Suppression of Anthracnose in Strawberry Using Water Extracts of Lamiaceae Herbs and Identification of Antifungal Metabolites

Hasib Ahmad¹ and Yoh-ichi Matsubara²*

¹The United Graduate School of Agricultural Science, Gifu University, Gifu 501-1193, Japan
²Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan

The present experiment was conducted to determine whether water extracts of lemon balm and oregano could suppress anthracnose in strawberry and to identify the important secondary metabolites responsible for such activity. Runner plants of three strawberry cultivars (Fragaria × ananassa Duch. ‘Sachinoka’, ‘Akihime’, and ‘Tochiotome’) were treated with water extracts (20%, w/v) of lemon balm (Melissa officinalis L.) and oregano (Origanum vulgare L.) separately and inoculated with Colletotrichum gloeosporioides (C. fructicola, CG1). Two weeks after CG1 inoculation, it was observed that the shoots and roots of the herb extract-treated plants had lower disease incidences and indices compared to those of the control regardless of the cultivar. Consequently, dry weights of the shoots of all the cultivars treated with the herb extracts were observed to be heavier than in the control; similarly, heavier dry weights of roots were also observed in herb extract-treated plants in ‘Sachinoka’ and ‘Tochiotome’. Upon analyzing the results of ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS), rosmarinic acid and luteolin in lemon balm and apigenin and protocatechuic acid in oregano were identified to be the metabolites with the highest concentration in their respective plants. In addition, the antifungal effect of all these compounds against CG1 was confirmed by in vitro tests. Thus, it can be concluded that water extracts of lemon balm and oregano could suppress anthracnose in strawberry plants, and the four identified compounds in the extracts could play key roles in the antifungal properties of these herbs.

Key Words: Colletotrichum gloeosporioides, lemon balm, oregano, rosmarinic acid, UPLC-MS/MS.

Introduction

The organisms belonging to the genus Colletotrichum are important plant pathogens that cause anthracnose in a wide range of plants worldwide (Cannon et al., 2012). In strawberry cultivation, C. gloeosporioides is a serious disease-causing organism that causes huge production loss in major strawberry-producing regions (Mori and Kitamura, 2003). It can infect several parts of strawberry plants, and its symptoms include crown and stolon necrosis (Howard et al., 1992), black leaf spot (Howard and Albregts, 1983), and fruit lesions (Howard and Albregts, 1984). It can also affect the production cycle, leading to up to 60–70% of yield loss (Legard et al., 2003; Smith, 2008). However, it is difficult to control the disease as mother plants with latent infection are often used for runner production. Moreover, the difficulty in developing cultivars due to polyploidy, incomplete resistance of the developed cultivars and inadequate control through cultural control methods makes this disease a serious problem in strawberry cultivation. Generally, the use of synthetic fungicides is the primary control measure used against this disease at the producer level. However, these chemicals pose a major threat to the environment, as well as to humans, because of their low selectivity and lack of biodegradability (Gao et al., 2017). Furthermore, the development of resistance by microorganisms to these chemical compounds results in higher dose-dependence, which increases the production cost, as well as food safety problems (Jílková et al., 2015). Hence, a search for an alternative and environment-friendly approach for disease control has become the latest challenge in crop production.

Lamiaceae herbs contain several phenolic com-
pounds, terpenoids, and glucosides as secondary metabolites, with beneficial effects such as antimicrobial and antioxidant activities (Martino et al., 2009; Stanojevic et al., 2010; Weerakkody et al., 2011). The antimicrobial and preservative activities of the essential oils (EOs) in herbs are well documented, primarily for agri-foods (Teixeira et al., 2013; Gomes et al., 2014). In addition, the in vitro antioxidant and antifungal effects of the EOs on plant pathogens have also been reported in a few studies (Isman, 2000; Quintanilla et al., 2002). However, the antifungal effects of Lamiaceae herbs on plant disease control remain unclear.

