

Antibacterial Activity of Various Plant Extracts Obtained from Duzce Province Against *Clavibacter michiganensis* subsp. *michiganensis*

Düzce İli'nden Elde Edilen Çeşitli Bitki Ekstraktlarının *Clavibacter michiganensis* subsp. *michiganensis*'e Karşı Antibakteriyel Aktivitesi

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ABSTRACT

Tomato bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), can result in yield losses of up to 60% and is increasingly difficult to manage due to environmental concerns and resistance associated with copper based bactericides. To identify ecofriendly and locally sourced alternatives, this study evaluated the *in vitro* antibacterial activity of ethanolic extracts from 18 plant species collected in Düzce province against Cmm using the agar well diffusion method at concentrations of 10, 20, and 30 mg/mL. Significant variation in antibacterial efficacy was observed among plant extracts and concentrations. Peppermint (*Mentha × piperita* L.) exhibited the strongest inhibitory effect across all concentrations, followed by Black Grape (*Vitis vinifera* L.), Bay Laurel (*Laurus nobilis* L.), Eucalyptus (*Eucalyptus globulus* Labill.), and Aloe Vera (*Aloe vera* (L.) Burm.f.). Some extracts, such as Lemon (*Citrus limon* (L.) Osbeck), Grape Seed (*Vitis vinifera* L. semen), and Ginger (*Zingiber officinale* Roscoe), showed dose-dependent activity, while Tea Tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) and Southernwood (*Artemisia abrotanum* L.) had the lowest antibacterial effects. These findings highlight the potential of locally sourced botanical extracts as sustainable alternatives to conventional copper based bactericides for managing tomato bacterial canker. Further research is recommended to determine minimum inhibitory concentrations, isolate active compounds, and assess *in vivo* efficacy for integrated disease management applications.

Keywords: Plant Extract, Antibacterial Activity, Biocontrol, Tomato Bacterial Canker

ÖZ

Clavibacter michiganensis subsp. *michiganensis* (Cmm) bakterisinin neden olduğu domates bakteriyel yanıklığı, %60'a varan verim kayıplarına yol açabilmekte ve çevresel kaygılar ile bakır bazlı bakterisitlere karşı gelişen direnç nedeniyle kontrol edilmesi giderek zorlaşmaktadır. Bu çalışma; çevre dostu ve yerel kaynaklı alternatifler belirlemek amacıyla, Düzce ilinden toplanan 18 bitki türünün etanolik ekstraktlarının Cmm'ye karşı *in vitro* antibakteriyel aktivitelerini 10, 20 ve 30 mg/mL konsantrasyonlarda agar kuyu difüzyon yöntemi kullanarak değerlendirmiştir. Bitki ekstraktları ve konsantrasyonları arasında antibakteriyel etkinlik bakımından önemli farklılıklar gözlemlenmiştir. Tüm konsantrasyonlarda en güçlü inhibitör etkiyi Nane (*Mentha × piperita* L.) göstermiş; onu sırasıyla Siyah Üzüm (*Vitis vinifera* L.), Defne (*Laurus nobilis* L.), Okaliptüs (*Eucalyptus globulus* Labill.) ve Aloe Vera (*Aloe vera* (L.) Burm.f.) izlemiştir. Limon (*Citrus limon* (L.) Osbeck), Üzüm Çekirdeği (*Vitis vinifera* L. semen) ve Zencefil (*Zingiber officinale* Roscoe) gibi bazı ekstraktlar doza bağlı aktivite gösterirken, Çay Ağacı (*Melaleuca alternifolia* (Maiden & Betche) Cheel) ve Pelin Otu/Sivri Otu (*Artemisia abrotanum* L.) en düşük antibakteriyel etkiyi sergilemiştir. Bu bulgular, yerel kaynaklı botanik ekstraktların domates bakteriyel kanseriyle mücadelede geleneksel bakır bazlı bakterisitlere sürdürülebilir bir alternatif olma potansiyelini vurgulamaktadır. Minimum inhibitör konsantrasyonlarının belirlenmesi, aktif bileşiklerin izole edilmesi ve entegre hastalık yönetimi uygulamaları için *in vivo* etkinliği değerlendirilmesi amacıyla ileri araştırmalar önerilmektedir.

