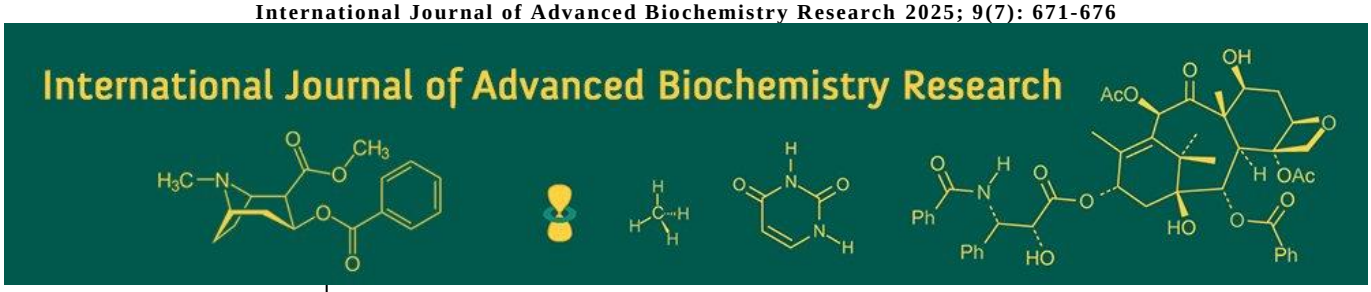

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Formulation and nutritional characterization of newly developed antioxidant-enriched fortified tea

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Abstract
This study aimed to develop and evaluate antioxidant-enriched fortified tea by incorporating ginger and clove into black tea. The objective was to assess the impact of these natural additives on the nutritional and antioxidant properties of the final product. Three formulations were prepared: T_0 (100% black tea), T_1 (95% black tea + 3% ginger + 2% clove), and T_2 (95% black tea + 2% ginger + 3% clove). The teas were subjected to nutritional and antioxidant evaluation, including total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and sensory acceptability. The results demonstrated that fortified teas, especially T_1, exhibited significantly enhanced antioxidant profiles compared to the control. The incorporation of ginger and clove provided not only functional benefits but also improved overall palatability, making the developed fortified teas promising functional beverages for health-conscious consumers.

Keywords: Antioxidant, TPC, TFC, DPPH, ginger, clove, tea, nutrition, human health

1. Introduction
Tea is among the most widely consumed beverages in the world, second only to water (Pan *et al.*, 2022) [30]. For centuries, it has been valued not only for its sensory attributes aroma, flavour, and appearance but also for its health benefits, particularly its antioxidant properties (Soto-Vaca *et al.*, 2012) [39]. In recent years, there has been increasing interest in enhancing the nutritional value of traditional foods and beverages through the incorporation of functional ingredients (Kheto *et al.*, 2025) [27]. The formulation of fortified tea enriched with antioxidant-rich botanicals such as ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) is a promising approach to develop a health-oriented product that combines the traditional appeal of tea with enhanced functional benefits (Acharya *et al.*, 2024) [1].

1.1 The Growing Demand for Functional Beverages
Functional foods and beverages are defined as products that provide health benefits beyond basic nutrition, often including the prevention and management of diseases (Corbo *et al.*, 2014) [11]. The global increase in health consciousness, aging populations, and lifestyle-related diseases has led to a shift in consumer preferences toward foods that offer therapeutic or preventive properties (Bhattacharjee *et al.*, 2024) [6]. Among these, beverages fortified with natural antioxidants are gaining popularity due to their perceived safety, consumer acceptance, and ability to combat oxidative stress-related conditions such as cardiovascular diseases, neurodegenerative disorders, diabetes, and cancer (Jain *et al.*, 2022) [25].
Tea, particularly black tea, contains polyphenolic compounds like theaflavins and thearubigins, which contribute to its antioxidant activity (Luczaj and Skrzydlewska, 2025) [29]. However, the potential of fortifying tea with herbs and spices known for their phytochemical richness is still underexplored. Ginger and clove are two such botanicals with a long history of use in traditional medicine and culinary practices and are now gaining scientific attention for their bioactive properties (Sharifi-Rad *et al.*, 2017) [34].