Lemon balm (Melissa officinalis L.) of the Lamiaceae family is an important medicinal herb that has been widely used in traditional medicines (Meftahizade et al., 2010); its essential oil is reported to possess antimicrobial activity (Romero et al., 2008), and its aqueous extract is reported to exhibit antiviral (Adorjan and Buchbauer, 2010), antioxidant (Spiridon et al., 2011), anti-inflammatory, antinoceptive (Birdane et al., 2007), and antidiabetic effects (Chung et al., 2010). The use of oregano (Origanum vulgare L.) has only increased in recent years because of the identification of several therapeutic properties of its extract including antioxidant, antimicrobial, anti-inflammatory (Oniga et al., 2018), antiviral (Zhang et al., 2014), anti-spasmodic (Gonceariuc et al., 2015), antiproliferative (Elshafie et al., 2016) effects. Quintanilla et al. (2002) reported that the EOs of herbs such as thyme, oregano, lemon balm, and peppermint inhibited the growth of Phytophthora infestans in in vitro plate assays. In addition, the EOs of lavender and rosemary were found to suppress the growth of Botrytis cinerea in vitro (Soylu et al., 2010). The volatile compounds in the EOs, which accumulate in closed environments under in vitro conditions, were responsible for inhibiting the fungi. However, the use of EOs in field conditions is impractical because they diffuse away from the applied surface, resulting in a decrease in the effective concentration, and enabling the disease-causing organism to resume growth (Letessier et al., 2001). In addition, EOs have been reported to possess phytotoxic effects in crops following foliar application at high concentrations (Letessier et al., 2001). A viable alternative to this could be the use of water extracts containing non-volatile secondary metabolites; this would constitute an environmentally-friendly disease control approach. Water extract preparation is a relatively easy and inexpensive process compared with that for EOs. In addition, as the extracts are non-volatile, they remain effective for longer periods than the EOs. The effectiveness of lemon balm water extract in controlling Fusarium wilt in strawberry was already confirmed in our previous study (Ahmad and Matsubara, 2020), with the water extract showing considerable suppression of fungal propagation leading to lower disease incidences and indices; the secondary metabolites identified in the extract also suppressed the pathogen in vitro, thereby proving their antifungal potential. Therefore, we decided to test whether or not the extracts of Lamiaceae herbs could also suppress strawberry diseases like anthracnose that affect the upper parts. In this study, we evaluated the effect of water extracts of lemon balm and oregano on controlling anthracnose in three strawberry cultivars. We also identified the major secondary metabolites present in these water extracts and evaluated their antifungal potential.

Materials and Methods

Growing Lamiaceae herbs and preparing their extracts

Seeds of M. officinalis and O. vulgare were sown in plastic containers (31.9 cm × 26.4 cm × 15.3 cm) containing commercial soil (Supermix A; Sakata Seed Corporation, Yokohama, Japan) and grown in a greenhouse. Eight weeks after sowing, the plants were uprooted and the shoots were cryopreserved using liquid nitrogen. The frozen samples were ground in distilled water using a mixer while maintaining the concentration of the herbal extract at 20% (w/v). The extract was then filtered and the filtrate was used as the herb water extract.

Bioassay of herb extracts for Anthracnose control in strawberry

Strawberry (Fragaria × ananassa Duch.) runner plants of three cultivars (‘Sachinoka’, ‘Akihime’, and ‘Tochiotome’ that are susceptible to anthracnose) were grown in pots (10.5 cm in diameter, 0.5 L) containing autoclaved commercial soil (SM-2; Premier Tech., Canada) and fertilized using slow-releasing granular fertilizer (Long Total 70 day type, N:P:K = 13:9:11; JCAM Agri. Co., Ltd., Tokyo, Japan). After four weeks, water extracts (20%, w/v) of lemon balm and oregano leaves were sprayed (10 mL/plant) on the strawberry plants two times before pathogen inoculation. For control plants, distilled water was used. C. gloeosporioides (C. fructicola, CG1) was cultivated on potato dextrose agar medium and incubated in dark conditions at 25°C for two weeks to facilitate sporulation; it was then subcultured for 7–10 days to facilitate further sporulation. The spores were harvested in distilled water and the concentration was adjusted to 10⁵ conidia·mL⁻¹. Each strawberry plant of the three cultivars was sprayed with 10 mL of the conidial suspension immediately after the second spraying of the herb extracts, and they were covered with plastic films for the first week to maintain humid conditions around them to facilitate inoculation (28 ± 3°C). Ten plants per treatment in triplicates were grown in a growth chamber at 28 ± 3°C with a 12 h photoperiod (750–1000 μmol·m⁻²·s⁻¹) and 70–80% relative humidity. Two weeks after inoculation, the symptoms of anthracnose were assessed as described by Li et al. (2010), i.e., percentage of dis-
Eased leaves and petioles using five levels: Level 1, 0 < □ < 20%; Level 2, 20 < □ < 40%; Level 3, 40 < □ < 60%; Level 4, 60 < □ < 80%; Level 5, 80 < □ < 100%. The disease index was calculated using the following formula:

Disease index = \( \frac{\Sigma (\text{number of plants} \times \text{number of degree in symptoms})}{\text{Total number of plants} \times \text{maximum degree in symptoms}} \times 100 \)

Ten plants were randomly chosen from each treatment and separated to shoots (compatible leaves and petioles), crown and roots. The roots were cleaned very carefully under slow flowing tap water in a tray to remove soil and debris and prevent loss of fine roots. After that, they were dried using a constant temperature drier (ETTAS 600B; AS ONE Corporation, Osaka, Japan) at 80°C for two days. The dry weights of shoots and roots were then measured.

**Analysis of herb water extracts using UPLC-MS/MS**

From the cryopreserved samples of five plants, 0.6 g of lemon balm and oregano leaves were pulverized separately in a mortar with liquid nitrogen to a fine powder and mixed with 3 mL of ultrapure water to prepare a sample extract solution (20%, w/v) for each. The sample solutions were then centrifuged at 13,000 rpm for 15 min at 4°C using Nanosep 10K (Pall Corporation, Tokyo, Japan) to remove proteins in the extracts; the supernatants were then distributed separately in a mortar with liquid nitrogen to a fine powder. The samples were analyzed using UPLC-MS/MS (Waters Corporation, Milford, MA, USA). A reversed-phase column (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 x 100 mm; Waters Corporation) with a thermostat at 25°C was used for the analysis. The mobile phases comprised 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.4 mL/min. The sample injection volume was 10 μL. The gradient profile was as follows: 0–6 min, 95% A; 6–12 min, 75% A; 12–30 min, 65% A; 30–32.5 min, 5% A; and 32.5–35 min, 95% A. The mass range of electrospay ionization was analyzed in negative mode at 50–1000 m/z using a mass spectrometer (Xevo Q ToF MS; Waters Corporation), and MS/MS collision was performed at 30 V. A mass chromatogram of the m/z value of each component in the extract was prepared from the measurements obtained using the retention time.

**Evaluation of identified compounds for antifungal effect against CG1**

The four major compounds identified through UPLC-MS/MS analysis were evaluated for their efficacy against CG1 using the poisoned food technique (Gupta and Tripathi, 2011). Two milligrams of rosmarinic acid, luteolin (both identified in the water extract of lemon balm), protocatechuic acid, and apigenin (both identified in the water extract of oregano) were separately dissolved in 40 μL of ethanol, and 960 μL of distilled water was added to each of the four solutions. Then, 1 mL of each of these solutions was mixed with 19 mL of Czapek-Dox agar media separately and poured into 9-cm sterile Petri dishes; distilled water was used as a control. After solidification of the media, a mycelial disk (5 mm) of 1-week old CG1 was cut using a cork borer and placed on the center of the media of each petri dish. The experiment was performed in triplicate for each compound. The inoculated plates were then incubated at 28°C and the diameters of the colonies were measured every day for seven days. The percent inhibition for each compound was determined using the following formula:

\[
\text{Percent inhibition} = \frac{X - Y}{X} \times 100
\]

Where \(X\): diameter of fungal colony growth on control plate and \(Y\): diameter of fungal colony growth on plates containing identified compounds.

