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Selected Synergistic Health Benefits of Spices (Ginger and Turmeric)

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Abstract

Spices have been an integral part of culinary traditions worldwide, adding flavor, color, and aroma to dishes. Ginger (G) and Turmeric (T) contain phenolic substances with strong anti-inflammatory and antioxidant properties. Combining G&T may produce synergistic effects, enhancing bioactivity and utilization in the food industry. The objectives of this study were to determine the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and their antioxidant potential by 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Potential (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC), and Nitric Oxide Radical Scavenging Ability (NORS) and the activity of carbohydrate and lipid metabolizing enzymes (α-Glucosidase, α-Amylase, and Lipase). Ginger and Turmeric extracts in pure and combination samples [100% Ginger (100G), 100% Turmeric (100T), 50% Ginger + 50% Turmeric (50G + 50T), 75% Ginger + 25% Turmeric (75G + 25T), & 25% Ginger + 75% Turmeric (25G + 75T)] were extracted with aqueous and 80% ethanol solutions. Results indicate that ethanol extracts had higher TPC, TFC, FRAP, and DPPH, while aqueous extracts exhibited higher TEAC values. Additionally, the two spices showed varying levels of NORS depending on whether the extracts were aqueous or ethanol-based suggesting both hydrophilic and lipophilic bioactive components are present in Ginger and Turmeric. Spices with higher ginger concentration showed greater enzyme inhibition. The 75G-25T ethanol extracts demonstrated the highest inhibition, with 50.23% by α-amylase and 60% by lipase, indicating a synergistic effect between ginger and turmeric. This synergism (G&T) enhances antioxidant activity and inhibit metabolizing enzymes.

Keywords

Ginger, Turmeric, Antioxidant, Anti-Inflammatory, Total Phenolics, Total Flavonoids

1. Introduction

In recent years, the interest of consumers and researchers has increased regarding the potential benefits of food and diet to prevent and treat various diseases and enhance human health [1]. Since ancient times, spices have been added to food to improve its organoleptic qualities and flavor. Due to their high antioxidant activity and positive effects on human health, spices have been used extensively as preservatives and medicines [2]. Bioactive compounds in spices, including gingerol from ginger and curcumin from turmeric, are known to have gastroprotective, anti-inflammatory, and anti-proliferative qualities.

Ginger is a versatile spice known for its pungent, warm flavor and significant health-promoting properties [3]. Its major phytochemicals, gingerol and shogaol, stimulate digestive enzymes and exhibit strong antioxidative effects by scavenging free radicals and activating the Nrf2 signaling pathway, which enhances the expression of detoxifying enzymes such as glutathione peroxidase [4]. Similarly, turmeric is commonly used as a coloring and flavoring agent in foods such as butter, cheese, fruit drinks, and curry powder [5]. Its key active compound, curcumin, is known for its potent immunomodulatory effects and can suppress oxidative stress by inhibiting the NF-κB pathway, thereby reducing the production of pro-inflammatory cytokines [6]. In addition to their antioxidant properties, both ginger and turmeric have demonstrated inhibitory activity against metabolic enzymes such as α-amylase and lipase by binding to their active sites and limiting substrate interaction [6]. These mechanisms contribute to the regulation of metabolic processes and support their potential role in managing chronic conditions related to oxidative stress.

Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and reduced antioxidant activity, leading to cellular and tissue damage if untreated [7]. In contrast, ROS is crucial in essential cellular functions such as energy production. However, factors like inadequate nutrition, diets rich in carbohydrates and fats, stress, and other environmental influences that increase ROS levels can also contribute to the onset of chronic conditions, including cardiovascular disease, diabetes, and obesity [8]. Beyond causing oxidative damage, elevated ROS levels can disrupt metabolic homeostasis. For instance, oxidative stress impairs insulin signaling pathways and reduces glucose uptake, contributing to insulin resistance and the development of type 2 diabetes. Similarly, oxidative stress alters lipid metabolism by promoting lipid peroxidation and dysregulation of lipid storage and transport, increasing the risk of obesity and atherosclerosis [9]. These metabolic dysfunctions are closely linked to the onset and progression of various chronic diseases. Therefore, strategies aimed at reducing oxidative stress may offer metabolic benefits and disease prevention. To counter these risks, incorporating bioactive compounds from functional foods, such as ginger and turmeric, can be beneficial. These foods have antioxidant properties that help mitigate the effects of excessive ROS [10]. Antioxidants are essential for providing the body with a defense system to combat oxidative stress [11]. The phytochemicals in spices

demonstrate antioxidative properties by neutralizing various oxidizing enzymes and stabilizing lipid peroxidation [12]. Antioxidant assays, such as (2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Potential (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC), and Nitric Oxide Radical Scavenging Ability (NORS), are, therefore, essential instruments for determining the extent to which various substances can combat oxidative stress. Antioxidant assays play a crucial role in identifying compounds that can neutralize oxidative damage, offering valuable information about their potential health advantages and their applications in food, pharmaceuticals, and cosmetics [13]. Carbohydrate and lipid metabolizing enzymes, such as α-Amylase, α-Glucosidase, and Lipase, were used to determine inhibition potential by the spices.

Research on these species has been conducted, with positive results in preventing inflammatory diseases [14]. Ginger and Turmeric have not been used in combination, so it is important to know their combined or potential synergistic effects. Spices, in combination, may enhance their functional bioactive effects in the treatment or prevention of chronic diseases for their utilization in functional food product development. Recent studies have highlighted the growing interest in combining spices to enhance their health-promoting properties. Rani *et al.* (2023) demonstrated that mixtures containing spices such as ginger and turmeric exhibit greater antioxidant and enzyme-inhibitory effects compared to individual extracts. The observed enhancement in bioactivity may result from the complementary actions of various phytochemicals, such as flavonoids, phenolics, and organosulfur compounds present in spices, suggesting the involvement of synergistic mechanisms. These interactions, commonly found in traditional dietary patterns, have been associated with reduced risk of metabolic and chronic diseases [15]. These findings support the use of spice combinations as a promising approach in functional food development to maximize their potential health benefits.

