Turkish Journal of Agriculture - Food Science and Technology, 13(12): 4280-4285, 2025
DOI: https://doi.org/10.24925/turjaf.v13i12.4280-4285.8540

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Antifungal Activity of *Rosa damascena* Leaf Extracts Against *Botrytis cineria* and *Alternaria alternata*

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ARTICLE INFO

Research Article

Received : 18.11.2025
Accepted : 08.12.2025

Keywords:
Alternaria alternate
Antifungal activity
Botrytis cineria
R. damascena extract
In vitro

ABSTRACT

This study was conducted to determine the *in vitro* antifungal activities of *Rosa damascena* ethanol, methanol, and hexane extracts against important plant pathogens, *Botrytis cinerea* and *Alternaria alternata*. In the study, the rates of inhibition of fungal mycelial growth by the extracts were determined on PDA medium at concentrations of 500, 1000, and 2000 µg/ml. In addition, to quantitatively evaluate antifungal activity, Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values (ranging from 125 to 4000 µg/ml using two-fold serial dilutions) were determined to assess the fungicidal effects of the extracts. The highest antifungal activity against both fungal phytopathogens was obtained from the hexane extract (*B. cinerea* 78.5% ± 3.0, *A. alternata* 84.1% ± 1.3). The MIC and MFC values of the extracts were determined to be 225–358 µg/ml and 2517–3960 µg/ml for *B. cinerea*, and 183–308 µg/ml and 2130–3917 µg/ml for *A. alternata*, respectively. These findings indicate that, particularly, the hexane extract may possess strong antifungal potential against economically important phytopathogens such as *B. cinerea* and *A. alternata*.

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Introduction

Botanical extracts and natural compounds are gaining increasing importance among environmentally friendly and sustainable strategies for controlling agricultural pathogens. The widespread use of synthetic fungicides has led to significant problems, including environmental pollution, toxic residues, and the development of resistance in target pathogens (Bhardwaj & Sharma, 2019). Therefore, the development of plant-derived antimicrobial agents has become a priority research area in terms of both environmental sustainability and food safety (González & Escobar, 2019). *R. damascena* Mill., a plant species particularly rich in essential oils, flavonoids, tannins, and phenolic compounds, has long been used in traditional medicine, cosmetics, and the food industry (Majedi, Khorasani & Abedi, 2024). Owing to this diverse phytochemical profile, numerous studies have demonstrated its antimicrobial, antioxidant, and anti-inflammatory properties (Bari, Rahman & Uddin, 2024). In particular, flower extracts distinct from the essential oil have been reported to exhibit potential antifungal activity against various pathogens (Erken & Gül, 2021).

Among phytopathogenic fungi, *B. cinerea* and *A. alternata* are responsible for plant diseases affecting economically important crops. *B. cinerea* is an airborne plant pathogen that causes grey mold in more than 200 crops worldwide (Abbey et al., 2019). Botrydial and botcinic acid both sesquiterpenes are the two major phytotoxins involved in infections caused by this fungus (Dalmais et al., 2011). This pathogen can infect plant products from the seedling stage through fruit maturation. Even harvested products may deteriorate because the fungus can survive under extreme conditions in both the field and storage facilities in the form of sclerotia and dormant mycelium. Therefore, it is considered a high-risk pathogen and has been classified as the second most important plant pathogen by Dean et al. (2012). On the other hand, *A. alternata* affects a wide range of crops worldwide (such as potato, apple, and tomato) and is responsible for various postharvest diseases during storage (Troncoso-Rojas et al., 2014). The major mycotoxins that have been identified in infected and decayed products include alternariol, altenuene, and altertoxins (Fernández Pinto & Patriarca, 2017). These phytopathogens cause severe yield losses in grapes, tomatoes, strawberries, and

many other crops of economic importance (Yıldız & Karakaya, 2019). In the search for environmentally friendly solutions for pest control and environmental management, plants and their chemical biodiversity have gained increasing attention as sources of biopesticides for integrated pest management (Souto et al., 2021; Schnarr et al., 2022). Previous studies have reported the biological activity of various plant extracts against *B. cinerea* (Cosoveanu et al., 2013; Abbey et al., 2019; Ok & Şanlı, 2025) and *A. alternata* (Cosoveanu et al., 2013; Şanlı & Ok, 2023). Although conventional fungicide applications remain widespread against these pathogens, plant-based alternative solutions are being developed for environmental and economic reasons (González-Coloma et al., 2022). Studies on the antifungal effects of *R. damascena* have shown that its essential oil exhibits significant inhibitory activity against pathogens such as *Fusarium graminearum* (Rathore et al., 2021). Furthermore, rose oil and rose water have been reported to possess potential antifungal activity against phytopathogenic fungi such as *B. cinerea* and *A. alternata* (Erken & Gül, 2021). However, the number of studies specifically investigating the effects of *R. damascena* flower extracts on these two pathogens remains limited in the literature.