**Statistical analyses**

The mean values for the dry weights of shoots and roots and antifungal effects of the identified compounds were analyzed by Tukey’s multiple range test at \(P < 0.05\). As the data for disease index were non-normally distributed, they were analyzed using the Steel–Dwass multiple range test \((P < 0.05)\). All the analyses were conducted using XLSTAT 2012 pro statistical analysis software (Addinsoft Inc., New York, NY, USA).

**Results**

Two weeks after *C. gloeosporioides* (*C. fructicola*, CG1) inoculation, the strawberry plants treated with herb extracts showed a significant increase in their shoot dry weight compared to that of the control, except for ‘Akihime’, under oregano treatment (Fig. 1). No significant difference was observed between the two herb treatments in terms of shoot dry weights. The dry weights of roots increased in both the herb treatments compared to the control; however, in ‘Akihime’, the control plants had a heavier dry weight of roots compared to the extract-treated plants. Similar to shoot dry weight, no significant difference was observed between the two herb treatments in terms of root dry weights.

The incidence of anthracnose in control plants reached 100% in all the cultivars except for ‘Akihime’, and 25% of plants in all the cultivars had a severity level of 5 (Fig. 2A). On the contrary, plants treated with oregano exhibited lower disease incidence compared to that of the control, except for ‘Akihime’, showing a lower severity level in all cultivars. The lowest disease severity (level 1) in all the cultivars was observed in plants treated with lemon balm; the disease incidence was also considerably lower in lemon balm-treated plants.
plants compared to control plants. Both the herb extract-treated plants exhibited a significant decrease in their disease indices compared to the control, with the lowest indices observed in lemon balm-treated plants in all the cultivars (Fig. 2B).

An analysis of water extracts of lemon balm and oregano was conducted using UPLC-MS/MS and represented in the form of chromatograms and spectrum graphs (Fig. 3). From the chromatograms, the most promising region of compounds with high peaks was observed between a retention time of 5 and 15 min. Regarding the lemon balm extract, compounds with m/z values of approximately 359 and 295 were observed for the two major peaks found at 12.29 min and 10.47 min, respectively (Fig. 3A, B). After cross-referencing this data with the mass bank (https://massbank.eu/MassBank/), the compounds were identified to be rosmarinic acid and luteolin. Another peak at a retention time of 9.81 min was observed. This appeared to be a fragment of the rosmarinic acid compound; however, its content was lower than that of the rosmarinic acid at 12.29 min (Fig. 3A). Oregano extract exhibited the two highest peaks at 10.05 min and 11.30 min, with corresponding m/z values of approximately 269 and 153 (Fig. 3C, D). After comparing this data with the mass bank, the compounds were identified to be apigenin and protocatechuic acid. All four of these compounds were found to be stable and present at higher amounts than the other compounds present in the extracts; therefore, they were selected for in vitro antifungal evaluation against CG1 in the present study.

The antifungal evaluation of the major components identified from lemon balm and oregano against CG1 showed promising results as observable in Figure 4. Among the four identified compounds from the two herb extracts, rosmarinic acid (61.7%) exhibited maximum growth suppression, which was statistically similar to the values exhibited by apigenin and protocatechuic acid (58.7 and 58.4%, respectively). Although luteolin showed lower suppression among the four compounds, it was still able to suppress mycelial growth close to 50%.

Discussion

Lamiaceae herbs contain a large number of phenolic compounds with antibacterial, antifungal, and antiviral properties (Bais et al., 2002). However, most reports on...
the activities of herb extracts were from *in vitro* studies. Although *in vitro* studies are critical in the identification of plant extracts with potential agricultural applications, *in vivo* evidence is required for their adoption for commercial use (Gorris and Smid, 1995). In the present study, the incidence of anthracnose was considerably lower in all the strawberry cultivars under the herb treatments compared to that of the control. Even though the cultivars tested were quite susceptible to anthracnose disease, the application of herb extracts prevented the disease from reaching higher severity levels as compared to level of the control. Moreover, the disease indices were also lower in herb-treated strawberry plants. In addition, disease suppression also led to the healthy development of the plants as observed by heavier dry weights of their shoots and roots. Lemon balm and oregano extracts have already been reported to possess antimicrobial and preservative effects (Abdellatif et al., 2014; Oliva et al., 2015); however, these activities were attributed to the volatile EOs present in the herbs (Quintanilla et al., 2002; Vardar-Unlu et al., 2003; Soylu et al., 2010). In the present study, we showed that water extracts of the herbs also have the potential to suppress disease-causing organisms.