2. Materials and Methods

2.1. Sample Preparation

Powered organic Ginger and Turmeric were acquired from Monterey Bay Spice Company. Sample extracts were prepared using aqueous (deionized water) and ethanol (80% ethanol) solutions, utilizing 100 ml of each solution per 5 g of Ginger and Turmeric samples [pure (100%) and combinations (50%/50%, 75%/25%, 25%/75%)]. A solid-to-solvent ratio of 1:20 (w/v) and a 1-hour sonication period were selected based on previously published protocols involving spice extract preparation for antioxidant and enzyme inhibition assays [16] [17]. These conditions were found effective in obtaining consistent extraction yields and bioactive profiles. Both aqueous and ethanol extracts were subjected to one hour of ultrasound treatment in a Bransonic M5800H Ultrasound Sonic Bath. Temperature was not externally controlled during sonication; however, the unit typically operates at ambient to mildly elevated temperatures (~35˚C - 40˚C) under continuous ultrasonic activity. Extracts were then centrifuged at 1107 g force at 4˚C for 20 minutes.

The resulting supernatants were filtered using Whatman filter paper, evaporated using a rotary evaporator (Buchi Rotavapor R-215, USA). Extracts were reconstituted in ethanol/water at 10 mg/mL, and working concentrations of 1.0 mg/mL were used in all antioxidant and enzyme inhibition assays. The final samples were stored in a 4˚C cooler. The complete experimental procedure is illustrated in Figure 1.

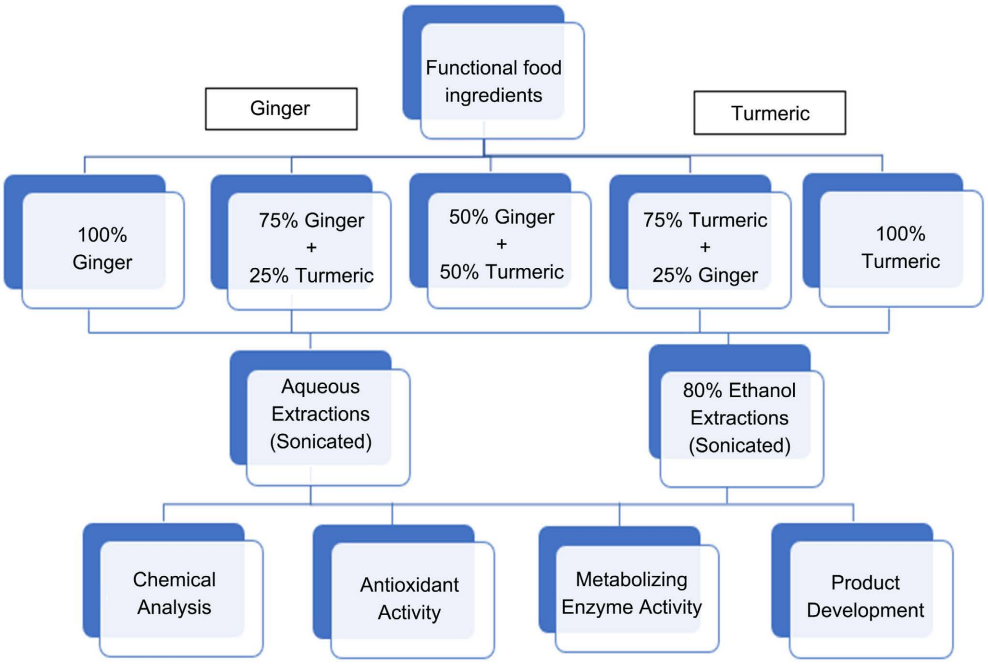

Figure 1. Experimental layout.

2.2. Chemical Analysis and Antioxidant Activities

The schematic representation of the experimental setup for the chemical analysis and assessment of antioxidant activity in the Ginger and Turmeric samples is illustrated in Figure 1. Chemical assays encompassed evaluations of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), while antioxidant assays involved tests for 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Potential (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC), and Nitric Oxide Radical Scavenging Ability (NORS).

2.2.1. Chemical Analysis Assays

The following assays were used to determine the phytochemical content of the ginger and turmeric extracts: total phenolic content (TPC) and total flavonoid content (TFC).

1) Total Phenolic Content (TPC)

The majority of plant tissue contains phenolic compounds, which are phytochemicals with benzene rings that have one or more hydroxyl substituents [18]. The total phenolic content of the Ginger and Turmeric extracts was calculated using the Folin-Ciocalteu method [19]. Dilutions of the extracts were mixed with

deionized water and Folin-Ciocalteu's reagent, using gallic acid as the standard, and then incubated for five minutes. The samples were treated with sodium bicarbonate and left to incubate for a total of 90 minutes. The samples were read using a Synergy HTX microplate reader at an absorbance of 750 nm.

2) Total Flavonoid Content (TFC)

According to Panche *et al.* (2016), flavonoids, a major class of phenolic compounds, are used in pharmaceutical, medicinal, and nutraceutical products that have positive effects on the body. Catechin is the method of determining total flavonoid content as the benchmark [20]. After adding 10% aluminum chloride to the mixture, diluted samples were incubated for an additional 5 minutes after being incubated for 5 minutes with deionized water and sodium nitrite. The samples were then treated with sodium hydroxide and extra deionized water, and the absorbance was measured at 510 nm.

2.2.2. Antioxidant Assays

Using the following assays, the antioxidant activity of the Ginger and Turmeric extracts was evaluated: Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Potential (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), Nitric Oxide Radical Scavenging Ability (NORS).

1) 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant potential of the Ginger and Turmeric sample extracts was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), a free radical scavenging assay [21]. The DPPH radical was added to diluted samples, and the results were read for 90 minutes, at 30-minute intervals, at an absorbance of 517 nm [22].