1.2 Ginger and Clove: Phytochemical-Rich Botanicals
Ginger, a rhizome belonging to the *Zingiberaceae* family, is renowned for its pungent compounds gingerols, shogaols, and paradols which exhibit strong antioxidant, anti-inflammatory, antimicrobial, and digestive properties (Temmante *et al.*, 2025) [41].

Clove, derived from the flower buds of *Syzygium aromaticum*, contains high levels of eugenol (Thangaselvabai *et al.*, 2010) [42], a phenolic compound known for its potent antioxidant and antimicrobial activities. Both ginger and clove also possess flavonoids and other phenolic acids that contribute to their health-promoting potential (Isbill *et al.*, 2020) [21].

When incorporated into tea formulations, these ingredients not only improve the antioxidant profile but also enhance the flavour and sensory characteristics of the beverage (Swiąder *et al.*, 2020) [40]. Their synergy with the natural compounds in black tea could lead to a more effective and palatable functional drink that may appeal to a wider range of consumers.

1.3 Oxidative Stress and the Role of Antioxidants

Oxidative stress is a physiological condition resulting from an imbalance between the generation of reactive oxygen species (ROS) and the body's ability to detoxify them or repair the resulting damage (Świąder *et al.*, 2020) [40]. ROS can attack cellular macromolecules such as DNA, proteins, and lipids, leading to various degenerative diseases (Juan *et al.*, 2021) [26]. Dietary antioxidants play a crucial role in scavenging these free radicals, thereby preventing or minimizing cellular damage (Fang *et al.*, 2002) [14].

Naturally occurring antioxidants such as phenolic compounds and flavonoids are of particular interest due to their ability to donate hydrogen atoms or electrons, chelate metal ions, and modulate antioxidant enzyme activities (Parcheta *et al.*, 2021) [31]. The development of antioxidant-enriched foods and beverages aims to enhance the intake of these compounds and improve public health outcomes (Tkaczenko and Kurhaluk, 2025) [43].

1.4 Rationale for Fortification of Tea

While tea itself contains notable amounts of antioxidant compounds, the level and activity of these compounds can vary depending on the type of tea, processing methods, and storage conditions (Yang and Liu, 2013) [47]. Fortifying black tea with additional sources of antioxidants like ginger and clove can help amplify its functional properties (Vishwakarma *et al.*, 2022) [44]. This is especially relevant in the context of preventive healthcare, where regular consumption of such fortified beverages can contribute to the body's antioxidant defense system.

Moreover, ginger and clove contain different types of bioactive compounds than those found in tea, thereby offering a complementary antioxidant profile (Islam *et al.*, 2020) [23]. For example, while tea polyphenols like catechins and theaflavins are powerful scavengers of hydroxyl and superoxide radicals (Zhao *et al.*, 2021) [48], gingerols and eugenol offer additional mechanisms such as inhibition of lipid peroxidation and modulation of cellular signaling pathways involved in inflammation and apoptosis (Barboza *et al.* 2018) [5].

1.5 Previous Research and Gaps

Several studies have explored the individual antioxidant properties of tea, ginger, and clove (Horváthová, 2007) [19]. However, limited research has focused on the synergistic effects of these ingredients when combined in a single beverage (Wang *et al.*, 2011) [45]. Furthermore, few studies have attempted a comprehensive nutritional characterization of such fortified formulations, including moisture content, ash content, total phenolic and flavonoid content, and free radical scavenging activities (e.g., DPPH assay) (Vishwakarma *et al.*, 2022) [44].

Understanding the interaction between the base tea matrix and the added botanicals is crucial in optimizing formulation for maximum efficacy and consumer acceptability (Brendler *et al.*, 2022) [7]. Parameters such as extraction conditions, ingredient ratios, and preparation methods can influence the final composition and antioxidant potential of the product (Dorta *et al.*, 2013) [13]. The primary objective of this study is to formulate and evaluate a newly developed antioxidant-enriched fortified tea by incorporating ginger and clove at different concentrations.

