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Evaluation of Antibacterial Activities of Medicinal and Aromatic Plant Extracts Against Pseudomonas *Syringae Pv*. Syringae in Citrus

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Abstract. Citriculture is one of the most important agricultural sectors worldwide, with citrus fruits being widely cultivated across various regions. However, plant diseases caused by a range of phytopathogens pose significant challenges to citrus production. Several bacterial pathogens are known to infect citrus crops, leading to substantial economic losses. The efficacy of current strategies for controlling bacterial diseases in citrus is limited. In this study, the antibacterial activities of aqueous extracts obtained from various medicinal and aromatic plants including *Allium sativum*, *Eucalyptus* sp., *Myrtus communis* L., *Nerium oleander*, *Origanum onites*, *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Thymbra spicata* L. subsp. *spicata*, and *Zingiber officinale* were evaluated against *Pseudomonas syringae* pv. *syringae* (*Pss*) using the **M**inimum **I**nhibitory **C**oncentration (**MIC**) tube dilution method. Among the tested plant extracts, *A. sativum*, *O. onites*, *T. vulgaris*, and *T. spicata* L. subsp. *spicata* exhibited antibacterial activity at concentrations of 5.50 µl/ml, 8.25 µl/ml, >64 µl/ml and >128 µl/ml, respectively. These findings suggest that certain plant extracts hold promise for the development of novel, environmentally friendly pesticides for the control of bacterial diseases in citrus cultivation.

Keywords: Citriculture, Plant Extracts, *Pseudomonas syringae* pv.*syringae*, MIC

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1 Introduction

Citrus production represents one of the most economically valuable sectors in the global fruit industry. Global citrus production is approximately 150 million tons, with oranges accounting for about 60%, mandarins 20%, and lemons 10% of the total, according to [1]. In Turkey, citrus production reached 5.3 million tons in 2023, positioning the country as a significant exporter [2]. Despite its economic importance, citrus production faces substantial challenges from various phytopathogens that adversely affect yield and fruit quality. Among bacterial pathogens, *Pseudomonas syringae* pv. *syringae* (*Pss*) is particularly destructive, causing necrotic lesions on flowers, shoots, and fruit surfaces, especially under cold and humid conditions [3,4]. The *P. syringae* species complex has a wide host range and can lead to significant economic losses in citrus through symptoms such as blossom blight and branch dieback [5, 6]. The management of bacterial diseases in citrus remains challenging due to the limited effectiveness of conventional control measures. Cultural practices, including pruning, sanitation, and crop rotation, are often insufficient to suppress pathogen spread, while chemical controls such as copper-based bactericides and antibiotics face reduced efficacy owing to the emergence of resistant pathogen strains [7,8]. Moreover, chemical applications have been reported to cause soil accumulation, phytotoxicity, and environmental residues [9,10].

Increasing public awareness and concern regarding pesticide residues and their environmental impacts have further intensified the demand for alternative and sustainable disease management strategies.In this context, plant-derived compounds and extracts from medicinal

and aromatic plants have emerged as potential eco-friendly alternatives. Compounds obtained from species such as *Allium sativum* (garlic), *Origanumonites* (Turkish oregano), *Thymus vulgaris* (thyme), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), and *Eucalyptus* spp. have demonstrated effective antibacterial activity against *Pseudomonas*, *Xanthomonas*, and *Erwinia* species [11, 12, 13]. The main active compounds responsible for these effects phenolic compounds, flavonoids, terpenes, and allyl sulfides are known to inhibit bacterial growth by disrupting cell membranes or interfering with enzymatic activity [14,15]. Additionally, the eco-friendly, non-toxic, and biodegradable nature of these natural compounds makes them attractive alternatives to chemical bactericides.

The present study aimed to evaluate the antibacterial activities of aqueous extracts from selected medicinal and aromatic plants namely *Allium sativum*, *Eucalyptus* sp., *Myrtus communis* L., *Nerium oleander*, *Origanum onites*, *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Thymbra spicata* L. subsp. *spicata*, and *Zingiber officinale*—against *Pseudomonas syringae* pv. *syringae*. Using the Minimum Inhibitory Concentration (MIC) method, this research seeks to identify plant extracts with significant antibacterial potential, thereby contributing to the development of environmentally friendly and sustainable strategies for controlling bacterial diseases in citrus cultivation.

2 Materials and Methods

2.1 Material

Pathogen Isolate: In the experiments, the Pss isolate coded Fk-15, which was obtained from citrus in 2022 and preserved in culture collections, was used in the study. Medicinal and aromatic plants: Extracts obtained from ten different medicinal and aromatic plant species were used in the experiments. These plants are shown in Table 1.