2) Ferric Reducing Antioxidant Potential (FRAP)

The test used to evaluate the antioxidant power of the samples used in this experiment is called Ferric Reducing Antioxidant Potential (FRAP), also referred to as Ferric Reducing Ability of Plasma and Ferric Reducing Antioxidant Power. Diluted samples were mixed with deionized water, 10 mM iron sulfate, and FRAP reagent, following a modification of Benzie & Strain [23]. The samples were then read at 593 nm.

3) Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) assay measures the extent to which an antioxidant can scavenge radicals like ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radicals [24]. As part of the process, ethanol-diluted ABTS radical stock solution (ABTS radical and potassium persulphate; 0.7 ± 0.025 absorbance at 734 nm) was added to diluted samples, and the absorbance was measured at 734 nm every minute for 6 minutes [25].

4) Nitric Oxide Radical Scavenging Ability (NORS)

The scavenging activity of the Ginger and Turmeric extracts against the nitric oxide free radical was assessed using the Nitric Oxide Radical Scavenging Ability (NORS) assay [26]. Diluted extracts were combined with ascorbic acid and 10mM sodium nitroprusside, and they were incubated at 25˚C for 150 minutes. The Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl eth-

ylene diamine dichloride) was used as the standard. The samples were then treated with Griess reagent to ensure the wavelength was measured at 546 nm [27].

2.3. Metabolizing Enzyme Assays

Enzyme assays for metabolizing carbohydrates and lipids, such as *α*-Amylase, *α*-Glucosidase, and Lipase, were used to determine enzymatic inhibition.

2.3.1. *α*-Amylase Activity

The enzyme *α*-Amylase converts starch to glucose. The enzyme assay method, which was adapted from Apostolidis *et al.* (2007), included combining diluted extracts with the *α*-amylase enzyme, 1% starch solution, and 3,5-Dinitrosalicylic (DNSA) for varying periods of time at 25˚C. The samples were then measured at 540 nm for absorbance [28].

2.3.2. *α*-Glucosidase Activity

To determine the *α*-glucosidase inhibition activity of Ginger and Turmeric extracts, a colorimetric assay called the *α*-glucosidase assay was conducted. Phosphate buffer (pH 6.8), *α*-glucosidase enzyme, and p-Nitrophenyl-a-D-glucopyranoside (PNPG) were combined with diluted extracts and incubated for 30 minutes at 37˚C. The mixture was then treated with 0.1M sodium carbonate, and the plates were read at an absorbance of 405 nm [28].

2.3.3. Lipase Activity

The lipase activity of the ginger and turmeric extracts was measured using the rapid, colorimetric lipase assay. Diluted samples were mixed with the lipase enzyme, potassium phosphate buffer, and Tween 80, following the modification of Mosmuller *et al.*, 1992, and incubated for 60 minutes at 30˚C. The samples were then treated with 25 mM 2,4 p-nitrophenyl butyrate (PNB) substrate, allowed to incubate for an extra 5 minutes, and the absorbance was measured at 405 nm [29].

2.4. Statistical Analysis

The experimental design for 1 and 2 objectives was a 5 × 2 factorial, including two extraction solvents (ethanol and aqueous) and 5 ingredient combinations (100% ginger, 100% turmeric, 50% ginger + 50% turmeric, 75% ginger + 25% turmeric, and 25% ginger + 75% turmeric). The data were presented as means ± SEM, and each experiment was conducted in triplicate. ANOVA (analysis of variance) was used for the statistical analysis. SAS 9.2 version was used to perform the One-way ANOVA statistical analysis. Tukey's studentized range test was used to identify significant differences between means; $p \leq 0.05$ was considered significant.

3. Results

3.1. Chemical Analysis & Antioxidant Activity

All the assays conducted compared 100% Ginger (100G) and 100% Turmeric (100T) to the following combination samples: 50% Ginger + 50% Turmeric (50G

+ 50T), 75% Ginger + 25% Turmeric (75G + 25T), and 25% Ginger + 75% Turmeric (25G + 75T). All spices were extracted using 80% ethanol (ET) and aqueous (AQ) as solvents.

3.1.1. Total Phenolic Content

Phenolic compounds found in spices belonging to the *Zingiberaceae* family, such as ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*), are recognized for their antioxidant potential. Ginger phenolics specifically contribute to regulating inflammation, obesity, diabetes, and cancer due to the active compounds, gingerols and shogaols [30]. A colorimetric assay (Total Phenolic Content—TPC) was utilized to determine the reducing capacity of a compound. The primary reagent used in this assay is the Folin-Ciocalteu reagent, which is paired with sodium carbonate and interacts with phenolic compounds [19]. The samples were read using a Synergy HTX microplate reader at an absorbance of 750 nm. **Table 1** shows the TPC of ginger and turmeric, alone and in combination, extracted using 80% ethanol (ET) and aqueous (AQ) as solvents. Significant differences ($p \leq 0.05$) were determined between the sample combinations and extraction solvents.

Table 1. Total Phenolic Content (TPC) of ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	TPC (mg G.A.E./100g DW)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	1947.83 ± 58.30^{cx}	635.46 ± 19.96^{ay}
100% Turmeric	3098.22 ± 52.96^{ax}	660.84 ± 13.29^{ay}
50% Ginger + 50% Turmeric	2396.91 ± 33.61^{bx}	565.97 ± 10.84^{cy}
75% Ginger + 25% Turmeric	2421.85 ± 52.26^{bx}	551.61 ± 3.31^{cy}
25% Ginger + 75% Turmeric	2733.66 ± 59.94^{abx}	595.72 ± 11.31^{by}

*G.A.E.—Gallic acid equivalent; DW—Dry weight. Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

The TPC of 100T (ET) (3098.22 ± 52.96 mg G.A.E./100g DW) was significantly ($p \leq 0.05$) higher compared to the 100G (ET) (1947.83 ± 58.30 mg G.A.E./100g DW). Combinations of 50G + 50T (2396.91 ± 33.61 mg G.A.E./100g DW) and 75G + 25T (2421.85 ± 52.26 mg G.A.E./100g DW) were significantly ($p \leq 0.05$) lower than 100T ET. However, combination 25G + 75T (ET) (2733.66 ± 59.94 mg G.A.E./100g DW) showed no significant difference compared to 100T (ET). Aqueous extracts of Spices, 100G and 100T, were both significantly ($p \leq 0.05$) higher compared to all combinations, 50G + 50T AQ (565.97 ± 10.84 mg G.A.E./100g DW), 75G + 25T AQ (551.61 ± 3.31 mg G.A.E./100g DW) and 25G + 75T AQ (595.72 ± 11.31 mg G.A.E./100g DW) respectively. When comparing combination extracts for the solvents (AQ and ET), the 25G + 75T displayed significantly higher ($p \leq 0.05$) TPC compared to 50G + 50T and 75G + 25T.

