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Evaluation of the chemical characteristics and control of the growth of spoilage fungi causing rot in grape fruit using ginger essential oil (*Zingiber officinale*)

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ABSTRACT

The growth of fungal pathogens on the grape fruit causes a decrease in its quality and shelf life. In this study, the antifungal activity of ginger (*Zingiber officinale*) essential oil was investigated against fungal pathogens that cause spoilage in grape fruit. Ginger essential oil was extracted using hydrodistillation method and the content of total phenol, total flavonoid, antioxidant activity based on the inhibition of DPPH and ABTS free radicals and its antifungal activity against *Aspergillus niger*, *Rhizopus stolonifer* and *Botrytis cinerea* strains based on disc diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum fungicidal concentration were evaluated. The results showed that ginger essential oil has 89.80 mg GA/g total phenol and 38.60 mg QE/g total flavonoid. Its antioxidant activity against DPPH and ABTS free radicals was 73.45 and 66.53 µg/mL, respectively. The results of antifungal activity showed that *A. niger* and *B. cinerea* were the most sensitive and resistant fungal strains to essential oil, respectively, and the diameter of the inhibition zone in the disc diffusion and well diffusion agar methods, the minimum inhibitory concentration, and the minimum fungicidal concentration for the *A. niger* strain was equal to 13.90 mm, 14.50 mm, 8 mg/mL and 64 mg/mL, respectively. In general, ginger essential oil can be used as an antioxidant and antimicrobial agent to increase the shelf life of agricultural products.

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1- Introduction

Grapes are primarily grown for fresh consumption and are popular all over the world. Grapes are a rich source of diverse nutrients, including vitamins, minerals, and antioxidants. These fruits are particularly abundant in polyphenols, which are associated with various health benefits, including reduced inflammation and a lower risk of chronic diseases. In addition, grapes are rich sources of vitamin C, potassium, and fiber, making them a healthy addition to any diet. Grapes also offer potential health benefits due to the presence of specific compounds like resveratrol, which is linked to improved cardiovascular health and a reduced risk of certain cancers [1, 2]. Moreover, regular grape consumption has been associated with improved blood sugar control and reduced inflammation in individuals with type 2 diabetes [3].

Nevertheless, fungal post-harvest infection in grapes can pose a significant challenge, leading to quality deterioration, reduced shelf life, and economic losses for producers and suppliers. Fungi such as *Botrytis cinerea* and *Penicillium species* are common pathogens that can cause post-harvest decay of grapes. Post-harvest treatment with fungicides and other anti-fungal agents can effectively control fungal infections in grapes [4].

The employment of chemical fungicides for grape preservation can entail several drawbacks. Firstly, excessive or improper fungicide usage can lead to the emergence of fungicide-resistant fungal strains, hindering future control of fungal infections. Secondly, concerns exist regarding potential health hazards associated with chemical fungicide use, particularly with prolonged exposure. Moreover, the employment of chemical fungicides can exert adverse effects on the environment [5, 6]. Consequently, growing concerns regarding the use of chemical fungicides have emerged, necessitating alternative strategies such as the employment of natural fungicidal agents, biological control, or the development of resistant grape cultivars.

In this context, essential oils have been reported to be effective in inhibiting mold growth on grapes after harvest. Numerous studies have demonstrated the antifungal properties of plant essential oils against a wide range of mold species [7, 8]. Essential oils contain bioactive compounds such as carvacrol, thymol, and cinnamaldehyde, which can disrupt the cell wall of molds and inhibit their growth [9]. Indeed, the application of essential oils to grape surfaces significantly reduces mold growth and extends the shelf life of grapes [10]. Furthermore, the utilization of essential oils as natural preservatives presents itself as a safe and sustainable alternative to synthetic preservatives. The antimicrobial properties of essential oils offer a promising solution to mitigate mold growth on grapes, thereby enhancing their quality and safety for consumption.