Table 1. Names and Plant Parts Used of the Plants Employed in the Experiments

Plant species	Plant parts used
Allium sativum	Bulb
Eucalyptus sp.	Green parts
Myrtus communis L.	Green parts
Nerium oleander	Green parts
Origanum onites	Above-ground parts
Rosmarinus officinalis	Flowering branches
Salvia officinalis	Leaf
Thymus vulgaris	Green parts
Thymba spicata L. subsp. spicata	Green parts
Zingiber officinale	Tuber

2.2 Metod

Preparation of Plant Extracts

One hundred grams of plant material from different parts of medicinal and aromatic plants were extracted in 100 ml of distilled water using an electric homogenizer. Since seeds were difficult to grind, they were soaked overnight in sterile water at a 1:1 ratio and then homogenized the following day. The homogenized plant extracts were filtered through cheesecloth to remove coarse particles. The remaining aqueous extract was centrifuged at 6,500 rpm for 15 minutes, after which the supernatant (upper phase) was collected and the pellet discarded. For vivo seed and pot experiments, the extracts were used immediately after the centrifugation step. For vitro Petri dish experiments, the extracts were sterilized by cold filtration. For this purpose, the extracts were first passed through a vacuum holder containing a 45 µm pore filter [16], and then through a Millipore 0.22 µm pore filter for sterilization [17,18]. Freshly prepared extracts were always used throughout the study.

Determination of the Minimum Inhibitory Concentrations of Different Plant Extracts Against Pseudomonas syringae pv. Syringae

The minimum inhibitory concentration (MIC) values of the plant extracts that were found to be effective against Pss in the paper disc diffusion assay were determined using the tube dilution method [19, 20, 21]. In the tube dilution method, 1 ml of a pathogen bacterial inoculum (Pss) with an approximate population of 10^6 cells/ml was added to glass test tubes containing NB broth medium. Then, different concentrations of freshly prepared plant extracts (0.125 µl/ml, 0.25 µl/ml, 0.5 µl/ml, 1 µl/ml, 2 µl/ml,

4 µl/ml, 8 µl/ml, 16 µl/ml, 32 µl/ml, 64 µl/ml, and 128 µl/ml) were added to the tubes at the specified ratios. In addition, a control tube containing only the pathogen bacteria (without plant extract) was used as a growth control, while a tube containing only the broth medium (without bacterial inoculation) was used as a medium control. After incubation at 25°C for 24 hours, the tubes were examined for turbidity as an indicator of bacterial growth. Subsequently, 100 µl from each tube was spread in triplicate onto KB agar plates using a sterile glass rod. The inoculated plates were incubated at 25°C for 24 hours. The lowest concentration of the plant extract that showed no visible turbidity and no bacterial growth on the medium was recorded as the MIC value.

3 Results and Discussion

In conclusion, the tested plant extracts, namely *A. sativum*, *O. onites*, *T. vulgaris*, and

T. spicata L. subsp. *spicata*, showed antibacterial activity against *Pss* at minimum inhibitory concentrations

of 5.50 µl/ml, 8.25 µl/ml, >64 µl/ml, and >128 µl/ml, respectively.

According to the results of the study, the strongest antibacterial effect was obtained from the *Allium sativum* extract. Review of previous studies shows that *Allium sativum* is one of the most extensively investigated plant species for its antibacterial activity against both human and plant pathogens. The effectiveness of *Allium sativum* is attributed to its sulfur-containing compounds, volatile oils (such as allicin, alliin, and ajoene), enzymes, carbohydrates, minerals, amino acids, bioflavonoids, vitamins, and more than 200 other chemical substances including beta-carotene [22]. Our results clearly demonstrate that *Allium sativum* extract exhibits significant antibacterial activity against *Pss*. The MIC value of *Allium sativum* for *Pseudomonas syringae* pv. *tomato* was determined to be 4.25 µl/ml [23]. When previous studies on MIC values are examined, a similar level of activity was reported against *Xanthomonas axonopodis* pv. *citri* and *Pseudomonas solanacearum*, with an MIC value of 40 mg/ml [24].

In another study, the MIC value of Origanum onites against Pss was determined to be >128 µl/ml, and its antibacterial activity was evaluated as low [25]. However, in our study, the MIC value of O. onites against Pss was recorded as 8.25 µl/ml. [26] reported that Origanum onites induces resistance in tomato plants. The specific compounds activated by Origanum onites in plants should be investigated in future studies.

Considering all the results, it would be beneficial to investigate the antibacterial effects of the most promising plant extracts through in vitro experiments against Pss, the causal agent of Bacterial Blast on Citrus. Before these environmentally friendly, plant-based antimicrobial substances can be commercialized, it is necessary to determine the optimum extraction method and formulation, identify the effective dosage levels, and develop methods to eliminate phytotoxicity.

4 Conclusion

The study aims to explore botanical agents as potential antibacterial resources, providing new perspectives for agricultural disease management. Considering all the results, this study has demonstrated that plant extracts can be used in the integrated management of Bacterial Blast on Citrus disease caused by Pseudomonas syringae pv. syringae. It would be beneficial to further investigate the successful plant extracts through different application methods. Before these environmentally friendly, plant-based antimicrobial substances can be commercialized, it is necessary to determine the optimum extraction method and formulation, identify the effective dosage levels, and develop methods to eliminate phytotoxicity.

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