Overall, TPC values of the ET extracts were significantly ($p \leq 0.05$) higher than those of the AQ Ginger and Turmeric (pure and combination) extracts, implying that the phytochemicals in these functional ingredients may be more soluble in lipophilic solvents than in hydrophilic ones. This suggests that the extractability of total phenolic content from Ginger and Turmeric (pure and combined) was higher in ET than in Water. Water extracts only hydrophilic compounds; however, ethanol extracts both lipophilic (predominantly) and hydrophilic phytochemicals.

3.1.2. Total Flavonoid Content

Table 2 presents the total flavonoid content (TFC) of ginger and turmeric extracts obtained using water and 80% ethanol. In the TFC assay, aluminum chloride interacts with the hydroxyl groups of flavonoids to form complexes. Additionally, an acid-sensitive reaction is triggered by the addition of sodium nitrite [20]. Significant differences ($p \leq 0.05$) were determined between the sample combinations and extraction solvents.

Table 2. Total Flavonoid Content (TFC) of ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	TFC (mg C.E./100g DW)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	932.07 ± 8.39^{bx}	559.1 ± 10.84^{by}
100% Turmeric	995.36 ± 6.39^{ax}	515.18 ± 3.75^{by}
50% Ginger + 50% Turmeric	691.75 ± 13.92^{cx}	639.53 ± 3.29^{ax}
75% Ginger + 25% Turmeric	939.79 ± 4.28^{bx}	537.99 ± 6.98^{by}
25% Ginger + 75% Turmeric	967.06 ± 3.05^{ax}	660.22 ± 6.65^{ay}

*C.E.—Catechin equivalent; DW—Dry weight. Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

The TFC of 50G + 50T ET extract (691.75 ± 13.92 mg C.E./100g DW) had significantly ($p \leq 0.05$) lower flavonoid content compared to the 100T, 100G ET (995.36 ± 6.39 mg C.E./100g DW and 932.07 ± 8.39 mg C.E./100g DW, respectively) and other combination extracts. Among combinations, 25G + 75T (ET) extract (967.06 ± 3.05 mg C.E./100g DW) had the highest TFC value compared to its counterparts.

Among the aqueous extracts, 100G AQ (559.1 ± 10.84 mg C.E./100g DW) extracts had a higher TFC value compared to 100T AQ (515.18 ± 3.75 mg C.E./100g DW). The highest TFC values were observed in the combination samples, ranging from 537.99 ± 6.98 (75G + 25T) to 660.22 ± 6.65 (25G + 75T). Among the TFC AQ values, 50G + 50T and 25G + 75T were significantly ($p \leq 0.05$) higher than 100G, 100T, and 75G + 25G samples. A synergistic effect is evident in all combinations, with greater TFC seen in samples with higher turmeric concentrations

(50G + 50T and 25G + 75T) compared to the 75G + 25T extracts.

The total flavonoid content (TFC) of extracts from Ginger and Turmeric varied depending on the solvent used. A significant difference was observed with 100T, with a higher TFC in 100T ET (995.36 ± 6.39 mg C.E./100g DW) compared to the lowest TFC in 100T AQ (515.18 ± 3.75 mg C.E./100g DW). Overall, in a nonpolar solvent such as ethanol, the Ginger and Turmeric (pure and combination) samples yielded higher total flavonoid content compared to water.

3.2. Antioxidant Assays

3.2.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Antioxidant Activity

Table 3 shows the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of ginger and turmeric extracts (water and 80% ethanol). DPPH is a stable free radical commonly used in research to assess the free radical scavenging capacity of various food products. The shift from dark purple to pale yellow in the DPPH assay indicates that the sample may have reduced the free radical to its inactive form [31]. In this assay, the primary role of the spices is to donate hydrogen atoms to neutralize the DPPH radical [32]. The absorbance was read at 517 nm [22]. Significant differences ($p \leq 0.05$) were determined between the sample combinations and extraction solvents.

Table 3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition of ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	DPPH % Inhibition (μg/ml)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	86.47 ± 0.45^{bx}	61.23 ± 2.33^{ay}
100% Turmeric	80.92 ± 1.09^{cx}	31.63 ± 0.34^{cy}
50% Ginger + 50% Turmeric	89.62 ± 0.23^{ax}	32.40 ± 0.28^{cy}
75% Ginger + 25% Turmeric	85.16 ± 2.81^{bx}	51.96 ± 2.04^{by}
25% Ginger + 75% Turmeric	87.03 ± 0.89^{abx}	9.72 ± 1.25^{dy}

Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

In the 80% ethanol extracts, 100G demonstrated the highest DPPH inhibition (86.47% ± 0.45%), closely followed by 100T with a significantly ($p < 0.05$) lower inhibition value (80.92% ± 1.09%). When combined in equal proportions, 50G + 50T ET demonstrated the highest DPPH inhibition (89.62 ± 0.23 μg/ml), which was significantly ($p \leq 0.05$) higher than spices (100G and 100T ET), and the 75G + 25T combination (85.16% ± 2.81%). This suggests a synergistic effect of the two spices when extracted together. Although 75G + 25T ET was slightly lower, it was still significantly ($p \leq 0.05$) greater than the inhibition seen in pure turmeric extracts. The DPPH inhibition for 25G + 75T (ET) (87.03% ± 0.89%) was nearly equivalent to that of 50G + 50T (ET) (89.62 ± 0.23), with no significant difference

observed between them, and was closely followed by 100G (ET) with a slightly lower inhibition of 86.47% ± 0.45%.