Ginger (*Zingiber officinale*), a well-known spice, has been utilized for centuries due to its medicinal and culinary properties. Ginger essential oil has been demonstrated to exhibit antifungal activity against various fungal species [11, 12]. This activity is attributed to its high content of bioactive compounds, including zingiberene, curcumin, and sesquifellandrene [13]. These compounds are believed to disrupt the cell membrane of fungal cells, leading to their death [14]. Furthermore, ginger

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essential oil has been demonstrated to effectively inhibit mold growth on food products, including grapes and strawberries [15, 16]. Therefore, the objectives of this study were to extract ginger essential oil, determine the total phenolic and flavonoid contents, investigate its antioxidant activity, and finally evaluate its antifungal activity against fungal strains responsible for post-harvest grape spoilage.

2- Materials and Methods

2-1- Materials

The materials used in this study were Sabouraud dextrose agar (SDA) medium and broth (Merck, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, America), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma, America), gallic acid (Sigma, America), quercetin (Sigma, America), and Folin-Ciocalteu reagent (Sigma, America).

2-2- Essential Oil Extraction

The plant material was dried in a controlled-temperature (30°C) drying chamber with constant air circulation and optimal humidity conditions. The plant was then packed in sealed containers to preserve its flavor, color, and aroma. The laboratory sample was ground in a blender for 1 min at room temperature, homogenized, and stored in a dark place prior to analysis. Essential oil extraction was performed using the conventional steam distillation method in a Clevenger apparatus. For this purpose, 100 g of the ground plant material was weighed and 600 mL of double-distilled water was added. The heating temperature was set to 130°C, and the essential oil was extracted under these conditions for 3 h [17].

2-3- Total Phenol Content

The total phenolic content of the essential oil was estimated using the Folin-Ciocalteu method [18]. A volume of 125 µL of the sample was transferred to a test tube, and 500 µL of distilled water was added. Then, 125 µL of Folin-Ciocalteu reagent was added, and the mixture was incubated for 6 min. Then, 1.25 mL of 7% sodium carbonate and 1 mL of distilled water were added. The samples were incubated for 90 min, and the absorbance of the samples was recorded using a spectrophotometer at a wavelength of 760 nm. Gallic acid was used as the standard along with the samples. The total phenolic content was expressed as milligrams of gallic acid per gram of essential oil (mg GA/g).

2-4. Total flavonoid content

To measure the total flavonoid content, 0.1 mL of ginger essential oil were added to 0.3 mL of 5% $NaNO_2$ solution. The resulting solution was then mixed for 5 min and mixed with 0.3 mL of 10% $AlCl_3$ solution. After adding 2 mL of 1 M NaOH, the absorbance was read at a wavelength of 510 nm. Quercetin was used as the standard compound. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of essential oil (mg QE/g) [19].

2-5. Antioxidant activity

2-5-1. DPPH radical scavenging activity

The DPPH radical scavenging capacity was evaluated according to the method of Bellik et al. (2013). For this purpose, 2 mL of different concentrations of ginger essential oil were added to 0.4 mL of methanolic solution of DPPH radical (0.5 mM). The mixture was incubated in the dark for 30 min, and then the absorbance of the resulting solution was recorded at a wavelength of 517 nm. The DPPH radical scavenging activity was calculated as a percentage as follows:

[DOI: 10.22034/FSCT.21.154.151]

% Radical scavenging activity = (AB - AA) / AB × 100

where AB is the absorbance of the control reaction (containing all reagents except the tested compound) and AA is the absorbance of the tested compound. Finally, the antioxidant activity of the essential oil was reported as IC50 (µg/mL) [20].

2-5-2. ABTS radical scavenging activity

The method of Ivanović et al. (2021) with the necessary modifications was used to evaluate the essential oil's ABTS radical scavenging activity. The ABTS cationic radical solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution and incubating it in a dark place at room temperature for 12-16 hours. Before use, this solution was further diluted with absolute ethanol to an absorbance of 0.700 at a wavelength of 734 nm. Then, 3950 µL of the reaction solution were mixed with 50 µL of the essential oil, and after incubation in the dark at room temperature for 30 min, its absorbance was measured at 734 nm against the control solution. The inhibitory effect, expressed as a percentage, was calculated using the following equation:

% Radical scavenging activity = (AB - AA) / AB × 100

In this equation, AB represents the absorbance of the control and AA represents the absorbance of the sample. The antioxidant activity of the essential oil was reported as IC_{50} (µg/mL) [17].