In this study, it was aimed to determine the *in vitro* antifungal effects of ethanol, methanol, and hexane extracts of *R. damascena* against *B. cinerea* and *A. alternata*, and to evaluate the potential of rose petal extracts as natural antifungal agents for controlling these phytopathogens.

Materials and Methods

Preparation of Extracts

The study was conducted in the laboratories of the Faculty of Agriculture at Isparta University of Applied Sciences in 2024. Ethanol, methanol, and hexane extracts of *R. damascena* flower were used as experimental materials. *R. damascena* flowers, collected during the rose processing season (May and June) in the early morning hours, were dried in the shade and ground prior to extraction. Ground flower samples (100 g) were extracted in a Soxhlet apparatus using ethanol, methanol, and hexane separately at 60 °C for 4 hours each. The obtained extracts were filtered through Whatman No. 1 filter paper, after which the solvents were removed using a rotary evaporator at 60 °C (Pizzale et al., 2002; Tian et al., 2011). The resulting extracts were stored at –20 °C for use in determining their antifungal activities (Tian et al., 2011).

Determination of Antifungal Activity

The antifungal activities of the extracts against the phytopathogens *B. cinerea* and *A. alternata* were determined *in vitro* at concentrations of 500, 1000, and 2000 µg/mL. The fungal plant pathogens used in the study were obtained from stock cultures maintained in the Phytopathology Laboratory of the Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences. The *in vitro* experiments were designed with three replications, each consisting of four petri dishes. Fungal cultures were initially grown on 90 mm petri dishes containing 20 mL of potato dextrose agar (PDA) at 24±2°C for 7 days before use. PDA medium was dispensed into erlenmeyer flasks, autoclaved at 121 °C for 15 minutes, and cooled to 40 °C. The extracts were prepared at final concentrations of 500, 1000, and 2000 µg/mL using dissolved in dimethylsulfoxide (DMSO) and added to 90 mL of PDA. The PDA–extract mixtures were shaken until homogenized and then poured into sterile 60 mm petri dishes at 10 mL per plate. A 5 mm mycelial plug of each pathogen was placed at the center of the petri dish, and the plates were incubated at 24±2 °C for 7 days (Kordali et al., 2016). The antifungal effects of the extracts were determined by measuring the mycelial growth diameter of the fungi and calculating the inhibition rate using the following equation (Deans & Soboda, 1990):

$$MGI(\%) = \frac{(MDCC - MDTC)}{MDTC} \times 100$$

MDCC: Mycelial diameter of control colony
MDTC: Mycelial diameter of treatment colony

Sterile deionized water was used as the control. The MGI values were fitted to a cubic regression model, and the effective concentration (EC_{50}) values defined as the concentration of extract required to inhibit 50% of mycelial growth were determined (Chen et al., 2023).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC values of the extracts against both phytopathogens were determined using the microtiter broth dilution technique described by Chen et al. (2023). Two-fold serial dilutions of each extract, ranging from 125 to 4000 µg/mL, were prepared in 10% DMSO, and 100 µL of each extract solution was pipetted into the first row of a 96-well microtiter plate. All other wells were filled with 50 µL of PDA broth containing DMSO. Serial dilutions were performed by transferring 50 µL from one well to the next to obtain decreasing concentrations of the test material. Subsequently, 50 µL of fungal suspension (5.0×10^6 cfu/mL) was added to each well. Each column of wells thus represented a series of dilutions inoculated with a single extract and a single fungal isolate. The plates were sealed with transparent film and incubated at 24±2 °C for 24–36 hours. After incubation, 15 µL of 2,3,5-triphenyl tetrazolium chloride (TTC, 0.18%) solution was added to each well, followed by incubation at 37 °C for 1 hour. The MIC value was recorded as the lowest concentration of the extract that showed no color change (clear well), indicating complete inhibition of visible fungal growth. The minimum fungicidal concentration (MFC) was determined by subculturing 10 µL of the contents from wells at or above the MIC value onto PDA plates. After 24 hours of incubation, plates were examined for fungal growth. The lowest concentration of extract showing no visible growth on the agar surface was considered the MFC (Akinduti et al., 2019).