The aqueous extracts showed much lower DPPH inhibition compared to ethanol extracts ranging from 9% - 61%. The 100G showed the highest DPPH inhibition (61.23% ± 2.33%), while 100T had significantly ($p \leq 0.05$) lower inhibition (31.63% ± 0.34%). The 50G + 50T combination showed minimal improvement (32.40% ± 0.28%), indicating no synergistic effect. The 75G + 25T had moderate inhibition (51.96% ± 2.04%), and 25G + 75T showed the lowest inhibition (9.72% ± 1.25%), highlighting turmeric's reduced antioxidant activity in aqueous extracts.

Ethanol extracts showed significantly ($p \leq 0.05$) higher DPPH inhibition compared to aqueous extracts, demonstrating ethanol's superior ability to extract antioxidants from ginger and turmeric. A synergistic effect was observed in the 50/50 ethanol combination, which had the highest DPPH inhibition, but this was not seen in aqueous extracts. Overall, pure ginger consistently showed stronger antioxidant activity than turmeric in both solvents.

3.2.2. Ferric Reducing Antioxidant Potential (FRAP)

The ferric reducing antioxidant potential (FRAP) method relies on antioxidants in an acidic medium reducing a ferroin analog, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$, to the extremely blue-colored Fe^{2+} complex $Fe(TPTZ)^{2+}$ [33]. Table 4 displays the FRAP of the spices extracted with 80% ethanol and water. Significant differences ($p \leq 0.05$) were determined between the different sample combinations and extraction solvents.

Table 4. Ferric Reducing Antioxidant Potential (FRAP) of ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	FRAP (mM Fe(II)/100g DW)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	553.90 ± 15.49^{cx}	381.81 ± 11.72^{ay}
100% Turmeric	801.57 ± 23.93^{bx}	110.65 ± 9.25^{dy}
50% Ginger + 50% Turmeric	1178.55 ± 46.56^{ax}	172.19 ± 7.11^{by}
75% Ginger + 25% Turmeric	860.62 ± 31.77^{bx}	202.55 ± 11.23^{by}
25% Ginger + 75% Turmeric	1032.15 ± 48.98^{ax}	144.59 ± 10.85^{bcy}

*Fe(II)—Ferric iron; DW—Dry weight. Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

The Ferric Reducing Antioxidant Potential (FRAP) of the combinations, 50G + 50T and 25G + 75T ET were significantly ($p \leq 0.05$) higher than the spices alone, 100G and 100T ET. Ethanol extracts for combination spices ranged from 553.90 ± 15.49 to 1178.55 ± 46.56 mM Fe (II)/100g DW. The FRAP value of the combination 75G + 25T (860.62 ± 31.77 mM Fe (II)/100g DW) was significantly ($p \leq 0.05$) higher than individual spice extracts, 100T ET (801.57 ± 23.93 mM Fe

(II)/100g DW), and 100G (552.90 ± 15.49 mM Fe (II)/100g DW).

The combination samples followed a similar pattern as the 100T extracts, showing higher FRAP values in ethanol (ET) extracts compared to aqueous (AQ) extracts. Overall, samples extracted in 80% ethanol and those with a higher proportion of Turmeric exhibited higher FRAP values, which were significantly ($p \leq 0.05$) greater than those of the aqueous extracts. The FRAP values of AQ extracts of (single & combinations) ranged from 110.65 ± 9.25 (100% T) to a high of 381.81 ± 11.72 (100% G). The ET extract of the 100T sample was about 8-fold higher compared to its AQ counterpart. Similarly, the combination samples of ET extracts were 4-9-fold higher than their AQ counterparts.

3.2.3. Nitric Oxide Radical Scavenging (NORS) Ability

Nitric oxide (NO) is a free radical produced from L-arginine in the endothelial cells of blood vessels. The nitric oxide radical scavenging (NORS) assay involves the reaction between nitric oxide radical and sodium nitroprusside. In the presence of an antioxidant, the production of NO_3^- and NO_2^- is prevented [27]. **Table 5** shows the NORS ability of the Ginger and Turmeric extracts. Significant differences ($p \leq 0.05$) were determined between the sample combinations and extraction solvents.

Table 5. Nitric Oxide Radical Scavenging Ability (NORS) of ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	NORS (mM NO/100g DW)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	62923.9 ± 218.2^{dy}	109,264 ± 126.6^{cx}
100% Turmeric	129,116 ± 270.3^{bx}	107,901 ± 131.7^{cy}
50% Ginger + 50% Turmeric	124,898 ± 229.7^{bx}	115,516 ± 185.0^{by}
75% Ginger + 25% Turmeric	98,286 ± 258.3^{cy}	108,493 ± 295.92^{cx}
25% Ginger + 75% Turmeric	169,575 ± 205.8^{ax}	136,567 ± 162.9^{ay}

*NO—Nitric oxide; DW—Dry weight. Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abcd". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

Nitric Oxide Radical Scavenging Ability (NORS) of 25G + 75T ET (169,575 ± 205.8 mM NO/100g DW) was significantly ($p \leq 0.05$) higher than 100G and 100T ET and also significantly ($p \leq 0.05$) highest among the other combinations, 50G + 50T ET (124,898 ± 229.7 mM NO/100g DW) and 75G + 25T ET (98,286 ± 258.3 mM NO/100g DW). Within the ET extracts, 100T (129,116 ± 270.3 mM NO/100g DW) showed a significantly ($p \leq 0.05$) higher NORS value compared to the lowest 100G (62923.9 ± 218.2 mM NO/100g DW) sample.