Anahtar Kelimeler: Bitki Ekstraktı, Antibakteriyel Aktivite, Biyokontrol, Domates Bakteriyel Kanseri

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and economically significant vegetable crops globally, playing a vital role in both international agricultural markets and food security (Kimura & Sinha, 2008). Tomatoes are recognized as functional foods with significant benefits for human health, including the prevention of cardiovascular diseases and certain types of cancer, owing to their high content of essential nutrients, such as vitamins C and E, β -carotene, flavonoids, and particularly the potent antioxidant lycopene (Dorais et al., 2008; Maurya et al., 2025). Beyond fresh consumption, its versatility in the food processing industry for products such as sauces, pastes, and juices, as well as its applications in the pharmaceutical and cosmetic sectors, underscores its multifaceted industrial importance (Sattar et al., 2024). The global tomato industry is characterized by a high production volume, reaching approximately 180 million tons annually. While a significant portion of this yield is destined for fresh consumption, around 20% is channelled into industrial processing (García et al., 2019). Although major players like China, India, and the USA continue to dominate the sector, recent shifts have been observed; for instance, while California's industrial production saw a notable increase, China experienced a significant decline of nearly 32% in recent periods (García et al., 2019). Within this global framework, Türkiye maintains a strategic position as the fourth largest producer and the sixth largest exporter worldwide (Aksoy & Kaymak, 2016). Production is heavily concentrated in the Marmara and Aegean regions, with Bursa, Manisa, and Izmir serving as primary hubs. Economically, the sector is a cornerstone of Turkish agricultural exports, with a substantial processing industry where 80% of the industrial crop is converted into tomato paste (Ertürk & Çirka, 2015). Despite its economic importance, global tomato production is significantly hindered by various biotic constraints, primarily diseases caused by fungal, viral, and bacterial pathogens (Eryigit et al., 2025). Among these, *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) stands out as a gram positive bacterium responsible for bacterial canker and

wilt, one of the most economically devastating diseases worldwide. Cmm is a systemic pathogen that primarily invades xylem vessels through wounds or natural openings, leading to vessel blockages that manifest as unilateral leaf wilting, stem cankers, and characteristic "bird's eye" lesions on the fruit (Meletzus et al., 2000; Peritore-Galve et al., 2021). The pathogen's high economic impact, which can reach up to 60% yield loss in commercial production, is exacerbated by its seed-borne nature. Cmm can persist both internally and externally in seeds, facilitating its rapid global dissemination and making it a major quarantine organism in many regions (Jahr et al., 1999; Peritore-Galve et al., 2021). The management of Cmm remains a complex challenge, relying on an integration of physical, chemical, and cultural practices. Physical methods, such as heat treatment of seeds, are employed to eradicate seed-borne inoculum; however, these techniques require precise control to avoid compromising seed viability and germination rates (Nandi et al., 2018). Chemical management traditionally relies on copper based compounds. Nevertheless, the long-term efficacy of these chemical treatments is increasingly compromised by the emergence of resistant bacterial strains and growing environmental concerns regarding soil pollution (Karakus et al., 2026). Cultural practices, including the use of certified pathogen-free seeds, crop rotation, and rigorous sanitation, form the backbone of integrated disease management (Obradovic et al., 2008). Furthermore, the limited availability of commercial tomato cultivars with robust resistance exacerbates the reliance on these preventive measures (Sen et al., 2015). Given the environmental risks and the rapid evolution of resistance associated with current chemical controls, there is a critical and urgent need for sustainable alternatives. Current research is increasingly focused on ecofriendly approaches, such as biological control agents and systemic acquired resistance inducers, to provide more resilient and environmentally compatible solutions for managing bacterial disease in the future (Sanver, 2026). Botanical extracts and essential oils have emerged as promising biorational tools due to their diverse antibacterial mechanisms. For instance, extracts from *Polygonum orientale* and its active compounds, such as dihydroquercetin, have been shown to severely damage the cell wall and membrane integrity of Cmm, leading to