Statistical Analysis

All the tests were carried out in triplicate. Data were subjected to analysis of variance by SAS (2009) statistical program and means were compared by Duncan's test at $P< 0.05$ significance level.

Results and Discussion

The antifungal activities of ethanol, methanol, and hexane extracts obtained from *R. damascena* petals against *B. cinerea* and *A. alternata* were examined at different doses, and the results are summarized in Table 1. The findings indicate that antifungal activity varied significantly depending on the type of extract and the applied dose. The ethanol extract exhibited biocontrol activity ranging from 16.7% to 54.2% against *B. cinerea* and from 29.1% to 48.1% against *A. alternata*. With increasing dose, the effectiveness against both pathogens increased markedly; notably, at 2000 µg/mL, the ethanol extract achieved 54.2% mycelial growth inhibition for *B. cinerea* and 48.1% for *A. alternata*. The methanol extract demonstrated higher antifungal activity than the ethanol extract against both pathogens. As the dose increased, inhibition rates rose from 28.2% to 68.8% for *B. cinerea* and from 35.1% to 63.1% for *A. alternata*. Among the tested extracts, the hexane extract exhibited the highest antifungal activity against both phytopathogens. Biocontrol effectiveness ranged from 30.9% to 78.5% for *B. cinerea* and from 38.7% to 84.1% for *A. alternata*. In particular, at 2000 µg/mL, the hexane extract showed strong antifungal activity against *A. alternata* (Table 1).

The antifungal effects of different solvent extracts of *R. damascena* against *B. cinerea* and *A. alternata* were evaluated based on their EC_{50}, MIC, and MFC values, and the results are presented in Table 2. For both phytopathogens, the lowest EC_{50}, MIC, and MFC values were obtained from the hexane extract. For *B. cinerea*, the lowest EC_{50} value was determined for the hexane extract (EC_{50}: 677 ± 20.5 µg/mL), followed by the methanol extract (EC_{50}: 808 ± 11.8 µg/mL). The EC_{50} value of the ethanol extract was considerably higher than those of the other extracts (EC_{50}: 1708 ± 31.2 µg/mL). The hexane extract also exhibited the lowest MIC (225 ± 20.4 µg/mL) and MFC (2517 ± 23.6 µg/mL) values. The methanol extract showed lower MIC (312 ± 30.6 µg/mL) and MFC (2933 ± 94.3 µg/mL) values compared to the ethanol extract (MIC: 358 ± 11.8 µg/mL; MFC: 3950 ± 70.7 µg/mL) (Table 2). Similarly, for *A. alternata*, the hexane extract was identified as the most effective extract. It exhibited both the lowest EC_{50} (758 ± 31.2 µg/mL) and MIC (183 ± 31.2 µg/mL) values. The MFC value was also lower than those obtained from the other extracts (2130 ± 94.3 µg/mL). The methanol extract was more effective than the ethanol extract, with lower EC_{50} (657 ± 9.4 µg/mL) and MIC (267 ± 23.6 µg/mL) values. The ethanol extract showed the highest EC_{50} (1767 ± 82.5 µg/mL) and MFC (3917 ± 117.9 µg/mL) values against *A. alternata* (Table 2).