The NORS ability of aqueous extracts had a similar trend for combination of spices where 25G + 75T AQ (136,567 ± 162.9) was significantly ($p \leq 0.05$) higher than pure ginger and turmeric extracts or in combination samples. The NORS of

the 50/50 combination (115,516 ± 185.0 mM NO/100g DW) was significantly ($p \leq 0.05$) lower than 25G + 75T, but significantly ($p \leq 0.05$) higher than all other values. However, combination 75G + 25T AQ (108,493 ± 295.92 mM NO/100g DW) showed no significant difference with the pure spice extracts 100G AQ (109,264 ± 126.6 mM NO/100g DW) and 100T AQ (107,901 ± 131.7 mM NO/100g DW). The AQ spice combinations suggest the potential of having a synergistic effect as it relates to NORS ability, due to two combinations being significantly ($p \leq 0.05$) higher than the spices, ginger, and turmeric extracted alone, and one combination 75G + 25T AQ) being slightly higher than pure turmeric.

Overall, NORS of ginger AQ extracts were significantly ($p \leq 0.05$) higher than ET extracts, whereas turmeric ET extracts were significantly ($p \leq 0.05$) higher than the AQ extracts. The NORS ability of ET extracts of two combinations (50G + 50T) and (25G + 75T) was significantly ($p \leq 0.05$) higher than the AQ extracts. However, that was not seen in the 75G + 25T (ET) samples, which also had the lowest (98286 ± 258.3) NORS compared to its counterparts.

3.2.4. Trolox Equivalent Antioxidant Capacity (TEAC)

The ability of the samples to scavenge the cation ABTS radical is determined by utilizing the Trolox vitamin E analog in the electron-based TEAC assay. The unstable form of the ABTS radical (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) is present as a blue-green solution. The blue-green hue changes to a pale blue color when an antioxidant is present, as the ABTS will accept an electron from the antioxidant [24]. **Table 6** shows the TEAC of the Ginger and Turmeric extracts. Significant differences ($p \leq 0.05$) were determined between the sample combinations and extraction solvents.

Table 6. Trolox Equivalent Antioxidant Capacity (TEAC) of ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	TEAC (MM T.E./100 g DW)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	75.89 ± 1.12^{ay}	81.48 ± 0.60^{cx}
100% Turmeric	49.32 ± 5.3^{cy}	80.04 ± 3.2^{cx}
50% Ginger + 50% Turmeric	50.72 ± 1.7^{cy}	89.07 ± 0.78^{ax}
75% Ginger + 25% Turmeric	62.45 ± 4.9^{by}	79.88 ± 1.98^{cx}
25% Ginger + 75% Turmeric	65.51 ± 4.4^{by}	84.00 ± 0.86^{bx}

*T.E.—Trolox equivalent, DW—Dry weight. Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

The TEAC of ET 100G (75.89 ± 1.12 MM T.E./100g DW) is significantly ($p \leq 0.05$) higher than ET 100T (49.32 ± 5.3 MM T.E./100g DW) and the combined spices extracts. Among combination samples, 25G + 75T ET (65.51 ± 4.4 MM T.E./100g DW) was slightly higher than 75G + 25T ET (62.45 ± 4.9 MM T.E./100g

DW), but significantly ($p \leq 0.05$) higher than 50G + 50T ET (50.72 ± 1.7 MM T.E./100g DW).

The TEAC of AQ samples follow a different trend, where 50G + 50T (89.07 ± 0.78 MM T.E./100g DW) was significantly ($p \leq 0.05$) higher than 100G (81.48 ± 0.60 MM T.E./100g DW) and 100T (80.04 ± 3.2 MM T.E./100g DW), however, the pure spice extracts (100G and 100T) showed no significant differences. The TEAC values of AQ spice combinations, 75G + 25T and 25G + 75T were 79.88 ± 1.98 and 84.00 ± 0.86 MM T.E./100g DW respectively, and were significantly ($p \leq 0.05$) lower than 50G + 50T (89.07 ± 0.78 MM T.E./100g DW). The trends for 25G + 75T and 75G + 25T ET remain similar, showing no significant difference, as these combinations display a potential for some synergistic interaction between the spices.

Overall, the TEAC in the AQ extracts was significantly ($p \leq 0.05$) higher than ET for pure and combination samples, suggesting that Ginger and Turmeric (pure and combination) have higher extractability of antioxidant phytochemicals in a polar solvent. This can be attributed to Trolox, a water-soluble vitamin E analog, exhibiting higher TEAC values in aqueous solutions, enhancing the antioxidant activity in these extracts.

3.3. Metabolizing Enzyme Assays

3.3.1. *α*-Amylase Activity

α-Amylase is an enzyme that plays a major role in carbohydrate metabolism, which is significant considering the high presence of simple carbohydrates in the Western diet. It breaks down complex carbohydrates such as amylose into simple sugars by hydrolyzing the glycosidic bonds. The 1% Starch was utilized as a substrate in this assay, along with 3,5-Dinitrosalicyclic (DNSA), *α*-amylase enzyme, and the spice extracts. A color change measured the amount of simple carbohydrates released from the starch and was read at an absorbance of 540 nm [28]. Table 7 shows the inhibition of *α*-amylase enzyme activity by the Ginger and Turmeric extracts.

