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Original Article

Effects of Salicylic Acid and Chitosan Foliar Application on Extract Components and the Antibacterial Activity of Melissa officinalis L.

Negin Safari Kamal Abadi[1], Naser Mohebalipour[2*], Mehdi Oraei[1], Hassan Nourafkan[3], Assad Assadi[4]

[1] Department of Horticulture, Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran
[2] Department of Agronomy, Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran
[3] Department of Horticulture, Medicinal Plants and Organic Products Research Center, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran
[4]Department of Veterinary Medicine, Faculty of veterinary, Medicinal Plants and Organic Products Research Center, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran

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Abstract

Background and Aim: *Melissa officinalis* L. is a medicinal herb with antibacterial properties. This research was carried out to investigate the effects of the foliar application of salicylic acid (SA) and chitosan (Ch.) on ethanolic extracts components and antibacterial activity of *M. officinalis*L.in the flowering stage.

Materials and Methods: The antibacterial activities of the ethanol extracts were investigated using the agar dilution method, minimal inhibitory concentration (MIC), and minimal bactericidal concentration against five bacteria, i.e., *S. aurous*, *B. subtilis*, *E. coli*, *S. enterica* and *P. mirabilis*.

Results: The major components were citronellal, Z-Citral, E-Citral, caryophyllene oxide, caryophyllene, linalool, carvacrol, α-Pinene and geraniol. The result showed that distinct concentration of Ch. and SA had remarkable effects on the chemical constituents of *M. officinalis* L. extract. All the examined bacteria were sensitive to the extracts of *M. officinalis* L. and antimicrobial activities of extracts against the examined bacteria depending on the concentration of the foliar application. The highest and lowest diameters of inhibition zones of the plant extracts were on *S. aurous* and *P. mirabilis* respectively. The MIC values of the extracts ranged from 78.25 ppm to 1250 ppm, whereas the MBC values ranged from 156.5 ppm to 1250 ppm.

Conclusion: *S. aurous* and *E. coli* were the most sensitive bacteria in Gram-positive bacteria and Gram-negative bacteria respectively. It was also observed that Ch. treatments were more effective on Gram-negative bacteria, while SA treatments were more influential on Gram-positive bacteria.

Keywords: *Melissaofficinalis* L.**,** Inhibitory, Agar diffusion, Chemical composition, Foliar application

***Corresponding Author:** Naser Mohebalipour, Department of Agronomy, Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran. Email: n.mohebalipour@gmail.com.

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Introduction

Toxicity and carcinogenicity of synthetic additives are two characteristics of these additives that have encouraged scientists and researchers in the food industry to look for alternatives, especially naturally-occurring antimicrobial agents (1). Medicinal herbs utilized in traditional medicine in the treatment of infectious illnesses seem to be a plentiful source of new bioactive secondary metabolites. Hence, several medicinal plants and plant extracts have been investigated with regard to their antibacterial activity in recent years (3,4). Essential oils and extracts are indeed natural products of by aromatic herbs. Their major constituents are terpenes and terpenoids (4).

It has been indicated that *M. officinalis* L. extracts have remarkable antimicrobial activities against viral, bacterial and fungal infections (5, 6). Lemon balm extract that has been externally used as a cream reduces the healing time of herpes mouth sores (recurring herpes labialis). Moreover, this extract hinders their spread and alleviates the symptoms of itching (7). Furthermore, the aqueous extract of *M. officinalis* L. has significant anti-HIV-1 activity (8). *M. officinalis* L. was also of great interest because of its exhibition of antioxidant (9), anti-inflammatory (10), sedative (11), hypolipidemic (12), and antiulcer genic properties (13). Application of herbal extracts and phytochemicals that have antimicrobial activities can be highly significant in therapeutic treatments. In the last few years, many studies have conducted on the significance of bioactive herbal extracts and pure isolated compounds in improving the in vitro efficacy of widely used antibiotics against different kinds of microorganisms (14-16).

The use of *M. officinalis* L. (Lemon balm) in traditional medicine in the treatment of insomnia, anxiety, gastric disorders, psychiatric disorders, migraines, hypertension and bronchial constrictions has been reported (17). Its antihyperlipidemic, anti-inflammatory and antioxidant characteristic have been recently indicated (18, 19). Moreover, it has been shown that the *M. officinalis* L. leaf extract has antiviral properties because of the content of phenolic acids, whereas its essential oil has antibacterial, antifungal, and antihistaminic activities (20, 21).

The factors that may affect the secondary metabolites of this plant are the type of species, geographical location, plant growth site (soil type, weather, altitude and water content), harvest time, the genetic structure of the plant, growth process, fertilization etc. (22,23).

The foliar application of nutrient elements method as a supplement to the soil method is an effective way of using low and high-consumption elements, amino acids, humic and fulvic acids, plant growth hormones, seaweed extracts and carbohydrates. Salicylic acid and chitosan are environmentally friendly immune stimulants that improve the morpho-physiological properties of plants through foliar application (24-27).

Salicylic acid, or ortho-hydroxybenzoic acid, is a type of phenolic compound. The compounds of this group can act as plant growth regulators (28). Salicylic acid as an elicitor can produce secondary metabolites in the plant (29). As a bio-polymer, chitosan is one of the most important derivatives of chitin. In contrast to synthetic polymer compounds, chitosan is non-toxic and completely compatible with living tissues, and it is decomposable in the environment (30).

However, limited knowledge exists regarding the impacts of salicylic acid and chitosan's foliar application on antibacterial activity. Hence, the aim of this study was to examine the effects of salicylic acid and chitosan on the antimicrobial activity of *M. officinalis.* The present research was conducted to investigate the effects of foliar application of SA and Ch. on the ethanolic extracts components and antibacterial activity of *M. officinalis*L.in the flowering stage.