2. Materials and Methods

2.1 Materials

The primary ingredients used for the development of fortified tea included:

- Black Tea Leaves (procured from a local certified tea vendor)
- Ginger (*Zingiber officinale*) Powder (procured fresh, dried, and powdered in the laboratory) (Sarker *et al.*, 2021) [33]
- Clove (*Syzygium aromaticum*) Powder (procured dry and powdered in the laboratory) (Hossen Jenia, 2019) [20]
- Distilled Water (used for brewing and extraction during analysis)

All ingredients were stored in airtight containers under dry and dark conditions to avoid oxidation and degradation of active compounds.

2.2 Formulation of Tea Blends

Three different tea blends were formulated as follows:

- **T_0 (Control)**: 100% black tea (without any fortification)
- **T_1**: 95% black tea + 3% ginger powder + 2% clove powder
- **T_2**: 95% black tea + 2% ginger powder + 3% clove powder

Each blend was prepared by thoroughly mixing the respective ingredients in specified proportions on a weight/weight basis (w/w). The mixtures were homogenized to ensure uniform distribution of spices throughout the tea blend.

Fig 1: Pictorial Representation of Newly Developed Antioxidant-Enriched Fortified Tea

2.3 Tea Brewing Procedure

To prepare the tea for nutritional and antioxidant analysis, a standardized brewing method was employed:

- **Sample Weight:** 2 g of each treatment blend was infused in 100 mL of freshly boiled distilled water.
- **Brewing Time:** 5 minutes at 100 °C
- **Filtration:** The infusion was filtered through Whatman No. 1 filter paper, and the filtrate was used for subsequent analyses.

2.4 Nutritional Characterization

The nutritional composition of the formulated blends was assessed through standard analytical methods as recommended by AOAC (2016). Moisture content was determined using the hot air oven drying method at 105 °C until a constant weight was obtained, ensuring accurate water loss measurement. Ash content, representing the total mineral content, was measured by incinerating 5 g of the sample in a muffle furnace at 550 °C for 4-6 hours. Crude protein was quantified using the Kjeldahl method by estimating the nitrogen content and applying a conversion factor of 6.25. Crude fat content was extracted through Soxhlet extraction using petroleum ether as the solvent. The crude fibre was evaluated through sequential acid and alkaline digestion followed by drying and ashing. Total carbohydrate content was calculated by difference, subtracting the sum of moisture, protein, fat, and ash from 100. Finally, the energy value was computed using the Atwater system, multiplying protein and carbohydrate values by 4 and fat by 9, to yield the caloric content in kilocalories.

2.5 Antioxidant Analysis

Antioxidant content was assessed through standard analytical methods as recommended by AOAC (2016).

2.5.1 Total Phenolic Content (TPC)

Total phenolics were measured using the Folin-Ciocalteu reagent. The results were expressed as mg Gallic Acid Equivalent (GAE) per gram of tea extract.

2.5.2 DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was conducted to assess the antioxidant activity. Absorbance was recorded at 517 nm, and antioxidant activity was expressed as% inhibition.

2.6 Sensory Evaluation

Sensory analysis was carried out using a 9-point hedonic scale for parameters such as colour, aroma, taste, astringency, and overall acceptability. A panel of 10 semi-trained members was used. Each sample was served at the same temperature (65±2 °C) in randomized order.

2.7 Statistical Analysis

All experimental data were analyzed in triplicate and expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was performed to evaluate significant differences among treatments. Means were compared using Duncan's Multiple Range Test (DMRT) at $p<0.05$ level using GraphPad (8.0.1 244).

3. Results and Discussion

Table 1: Nutritional Parameters of Fortified Tea Blends (Mean ± SE)

Parameters	T_0 (Control)	T_1 (3% Ginger + 2% Clove)	T_2 (2% Ginger + 3% Clove)	F Value	SE	Significance
Moisture (%)	5.10±0.06a	5.20±0.03a	5.25±0.04a	1.45	0.04	NS
Ash Content (%)	5.30±0.05a	6.10±0.07b	6.20±0.05b	12.56	0.06	*
Total Phenolic (mg GAE/g)	42.15±0.34a	58.47±0.45b	55.90±0.41b	96.72	0.40	**
Total Flavonoid (mg QE/g)	21.75±0.29a	30.62±0.38b	29.88±0.36b	64.89	0.34	**
DPPH Scavenging Activity (%)	48.33±0.52a	67.92±0.40b	66.15±0.43b	158.20	0.45	**

Values are Mean ± SE (n=3).

