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Nutritional, Antioxidative and Aflatoxin Safety Assessment of Ginger-Turmeric Tea: A Functional Herbal Beverage Tea

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ABSTRACT

Turmeric and ginger belong to the family *Zingiberaceae*, which contains bioactive chemicals with a variety of uses. Ginger *(Zingiber officinale)* has several bioactive components, such as gingerols, zingiberene, caffeic acid, salicylate and capsaicin. It is utilized in a wide range of food and beverages. Turmeric *(Curcuma longa)* contains numerous physiologically active substances, including Curcumin, dimethoxycurcumin and bis-demethoxycurcumin.Both ginger and turmeric have been used to treat nausea, indigestion, stomachaches, toothaches, insomnia, asthma, diabetes, infertility, neurological issues, rheumatism and memory loss. Apart from water, tea is the most popular beverage in the world. **Objectives:** To develop ginger-turmeric tea and assess of nutritional facts, aflatoxin and antioxidant potential. **Methods:** The nutritional facts and aflatoxin were determined by standard AOAC methods, and the antioxidant activity of the ginger-turmeric tea was evaluated by DPPH assay. **Results:** The results of this study revealed that ginger-turmeric tea has 7.5 ± 0.84% moisture, 5.12 ± 0.65 ash, 0.22 ± 0.01% fat, 14.75 ± 1.67% fiber, 8.10±0.82% protein, carbohydrates 64.25 ± 3.90, and energy 292 ± 5.30 kcal/100g, while the results of aflatoxin study showed that aflatoxins (B1, B2, G1 and G2) were absent. The significant antioxidant capacity DPPH (% inhibition) was found to range from 25.50 ± 1.20–74.80 ± 2.77% at concentrations of 0.2–1.0 mg/mL, significant differences in various concentrations $p<0.05$ were found. **Conclusions:** This study concluded that the developed ginger-turmeric tea is a good source of protein, fiber and has a high content of natural antioxidants, making it advantageous for treating oxidative stress.

INTRODUCTION

Herbs and spices have long been used to improve the flavor of food and are also used medicinally to cure a variety of illnesses in many civilizations worldwide. Polyphenols and flavonoids are abundant in many plants used in traditional medicine, and these compounds represent a significant source of phytochemicals for both human and animal health [1]. Most spices have a variety of possible health benefits, including positive effects on lipid metabolism, anti-diabetic effects, stomach-stimulating properties and probable anti-inflammatory and antioxidant properties [2]. Ginger, often known as ginger root, is the rhizome of the *Zingiber officinale* and is used as a spice, medicinal or delicacy. Ginger contains chemical compounds, especially gingerol and shogaol, which are thought to have functions

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as anti-cancer, anti-inflammatory, anti-hypertensive, anti-diabetic, anti-hyperlipidemic and antioxidant [3]. The *Zingiberacea* family includes the rhizomatous herbaceous perennial plant known as turmeric. Turmeric has been shown to contain around 250 phytochemicals, mostly terpenoids and phenolic compounds including curcumins, turmerone, α-zingiberene, carotene, syringic, p-coumaric, ascorbic acid, ferulic acid, nd vanillic acid [4]. Curcumin is its active element that has garnered attention due to its potential to treat a wide range of disorders, such as diabetes, cancer, Alzheimer's disease, allergies, arthritis, and other chronic conditions [5]. Following harvest, both ginger and turmeric are dried and kept in extremely unsanitary circumstances, which increases the risk of mycotoxins and aflatoxins. Aflatoxins are fungal metabolites mostly produced by the *Aspergillus flavus* and *Aspergillus parasiticus* species. AFB1 is the most poisonous of the four main aflatoxins, which are AFB1, AFB2, AFG1, and AFG2. Hepatocellular cancer, acute aflatoxicosis, immunological suppression, malnourishment and growth deficits are all associated with AFB1 [6]. Numerous pre-harvest and post-harvest factors, such as agronomic and agricultural practices, transportation, storage and processing techniques, can result in aflatoxin contamination along the supply chain. As a result, aflatoxins should not be present in the raw material. The medicinal qualities of plants have been thoroughly investigated in the current scientific environment due to their strong antioxidant qualities, lack of side effects and viability from an economic standpoint. With a large percentage of the world's population depending on herbal medicines, herbal therapy is essential to maintaining human health and prosperity [7]. Furthermore, the usage of natural elements produced from plants has been essential since the start of the global COVID-19 epidemic. As a result, the COVID-19 pandemic has brought attention to the necessity of natural antioxidants that improve immunity, improve human health and possibly even stop oxidative stress [8].