the leakage of vital intracellular components (Cai et al., 2023). Additionally, extracts from plants like *Tamarix ramosissima* and *Rosmarinus officinalis* exhibit anti-biofilm activity, which is crucial for preventing the persistent colonization of the pathogen within the host (Ichim et al., 2017). Essential oils from *Lavandula angustifolia*, *Ocimum basilicum* and *Thymus* spp. also demonstrate potent bactericidal effects, with *Thymus vulgaris* oil showing up to 99.39% growth inhibition (Belgüzar et al., 2016; Moglia et al., 2025). The practical application of these natural compounds as seed and foliar treatments has shown significant potential in vivo; Notably, sugar maple leaf extracts have provided high levels of protection against Cmm in greenhouse trials, sometimes exceeding the efficacy of traditional copper-based treatments (Tremblay et al., 2024). While challenges remain in standardizing these extracts for large scale agricultural use, they represent a vital frontier in the development of integrated and sustainable management strategies for tomato bacterial canker.

Despite the promising potential of various botanical sources, there remains a significant gap in our knowledge regarding the efficacy of many specific and under-investigated plant species against Cmm. Many regional flora and unique plant extracts remain unexplored for their potential to inhibit this devastating pathogen, particularly regarding their specific minimum inhibitory concentrations and mechanisms of action. This lack of comprehensive screening limits the diversity of available biorational tools for integrated pest management. Therefore, this study aimed to evaluate the in vitro antibacterial activity of several unique plant extracts against Cmm. By identifying novel botanical candidates with high inhibitory potential, this study addresses the critical need for eco-friendly alternatives and contributes to the development of more sustainable strategies for managing bacterial canker in tomato production.

MATERIAL AND METHODS

Plant Material

The plant materials used in this study were obtained from two sources: some species were collected from

natural habitats in Düzce Province in 2024, whereas others were purchased from local herbalists and markets in the same region (Table 1). All plant samples were used in their raw forms. The collected plant materials were cleaned of foreign matter, air-dried at room temperature away from direct sunlight, and stored in paper bags until use (Harborne, 1998).

Table 1. Plant species used in the study

Plant species	Scientific names
Grapefruit	<i>Citrus × paradisi</i> Macfad.
Peppermint	<i>Mentha × piperita</i> L.
Grape	<i>Vitis vinifera</i> L.
Mandarin	<i>Citrus reticulata</i> Blanco
Eucalyptus	<i>Eucalyptus globulus</i> Labill.
Mustard	<i>Sinapis alba</i> L.
Aloe vera	<i>Aloe vera</i> (L.) Burm.f.
Southernwood	<i>Artemisia abrotanum</i> L.
Horse chestnut	<i>Aesculus hippocastanum</i> L.
Bay Laurel	<i>Laurus nobilis</i> L.
Wheat	<i>Triticum aestivum</i> L.
St. John's Wort	<i>Hypericum perforatum</i> L.
Grape Seed	<i>Vitis vinifera</i> L. (semen)
Tea Tree	<i>Melaleuca alternifolia</i> (Maiden & Betche) Cheel
Ginger	<i>Zingiber officinale</i> Roscoe
Lemon	<i>Citrus limon</i> (L.) Osbeck
Walnut	<i>Juglans regia</i> L.
Orange	<i>Citrus sinensis</i> (L.) Osbeck

Preparation of Plant Extracts

The dried plant materials were ground into a fine powder using a laboratory grinder. Extraction was carried out using the maceration method: 10 g of each plant powder was dissolved in 100 mL of 80% ethanol and kept on a shaker at room temperature for 48 h (Harborne, 1998). The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was evaporated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extracts were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 10, 20, and 30 mg/mL and stored at -20°C until use (Trease & Evans, 2002).