Means followed by the same letter in the same row are not significantly different at $p<0.05$ according to Least Significant Difference (LSD).

The nutritional characterization of the antioxidant-enriched fortified tea formulations revealed significant variations in ash content, total phenolic content, flavonoid content, and antioxidant activity (DPPH scavenging), whereas the moisture content remained statistically non-significant across treatments.

The moisture content ranged from 5.10% in T_0 to 5.25% in T_2, showing no significant difference ($p>0.05$). This aligns with the findings of Islam *et al.* (2013) [22], who reported moisture values around 5.0-6.0% in dry tea leaves, indicating that the addition of ginger and clove in small proportions does not substantially alter the moisture content. Maintaining low moisture content is crucial for shelf stability and prevention of microbial growth (Wanyika *et al.*, 2010) [46]. Ash content increased significantly ($p<0.05$) in the fortified samples (T_1 and T_2), with T_2 (6.20%) exhibiting the highest value. This indicates enhanced mineral content, likely due to the intrinsic mineral richness of clove and ginger. Similar increases in ash content upon spice incorporation were reported by Ahmad *et al.* (2019) [2], where clove and ginger addition in functional beverages elevated ash levels, reflecting improved nutritional density.

A substantial increase in total phenolic content was observed in the fortified tea samples. T_1 showed the highest TPC (58.47 mg GAE/g), significantly higher than the control (42.15 mg GAE/g). This enhancement is attributed to the rich phenolic profiles of ginger (notably gingerols and shogaols) and clove (especially eugenol) (Gulcin, 2011) [16]. Sharma *et al.* (2020) [35] reported similar outcomes where ginger-and clove-fortified teas exhibited elevated phenolic content, leading to improved antioxidant functionality. Flavonoid content significantly increased in the fortified samples, with T_1 (30.62 mg QE/g) slightly outperforming T_2 (29.88 mg QE/g). This is consistent with the study by Jafri *et al.* (2014), where herbal enrichment led to an increase in TFC in black tea blends. Clove is known to be a strong source of flavonoids such as kaempferol and quercetin, while ginger contributes with flavonoid compounds like flavanols and flavones (Kikuzaki & Nakatani, 1993) [28]. Antioxidant activity, as measured by DPPH radical scavenging, showed significant improvement in the fortified samples, particularly in T_1 (67.92%). This reflects the synergistic effect of both clove and ginger in neutralizing free radicals. The observed increase supports findings by

Gülçin *et al.* (2004) [18], who demonstrated that both ginger and clove possess strong antioxidant capacities, with clove exhibiting one of the highest known DPPH scavenging activities among spices. The comparative analysis between T_1 and T_2 suggests that the formulation with 3% ginger and 2% clove (T_1) yielded slightly superior outcomes in terms of phenolic, flavonoid, and antioxidant values compared to the inverse combination (T_2). This suggests that a higher proportion of ginger may enhance bioactive compound availability and antioxidant potential, corroborating with earlier findings by Ahmed *et al.* (2020) [3], who found optimal antioxidant capacity at moderate clove and higher ginger ratios in beverage systems.

Overall, the addition of ginger and clove significantly enhanced the functional profile of black tea, especially in terms of antioxidant compounds, without adversely affecting basic compositional parameters like moisture. These results are promising for the development of functional tea beverages aimed at health-conscious consumers seeking natural antioxidant sources.