2-6. Antifungal activity

The antifungal activity of ginger essential oil against the fungal strains *Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer* was evaluated using the methods of disc diffusion (agar disc diffusion), well diffusion (agar well diffusion), minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC).

2-6-1. Disc diffusion method

The method of Alizadeh Behbahani et al. (2017) was used to evaluate the antifungal activity of the essential oil. In this assay, sterile essential oil was gently added to blank discs and incubated at room temperature for 15 min. The blank discs were then placed on Sabouraud dextrose agar medium inoculated with fungal strains. Incubation was carried out at 27°C for 72 h, and then the diameter of the zone of inhibition around the discs was measured (in millimeters) and reported as the antifungal activity of ginger essential oil [21].

2-6-2. Well diffusion method

This assay was performed using a microbial suspension with a standard turbidity of 0.5 McFarland. Ginger essential oil was sterilized using a 0.22 µm syringe filter. Wells with a diameter of 6 mm were created on Sabouraud dextrose agar medium, and fungal strains and essential oil were added to the wells. The culture medium was incubated at 27°C for 72 h, and the antimicrobial effect was reported as the diameter of the inhibition zone around the wells (millimeters) [22].

2-6-3. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

To perform this assay, serial dilutions of ginger essential oil (4-512 µg/mL) were prepared in 10 mL test tubes, and then 20 µL of microbial suspension was added to each tube. Incubation was carried out at 27°C for 72 h, and the growth of the strains was visually assessed by the turbidity in the test tubes. The first test tube without turbidity (microbial growth) was recorded as the MIC of the essential oil. Then, surface culture from the turbidity-free tubes was used on Sabouraud dextrose agar medium to determine the MFC. Incubation

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was repeated under the same conditions, and the concentration of essential oil that prevented the growth of the fungal strains was determined as the MFC [23].

2- 7- Statistical analysis

All experiments were performed in triplicate. Data were analyzed using SPSS software (version 18) and one-way ANOVA. The difference in means was determined by Duncan's test at $p<0.05$.

3- Results and Discussion

Ginger essential oil, extracted from the rhizome of the Zingiber officinale plant, has been recognized for its diverse biological properties, including antimicrobial, antioxidant, and anti-inflammatory effects. Ginger essential oil contains various bioactive compounds, including phenols and flavonoids, which contribute to its therapeutic properties. The total phenolic and flavonoid content of ginger essential oil was 89.80 mg GA/mg and 38.60 mg QE/g, respectively (Figure 1). Phenolic and flavonoid compounds possess antioxidant activity and are capable of scavenging free radicals and protecting against oxidative stress.