Despite the recognized health benefits of ginger, turmeric, and green tea, there is limited research on their combined effects in a single herbal tea formulation, particularly regarding nutritional value, antioxidant potential, and aflatoxin safety. Most previous studies have focused on individual ingredients, leaving a gap in understanding the synergistic properties of such a combination. Moreover, aflatoxin contamination remains a concern due to improper handling and storage, emphasizing the need for evaluating safety in newly developed herbal beverages. This study aims to develop the ginger-turmeric tea and assessment of its nutritional facts, aflatoxin and antioxidant activity.

METHODS

This experimental study was conducted in March-May 2025 at the Food and Biotechnology Research Centre, PCSIR, Labs complex, Lahore. Before being utilized, the raw materials green tea *(Camellia sinensis)*, ginger *(Zingiber officinale)*, and turmeric were collected from the local market in Lahore, Pakistan and kept at room temperature (25 ± 2°C). After washing, chopping and three hours of oven drying at 70°C, the three materials were blended until a consistent powder was obtained. After that tea was prepared by properly combining these ingredients with a small cardamom and filling it in a tea bag. The ginger-turmeric tea was prepared by immersing the tea bag in hot water. 50g of the ground raw samples and the developed tea were added to 500mL of the conical flask. Add 200 mL of dichloromethane and 10 mL of water, respectively and shake it a half-hour using a shaker. In a 300 mL glass flask containing 10g of Na_2SO_4, the mixture was filtered via filter paper. After letting the contents settle gently, the solution was re-filtered into a 100 mL beaker using Whatman filter paper. On TLC plates, samples were spotted. Before being used for analysis, the TLC plates, which included a coated gel of silica on a glass plate, were activated in a hot air oven for an hour at 80°C. After drying, this plate was placed in a diethyl ether-containing TLC tank (first mobile phase) and then, for 30 minutes, it was placed in a second mobile phase tank that contained acetone and chloroform in an 88:12 (v/v) ratio. Blue fluorescence under UV light after plate development verified the presence of aflatoxin, which was then compared to standard AFB1(2µg/ml), B2 (0.508µg/ml), G1 (2.01µg/ml) and G2 (0.515µg/ml) Biopure Romar Lab Austria (Product-10003656; Lot: 1000046444). The spray of aqueous sulphuric acid (50/50 v/v) was done on the TLC plate for the confirmation of aflatoxins presence [9]. The aflatoxins were determined by using a formula. Total aflatoxins(µg/Kg) = $S \times Y \times V / W \times Z$

Where:

S = volume of aflatoxins standard in µL of equivalent intensity to Z (µL of sample).

Y = aflatoxins standardconcentration in µg/mL.

V = volume of solvents in µLrequired to dilute final extract.

W = weight of original sample in g contained in final extract.

Z = volume of sampleextract (µL) require to give fluorescence intensity comparable to that of S. Fs total = concentration of AFB1 + AFB2 + AFG1 + AFG2.

Nutritional profile (moisture, ash, fat, crude fiber, crude protein and carbohydrate contents were determined for raw ginger, turmeric and tea samples developed by AOAC standard methods [10]. By immersing a 1.5g tea sachet sample in 100ml of boiling water, the tea sample's aqueous

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extract was produced. These were left for one to two minutes. Additionally, their level of antioxidant activity was evaluated as well. The approach adapted by Saeed *et al.* was used to test the extracts' scavenging capabilities on the stable free radical DPPH [11]. 2.9 mL of methanolic 2,2-diphenylpicrylhydrazyl radical scavenging capability (DPPH) solution was combined with 0.1 mL of tea water extract. Each sample was incubated in the dark for 30 minutes, and its absorbance at 517 nm was compared to a blank. By measuring the sample's and standard BHT absorbance and using the following formula, the proportion of percentage inhibition (DPPH) was determined: Percentage inhibition (DPPH) = [1 – (As/A0)] × 100. Where A0 represents the absorbance of the DPPH solution, and as represents the absorbance of the samples. The results were statistically analyzed using SPSS Minitab software version 25. Data were represented as mean ± SD, and analysis of variance (ANOVA) was performed. Statistical significance was defined at $p<0.05$.

RESULTS

The moisture, ash, fat, fiber, protein, carbohydrate contents and energy of ginger, turmeric and ginger-turmeric tea were determined by AOAC standard methods. According to this study, turmeric had the highest moisture content (13.20%), followed by ginger (10.60%) and ginger-turmeric tea (7.50%). Turmeric had the lowest amount of ash (3.10%), followed by developed tea (5.12%) and ginger (5.80%). Turmeric had the highest lipid content (4.70%), followed by ginger (3.70%) and developed tea (0.22%). According to the fiber analysis, developed tea had the highest fiber content (14.75%), followed by ginger (13.50%), and turmeric had the lowest (2.78%). The highest protein level was found in ginger (8.90%), followed by developed tea (8.10%) and turmeric (5.85%). Ginger had carbohydrate content (57.50%), while developed tea (64.25%) and turmeric had the highest (68.37%). Turmeric has the maximum energy 343 ± 5.80Kcal/100g, followed by ginger 299 ± 5.38Kcal/100g and developed tea 292 ± 5.30 Kcal/100g (Table 1).