Antimicrobial Activity Assay

The antimicrobial activity of the plant extracts was

evaluated against *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) isolate 633, obtained from the stock collection of the Department of Plant Protection, Faculty of Agriculture, Düzce University, Türkiye. The antimicrobial activity was assessed using the agar well diffusion method (Bauer et al., 1966). Bacterial suspensions were prepared in sterile distilled water and adjusted to an optical density (OD) of 0.1 at 600 nm using UV-spectrophotometry (PG Instrument T60). The suspensions were spread uniformly onto nutrient agar plates, and wells of 6 mm diameter were punched into the agar. Subsequently, 100 µL of each plant extract at the tested concentrations (10, 20, and 30 mg/mL) was added to each well. The plates were incubated at 28°C for 48 h, after which the inhibition zone diameters were measured in millimeters (Bauer et al., 1966). Each treatment was performed in three independent replicates.

Statistical Analysis

All data were subjected to two-way analysis of variance (Two-way ANOVA) to determine the effects of plant

extract type, concentration, and their interaction on the inhibition zone diameters. Mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$. All statistical analyses were conducted using IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA). Results are expressed as mean $\pm$ standard deviation (SD) of three independent experiments.

RESULTS

The in vitro antibacterial activity of 18 plant extracts against *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) isolate 633 was evaluated at three concentrations (10, 20, and 30 mg/mL) using the agar-well diffusion method. Two-way ANOVA revealed that both plant extract type ($F=9.245$, $df=17$, $p<0.001$) and concentration ($F=4.788$, $df=2$, $p<0.05$) had statistically significant effects on the inhibition zone diameters (Table 2). The interaction between the plant extract and concentration was not statistically significant ($F=1.399$, $df=34$, $p>0.05$) (Table 2).

Table 2. Two-way ANOVA results for the effects of plant extract type, concentration, and their interaction on inhibition zone diameters (mm) against *Clavibacter michiganensis* subsp. *michiganensis*

Source of variation	Sum of squares	Df	F Value	P Value	Significance
Plant extract	51.7094	17	9.245	<0.001	***
Concentration (mg/mL)	3.1505	2	4.7878	0.010	**
Plant $\times$ Concentration	15.654	34	1.3994	0.099	ns
Residual (Error)	35.5333	108			

*** $p<0.001$; ** $p<0.01$; ns=not significant

At 10 mg/mL, the highest inhibition zone diameters were recorded for peppermint (3.20 ± 1.00 mm), Black Grape (2.17 ± 0.55 mm), orange (2.17 ± 1.76 mm), mandarin (2.13 ± 0.51 mm), and Aloe Vera (1.73 ± 0.71 mm), all of which were assigned to Tukey group “a”. Mustard (1.03 ± 0.95 mm), grapefruit (0.90 ± 0.44 mm), ginger (0.87 ± 0.06 mm), walnut (0.80 ± 0.10 mm), Tea Tree (0.80 ± 0.00 mm), Grape Seed (0.67 ± 0.06 mm), southernwood (0.67 ± 0.12 mm), and lemon (0.23 ± 0.06 mm) produced significantly lower inhibition zones and were assigned to Tukey group “b” at this concentration. At 20 mg/mL, peppermint again demonstrated the

highest activity (2.73 ± 0.64 mm), followed by Bay Laurel (2.27 ± 0.23 mm), eucalyptus (2.27 ± 0.49 mm), Aloe Vera (2.13 ± 0.46 mm), and Black Grape (1.77 ± 0.25 mm), all in Tukey group “a”. In contrast, ginger (1.13 ± 0.06 mm), St. John's Wort (0.73 ± 0.12 mm), Grape Seed (0.87 ± 0.12 mm), southernwood (0.87 ± 0.06 mm), Tea Tree (0.77 ± 0.06 mm), and lemon (0.47 ± 0.06 mm) showed significantly lower activity and were classified as Tukey group “b” at this concentration.