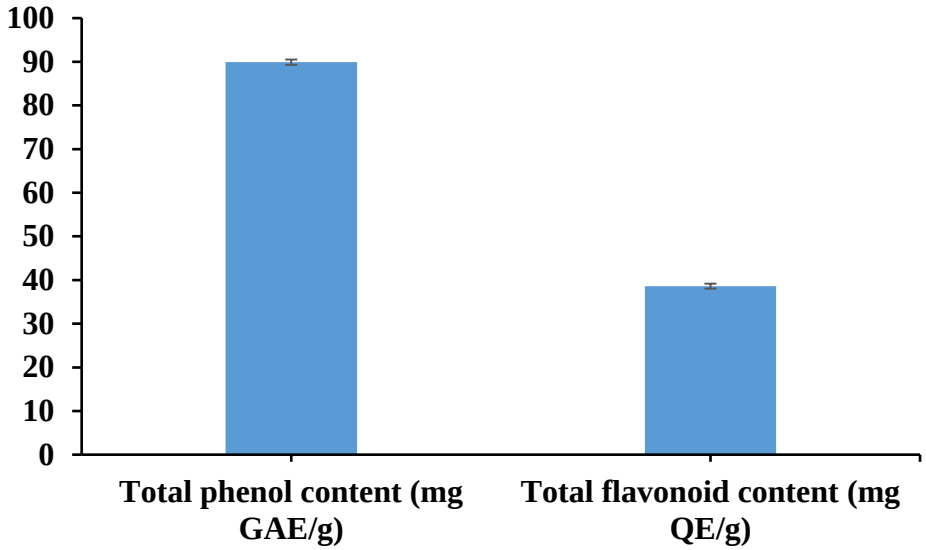

Figure 1. Total phenol and flavonoid contents of ginger essential oil.

The antioxidant activity of ginger essential oil is presented in Figure 2. Ginger essential oil exhibited free radical scavenging capacity use of DPPH and ABTS radicals of 73.45 and 66.53 µg/mL, respectively.

[DOI: 10.22034/FSCT.21.154.151]

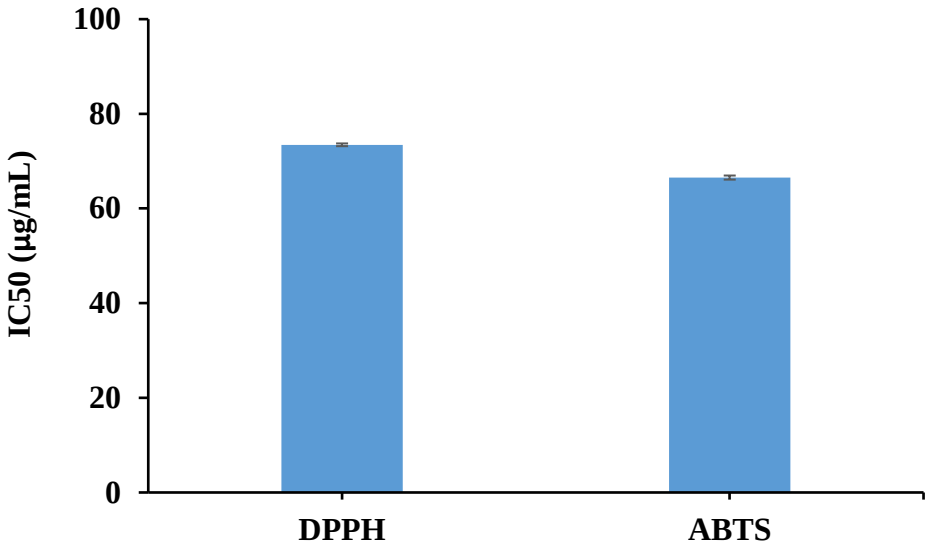

Figure 2. Antioxidant activity of ginger essential oil based on DPPH and ABTS radical scavenging methods.

The total phenolic and flavonoid content, as well as the antioxidant activity of ginger essential oil, can vary depending on the extraction conditions and plant source. Bellik et al. (2013) reported that ginger essential oil has a total phenolic content of 67.6 mg GA/g, and the DPPH radical scavenging effect significantly increased with increasing essential oil concentration [20]. Additionally, total phenolic content of ginger methanol and hexane extracts ranged from 10.6 to 95.2 mg GA/g, and concentration-dependent DPPH radical scavenging activity (10-90% within a concentration range of 40-240 µg/mL) was observed in ginger essential oil [18]. Ali et al. (2018) reported that the methanol-chloroform extract of ginger contained 40.25 mg QE/g total flavonoids and 60.34 mg GA/g total phenols, and the extract exhibited remarkable ability to scavenge DPPH and ABTS radicals [24]. The antioxidant activity of plant extracts containing polyphenolic components is well established due to their oxidation-reduction properties. These components act as hydrogen or electron donors, capable of scavenging free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [20, 25-27]. Antioxidants are compounds that can neutralize free radicals and protect the immune system from oxidative stress, which is associated with various diseases such as cancer, cardiovascular diseases, and neurodegenerative diseases [28, 29].