The biocontrol potential of *R. damascena* ethanol, methanol, and hexane extracts against the phytopathogens *B. cinerea* and *A. alternata* was evaluated based on both inhibition rates (%) and EC_{50}, MIC, and MFC values. The antimicrobial activity of plants is primarily determined by their capacity to inhibit or eliminate microbial growth. As part of their defense mechanisms against microbial infections, plants synthesize a wide array of chemical compounds. These bioactive compounds, including alkaloids, flavonoids, tannins, essential oils, and other secondary metabolites, exhibit antimicrobial properties, making them potential sources for the development of novel antimicrobial agents or drugs (Vaou et al., 2021; Alibi et al., 2021). Extracts obtained from the petals and sepals of *R. damascena* contain a large number of active compounds, reportedly over 300 different types, with the most abundant and effective components being stearopten (20–25%), citronellol (40–60%), geraniol (30–40%), and linalool (20–30%) (Zarghami et al., 2001). Other important bioactive compounds isolated from *R. damascena* include glycosides, anthocyanins, flavonoids, and terpenes. Additionally, *R. damascena* extracts have been reported to contain quercetin, carboxylic acids, camphor, and myrcene (Jager et al., 2007; Katayoun & Mehdi, 2015). In particular, phenolic compounds such as kaempferol and quercetin have been shown to exhibit potential antimicrobial properties, highlighting *R. damascena* as a promising candidate for natural antimicrobial agents, as supported by several studies (Chroho et al., 2022; Piotrowicz et al., 2021; Trendafilova et al., 2023).

Table 1. Biocontrol Activities (%) of *R. damascena* Ethanol, Methanol, and Hexane Extracts Against *B. cinerea* and *A. Alternata*

Extracts	Doses (µg/ml)	B. cineria	A. alternata
Ethanol	500	$16.7^{g} \pm 0.9$	$29.1^{f} \pm 1.1$
	1000	$39.3^{e} \pm 1.7$	$46.4^{d} \pm 0.7$
	2000	$54.2^{d} \pm 2.9$	$48.1^{d} \pm 2.0$
Methanol	500	$28.2^{f} \pm 1.6$	$35.1^{ef} \pm 1.2$
	1000	$63.0^{c} \pm 1.7$	$60.2^{c} \pm 2.8$
	2000	$68.8^{b} \pm 1.1$	$63.1^{c} \pm 2.6$
Hexane	500	$30.9^{f} \pm 1.0$	$38.7^{e} \pm 2.3$
	1000	$75.2^{a} \pm 0.7$	$71.3^{b} \pm 2.0$
	2000	$78.5^{a} \pm 3.0$	$84.1^{a} \pm 1.3$

Table 2. EC_{50}, Minimum Inhibitory Concentrations (MICs), and Minimum Fungicidal Concentrations (MFCs) of Different *R. damascena* Petal Extracts Against *B. cinerea* and *A. Alternata*

Extracts	B. cineria			A. alternata		
	EC_{50}	MIC	MFC	EC_{50}	MIC	MFC
Ethanol	$1708^{a} \pm 31.2$	$358^{a} \pm 11.8$	$3950^{a} \pm 70.7$	$1667^{a} \pm 82.5$	$308^{a} \pm 31.2$	$3917^{a} \pm 117.9$
Methanol	$808^{b} \pm 11.8$	$312^{a} \pm 30.6$	$2933^{b} \pm 94.3$	$657^{c} \pm 9.4$	$267^{a} \pm 23.6$	$2620^{b} \pm 102.7$
Hexan	$677^{c} \pm 20.5$	$225^{b} \pm 20.4$	$2517^{c} \pm 23.6$	$758^{b} \pm 31.2$	$183^{b} \pm 31.2$	$2130^{c} \pm 94.3$