Table 7. Inhibition (%) of α-Amylase Activity by ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	*α*-Amylase (% Inhibition)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	27.55 ± 0.27^{cy}	51.02 ± 0.07^{bx}
100% Turmeric	28.08 ± 0.11^{cy}	76.53 ± 0.05^{ax}
50% Ginger + 50% Turmeric	39.9 ± 0.28^{by}	41.33 ± 0.10^{cx}
75% Ginger + 25% Turmeric	50.23 ± 0.08^{ax}	33.16 ± 0.13^{dy}
25% Ginger + 75% Turmeric	11.82 ± 0.20^{dy}	38.78 ± 0.17^{cx}

Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

α-Amylase enzyme inhibition was found to be higher in the ET 100T (28.08% ± 0.11%) spice extract compared to 100G (27.55% ± 0.27%). The inhibition of the combination extracts is significantly different ($p \leq 0.05$), with the lowest % amylase inhibition found in the (ET) 25G + 75T (11.82 ± 0.20), followed by 50G + 50T (39.9% ± 0.28%) and the highest in 75G + 25T (50.23% ± 0.08%) combination. In the AQ extracts, 100T (76.53% ± 0.05%) had a significantly ($p \leq 0.05$) higher % inhibition compared to 100G (51.02% ± 0.07%) spice extract and the combination extracts. In the combination spice extracts (AQ), the percentage of enzyme inhibition ranged from 33.16% ± 0.13% (75G + 25T) to 41.33% ± 0.10% (50G + 50T). There were no significant differences observed between pure ginger and pure turmeric ethanol extracts, however, the aqueous (pure and combination) spice extracts showed significant differences among them.

In comparison to ET extracts, AQ extracts 100G (51.02% ± 0.07%) and 100T (76.53% ± 0.05%) showed a greater percentage inhibition of the *α*-amylase metabolizing enzyme (almost 2-fold). Comparable outcomes were observed in the AQ extracts of 25G + 75T (38.78% ± 0.17%) and 50G + 50T (41.33% ± 0.10%), whereas the ET extract demonstrated a greater percentage of amylase inhibition by 75G + 25T combination (50.23% ± 0.08%). The % inhibition for *α*-amylase was overall highest in the 75G-25T Ethanol extract (50.23% ± 0.08%), indicating a synergistic interaction between the two ingredients in ethanol, a more lipid-soluble solvent. Both ginger and turmeric (in pure form and combinations) showed inhibitory effects on the *α*-amylase enzyme; however, aqueous turmeric extracts exhibited higher inhibition, suggesting that turmeric may serve as a more effective functional ingredient for managing postprandial hyperglycemia and carbohydrate metabolism disorders such as type 2 diabetes.

The combination of Spices (Cinnamon, Cardamom, Cloves, and Turmeric) showed a higher inhibition by alpha-amylase in aqueous extracts (91.70 ± 2.62) compared to methanol extracts [34]. The highest level of alpha-amylase inhibitory activity was observed in the water extract, which was subsequently followed by the ethanol and hydroalcoholic extracts [35].

3.3.2. *α*-Glucosidase Activity

α-Glucosidase enzyme inhibition assay evaluates the potential antioxidant activities and mechanisms by which these spices inhibit the carbohydrate-metabolizing enzyme, *α*-glucosidase. Inhibiting this enzyme may aid in preventing chronic conditions such as type 2 diabetes and obesity. p-Nitrophenyl-*α*-D-glucopyranoside (PNPG) was used as the substrate, and as a result, the release of p-Nitrophenyl produced a yellow color. The absorbance is read at 540 nm [28]. **Table 8** shows the *α*-glucosidase enzyme activity by the Ginger and Turmeric extracts.

α-Glucosidase enzyme inhibition was significantly ($p \leq 0.05$) higher in the ET 100G (54.31% ± 0.06%) spice extract compared to 100T (31.00% ± 0.10%). In the combination samples, the % inhibition of Glucosidase showed significant ($p \leq 0.05$) differences from a low of 28.79% ± 0.12% (25G + 75T) to a high of 41.46 ± 0.10 (75G + 25T). In the AQ extracts, 100G (46.17% ± 0.19%) resulted in a signif-

icantly ($p \leq 0.05$) higher inhibition compared to 100T (27.8% ± 0.43%) and among the combination samples. The 75G + 25T (45.32% ± 0.04%) had the highest % inhibition of the Glucosidase enzyme compared to other combinations, followed by 50G + 50T (44.88% ± 0.07%).

Table 8. Inhibition (%) of *α*-Glucosidase Activity by ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	*α*-Glucosidase (% Inhibition)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	54.31 ± 0.06^{ax}	46.17 ± 0.19^{ay}
100% Turmeric	31.00 ± 0.10^{dx}	27.8 ± 0.43^{cy}
50% Ginger + 50% Turmeric	35.37 ± 0.13^{cy}	44.88 ± 0.07^{ax}
75% Ginger + 25% Turmeric	41.46 ± 0.10^{by}	45.32 ± 0.04^{ax}
25% Ginger + 75% Turmeric	28.79 ± 0.12^{dy}	37.78 ± 0.14^{bx}

Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

A significantly ($p \leq 0.05$) lower *α*-glucosidase % inhibition is observed by 100G and 100T AQ extracts compared to their ET extracts. However, AQ combination spice extracts resulted in a higher % of inhibition compared to the ET samples. Overall, the % inhibition for *α*-glucosidase was highest by the (AQ) 75G-25T, suggesting a synergistic interaction between the two ingredients in a water-soluble solvent. Among both ET and AQ extracts, the highest % inhibition of *α*-glucosidase was demonstrated by the 100G (ET), however the lowest was seen by the 100T (AQ) extracts.

3.3.3. Lipase Activity

A fat-metabolizing enzyme, lipase, breaks down triglycerides into glycerol and free fatty acids. In this assay, the ability of the antioxidants in the spices to inhibit the enzyme is evaluated. A colorimetric test is designed to measure the lipase enzyme activity in selected ingredients. It uses Tween 80 and 2,4-p-nitrophenyl butyrate (pNB) as substrates, with the results read at an absorbance of 405 nm. The release of butyric acid from pNB is assessed by observing the color change [29]. **Table 9** shows the inhibition of lipase enzyme activity by the Ginger and Turmeric extracts.