Essential oils exhibit antifungal properties against various fungal species, making them potential alternatives to conventional antifungal agents. The antifungal activity of essential oils is attributed to their ability to disrupt fungal membranes, inhibit fungal enzymes, and interfere with fungal cell replication [23, 30]. The antifungal activity of ginger essential oil against *Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer* strains was evaluated using the disc diffusion method and the results are presented in Figure 3. The zone of inhibition diameters ranged from 10.40 to 13.90 mm. *Aspergillus niger* was the most susceptible strain with a zone of no growth of 13.90 mm, while *Botrytis cinerea* was the most resistant strain with a zone of inhibition of 10.40 mm.

[DOI: 10.22034/FSCT.21.154.151]

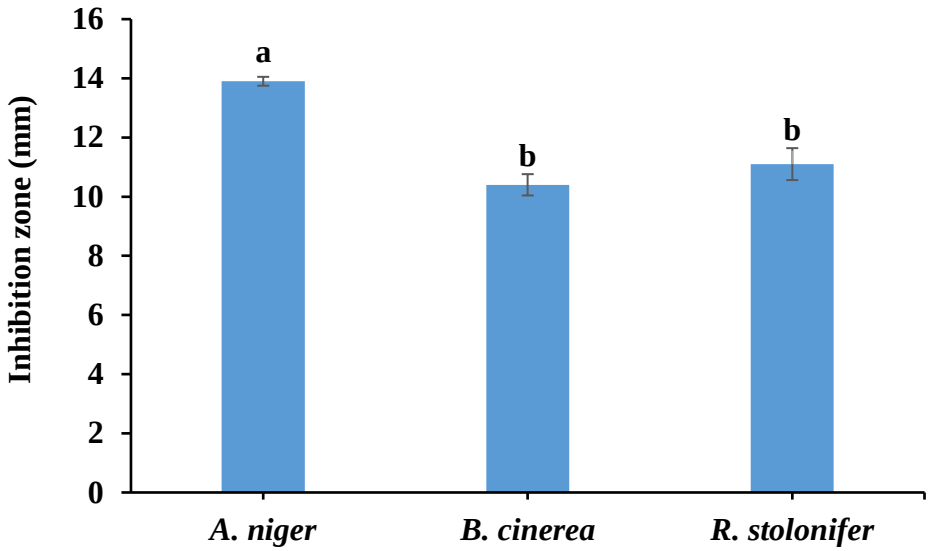

Figure 3. Antifungal activity of ginger essential oil based on disc diffusion agar method. Means with different superscripts are significantly different ($p<0.05$).

Figure 4 shows the results of the antimicrobial activity of ginger essential oil using the well diffusion method. The zones of no growth for the fungal strains ranged from 11.50 to 14.50 mm. *Aspergillus niger* was the most susceptible fungal strain to ginger essential oil, with a zone of no growth of 14.50 mm. *Botrytis cinerea* was the most resistant strain, with a zone of no growth of 11.50 mm. It is noteworthy that the average zone of no growth diameter was larger in the agar well diffusion method compared to the agar disk diffusion method. This is attributed to the direct contact between the essential oil and the fungus in the agar well diffusion method, whereas the antimicrobial activity of the bioactive compound in the agar disk diffusion method is due to the diffusion of the antimicrobial agent from the disk into the culture medium [26, 31, 32].