The analysis of lemon balm and oregano water extracts using UPLC-MS/MS was represented as chromatograms and the two highest peaks and their corresponding m/z values were cross-referenced with mass bank; the two compounds related to the two high-
The presence of rosmarinic acid in lemon balm has been reported previously in various studies (Tóth et al., 2003; Miron et al., 2013), and its content was found to be higher in lemon balm than that in most other Lamiaceae herbs (Zgórka and Glowiak, 2001). The presence of luteolin in lemon balm has also been reported in several studies (Patora and Klimek, 2002; Ordaz et al., 2018), and it is one of the common flavonoids found in both methanolic as well as aqueous extracts. The presence of rosmarinic acid and luteolin in a water extract of lemon balm was also confirmed in our previous study (Ahmad and Matsubara, 2020). In addition, the presence of apigenin and protocatechuic acid has also been reported in several species of oregano and they are common in both aqueous and non-aqueous extracts (Gutiérrez-Grijalva et al., 2017). The high content of these four compounds in lemon balm and oregano, respectively, makes them the likely principle components of the extracts responsible for suppressing disease in this study; therefore, to confirm their antifungal activities, we performed further in vitro antifungal assays.

The four identified compounds showed considerable suppression of C. gloeosporioides (C. fructicola, CG1) in an in vitro antifungal assay. It has been hypothesized that phenolic acids such as rosmarinic acid act as phytoanticipins in plants (Dixon, 2001). Bais et al. (2002) reported that the antifungal activity of rosmarinic acid is exerted through breakage of the interseptum of the fungal mycelia and damage to the fungal cell surface by pilferage. Such specific activity of rosmarinic acid against microorganisms makes it a potent and novel antimicrobial agent. Flavonoids such as luteolin and apigenin have been reported to possess membrane disruption ability (Górnia et al., 2019). Ollila et al. (2002) reported that flavones such as apigenin cause destabilization of the membrane structure by disordering and disorienting the membrane lipids, inducing leakage. Furthermore, flavonoids have also been reported to cause bacterial aggregation by partially lysing them, leading to membrane fusion and consequently reducing the active nutrient uptake due to a smaller membrane (Górnia et al., 2019). Apigenin was also found to have antifungal activity against bacteria (Awolola et al., 2014). Xie et al. (2014) linked the antimicrobial effects of flavonoids to their capacity to form complexes with extracellular and soluble proteins and with the cell wall. Luteolin was reported to possess antifungal activity against Aspergillus niger, Trichophyton mentagrophytes, and Candida albicans (Abad et al., 2007). Protocatechuic acid was reported to have considerable antimicrobial activity against several gram-positive and -negative bacteria (Alves et al., 2013). Therefore, the presence of rosmarinic acid and luteolin in lemon balm extract, as well as apigenin and protocatechuic acid in oregano extract, could be responsible for the antifungal effect of these extracts. Furthermore, the compounds could have worked synergistically in suppressing the pathogen when applied as water extracts of the herbs, which indicates their potential as antimicrobial agents. Most importantly, combining the findings of our previous study (Ahmad and Matsubara, 2020), which evaluated the effect of major compounds in lemon balm extract on Fusarium oxysporum f. ap. fragariae, and the present study, we can conclude that the major compounds in lemon balm extract, especially rosmarinic acid and luteolin, suppress the growth of both F. oxysporum and C. gloeosporioides. Therefore, it can be hypothesized that lemon balm extract may exert a dual suppression effect against both these diseases. Considering the severe detrimental effect of synthetic agrochemicals, it could potentially provide an economically viable solution to commercial farmers against not one, but two major diseases; however, further studies are required to clarify this hypothesis. The absence of caffeic acid in the lemon balm extract in the present study is in stark contrast to our previous study and could be the result of fluctuating levels observed in supplemental analysis of the extract in the previous study (Ahmad and Matsubara, 2020). However, it did not prevent the extract from exhibiting disease suppression due to the stable presence of rosmarinic acid and high luteolin content. Moreover, the identified compounds in oregano also showed suppression of anthracnose in this study, giving an alternative choice of extract for use against this disease and indicating the potential versatile applicability of different herb extracts from the Lamiaceae family.

