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Antioxidant and antifungal activity of ginger (Zingiber officinale) methanolic extract

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Abstract

Ginger is a medicinal herb that contains bioactive components, and it is now widely used for its therapeutic properties. This study aims to demonstrate the correlation between the phytochemical constituents and antioxidant and antifungal activities of methanolic crude extracts obtained from ginger rhizomes. In this study, we estimated the total phenolic compounds (TPCs) and total flavonoids (TFs) present in the methanolic extract of ginger rhizomes. Antioxidant and antifungal activities against *F. solani* and *R. solani* were evaluated for this extract. The methanol extraction process of ginger rhizomes produced a yield of 8g/100g. The methanolic extract obtained from ginger rhizome flour had a total phenol (TPCs) content of 152.77 mg GAE/g dry extract. The extract also had a total flavonoid (TFCs) content of 10.14 mg QE/g dry extract. In DPPH assay, the methanolic extract of ginger rhizomes has been observed to have increased antioxidant activity with increasing concentrations. As the extract concentration increases from 20 to 640 µg/mL, the DPPH radical scavenging activity also increases from 22.23% to 85.5%. The results obtained using the FRAP method were identical to the DPPH assay. The methanolic extract of ginger rhizome was found to inhibit the growth of *F. solani* and *R. solani* mycelium in a concentration-dependent manner on Petri dishes containing solid agar medium. Our findings indicate that ginger could serve as a promising source of natural antioxidants and antifungal agents.

Keywords: Ginger rhizomes, DPPH, FRAP, Phenolic compounds, *R. solani*, *F. solani*.

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1. Introduction

Plants contain biologically active compounds known as phytochemicals. There is a growing interest in their potential health benefits and ability to prevent diseases [1]. Ginger is also a rich source of phytochemicals [2]. Ginger is a plant that belongs to the family *Zingiberaceae*. It is cultivated in many countries and sold in different parts of the world. The dried rhizomes of the ginger plant are used as a spice and flavoring agent. They are also believed to have several medicinal properties [3]. Ginger has been gaining popularity lately due to its potential antioxidant, antibacterial, antifungal, anti-inflammatory and antidiabetic properties [4,6].

Potential sources of antioxidants have been searched for in various plant materials such as fruits, vegetables, leaves, oilseeds, cereal crops, barks and roots, spices, herbs, and crude plant drugs [7]. Natural antioxidants are derived from plants and include vitamins, phenolic compounds, and flavonoids [8]. Recent research is centered on discovering natural sources of antioxidants. Consumers are more health-conscious, and synthetic antioxidants are now limited due to their carcinogenic properties [9]. There is a growing trend of searching for natural antioxidants, and spices are an excellent source of antioxidants, some of which outperform synthetic antioxidants and are safer from a health point of view [10]. Phenolic compounds are commonly present in both edible and non-edible plants. These compounds have been reported to have multiple biological effects such as antioxidant activity. The food industry is increasingly interested in crude extracts of fruits, herbs, vegetables, cereals, and other plant materials that are rich in phenolics. This is because they help to slow down the oxidative degradation of lipids, thereby increasing the nutritional value and quality of food. The importance of the antioxidant constituents of plant materials in maintaining good health and protecting against coronary heart disease and cancer is also gaining interest among scientists, food manufacturers, and consumers. As a result, the trend of the future is moving towards functional food with specific health effects [11].

Antimicrobial chemicals, including benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors, are frequently utilized in agriculture to control plant diseases. However, these chemicals face several issues in areas where fungi have developed resistance, which limits their effectiveness [12]. In order to address this issue, higher concentrations of the chemicals were used, but this increased the risk of toxic residues in the products, which could have adverse effects on both humans and the environment [13]. There has been growing interest in using plant extracts to control pests and diseases in agriculture, which are less harmful to human health and the environment [14]. This study aims to demonstrate the correlation between phytochemical constituents and the antioxidant and antifungal activities of methanolic crude extracts obtained from ginger rhizomes.

2. Materials and Methods

2.1. Plant materials

Rhizomes of ginger were purchased from the local market in Zagazig City (Egypt), dried, and ground into a fine powder.