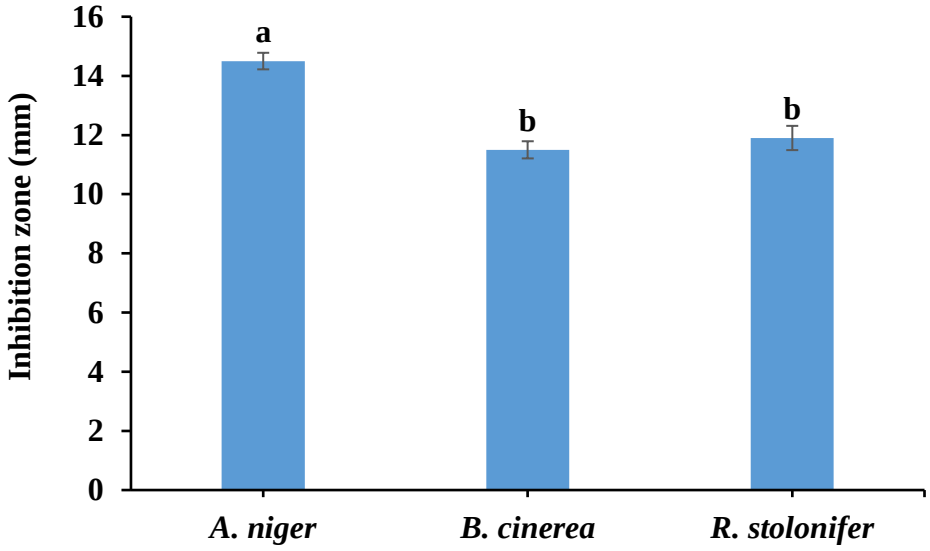

Figure 4. Antifungal activity of ginger essential oil based on well diffusion agar method. Means with different superscripts are significantly different ($p<0.05$).

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The results of the minimum inhibitory concentration (MIC) assay for the essential oil against the fungal strains are presented in Table 1. The essential oil at a concentration of 4 mg/mL did not exhibit any antimicrobial activity against any of the strains. The *Botrytis cinerea* and *Rhizopus stolonifer* strains were able to grow in the presence of essential oil concentrations of 8 and 16 mg/mL, respectively, but the *Aspergillus niger* strain was sensitive to essential oil concentrations above 4 mg/mL. It is worth noting that no microbial growth was observed at essential oil concentrations of 32-512 mg/mL. Overall, *Aspergillus niger* was the most susceptible fungal strain to the essential oil. The minimum inhibitory concentrations (MICs) for *Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer* were 8, 32, and 32 mg/mL, respectively.

Table 1. Antifungal activity of ginger essential oil based minimum inhibitory concentration (MIC) method.

Fungi	**Concentration (mg/mL)**								
	4	8	16	32	64	128	256	512	Control
A. niger	+	-	-	-	-	-	-	-	-
B. cinerea	+	+	+	-	-	-	-	-	-
R. stolonifer	+	+	+	-	-	-	-	-	-

-: inactive, +: active

Table 2 presents the results of the minimum fungicidal concentration (MFC) assay for the essential oil against the fungal strains. Essential oil concentrations of 4-32 mg/mL did not exhibit any fungicidal activity against any of the fungal strains. Higher concentrations of the essential oil showed fungicidal activity against the *Aspergillus niger* strain, while the *Rhizopus stolonifer* strain was resistant to an essential oil concentration of 128 mg/mL. Among the fungal strains studied, *Botrytis cinerea* exhibited the highest resistance to the essential oil, with a minimum fungicidal concentration (MFC) of 512 mg/mL. Overall, the MFCs for *Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer* were 64, 512, and 256 mg/mL, respectively.

Table 2. Antifungal activity of ginger essential oil based minimum fungicidal concentration (MFC) method.

Fungi	**Concentration (mg/mL)**								
	4	8	16	32	64	128	256	512	Control
A. niger	+	+	+	+	-	-	-	-	-
B. cinerea	+	+	+	+	+	+	+	-	-
R. stolonifer	+	+	+	+	+	+	-	-	-

-: inactive, +: active

The antifungal activity of ginger essential oil and extract has been reported in various studies. Consistent with the findings of this study, the antifungal activity of ginger essential oil against *Aspergillus niger* and *Rhizopus stolonifer* strains was also reported by El-Baroty et al. (2010) [33].