Lipase enzyme inhibition (%) was significantly ($p \leq 0.05$) higher by the ET 100G (62.14% ± 0.07%) spice extract compared to 100T (49.30% ± 0.07%). The lipase inhibition (%) by the combinations are significantly different ($p \leq 0.05$), with the lowest % lipase inhibition seen by the ET 25G + 75T (45.54% ± 0.17%), followed by 50G + 50T (54.65% ± 0.04%) and the highest by the 75G + 25T (60.23% ± 0.03%) combination. In the AQ extracts, 100G (61.43% ± 0.09%) resulted in a significantly ($p \leq 0.05$) higher % inhibition compared to 100T (56.22% ± 0.04%)

spice extract. In the combination spice extracts (AQ), the percentage of enzyme inhibition ranged from a low of 22.27% ± 0.09% (75G + 25T) to 57.88% ± 0.07% (50G + 50T). There were significant differences in the lipase inhibition (%) between (pure and combination) ethanol spice extracts and the aqueous (pure and combination) spice extracts.

Table 9. Inhibition (%) of Lipase Activity by ginger and turmeric extracts (Ethanol & Aqueous).

Ginger and Turmeric Combinations	Lipase (% Inhibition)	
	80% Ethanol Extracts	Aqueous Extracts
100% Ginger	62.14 ± 0.07^{ay}	61.43 ± 0.09^{ax}
100% Turmeric	49.30 ± 0.07^{cy}	56.22 ± 0.04^{bx}
50% Ginger + 50% Turmeric	54.65 ± 0.04^{by}	57.88 ± 0.07^{bx}
75% Ginger + 25% Turmeric	60.23 ± 0.03^{ax}	22.27 ± 0.09^{dy}
25% Ginger + 75% Turmeric	45.54 ± 0.17^{cy}	53.22 ± 0.09^{bcx}

Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of Ginger and Turmeric combinations, shown in columns, indicated by letters "abc". Significant differences ($p \leq 0.05$) of extraction solvents, shown in rows, indicated by letters "xy".

The inhibition of the lipase enzyme by the 100G was higher in ET extracts and the inhibition by the 100T was higher in AQ extracts. Comparable outcomes were observed in the AQ extracts ranging from a significant ($p < 0.05$) lower inhibition in 75G + 25T (22.27% ± 0.09%) to higher inhibition in 50G + 50T (57.88% ± 0.07%), whereas there was no significant difference observed between 100T (56.22% ± 0.04%), 50G-50T (57.88% ± 0.07%), 25G-75T (53.22% ± 0.09%). Overall, the ET extract demonstrated a greater percentage of lipase inhibition by the 75G + 25T combination (60.23% ± 0.03%), which suggests that ginger and turmeric have better lipase inhibition in non-polar solvents, whereas the % inhibition was lower by the polar (hydrophilic) solvent. Based on the results, there may have been a synergistic interaction between the two spices, when Turmeric and higher concentrations of Ginger were combined, as shown by the higher percentage of inhibition.

Bae and Kim (2011) showed similar results in their study, where the anti-obesity effect of the Ginger spice extract was assessed using the porcine pancreatic lipase assay. Specifically, the ethanol-soluble portion of *Z. officinale* showed a significantly higher pancreatic lipase inhibitory activity compared to the other solvent-soluble portions of *Z. officinale*. These results indicate that *Z. officinale* may have therapeutic potential in the development of anti-obesity agents or their precursors.

4. Discussions

The variations observed between ethanol and aqueous extracts in total phenolic content (TPC), total flavonoid content (TFC), and antioxidant assays can be at-

tributed to differences in solvent polarity and the chemical nature of the extracted phytoconstituents. Ethanol, being a semi-polar solvent, is more efficient at extracting both hydrophilic and lipophilic compounds, thereby enhancing the extraction efficiency of a broader spectrum of phenolics and flavonoids. This correlates with the elevated values observed in TPC, TFC, DPPH, and FRAP assays for ethanol extracts. Conversely, water, a highly polar solvent, primarily extracts hydrophilic constituents, which may contribute to the relatively higher Trolox Equivalent Antioxidant Capacity (TEAC) observed in aqueous extracts. This may also be influenced by the use of Trolox, a water-soluble analog of vitamin E, as the reference standard in the assay, enhancing the antioxidant activity in polar extraction systems [36]. These observations highlight the importance of aligning solvent polarity with the chemical nature of both phytochemicals and the assay reagents to optimize antioxidant evaluation and extract bioactivity.

Despite the promising outcomes, this study has certain limitations. The phytochemical evaluation was limited to total phenolic and flavonoid content without detailed identification or quantification of individual bioactive compounds. Additionally, antioxidant and enzyme inhibitory effects were assessed using *in vitro* chemical assays, which do not fully reflect physiological complexity, including absorption, metabolism, and cellular interactions. Future studies should incorporate compound-specific profiling through techniques such as high-performance liquid chromatography (HPLC) to better characterize active constituents [37] [38]. Furthermore, follow-up research involving cell culture models could help elucidate the biological relevance of antioxidant and enzyme-inhibitory activities and provide mechanistic insight into how ginger and turmeric combinations influence metabolic pathways. *In vivo* studies or clinical trials are also warranted to confirm the synergistic potential and therapeutic applicability of these spice extracts in metabolic disease management.

5. Conclusion

The study aimed to investigate the potential health benefits of ginger and turmeric to help prevent type 2 diabetes and obesity. The results indicate that ethanol extracts had higher levels of TPC, TFC, FRAP, and % DPPH inhibition, while aqueous extracts exhibited higher TEAC values. This difference may be attributed to the presence of Trolox, a water-soluble analog of vitamin E, which enhances antioxidant activity in aqueous extracts. Additionally, the two spices showed varying levels of NORS depending on whether the extracts were aqueous or ethanol-based. Thus, the study suggests that both hydrophilic and lipophilic bioactive components are present in Ginger and Turmeric extracts. The combination of the spices, especially with higher ginger concentrations, resulted in greater inhibition (%) of metabolizing enzymes, such as amylase, glucosidase, and lipase. Overall, the synergistic effects of ginger and turmeric enhance antioxidant activity and inhibit metabolizing enzymes, as demonstrated by the similar interactions observed in the phytochemical and antioxidant assays.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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