2.2. Proximate analysis

The moisture, ash, fat, fiber, and protein content of ginger rhizomes were determined using AOAC methods [15]. To determine the amount of moisture in the sample, it was dried until it reached a constant weight at a temperature of 105 °C. The crude lipid content was extracted from the sample using a Soxhlet apparatus with petroleum ether. The method used to determine the protein content was micro-Kjeldahl. The carbohydrate content was calculated by subtracting ash, fat, fibre, and protein from the total dry weight to obtain the carbohydrate content in g/100g. Moisture content was presented as a percentage of fresh weight (g/100 g), while other contents were presented as a percentage of dry weight (g/100 g).

2.3. Methanolic extract preparation

Twenty grams of pulverized ginger rhizomes were extracted with methanol 80% (200 ml) for three hours at 25 °C ±3 °C using a magnetic stirrer. The solution was filtered through Whatman filter paper. No.1 Re-extraction of the remains occurred twice more under identical conditions. The methanol was filtered through a vacuum rotary evaporator (BüCHI-water bath-B-480), and the residual water was lyophilized using a freeze drier (Thermo-electron Corporation–Heto power dry LL 300). Refrigeration was used to preserve the extract for subsequent analysis [16].

2.3.1. Total phenolic compounds (TPCs) estimation

The total phenolic compounds (TPCs) were assessed using the Folin-Ciocalteu reagent method, as outlined by [17]. To perform the test, take 1 ml of the sample containing 1000 µg/mL and add it to a test tube with 5 ml of Folin-Ciocalteu reagent diluted with water 1:10 (V/V). Then, add 4 mL of sodium carbonate (75 g/L) to the same test tube. Mix the contents of the tube well for 15 seconds by vortexing, and let it stand for 30 min at a temperature of 40 °C for the color to develop. Finally, measure the absorbance of the sample at 765 nm using a spectrophotometer. A standard calibration curve was produced using Gallic acid in concentrations ranging from 0 to 200 µg/L. The total amount of phenolic compounds was expressed as milligrams of gallic acid equivalents (GAE) per 10 gm of extract.

2.3.2. Total flavonoids (TFs) estimation

Total flavonoids (TFs) were estimated according to the protocol of Ordonez et al. [18]. One mL of 1000 µg/mL extract was blended with two milliliters of 20 g/L $AlCl_3$ ethanol solution. After 60 min, the absorbance at 420 nm was measured. A standard calibration curve was produced using quercetin in concentrations ranging from 0 to 200 µg/L. The total amount of flavonoid was expressed as mg of quercetin equivalents (QE) per 10 gm of extract.

2.3.3. Antioxidant activity (DPPH-assay)

The antioxidant activity of ginger rhizomes methanolic extract was estimated by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay [19]. 2500 µl of a 0.1 mM DPPH solution dissolved in methanol was mixed with 500 µl of extract at various concentrations (20, 40, 80, 160, 320, and 640 µg extract/1ml methanol). Following a 30-minute incubation at 27 °C ± 3 °C, the absorbance at 517 nm was measured in conjunction with the control [20].

The antioxidant potential of DPPH radicals (%) was studied as follow:

$$Inhibition\ (\%) = [(Abs\ control - Abs\ sample)/Abs\ control]x\ 100$$

Where Abs. control is the absorbance of the control and Abs. sample is the absorbance in the presence of ginger rhizomes methanolic extract.

2.3.4. Ferric reducing antioxidant power (FRAP)

The reducing power of ginger rhizomes methanolic extract (20, 40, 80, 160, 320, and 640 µg extract/1mL methanol) was estimated by recording the absorption of Perl's Prussian blue complex resulting from the reduction of Fe^{3+} to Fe^{2+} at 700 nm as described by [21]. An increase in absorbance was considered as an indication of an increase in ferric reducing activity.

2.3.5. **In vitro** *antifungal bioassay*

The effect of ginger rhizomes methanolic extract at different concentrations (0, 100, 200, 400, and 800 µg/ml) was tested on the linear growth of *F. solani* and *R. solani* using a potato dextrose agar (PDA) medium. Plates were incubated at 25 °C in an incubator. Colony diameters were measured daily until the fungal growth covered the control Petri plates. The following equation was used to calculate linear growth.

$$\text{Linear growth reduction}(\%) = \frac{\text{Control growth} - \text{Treat. growth}}{\text{Control growth}} \text{ X } 100$$

2.3.6. Statistical analysis

The experimental design was factorial and utilized a completely randomized design (CRD). We performed an analysis of variance (ANOVA) on every single piece of data for a completely randomized design. The concentration differences were identified using Tukey's range test ($P \leq 0.05$).