At 30 mg/mL, Peppermint maintained its superior activity (2.93 ± 0.38 mm), with Black Grape (2.43 ± 0.67

mm), Bay Laurel (2.40 ± 0.20 mm), Horse Chestnut (2.23 ± 0.15 mm), and Eucalyptus (1.90 ± 1.14 mm) also showing high inhibition zones, all within Tukey group “a”. Notably, several extracts that showed lower activity at 10 mg/mL exhibited markedly increased inhibition at 30 mg/mL; Lemon increased from 0.23 ± 0.06 mm to 1.93 ± 0.72 mm, Grape Seed from 0.67 ± 0.06 mm to 1.63 ± 0.06 mm, and Ginger from 0.87 ± 0.06 mm to 1.87 ± 0.06 mm, all reaching Tukey group “a” at this concentration. Conversely, Tea Tree showed an inverse trend, declining from 0.80 ± 0.00 mm at 10 mg/mL to 0.33 ± 0.06 mm at 30 mg/mL, remaining in Tukey group “b” throughout all concentrations.

When the overall mean inhibition zones were compared across all concentrations, peppermint (*Mentha × piperita* L.) ranked first with 2.96 ± 0.66 mm, followed

by Black Grape (*Vitis vinifera* L.; 2.12 ± 0.54 mm), Bay Laurel (*Laurus nobilis* L.; 2.06 ± 0.50 mm), eucalyptus (*Eucalyptus globulus* Labill.; 2.00 ± 0.77 mm), and Aloe Vera (*Aloe vera* (L.) Burm.f.; 1.94 ± 0.52 mm), all forming Tukey group “a”. Horse Chestnut, Orange, Mandarin, Wheat, Mustard, Grapefruit, Ginger, St. John's Wort, Walnut, Grape Seed, and Lemon constituted Tukey group b with overall means ranging from 0.88 to 1.82 mm. The lowest overall antibacterial activity was recorded for southernwood (*Artemisia abrotanum* L.; 0.79 ± 0.12 mm) and Tea Tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel; 0.63 ± 0.23 mm), both assigned to Tukey group “c” (Table 3) (Figure 1).

Table 3. Mean inhibition zone diameters (mm±SD) of plant extracts at different concentrations against *Clavibacter michiganensis* subsp. *michiganensis* isolate 633, with Tukey HSD grouping letters

Plant Extract	10 mg/mL	20 mg/mL	30 mg/mL	Overall
Peppermint	3.20 ± 1.00^a	2.73 ± 0.64^a	2.93 ± 0.38^a	2.96 ± 0.66^a
Black Grape	2.17 ± 0.55^a	1.77 ± 0.25^a	2.43 ± 0.67^a	2.12 ± 0.54^a
Bay Laurel	1.50 ± 0.44^a	2.27 ± 0.23^a	2.40 ± 0.20^a	2.06 ± 0.50^a
Eucalyptus	1.83 ± 0.81^a	2.27 ± 0.49^a	1.90 ± 1.14^a	2.00 ± 0.77^a
Aloe Vera	1.73 ± 0.71^a	2.13 ± 0.46^a	1.97 ± 0.51^a	1.94 ± 0.52^a
Horse Chestnut	1.87 ± 0.31^a	1.37 ± 0.06^a	2.23 ± 0.15^a	1.82 ± 0.41^b
Orange	2.17 ± 1.76^a	1.47 ± 0.06^a	1.77 ± 0.06^a	1.80 ± 0.93^b
Mandarin	2.13 ± 0.51^a	1.27 ± 1.76^a	1.83 ± 0.15^a	1.74 ± 1.00^b
Wheat	1.57 ± 0.06^a	1.70 ± 0.00^a	1.60 ± 0.00^a	1.62 ± 0.07^b
Mustard	1.03 ± 0.95^b	1.67 ± 1.20^a	1.63 ± 1.12^a	1.44 ± 1.00^b
Grapefruit	0.90 ± 0.44^b	1.33 ± 0.55^a	1.90 ± 0.62^a	1.38 ± 0.64^b
Ginger	0.87 ± 0.06^b	1.13 ± 0.06^b	1.87 ± 0.06^a	1.29 ± 0.45^b
St. John's Wort	1.43 ± 0.21^a	0.73 ± 0.12^b	1.17 ± 0.06^b	1.11 ± 0.33^b
Walnut	0.80 ± 0.10^b	1.53 ± 0.40^a	0.87 ± 0.15^b	1.07 ± 0.42^b
Grape Seed	0.67 ± 0.06^b	0.87 ± 0.12^b	1.63 ± 0.06^a	1.06 ± 0.45^b
Lemon	0.23 ± 0.06^b	0.47 ± 0.06^b	1.93 ± 0.72^a	0.88 ± 0.88^b
Southernwood	0.67 ± 0.12^b	0.87 ± 0.06^b	0.83 ± 0.06^b	0.79 ± 0.12^c
Tea Tree	0.80 ± 0.00^b	0.77 ± 0.06^b	0.33 ± 0.06^b	0.63 ± 0.23^c
Control	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b