The highest biocontrol activity against *B. cinerea* and *A. alternata* was observed with the hexane extract. At a concentration of 1000 µg/mL, the hexane extract exhibited 75.2% inhibitory effect on *B. cinerea* and 71.3% on *A. alternata*, reaching a strong inhibition of 84.1% for *A. alternata* at 2000 µg/mL. This extract also showed the lowest EC_{50}, MIC, and MFC values among the tested extracts. These results indicate that the hexane extraction process enriches lipophilic compounds such as terpenes, aliphatic hydrocarbons, and phenolics in rose leaves, which exert strong fungistatic and fungicidal effects on *B. cinerea* and *A. alternata*. The type and polarity of the solvent can affect extract quality, yield, extraction rate, inhibitory compounds, toxicity, and other biological activities (Eloff, 1998; Zhang et al., 2019). Rafińska et al. (2019) also reported that the total secondary metabolites and their antioxidant capacities largely depend on the solvent used for extraction. Similarly, Wakeel et al. (2019) demonstrated that extracts obtained from the same plant using different solvents have distinct chemical compositions. In agreement with our findings, Shohayeb et al. (2014) reported that *R. damascena* essential oil and extracts obtained with water, hexane, and ethanol exhibited antifungal and antibacterial activity, with MIC and MBC values ranging from 0.125–2.0 mg/mL and 0.4–0.5 mg/mL, respectively, indicating moderate antimicrobial activity. Similarly, Özsoy et al. (2025) determined the EC_{50} of *R. damascena* essential oil against *Fusarium graminearum* as 604 µg/mL. Numerous studies have also shown that some plant essential oils and extracts exert fungicidal effects against *B. cinerea* and *A. alternata* by disrupting pathogen membrane integrity (Wang et al., 2010; Hou et al., 2020; Chen et al., 2025). Furthermore, antifungal studies with *R. damascena* essential oil and different extracts have identified specific components with strong antifungal activity, which damage the lipid structures of pathogen membranes (Halawani, 2014; Antoniadou et al., 2024; Özsoy et al., 2025). The methanol extract also exhibited considerable antifungal activity against both phytopathogens, achieving 68.8% inhibition of *B. cinerea* and 63.1% inhibition of *A. alternata* at 2000 µg/mL. The methanol extract exhibited moderate-to-high EC_{50} values (808 µg/mL for *B. cinerea* and 657 µg/mL for *A. alternata*) and MIC values. This indicates that methanol, similar to hexane, effectively extracts semi-polar compounds such as flavonoids, phenolic acids, and tannins. Tsai et al. (2008) reported that the methanolic extract of *R. damascena* showed MIC values above 8 mg/mL against *S. mutans*, *S. sobrinus*, and *S. sanguis*. Halawani (2014), in a study evaluating the antibacterial and antifungal activities of ethanol, methanol, hexane, and aqueous extracts of *R. damascena*, reported MIC values ranging from 62.5 to 250 µg/mL and MBC values from 125 to 500 µg/mL, with the strongest antifungal activity observed for the ethanol extract (MIC and MFC of 62.5 µg/mL). Decoctions, essential oil, absolutes, and methanol and ethanol extracts of rose leaves have been reported to exhibit moderate-to-strong antioxidant activity in various systems (Boskabady, 2011; Mahboubi, 2016), which consequently inhibits the growth of many pathogens (Gochev et al., 2008; Özkan et al., 2004). In contrast, the ethanol extract showed low biocontrol activity, consistent with its high EC_{50}, MIC, and MFC values. This suggests that ethanol, due to its extraction profile, moderately dissolves both lipophilic and polar compounds, whereas the active antifungal components in *R. damascena* leaves are likely non-polar or semi-polar rather than highly polar. In this study, the antifungal activity and fungistatic/fungicidal potential of the extracts, as indicated by EC_{50}, MIC, and MFC values, were consistent with their observed biocontrol activity. The hexane extract, which exhibited the lowest EC_{50} and MIC values, correspondingly showed the highest biocontrol activity. Similarly, the methanol extract demonstrated moderate EC_{50} and MIC values and showed medium-to-high biocontrol activity. The ethanol extract, with the lowest fungistatic and fungicidal effect, also exhibited the lowest inhibition rates. Dehghan Kashani et al. (2010) reported that both ethanol and aqueous extracts of *R. damascena* showed antimicrobial activity only against *S. aureus*, while activity against other pathogens such as *E. faecalis*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* was very low.

Conclusion

This study demonstrated that the antifungal efficacy of *R. damascena* flower extracts against *B. cinerea* and *A. alternata* is strongly influenced by the type of solvent used for extraction. Among the tested extracts, the hexane extract exhibited the highest biocontrol potential, achieving the greatest inhibition rates and the lowest EC_{50}, MIC, and MFC values against both pathogens. The methanol extract also showed considerable inhibitory. In contrast, the ethanol extract exhibited relatively weak antifungal properties, consistent with its higher EC_{50}, MIC, and MFC values. Overall, the findings highlight that solvent types and polarity significantly affects the bioactive antifungal compounds from *R. damascena*. The strong fungistatic and fungicidal effects observed for the hexane extract suggest its potential application as a natural antifungal agent for managing diseases caused by *B. cinerea* and *A. alternata*. Future research should focus on identifying and characterizing the specific active constituents within these extracts and evaluating their efficacy under greenhoue and field conditions to support the development of plant-based antifungal formulations.

Declarations

This study was presented at the III. International Biological and Life Sciences Congress (Antalya, BIOLIC 2025)

Conflict of interest: Authors declare that there is no conflict of interest

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