Table 1: Nutrition Information of Raw Material and Ginger-Turmeric Tea

Parameters	Ginger	Turmeric	Ginger-turmeric Tea
Moisture	10.60 ± 1.44	13.20 ± 1.56	7.50 ± 0.84
Ash	5.80 ± 0.69	3.10 ± 0.40	5.12 ± 0.65
Fat	3.70 ± 0.43	4.70 ± 0.50	0.22 ± 0.01
Fiber	13.50 ± 1.61	2.78 ± 0.67	14.75 ± 1.67
Protein	8.90 ± 0.84	5.85 ± 0.56	8.10 ± 0.82
Carbohydrates	57.50 ± 3.80	68.37 ± 3.98	64.25 ± 3.90
Energy (Kcal/100g)	299 ± 5.38	343 ± 5.80	292 ± 5.30

Aflatoxin was measured in this study using thin-layer chromatography (TLC), and the findings indicated that all samples of raw ginger, turmeric, and developed ginger-turmeric tea were free from aflatoxins. All the samples are fit for human consumption according to the EU limit (2023), i.e. 5-10 ppb for ginger and turmeric, while it is 0-20 ppb for developed tea (Table 2).

Table 2: Determination of Aflatoxin Contamination in Raw Ingredients and Ginger-Turmeric Tea

Samples	Aflatoxins (ppb) B1, B2, G1 and G2	Total Aflatoxins	Fit/ Unfit	EU Limit (2023)
Raw Material Ginger Powder	Not Detected	Not Detected	Fit	5-10 ppb
Raw Material Turmeric Powder	Not Detected	Not Detected	Fit	5-10 ppb
Raw Material Green Tea	Not Detected	Not Detected	Fit	0-20 ppb
Ginger-turmeric Tea	Not Detected	Not Detected	Fit	0-20 ppb

Five distinct concentrations, ranging from 0.2 to 1.0 mg/ml, were employed to assess the antioxidant activities of the developed ginger-turmeric tea. After 30 minutes, measurements were made. The findings show that the % inhibition (DPPH), ranging from 25.50 ± 1.20 to 74.80 ± 2.77, increased with increasing concentration based on the ANOVA test. it concluded that the antioxidant activity of the developed tea was significantly different at different concentrations ($p<0.05$). The highest free radical scavenging activity was seen at a concentration of 1 mg/ml; however, all tea extracts demonstrated a notable capacity to eliminate free radicals (Figure 1).

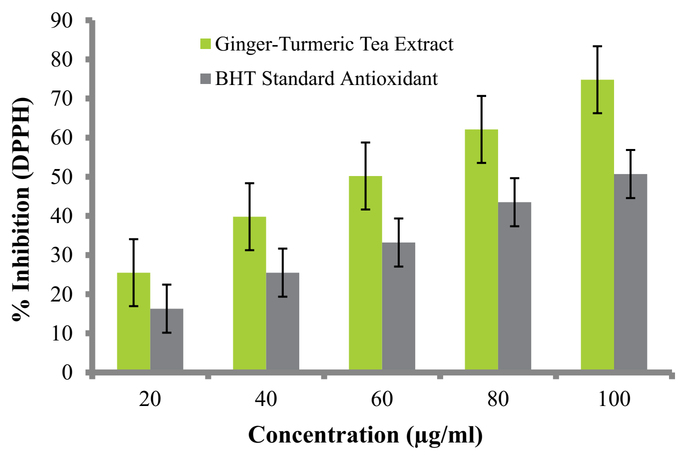

Figure 1: Antioxidant Activity % Inhibition (DPPH) Ginger-Turmeric Tea and BHT

DISCUSSION

According to reports, dried ginger has the following contents: 7.0–10.90% moisture, 8.50–12.4% protein, 70–72% carbohydrate, 5.70–6.64% ash, 7.17–14.1% fiber, and 1.8–4.0% fat. While the turmeric's overall composition is between 11 and 13% moisture, 6 to 9% protein, 5 to 10% fat, 60 to 70% total carbohydrates, 3 to 7% ash and 2 to 7% fiber [12]. The 8.85% crude protein content of ginger-turmeric tea suggests that it may be a useful source of