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Furthermore, Abdullahi et al. (2020) reported that ginger essential oil exhibited antimicrobial activity against plant pathogens *Fusarium oxysporum*, *Pyricularia oryzae*, *Colletotrichum falcatum*, *Ganoderma boninense*, and *Rigidoporus microporus*, with the effect being concentration-dependent [34]. The antibacterial activity of ginger essential oil against pathogenic bacteria was reported in the study by Mesomo et al. (2013), and this effect was attributed to the presence of phenolic compounds in the oil [35]. Rahmati Joneidabad et al. (2021) investigated the antifungal activity of Khuzestani savory essential oil against fungal strains causing strawberry spoilage and mold (*Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer*) and reported that the antifungal properties of the essential oil are attributed to the presence of bioactive compounds such as ketones, aldehydes, and phenols. These compounds, having an aromatic nucleus and a phenolic hydroxyl group, can form hydrogen bonds with sulfhydryl groups in the active site of fungal enzymes, thereby inactivating them. The researchers also reported that the low molecular weight and lipophilic nature of the essential oil allow it to be easily absorbed by fungal mycelium and inhibit fungal cell growth [23]. The results of the present study were consistent with other studies conducted on the antimicrobial effect of essential oils and plant extracts [45-36]. Based on the findings presented above, ginger essential oil can be effectively employed to control the growth of fungal strains responsible for grape spoilage.

4- Conclusion

The findings of this study demonstrated that ginger essential oil possesses a significant content of phenols and flavonoids, which contribute to its antioxidant and antimicrobial activities. Ginger essential oil was capable of inhibiting the growth of post-harvest grape spoilage-causing fungal pathogens (*Aspergillus niger*, *Rhizopus stolonifer*, and *Botrytis cinerea*). The overall findings of this study demonstrate that ginger essential oil can be employed as a novel antimicrobial agent to suppress the growth of plant fungal pathogens and serve as a potential alternative to synthetic fungicides for sustainable agricultural production.

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مقاله علمی–پژوهشی

ارزیابی ویژگی‌های شیمیایی و کنترل رشد قارچ‌های عامل پوسیدگی پس از برداشت میوه انگور با استفاده از اسانس زنجبیل (*Zingiber officinale*)

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چکیده

رشد پاتوژن های قارچی روی میوه انگور سبب افت کیفیت و کاهش ماندگاری آن می‌گردد. در این مطالعه، فعالیت ضد قارچی اسانس زنجبیل در برابر پاتوژن‌های قارچی عامل فساد پس از برداشت میوه انگور مورد بررسی قرار گرفت. اسانس زنجبیل با استفاده از روش تقطیر با آب استخراج گردید و محتوای فنول کل، فلاونوئید کل، فعالیت آنتی‌اکسیدانی بر پایه مهار رادیکال‌های آزاد DPPH و ABTS و فعالیت ضد قارچی آن در برابر سویه‌های *آسپرژیلوس نایجر*، *ریزوپوس استولونیفر* و *بوتریتیس سینه‌را* بر اساس روش‌های انتشار در دیسک، انتشار در چاهک، حداقل غلظت مهارکنندگی رشد و حداقل غلظت کشندگی مورد ارزیابی قرار گرفت. نتایج نشان داد که اسانس زنجبیل دارای ۸۹/۸۰ mg GA/g فنول کل و ۳۸/٦۰ mg QE/g فلاونوئید کل می‌باشد. فعالیت آنتی‌اکسیدانی آن در برابر رادیکال‌های آزاد DPPH و ABTS به ترتیب برابر با ۷۳/٤۵ و ٦٦/۵۳ میکروگرم در میلی‌لیتر بود. نتایج فعالیت ضد قارچی نشان داد که *آسپرژیلوس نایجر* و *بوتریتیس سینه‌را* به ترتیب حساس‌ترین و مقاوم‌ترین سویه‌های قارچی نسبت به اسانس بودند و قطر هاله عدم رشد در روش انتشار در دیسک و انتشار در چاهک، حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی برای سویه *آسپرژیلوس نایجر* به ترتیب برابر با ۱۳/۹۰ میلی‌متر، ۱٤/۵۰ میلی‌متر، ۸ میلی‌گرم در میلی‌لیتر و ٦٤ میلی‌گرم در میلی‌لیتر بود. بطور کلی، اسانس زنجبیل قابلیت استفاده بعنوان عامل آنتی‌اکسیدان و ضد میکروب جهت افزایش عمر نگهداری محصولات کشاورزی را دارا می‌باشد.

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