*Letters following Mean±SD values indicate Tukey HSD grouping. Means followed by different letters are significantly different ($p \leq 0.05$). Values represent mean±SD of three independent replicates.

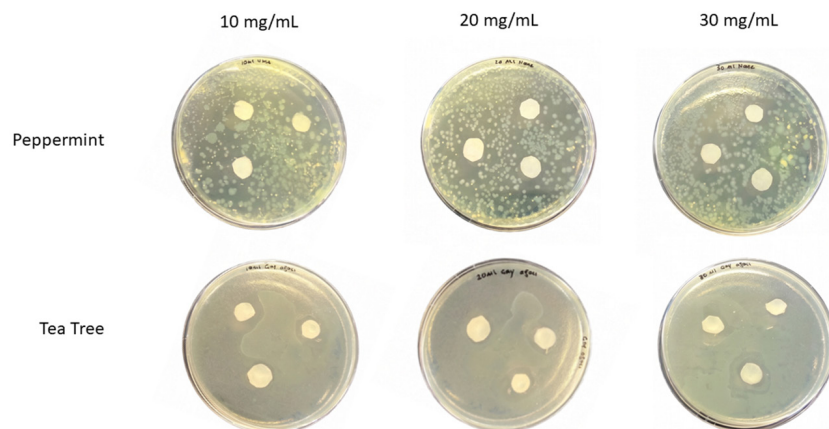


Figure 1. Inhibition zones formed by the most effective extract, Peppermint (*Mentha × piperita* L.), and the least effective extract, Tea Tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel), against Cmm in agar well diffusion assay at concentrations of 10, 20, and 30 mg/mL

DISCUSSION

The present study demonstrated that the ethanolic extracts of 18 plant species collected from Düzce province exhibited varying degrees of *in vitro* antibacterial activity against *Clavibacter michiganensis* subsp. *michiganensis*. Cmm. The significant differences observed among plant extracts and concentrations confirm that both the botanical source and the applied concentration are critical determinants of antibacterial efficacy.

Peppermint (*Mentha × piperita* L.) demonstrated the highest overall antibacterial activity across all tested concentrations, consistent with the well-documented antimicrobial properties of *Mentha*. The antibacterial potential of peppermint is primarily attributed to its high content of phenolic compounds, flavonoids, and terpenoids, particularly menthol and menthone, which disrupt bacterial cell membrane integrity and inhibit key metabolic enzymes (Burt, 2004). Although most previous studies have evaluated peppermint essential oil rather than ethanolic extracts, the inhibitory activity observed in the present study suggests that non-volatile phenolic fractions also contribute significantly to its antibacterial properties. The consistent activity of peppermint across all three concentrations further underscores its potential as a reliable botanical candidate against gram-positive pathogens such as Cmm.

