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Evaluation of the antioxidant activity and total phenolic content of ginger root extract (*Zingiber officinale* L.)

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Abstract

Ginger is a medicinal and nutritional plant characterized by its rich composition of bioactive compounds. It contains approximately 40 antioxidant and anti-inflammatory compounds, including phenols. Therefore, the current study aimed to evaluate the antioxidant efficacy of ginger's alcoholic extract and to estimate its phenolic compound content. The alcoholic extraction of ginger root was performed by soaking it in 70% ethanol. Using DPPH and ABTS assays, the antioxidant activity of the plant extract was evaluated in vitro. The total phenolic content was estimated using the Follin-Cioalto method, and IC_{50} values were calculated based on dose-response curves. Our results indicate that the alcoholic ginger extract exhibited concentration-dependent antioxidant activity in both the DPPH and ABTS assays, the highest concentration (400 micrograms/ml) recorded inhibition rates of 82.2% in the DPPH and 87.8% in the ABTS.

IC_{50} values were recorded at 93.6 ± 3.9 µg/mL for the DPPH assay and 79.2 ± 3.3 µg/mL for the ABTS assay, reflecting the extract's higher efficiency in neutralizing the ABTS free radical. The total phenolic content yielded 78.5 ± 2.7 mg gallic acid equivalent/g dry weight, indicating the major role of phenolic compounds in the antioxidant activity. These results, taken together, indicate the potential use of ginger root extract in food and pharmaceutical applications and confirm that it is a promising natural source of antioxidants.

Key words: Phenols, antioxidant, DPPH, ABTS, ginger

Introduction

Ginger (*Zingiber officinale* L.) has been used as a spice in cooking to add a distinctive flavor for thousands of years. Ginger is also used in food supplements, cosmetics, traditional medicine, and pharmaceuticals due to its multiple biological properties, including its antioxidant, anti-inflammatory, antibacterial, and antidiabetic properties, as it contains active chemical compounds (Zhukovets and Özcan, 2020).

This effect is mainly due to its composition of biologically active compounds such as phenols (gingerol, shogaol, zingerone, and paradol) and a mixture of essential oils, including volatile and non-volatile oils, which play a role in inhibiting free radicals and reducing oxidative stress (Ijaz *et al*., 2025).

Oxidative stress, resulting from the accumulation of free radicals in the body, is an important factor in the development of many chronic diseases, including cancerous, cardiac, and inflammatory diseases (Jomova *et al*., 2023).

The most common antioxidant assayss in food and supplement analysis are the diphenylpicrylhydrazyl (DPPH) free radical assay, the iron reduction capacity assay (FRAP), and the 2,2′-azenobis(3-ethylbenzothiazolene-6-sulfonate) assay (ABTS) (Parcheta *et al*., 2021). The DPPH assay can be used to assess the antioxidant activity of functional foods, such as plant extracts due to its high stability, ease of practical application, and low cost of the DPPH radical (Han *et al*., 2017). The unpaired valence electron of the nitrogen atom in the DPPH radical is reduced by a hydrogen atom from the antioxidant molecule, resulting in the formation of DPPH-H hydrazine (Sirivibulkovit *et al*., 2018). The total phenolic compound (TPC) content was also estimated using the Folin-Ciocalteu method, as it is an important chemical indicator that shows a positive correlation with antioxidant activity (Asem *et al*., 2020).

Ghasemzadeh et al. (2010) reported that ethanolic ginger extract showed a total phenolic content of 65.4–89.7 mg GAE/g, and in the DPPH assay, it achieved free radical scavenging activity exceeding 80% at high concentrations. Furthermore, the IC_{50} values for the DPPH assay were recorded at 90–105 µg/mL. Ginger extract showed higher ABTS capacity compared to DPPH, with inhibition values of more than 85% at high concentrations, with IC_{50} values less than 85 µg/mL, indicating higher efficiency in neutralizing soluble free radicals in

the aqueous and lipid phases (Mošovská *et al*., 2016).

This research aims to evaluate the antioxidant activity of ginger root extract using DPPH and ABTS assayss, estimate the total phenolic compound content, and calculate IC_{50} values extracted from dose-response curves.

Materials and Methods

1. Plant Materials

Ginger roots were selected from the local market, where impurities were removed, then they were cut into thin slices and dried thoroughly at room temperature away from sunlight. The samples were then ground into a fine powder and stored in airtight containers.

2. Preparation of the Plant Extract

10 grams of dried and ground ginger were weighed and mixed with 100 ml of 70% ethanol. The mixture was mechanically shaken for 24 hours at room temperature. The extract was then filtered using filter paper and centrifuged at 4000 rpm for 10 minutes. Finally, the clear filtrate was collected and used directly in spectroscopic analyses (Harborne, 1998).