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protein. Turmeric's 5.12% ash percentage indicates that it has a respectable amount of minerals. By eliminating possible carcinogens from the body and preventing the absorption of excess cholesterol, the fiber (14.75%) in the developed product will aid in cleansing the consumer's digestive tract. Additionally, fiber gives meals volume and keeps people from consuming too many starchy foods, which may protect against metabolic diseases, including diabetes mellitus and hypercholesterolemia [13]. The developed product has 64.25% carbohydrate content, according to the current investigation. It has a high calorie value and was suggested by the high carbohydrate content [14]. The Food and Agriculture Organization (FAO) estimates that mycotoxins contaminate about 25% of the world's food crop supply each year, with aflatoxins being the most dangerous [15]. It is concerning to note that over 5 billion individuals globally are regularly exposed to elevated quantities of aflatoxin, surpassing 1000 parts per billion (ppb) [16]. More than 100 countries and regions have put in place unique aflatoxin threshold requirements for different food products in order to guarantee food safety. In this regard, the European Union (EU) has imposed comparatively strict regulations. They specifically state that the overall amounts of aflatoxins (AFB1, AFB2, AFG1 and AFG2) in all cereals should not be more than 4 ppb, while the values for spices should be between 5 and 10 ppb and for raw green tea powder, 20 ppb [17]. Aflatoxins are a major contributor to the formation of primary hepatocellular carcinoma, a form of malignant liver tumor and can seriously harm the liver. According to studies, aflatoxins are responsible for over half of the risk of liver cancer in Asia and Africa [18, 19]. Both our novel tea and its raw material are safe for human consumption and free of aflatoxins. The sample's ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a gauge of its antioxidant capacity. In the presence of oxidants, DPPH is reduced and loses its purple-blue blue when it takes an electron provided by an antioxidant component, which can be measured by changes in absorbance [20]. Because ginger, a raw ingredient in ginger-turmeric tea, includes non-nutritional chemicals with antioxidant qualities, it helps strengthen the body's immune system. Mahmudati *et al.* investigated the antioxidant activity of three different types of ginger and depicted that red ginger had the maximum antioxidant activity by the infusion method, 79.83%, followed by elephant 70.43% and emprit ginger 61.70%, respectively [3]. On the other hand, the decoction of elephant ginger showed the highest antioxidant activity, 78.76%, followed by red ginger, 70.56% and elephant ginger, 60.93%. According to Priyanka *et al.* who examined the antioxidant activities of different varieties of turmeric, other ingredients of this tea also have significant antioxidant activity [20]. They showed that the Prathibha variety's higher antioxidant activity might be caused by a higher percentage of phenolics, flavonoids, curcuminoids and natural antioxidants. Antioxidants found in ginger help prevent free radicals from harming the body's immune system cells by preventing their entry. Additionally, antioxidants help to boost immuno-stimulatory activity, and cells help to maximize the immune system, and turmeric is less immune-stimulating than ginger [20]. To preserve the body's immune system, ginger can have a therapeutic effect by boosting DNA repair, increasing antioxidants, and reducing lipid peroxidation [21]. Green tea *(Camellia sinensis)*, a popular beverage high in polyphenols, was the third component of this innovative tea. Studies showing green tea's anti-carcinogenic, anti-microbial, antiviral, anti-hypercholesterolemic, anti-obesity, anti-inflammatory and antioxidant qualities have stoked interest in the beverage over time. Green tea's high catechin content, which makes up between 15 and 27% of the dry weight of the leaves, is thought to be responsible for its health benefits [22]. The inclusion of phytochemicals such as catechins, flavonoids, polyphenols, and tannins may be the cause of the developed novel tea's high level of antioxidant activity. The substantial antioxidant qualities of several plant-derived substances, including tannins, flavonoids, terpenoids, glycosides, alkaloids, and phenols, have also been demonstrated by earlier research [23]. As far as there isn't a tea like this in this combination, and it's a novel tea that was developed first time in Pakistan.

This study was limited to laboratory-scale preparation and evaluation of ginger-turmeric tea, without assessment of long-term storage stability or clinical efficacy in humans. Future research should focus on large-scale production, shelf-life studies, and in vivo investigations to validate health benefits and safety, potentially leading to commercial development of functional herbal beverages.

CONCLUSIONS

According to the findings of this study, a new ginger-turmeric tea has been developed that offers natural antioxidants in addition to two essential nutrients fiber and protein. Moreover, developed tea contains numerous bioactive compounds that can be utilized to prevent or cure a variety of human health conditions, including diabetes, inflammation, oxidative stress, immune system diseases and neurological disorders.

Authors' Contribution

Conceptualization: MKS
Methodology: MKS, SA
Formal analysis: HKA, AK, SJ
Writing and Drafting: NZ, AS,
Review and Editing: NZ, AS, HKA, AK, SJ, MKS, SA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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