The general application method adopted by farmers for controlling diseases such as anthracnose is usually by spraying agrochemicals. However, the innate volatile nature of the EOs prevents their application by spraying in field conditions as these spontaneously evaporate from the applied surface, rendering them useless (Letessier et al., 2001). Conversely, water extracts are nonvolatile and could remain effective for a longer time, allowing their practical use.

In the present study, the direct effects of the antifungal properties of lemon balm and oregano water extract on C. gloeosporioides (C. fructicola, CG1) and subsequent disease suppression were observed. From the findings, it can be concluded that Lamiaceae herb extracts have the potential to suppress major strawberry diseases like anthracnose and can be utilized as an eco-friendly control measure in field conditions. In addition, there may be some indirect defense mechanisms, such as induced systemic resistance through phytoalexins accumulation (Dalisay and Kuc, 1995; Colpas et al., 2009), which could also be playing a role in disease suppression. Furthermore, the antioxidants present in the herb extracts could also be playing a key role in dis-
ease resistance by suppressing pathogenesis-related reactive oxygen species production. Future experiments targeting all the above possible hypotheses will help us better understand the multifaceted mechanisms of these herb extracts and the possible dual suppression of anthracnose and Fusarium wilt in strawberry in field conditions. In addition, other herbs in the Lamiaceae family besides lemon balm and oregano must also be screened for the presence of similar properties and to determine whether they possess similar suppressive effects on CG1. Finally, evaluation of their antifungal properties against other top and soil-borne diseases and applications to other crops should also be considered in the future.

**Literature Cited**

Abad, M. J., M. Ansueategui and P. Bermejo. 2007. Active antifungal substances from natural sources. ARKIVOC 2007: 116–145.

Abdellatif, F., H. Boudjella, A. Zitouni and A. Hassani. 2014. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J. Appl. Microbiol. 115: 346–357.

Adorjan, B. and G. Buchbauer. 2010. Biological properties of essential oils: an updated review. Flav. Fragr. J. 25: 407–426.

Ahmad, H. and Y. Matsubara. 2020. Effect of Lemon balm water extraction on fusarium wilt control in strawberry and antifungal properties of secondary metabolites. Hort. J. 89: 175–181.

Alves, M., I. Ferreira, H. Froufe, R. Abreu, A. Martins and M. Pintado. 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J. Appl. Microbiol. 115: 346–357.

Awolola, G. V., N. A. Koobranally, H. Chenia, F. O. Shode and H. Bajnath. 2014. Antibacterial and anti-biofilm activity of flavonoids and triterpene isolated from the extracts of Melissa officinalis L. EXCLI J. 13: 772–781.

Dixon, R. A. 2001. Natural products and plant disease resistance. Nature 411: 843–847.

Elshafie, H. S., M. F. Armentano, M. Carmosino, S. A. Bufo, V. De Feo and I. Camele. 2017. Cytotoxic activity of Origanum vulgare L. on hepatocellular carcinoma cell line HepG2 and evaluation of its biological activity. Molecules 22: 1435.

Gao, Y. Y., L. F. He, B. X. Li, W. Mu, J. Lin and F. Liu. 2017. Sensitivity of Colletotrichum acutatum to six fungicides and reduction in incidence and severity of chili anthracnose using pyraclostrobin. Austraras. Pathol. Pathol. 46: 521–528.

Gird, C. E., L. E. Dutu, T. Costea, I. Nencu, M. L. Popescu and O. O. Tudorel. 2016. Preliminary research concerning the obtaining of herbal extracts with potential neuroprotective activity note I. Obtaining and characterization of a selective Origanum vulgare L. dry extract. Farmacia 64: 680–687.