Black Grape (*Vitis vinifera* L.), Bay Laurel (*Laurus*

nobilis L.), Eucalyptus (*Eucalyptus globulus* Labill.), and Aloe Vera (*Aloe vera* (L.) Burm.f.) also exhibited notable antibacterial activity. The inhibitory effect of *Vitis vinifera* extracts has been attributed to their high polyphenol content, including resveratrol, quercetin, and catechins, which exert antibacterial effects through multiple mechanisms including cell membrane disruption and inhibition of nucleic acid synthesis (Baydar et al., 2004). The activity of *Laurus nobilis* is consistent with previous reports highlighting the role of eugenol, linalool, and 1,8-cineole as key bioactive components responsible for its broad spectrum antimicrobial properties (Ramos et al., 2012). Similarly, the antibacterial activity of *Eucalyptus globulus* has been well established and is largely attributed to its rich content of 1,8-cineole (eucalyptol), terpinen-4-ol, and various polyphenolic compounds (Bachir & Benali, 2012).

Several extracts exhibited a pronounced, dose-dependent increase in antibacterial activity. Lemon (*Citrus limon* (L.) Osbeck), Grape Seed (*Vitis vinifera* L. semen), and ginger (*Zingiber officinale* Roscoe) showed significantly higher inhibition at 30 mg/mL than at lower concentrations. This dose-dependent response suggests that the active compounds in these extracts reach effective bactericidal concentrations only at higher doses, which may be related to their relatively lower content of primary antimicrobial compounds compared to more potent extracts such as peppermint. In contrast, Tea Tree (*Melaleuca alternifolia*

(Maiden & Bettle) Cheele) and southernwood (*Artemisia abrotanum* L.) exhibited the lowest overall antibacterial activity among all the tested extracts. The poor performance of the Tea Tree extract is particularly noteworthy, as this plant is widely recognized for its potent antimicrobial properties, primarily attributed to terpinen-4-ol in its essential oil (Carson et al., 2006). However, it should be noted that the present study utilized ethanolic extracts rather than essential oils; consequently, the volatile terpene compounds responsible for the characteristic antimicrobial activity of tea tree oil may not have been adequately captured during the maceration process. This highlights a critical methodological consideration: the extraction method profoundly influences the chemical composition of the resulting extract and, consequently, its biological activity of the extract. Therefore, future studies should directly evaluate the antibacterial potential of tea tree essential oil against Cmm.

The absence of a significant plant $\times$ concentration interaction indicates that the relative ranking of plant extracts remained generally consistent across concentrations, except for a few extracts that demonstrated clear dose-dependent responses. This finding simplifies the interpretation of the results for practical applications, as it suggests that the most effective extracts at lower concentrations tend to maintain their superiority at higher concentrations.

Compared to previously reported studies on the botanical control of Cmm, the inhibition zone diameters recorded in the present study were generally modest. For instance, *Thymus vulgaris* essential oil has been reported to achieve up to 99.39% growth inhibition of Cmm (Belgüzar et al., 2016) and sugar maple leaf extracts have demonstrated efficacy comparable to copper-based treatments in greenhouse conditions (Tremblay et al., 2024). The relatively low activity observed here may be attributable to the use of crude ethanolic extracts, which contain a complex mixture of compounds that may interact antagonistically, as well as to the specific isolate of Cmm used, which may possess distinct susceptibility characteristics. Nevertheless, the identification of Peppermint, Black Grape, Bay Laurel, Eucalyptus, and Aloe Vera as promising candidates warrants further investigation, including fractionation

studies to identify the specific active compounds, determination of minimum inhibitory concentrations, and evaluation of in vivo efficacy under greenhouse and field conditions.

CONCLUSION

In conclusion, this study provides the first comprehensive evaluation of 18 plant extracts collected from the Düzce province against Cmm, one of the most economically significant bacterial pathogens of tomato. The results clearly demonstrate that peppermint (*Mentha $\times$ piperita* L.) possesses the strongest in vitro antibacterial activity against Cmm among the tested extracts, followed by Black Grape, Bay Laurel, Eucalyptus, and Aloe Vera. These findings highlight the potential of locally sourced botanical extracts as sustainable and environmentally compatible alternatives to conventional copper-based bactericides for managing tomato bacterial canker. However, further studies, including minimum inhibitory concentration determination, bioactive compound identification, and greenhouse trials, are necessary to fully establish the practical applicability of these extracts in integrated disease management programs.

Ethical Approval: Ethical approval is not required for this study.

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