3. Results and Discussion

3.1. Proximate analysis

The results of moisture content and proximate composition (fat, protein, ash, fiber, and carbohydrates) for ginger rhizome are presented in Table 1. The moisture content of the sample was 87.15%. The study finding align with previous research [22]. According to a previous study [23], the moisture content of fresh ginger rhizome is 80% which is significantly lower than our results. The carbohydrate content was the second major component of the samples. Dried ginger showed the highest total carbohydrates, which were 97.27%. The ether extract (fat) value of 0.53% compared to 0.55% reported by [24]. According to the literature, the fat content appears to be relatively high compared to the results obtained in this study [25]. The ash content, which reflects the mineral elements, was found to be 0.18%. The protein content was 0.99%. The literature shows relatively high protein content compared to these results [23]. The crude fiber content was 1.03%.

Table 1: Proximate analysis of fresh ginger rhizome

Parameter	Concentration (%)
Moisture*	87.15 ±0.54
Carbohydrate**	97.27 ±0.49
Ash**	0.18 ±0.0.004
Fat**	0.53 ±0.006
Crude fiber**	1.03 ±0.003
Protein**	0.99 ±0.009

* The moisture content was measured based on the weight of the fresh sample.
** The content was measured based on the dry weight.

3.2. Extract yield, TPCs, and TFs

Table 2 presents the total phenol content, total flavonoid, and extraction yield of ginger rhizomes methanolic extract. The methanol extraction process yielded 8 gm of extract from every 100 gm of ginger rhizomes. Polyphenolic compounds are known for their antioxidant activity, and the extracts' activity is likely due to these compounds [26].The resulting methanolic extract from ginger rhizome flour contained 152.77 mg GAE g^{-1} dry extract of TPCs and 10.14 mg QE g^{-1} dry extract of TFCs. [27] examined the antioxidant activity of methanol extracts from ginger and estimated its phenolic content, which was 6.3 mg GAE/g sample dry weight. The literature shows relatively low TPCs and TF content compared to these results [28].

Table 2: Yield of extracted substances (g), TPCs (GAE g-1 dry extract), and TFs (QE g-1 dry extract)

Parameters	**Concentration**
Extraction yield	8 g/ 100g sample
TPCs	152.77 mg GAE g^{-1} dry extract
TFs	10.14 mg QE g^{-1} dry extract

TPCs: total phenolic compounds; TFs: total flavonoids

3.3. Antioxidant activity

Figure 1 shows the percentage of inhibition of ginger rhizome methanolic extract's antioxidant activity using the DPPH assay. The methanolic extract of ginger rhizomes exhibits antioxidant activity. The methanolic extract of ginger rhizomes has been observed to have increased antioxidant activity with increasing concentrations. As the extract concentration increases from 20 to 640 µg/mL, the DPPH radical scavenging activity also increases from 22.23% to 85.5%. [29] found that the alcohol extract of ginger from Vietnam inhibited the DPPH radical up to 90.1%, agreeing with our results.

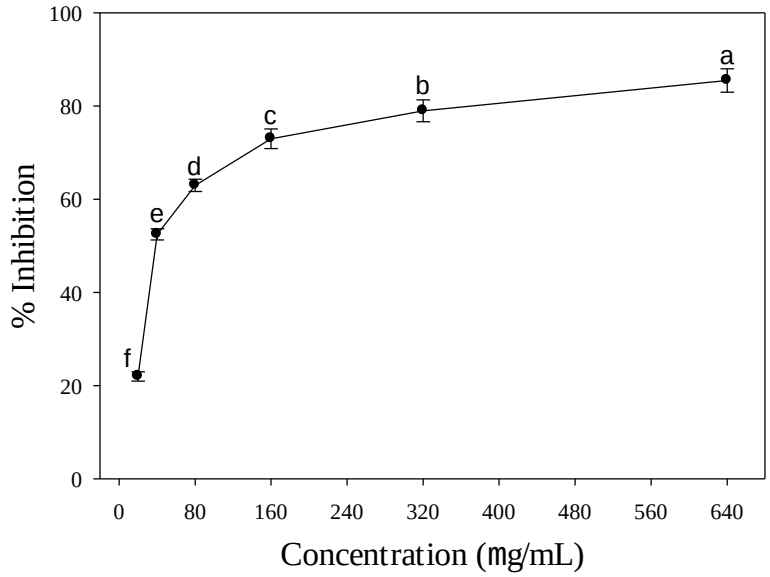

Figure 1: Antioxidant activity (% inhibition) of ginger rhizomes methanolic extract at different concentration (20, 40, 80, 16, 320, and 640 µg/mL). Bars with different letters are significantly different at $p < 0.05$. Data for each bar are expressed as the mean of three replicates ± SDs.