Gomes, M., M. Cardoso, M. Soares, L. Batista, S. Machado, M. Andrade, C. Azeredo, J. Resende and L. Rodrigues. 2014. Use of essential oils of the genus Citrus as biocidal agents. Am. J. Plant Sci. 5: 299–305.

Gonçalves, M., Z. Balmus, A. Benea, V. Barsan and T. Sandu. 2015. Biochemical diversity of the Origanum vulgare ssp. vulgare L. and Origanum vulgare ssp. hirtum (Link) etswaart genotypes from Moldova. J. ASL. Life Sci. 2: 92–100.

Górniai, I., R. Bartoszewski and J. Króliczewski. 2019. Comprehensive review of antimicrobial activities of plant flavonoids. J. Phytochem. Rev. 18: 241–272.

Gorris, L. G. M. and E. J. Smid. 1995. Crop protection using natural antifungal compounds. Pestic. Outlook 6: 20–24.

Gupta, S. K. and S. C. Tripathi. 2011. Fungitoxic activity of Solanum torvum against Fusarium sacchari. Plant Protect. Sci. 47: 83–91.

Gutiérrez-Grijalva, E. P., M. A. Picos-Salas, N. Leyva-López, M. S. Criollo-Mendoza, G. Vázquez-Ólivo and J. B. Heredia. 2017. Flavonoids and phenolic acids from oregano: occurrence, biological activity and health benefits. Plants 7: 2.

Howard, C. M. and E. E. Albregts. 1983. Black leaf spot phase of strawberry anthracnose caused by Colletotrichum gloeosporioides (C. fragariae). Plant Dis. 67: 1144–1146.

Howard, C. M. and E. E. Albregts. 1984. Anthracnose of strawberry fruit caused by Glomerella cingulata in Florida. Plant Dis. 68: 824–825.

Howard, C. M., J. L. Maas, C. K. Chandler and E. E. Albregts. 1992. Anthracnose of strawberry caused by the Colletotrichum complex in Florida. Plant Dis. 76: 976–981.

Isman, M. B. 2000. Plant essential oils for pest and disease management. Crop Prot. 19: 503–608.

Jilková, B., J. Víchová, R. Pokorný and K. Vježárka. 2015. Sensitivity of Colletotrichum acutatum isolates to selected fungicides. Acta Univ. Agric. et Silvic. Mendel. Brun. 63: 1111–1119.

Legard, D. E., S. J. MacKenzie, J. C. Mertley and C. K. Chandler. 2003. Evaluation of fungicides to control anthracnose fruit rot of strawberry, 2001–2002. Fungic. Nematicide Tests 58: SMF009. DOI: 10.1094/FN58.

Letessier, M. P., K. P. Svoboda and D. R. Walters. 2001. Antifungal activity of the essential oil of hyssop (Hyssopus officinalis). J. Phytopathol. 149: 673–678.

Li, Y., Y. Miyawaki, T. Okada and Y. Matsubara. 2010. Disease tolerance and changes in antioxidative abilities in mycorrhizal strawberry plants. J. Japan. Soc. Hort. Sci. 79: 174–178.

Martino, L. D., V. D. Feo and F. Nazzaro. 2009. Chemical composition and in vitro antimicrobial and mutagenic activities of seven Lamiaceae essential oils. Molecules 14: 4213–
Soylu, E. M., S. Kurt and S. Soylu. 2010. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. Int. J. Food Microbiol. 143: 183–189.

Spiridon, L., S. Colceru, N. Anghel, C. A. Teaca, R. Bodirlea and A. Armatu. 2011. Antioxidant capacity and total phenolic contents of oregano (Origanum vulgare), lavender (Lavandula angustifolia) and lemon balm (Melissa officinalis) from Romania. Nat. Prod. Res. 25: 1657–1661.

Stanojevic, D., L. Comic, O. Stefanovic and S. Suk dolak. 2010. In Vitro synergistic antibacterial activity of Salvia officinalis L. and some preservatives. Arch. Biol. Sci. 62: 175–183.

Teixeira, B., A. Marques, C. Ramos, C. Serrano, O. Matos, N. R. Neng, J. M. Nogueira, J. A. Saraiva and M. L. Nunes. 2013. Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oils. J. Sci. Food Agr. 93: 2707–2714.