3. Estimation of Antioxidant Activity Using the DPPH Assays

This activity was evaluated using the DPPH assays according to the standard spectroscopic method described by Brand-Williams et al. (1995). A 0.1 mmol/L DPPH solution was prepared in methanol, and 1 mL of the plant extract was mixed with 1 mL of the DPPH solution. The mixture was then left to stand in the dark for half an hour at room temperature. Photoabsorption was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm, with methanol used as a control solution.

The percentage of inhibition was calculated using the following equation: % inhibition= $Sample^{A}$ control $-A\backslash$ $control^{A}$ $\times 100$

Where:

SampleA: Light absorption of the sample containing the plant extract

ControlA: Light absorption of the free radical solution (DPPH or ABTS) without the addition of the extract

4. Estimation of Antioxidant Activity Using the ABTS Assays

Using the $ABTS^{\bullet+}$ assay, this activity was evaluated according to the method described by Re et al. (1999), whereby 20 µL of the ginger extract at several concentrations (25–400 µg/mL) were mixed with 980 µL of $ABTS^{\bullet+}$ solution, and the mixture was incubated for 6 minutes at room temperature in the dark. The optical absorption was measured at a wavelength of 734 nm using a UV-Vis spectrometer, and the percentage

of free radical inhibition (%) was calculated using the same equation as in the DPPH assay.

5. Estimation of Total Phenolic Content (TPC)

Using Folin–Ciocalteu reagent, the total phenolic content was determined according to the method described by (Singleton and Rossi, 1965). 0.5 mL of the ginger extract was added to 2.5 mL of diluted Folin–Ciocalteu reagent (1:10), and the mixture was left to stand for 5 minutes. 2 mL of 7.5% sodium carbonate solution was then added, and the mixture was incubated in the dark for 30 minutes at room temperature. The light absorption was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Gallic acid was used to establish the standard curve, and the results were expressed in mg GAE/g of dry sample.

6. Statistical Analysis

All analyses were performed with three replicates, and results are presented as mean ± standard deviation (Mean ± SD). One-way ANOVA was used, and values were considered statistically significant at $p < 0.05$ (Zar, 2010).

Using nonlinear regression analysis, IC_{50} values (the concentration required to inhibit 50% of free radicals) were calculated from dose-response curves (Motulsky & Christopoulos, 2004).

Results

Antioxidant activity of ginger extract

The results in Table No. (1) show that ginger root extract has antioxidant activity in the DPPH and ABTS assayss, which are dose-dependent. At the highest concentration used, the percentage of free radical inhibition gradually increased with increasing concentration of plant extract, reaching more than 80% in the DPPH assays and more than 85% in the ABTS assays. The results also (Figure 1) indicated that the inhibition rates in the ABTS assay were higher than those measured in the DPPH assay at all concentrations, suggesting greater efficiency of ginger extract in capturing the ABTS free radical.

This is clear evidence of the effectiveness of ginger extract, and is consistent with what Tung et al., (2017) found, who showed that alcoholic ginger extracts exhibit similar behavior in free radical capture assayss, at high concentrations with a significant increase in inhibition rates.

Table 1. Dose-dependent DPPH and ABTS radical scavenging activities of ginger root extract

Extract concentration (µg/mL)	DPPH inhibition (%) ± SD	ABTS inhibition (%) ± SD
25	21.5 ± 1.2	26.6 ± 1.3
50	34.6 ± 1.7	37.9 ± 1.7
100	52.7 ± 2.0	56.4 ± 2.2
200	68.8 ± 2.3	73.5 ± 2.4
400	82.2 ± 2.6	87.8 ± 2.3

Values are expressed as mean ± standard deviation (n = 3)

