exhibited the highest mortality, reinforcing that the ethanol, rather than the extracts, likely contributed to cytotoxicity at higher doses. Overall, the formulation displayed an acceptable cytotoxic profile at concentrations effective for antimicrobial activity.

Discussion

The results obtained in this study substantiate the hypothesis that black tea and ginger, when combined, exhibit potent antimicrobial activity with low cytotoxic risk in vitro. These findings align with previous research which suggests that both black tea polyphenols and gingerols exhibit membrane-disrupting properties that inhibit microbial growth [6,7].

The antibacterial efficacy is likely due to the synergistic interaction between catechins and flavonoids in black tea (e.g., theaflavins, thearubigins) and active terpenoids in ginger (e.g., gingerol, shogaol). These compounds are known to interfere with microbial quorum sensing, cell wall synthesis, and enzyme function [8–10].

The moderate antifungal effect against *Candida albicans* is notable, particularly since many plant extracts show limited activity against fungal species. This suggests that the formulation could have broad-spectrum applications, especially for oral pathogens in dental care products such as mouth rinses or medicated pastes [11,12].

The brine shrimp assay demonstrated minimal toxicity of the formulation at antimicrobial concentrations, suggesting its relative safety for further biological or preclinical investigation. The higher mortality seen at 40 µL may be attributed to concentration saturation of bioactives or residual ethanol, though the 80 µL dose interestingly showed reduced toxicity, possibly due to adaptive stabilization or precipitation of actives [13,14].

This study also complements recent trends in phytomedicine, where polyherbal formulations are favored for their multi-targeted action and reduced potential for resistance development [15–17]. From a pharmaceutical development perspective, black tea and ginger represent a cost-effective, sustainable, and consumer-acceptable combination.

While promising, the study is limited by the absence of detailed mechanistic evaluation (e.g., molecular docking, scanning electron microscopy), lack of standard antibiotic comparators, and the use of a preliminary cytotoxicity model (brine shrimp) rather than mammalian cell lines. These limitations should be addressed in follow-up studies using clinical isolates, quantitative MIC/MBC analysis, and cell-based cytotoxicity models (e.g., MTT, LDH release) [18–20].

Further formulation refinement (e.g., encapsulation, emulsification) may also enhance stability, bioavailability, and targeted delivery, opening avenues for development as oral rinses, wound gels, or bioadhesive dental films [21–23

Conclusion

This study provides preliminary evidence that black tea and ginger, when combined in equal proportion and extracted using ethanol, exhibit promising antimicrobial activity against common bacterial and fungal pathogens. The formulation also demonstrated low cytotoxicity in the brine shrimp model, indicating its safety at lower concentrations. These properties make it a viable candidate for development into herbal antimicrobial agents, especially for applications in oral health,

topical formulations, and dental therapeutics. Further studies involving mechanism analysis, biofilm disruption potential, and human cell line safety are recommended to validate clinical utility.

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