Tóth, J., M. Mrilianová, D. Tekefová and M. Koreňová. 2003. Rosmarinic acid—an important phenolic active compound of lemon balm (Melissa officinalis L.). Acta Fac. Pharm. Univ. Comenianae 50: 139–146.

Vardar-Unlu, G., F. Candan, A. Sokmen, D. Daferera, M. Polissiou, M. Sokmen, E. Dönmez and B. Tepe. 2003. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatus Fisch. et Mey. var. pectinatus (Lamiaceae). J. Agric. Food Chem. 51: 63–67.

Weerakkody, N. S., N. Caffin, L. K. Lambert, M. S. Turner and G. A. Dykes. 2011. Synergistic antimicrobial activity of galangal (Alpinia galanga), rosemary (Rosmarinus officinalis) and lemon iron bark (Eucalyptus staigerana) extracts. J. Sci. Food Agr. 91: 461–468.

Xie, Y., W. Yang, F. Tang, X. Chen and L. Ren. 2014. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr. Med. Chem. 22: 132–149.

Zgórka, G. and K. Głowniak. 2001. Variation of free phenolic acids in medicinal plants belonging to Lamiaceae family. J. Pharm. Biomed. Anal. 26: 79–87.

Zhang, Z., F. Vriese koop, Q. Yuan and H. Liang. 2014. Effects of nisin on the antimicrobial activity of D-limonene and its nano emulsion. Food Chem. 150: 307–312.

Smith, B. J. 2008. Epidemiology and pathology of strawberry anthracnose: A North American perspective. HortScience 43: 69–73.

Soylu, E. M., S. Kurt and S. Soylu. 2010. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. Int. J. Food Microbiol. 143: 183–189.

Spirdon, L., S. Colceru, N. Anghel, C. A. Teaca, R. Bodirlea and A. Armatu. 2011. Antioxidant capacity and total phenolic contents of oregano (Origanum vulgare), lavender (Lavandula angustifolia) and lemon balm (Melissa officinalis) from Romania. Nat. Prod. Res. 25: 1657–1661.

Stanojevic, D., L. Comic, O. Stefanovic and S. Suk dolak. 2010. In Vitro synergistic antibacterial activity of Salvia officinalis L. and some preservatives. Arch. Biol. Sci. 62: 175–183.

Teixeira, B., A. Marques, C. Ramos, C. Serrano, O. Matos, N. R. Neng, J. M. Nogueira, J. A. Saraiva and M. L. Nunes. 2013. Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oils. J. Sci. Food Agr. 93: 2707–2714.

Tóth, J., M. Mrilianová, D. Tekefová and M. Koreňová. 2003. Rosmarinic acid—an important phenolic active compound of lemon balm (Melissa officinalis L.). Acta Fac. Pharm. Univ. Comenianae 50: 139–146.

Vardar-Unlu, G., F. Candan, A. Sokmen, D. Daferera, M. Polissiou, M. Sokmen, E. Dönmez and B. Tepe. 2003. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatus Fisch. et Mey. var. pectinatus (Lamiaceae). J. Agric. Food Chem. 51: 63–67.

Weerakkody, N. S., N. Caffin, L. K. Lambert, M. S. Turner and G. A. Dykes. 2011. Synergistic antimicrobial activity of galangal (Alpinia galanga), rosemary (Rosmarinus officinalis) and lemon iron bark (Eucalyptus staigerana) extracts. J. Sci. Food Agr. 91: 461–468.

Xie, Y., W. Yang, F. Tang, X. Chen and L. Ren. 2014. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr. Med. Chem. 22: 132–149.

Zgórka, G. and K. Głowniak. 2001. Variation of free phenolic acids in medicinal plants belonging to Lamiaceae family. J. Pharm. Biomed. Anal. 26: 79–87.

Zhang, Z., F. Vriese koop, Q. Yuan and H. Liang. 2014. Effects of nisin on the antimicrobial activity of D-limonene and its nano emulsion. Food Chem. 150: 307–312.