Different concentrations of ginger rhizome methanolic extract ranging from 20 to 640 µg/mL were used to measure the ferric reducing antioxidant power of the sample. Absorbance measurements were recorded at different concentrations of the sample. When the absorbance level goes up, it means that the ferric-reducing activity has increased. Figure 2 shows the results of ferric reducing antioxidant power (FRAP) of ginger rhizomes' methanol extracts. The results indicate that all concentrations tested had a significant effect in reducing the ferric ion in ac concentration-dependent manner. The lowest absorbance value of 0.432 was observed at 20 µg/mL, while the maximum value of 1.332 was observed at 640 µg/mL. The results presented here are in agreement with the findings reported by [30]. It can be observed that all samples exhibited similar trends in both the DPPH radical scavenging and ferric reducing power (FRAP) methods when comparing their antioxidant activity.

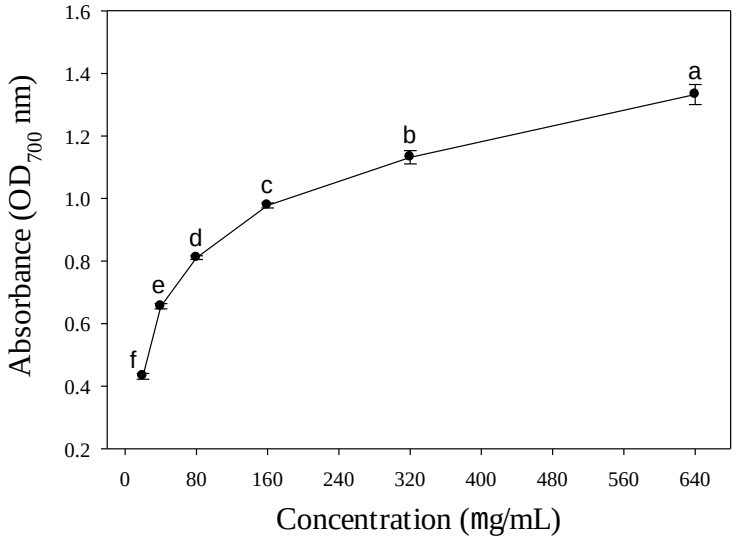

Figure 2: Ferric reducing antioxidant power (FRAP) of ginger rhizomes methanolic extract at different concentration (20, 40, 80, 16, 320, and 640 µg/mL). Bars with different letters are significantly different at $p < 0.05$. Data for each bar are expressed as the mean of three replicates ± SDs.

3.4. Antifungal activity

The methanolic extract of ginger rhizome was found to inhibit the growth of *F. solani* mycelium in a concentration-dependent manner on Petri dishes containing solid agar medium. This effect was observed after 7 days of incubation at 24°C, as shown in Figure 3. The highest reduction in growth of *F. solani* (85.55%) was observed at a concentration of 800 µg/mL.

Similarly, following 7 days of incubation at 24 °C, ginger rhizome methanolic extract suppressed *R. solani* mycelial growth in a concentration-dependent manner on solid agar medium Petri dishes (Figure 4). At 800 µg/mL, the greatest growth decrease of *R. solani* (83.33%) was noted. Recent studies have provided evidence supporting our findings [31,32]. The antifungal properties of ginger rhizomes methanolic extract are due to the presence of phenolic compounds such as gingerol, shagaol, and paradol [33].

4. Conclusions

The extract derived from ginger rhizomes has been found to possess inhibitory effects on fungal strains, making it a promising candidate for the development of new systemic and topical antifungal drugs. In addition, this extract also possesses natural antioxidant properties.