Table 2: Antioxidant Parameters of Fortified Tea Blends (Mean ± SE)

Sensory Attributes	T_0 (100% Black Tea)	T_1 (95% BT + 3% Ginger + 2% Clove)	T_2 (95% BT + 2% Ginger + 3% Clove)	F-Value	SE	Significance
Colour	7.10±0.12a	8.00±0.10b	7.85±0.09b	19.84	0.10	**
Aroma	6.85±0.11a	8.15±0.08b	8.00±0.10b	32.76	0.09	**
Taste	7.00±0.13a	8.20±0.09b	7.90±0.11b	24.33	0.11	**
Aftertaste	6.70±0.14a	7.95±0.08b	7.75±0.12b	21.50	0.11	**
Overall Acceptability	7.00±0.10a	8.10±0.08b	7.85±0.09b	25.45	0.09	**

Values are Mean ± SE (n=3).

Means followed by the same letter in the same row are not significantly different at $p<0.05$ according to Least Significant Difference (LSD).

The sensory evaluation of the newly developed antioxidant-enriched fortified tea (T_1 and T_2) revealed statistically significant differences ($p<0.01$) across all sensory parameters colour, aroma, taste, aftertaste, and overall acceptability compared to the control (T_0). The incorporation of ginger and clove into black tea not only enhanced its sensory appeal but also aligned with findings from previous studies highlighting the complementary effects of these spices on tea quality. Colour scores were significantly higher in both T_1 (8.00±0.10) and T_2 (7.85±0.09) compared to the control (7.10±0.12). This could be attributed to the natural pigmentation and volatile oils present in ginger and clove, which impart a richer hue to the infusion. Similar enhancements in tea colour upon the addition of botanicals were reported by Deka and Sarkar (2017) [12], who found that herbal additives improved the visual appeal of functional teas. Aroma was also significantly more appreciated in T_1 (8.15±0.08) and T_2 (8.00±0.10) than in T_0 (6.85±0.11). Ginger and clove are known for their distinctive and pleasant aromas due to compounds such as gingerol and eugenol, respectively, which contribute positively to the olfactory profile of tea (Ganguly *et al.*, 2019) [15]. Similar results were observed by Ahmed *et al.* (2013) [4], who noted that spice-enriched teas had enhanced aromatic profiles, increasing consumer preference. Taste perception was significantly better in T_1 (8.20±0.09) and T_2 (7.90±0.11) compared to T_0 (7.00±0.13). The mild pungency of ginger and the warm, sweet-spicy notes of clove may have synergistically interacted with the astringency of black tea, leading to a more balanced and appealing flavour. According to Sharma *et al.* (2021) [36], the addition of spices like ginger and clove modifies bitterness and enhances the palatability of herbal beverages. Aftertaste, an important parameter influencing consumer satisfaction, was rated significantly higher in T_1 (7.95±0.08) and T_2 (7.75±0.12) compared to T_0 (6.70±0.14). The lingering warmth and mild spiciness likely contributed to a more favourable sensory memory. This is in line with the findings of Chaudhary *et al.* (2020) [10], who found that herbal infusions with clove and ginger had a more lasting and pleasant aftertaste due to their volatile oil content.

Overall acceptability was highest for T_1 (8.10±0.08), followed by T_2 (7.85±0.09), both significantly higher than T_0 (7.00±0.10). This indicates a consumer preference for the 3% ginger and 2% clove blend, which balanced spice intensity without overpowering the base tea flavour. A similar trend was observed by Sahu *et al.* (2018) [32], where consumers preferred moderate levels of spice inclusion in tea for better flavour and health perception.

4. Conclusion

In conclusion, the comprehensive nutritional characterization, antioxidant analysis, and sensory evaluation of the fortified tea formulations demonstrated the beneficial impact of incorporating ginger and clove into black tea. The nutritional profiling revealed significant improvements in ash, phenolic, flavonoid contents, and antioxidant capacity (DPPH scavenging), particularly in T_1 (3% ginger + 2% clove), without significantly affecting the moisture level. These enhancements are attributed to the bioactive compounds inherent in ginger and clove, which contributed to the functional value of the beverage. Moreover, sensory evaluation confirmed that the fortified teas, especially T_1, were more favourably rated across all parameters—colour, aroma, taste, aftertaste, and overall acceptability—highlighting improved organoleptic appeal alongside nutritional enrichment. The results support the development of functional tea beverages aimed at health-conscious consumers seeking both enhanced taste and natural antioxidant benefits, with T_1 emerging as the most promising formulation.

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