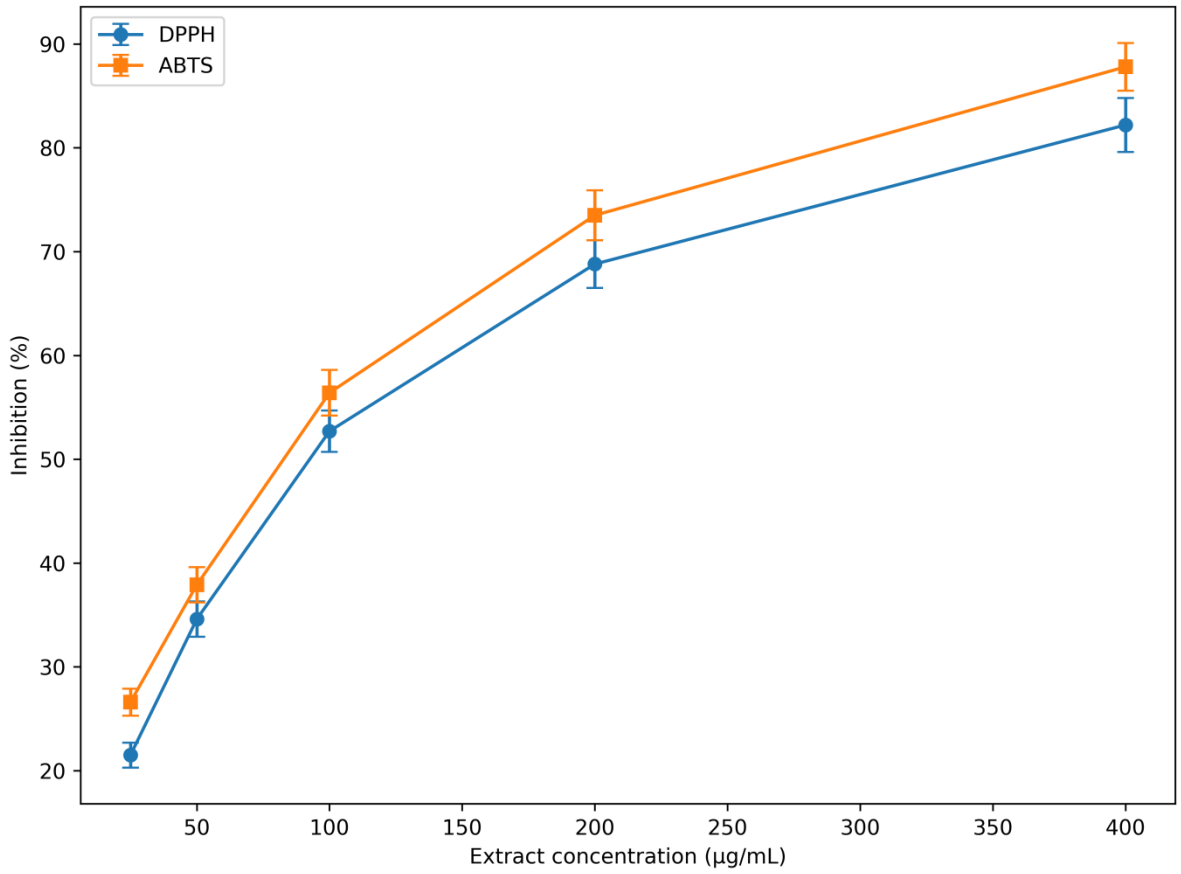

Figure 1. Dose–response curves of DPPH and ABTS radical scavenging activities of ginger extract

Total Phenolic Content (TPC)

Table (2) shows that the total phenolic content in the plant extract was 78.5 ± 2.7 mg GAE/g dry weight. This value indicates the efficiency of the extraction process, which falls within the published

range for alcoholic ginger extracts (65 and 90 mg GAE/g). This level of phenolic compounds is of great importance because of their major role in antioxidant activity, which is consistent with what Prakash et al., (2010) stated about the positive correlation between the total phenolic content and the ability to inhibit free radicals in plant extracts.

Accordingly, the total phenol content in ginger extract is a key factor in explaining its antioxidant efficacy, and supports the positive relationship between TPC and antioxidant activity in this study.

Table 2. Total phenolic content (TPC) of ginger root extract determined by the Folin–Ciocalteu method

Sample	Total phenolic content (mg GAE/g) ± SD
Ginger extract (ethanol 70%)	78.5 ± 2.7

The values represent the mean ± standard deviation (n = 3)

IC_{50} Values for DPPH and ABTS Assayss

The results in Table (3) indicate a clear ability of ginger root extract to inhibit free radicals. The IC_{50} value in the DPPH assays reached 93.6 ± 3.9 µg/mL, while the IC_{50} value in the ABTS assays was relatively lower at 79.2 ± 3.3 µg/mL. This lower IC_{50} value in the ABTS assays indicates a higher efficiency of ginger extract in inhibiting the $ABTS^{\bullet+}$ radical compared to the DPPH radical. This difference is attributed to the nature of the free radicals used and the different reaction mechanisms, which is consistent with what Wołosiak et al. (2021) noted, who confirmed that when evaluating plant extracts, the ABTS assays shows a higher sensitivity to antioxidant compounds compared to the DPPH assays. The results in Table (3) also confirm that ginger extract possesses quantifiable antioxidant activity.

Table 3. IC_{50} values of ginger root extract determined by DPPH and ABTS assays

Assay	IC_{50} (µg/mL)
DPPH	93.6 ± 3.9
ABTS	79.2 ± 3.3

IC_{50} values were calculated from dose–response curves using nonlinear regression analysis. Values are expressed as mean ± SD (n = 3)

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