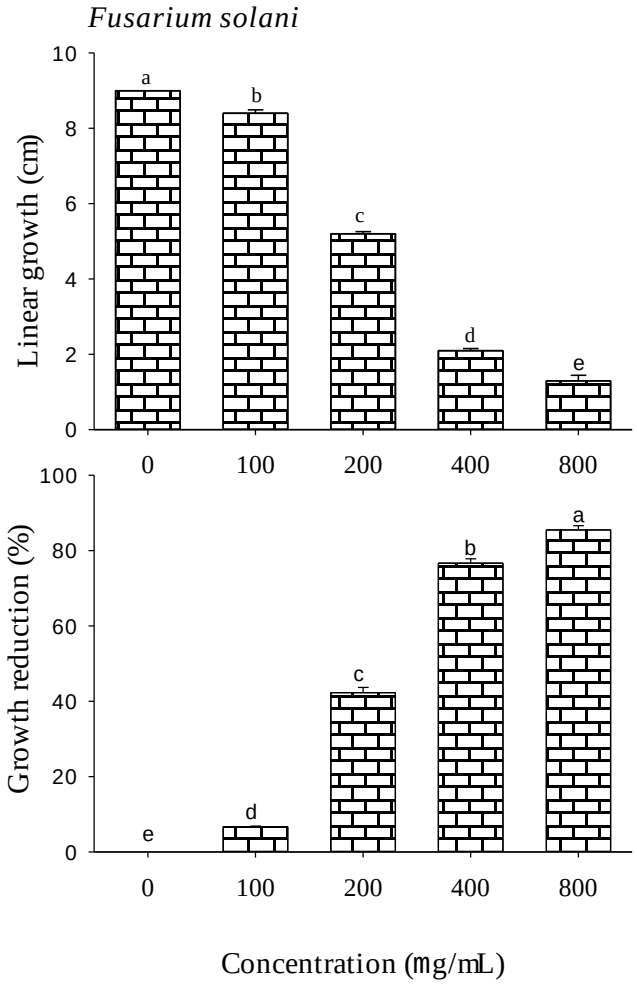

Figure 3: Antifungal activity of ginger methanolic extract against *F. solani* at different concentrations (100, 200, 400, and 200 µg/mL) compared to control (0 µg/mL). Bars with different letters are significantly different at $p < 0.05$. Data for each bar are expressed as the mean of three replicates ± SDs.

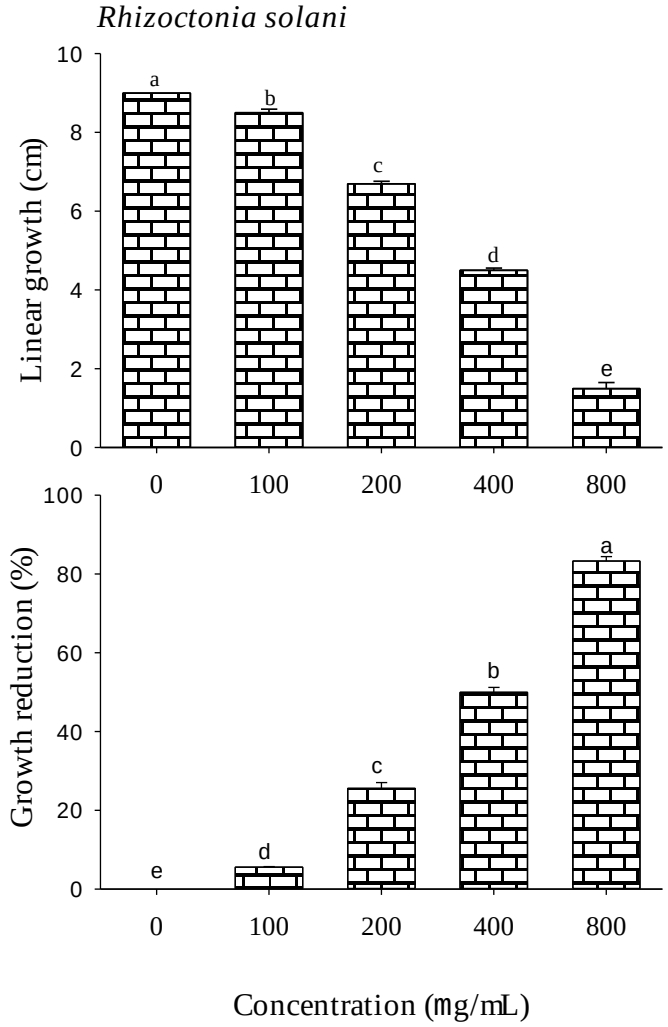

Figure 4: Antifungal activity of ginger methanolic extract against *R. solani* at different concentrations (100, 200, 400, and 200 µg/mL) compared to control (0 µg/mL). Bars with different letters are significantly different at $p < 0.05$. Data for each bar are expressed as the mean of three replicates ± SDs.

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