Materials and Methods

Experimental Sites

The experiment was conducted at the research farm of Agricultural and Natural Resources Research and Education Center, Kerman, Iran, in 2017.The soil sample was air-dried and tested for pH, electrical conductivity (EC), organic matter (through sulfuric acid method), soil texture (hydrometer method), nitrogen (Kjeldahl method), phosphorus (Olsen procedure) and potassium after extraction with ammonium acetate. The soil and water characteristics of the experimental sites have been shown in Table1. This study was extracted from a PhD thesis at Miyaneh Branch, Islamic Azad University, Miyaneh, East

Azarbaijan, Iran. The research code is 21850247951001.

Experimental Design

A factorial experiment was carried out on randomized complete block design with salicylic acid (Merck.co) in four concentrations (50, 100, 150 and 200 mg/l) (31). Chitosan (Merck.co) had four concentrations (50, 100, 150 and 200 mg/l) (32) and three replicates. The seeds of *M. officinalis* L. (Lemona F1 type) were harvested from Pakan Seed Company, Isfahan, Iran. The seeds of *M. officinalis* L. were cultivated in seedling trays, each including 100 cells with 50mm depth, 40mm diameter, and30 cm^3 volume that was filled with a mixture of cocopeat and sand, on March 15. The seedlings were transferred from the seedling trays to the pots before the four-leaf stage in early May. The first foliar application was performed in the four-leaf stage, and other steps were seven days after the previous step. Some plants were sprayed with distilled water, and the plants that were not suitable for foliar application were considered as control treatment. One week after the last foliar application the plants were harvested for extracting and photochemical evaluation. In the present research, no inorganic fertilizer and systemic pesticide were utilized throughout the experiment, and weed control was carried out manually.

Preparation of the Ethanolic Extract

Vegetative bodies of each plant were harvested and shadow dried at 22–27℃. After drying, 50 g of plant powder was added to 200 ml of 75% ethanol solution at 25℃ and the extract was evaporated by a vacuum rotary evaporator (IKA Germany Company, model RV 8) and filtered through Whatman No.41 paper to obtain particle free extract (40). The concentrated extract was dried in oven (Germany Company, model UF450) at 2 ℃ for 2 hr. Dimethyl Sulfoxide (DMSO) was used to dissolve the extract powder. The sterilized needle was used for sterilization and the filtered extracts were kept at-18℃ in sterile tubes until the time of testing (33).

GC-MS analysis

GC-MS analysis was performed using an Agilent 6890N GC-MS fitted with an Agilent 5973 capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The extract solvent used for the analysis of GC/MS was n-hexane. The GC oven temperature was programmed from 60-220 °C at the rate of 5 °C/min. The carrier gas was helium (99.999%) at a flow rate of 1 ml/min and ionization energy of 70 electron volts. The identification of compounds was carried out using the library's proposal fora gas chromatograph machine connected to a mass spectrometer, comparing compounds with values published in various sources, and using the information from the NIST library (34).

Detection of the Antibacterial Activity of the Extract

Bacterial Strains

We examined the antibacterial activity of the herbal extracts using Gram-positive (*S. aureus* ATCC 1787 and *B. cereus* ATCC 11778) and Gram-negative bacterial strains (*E. coli* ATCC 1399, *S. enterica* ATCC 13076 and *P. mirabilis* ATCC 43071). The reference strains were purchased from Persian Type Culture Collection in Iran.

Chemicals, Microbial Media and Inoculum Preparation

Dimethylsulphoxide (DMSO) used in this study was obtained from Sigma-Aldrich, USA. Ampicillin and gentamicin were also purchased from Padtan Teb Company, Iran. All microbial media, Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB), Nutrient Broth and Nutrient agar were provided by Merck, Germany. Investigated bacterial were prepared from Persian Type Culture Collection in Iran.

MIC and MBC Evaluations

The tube dilution method is an accurate and sensitive method which is to identify and evaluate the antimicrobial properties of herbal extracts (35,36). Minimum inhibitory concentration (MIC) is the minimum concentration of extracts that can inhibit bacterial growth at the rate of 90 percent, and a minimum bacterial concentration (MBC) is the minimum amount of extract that prevents the growth of bacteria at 99.9% (8). MIC and MBC of the antimicrobial activity can be determined using the tube dilution method. To determine the MIC of the *M. officinalis* L. extract, a series of 12 glass tubes were used. Nine test tubes were used to test various extract dilutions, a tube was used as a negative control (extract diluted plus medium), and finally another tube was used as a positive control (containing bacterial suspension plus medium). Moreover, a tube containing solvents,

Table 1: Soil physico-chemical properties and water characteristics of experimental sites.

Soil properties	Values	Water characteristics	Values
EC (dS/m)	2.65	EC[1] (dS/m)	2.8
pH	7.2	pH	7
Organic matter (%)	0.45	Ca^{2+} (mequiv/l)	11
Nitrogen (%)	0.07	Mg^{2+} (mequiv/l)	10
Phosphorus (mg/kg)	10.9	Na^{+} (mequiv/l)	6.9
Potassium (mg/kg)	202	SAR[1]	3.2
Soil texture	Loam-clay	Cl^{-} (mequiv/l)	16

the microbial suspension and culture was used to ensure the growth of bacteria in media containing the solvent used for extraction. The initial concentration was 5 mg/ml. By the serial dilution of 50% of tube No.1, its concentration reached 2.5 mg/ml, and a concentration of 0.00976 mg/ml was obtained for tube 9. Subsequently, 50 µl of bacterial suspension with 1.5×10^8 CFU/ml was added to all the tubes except tube No. 10 (negative control). Extract dilution for all the bacteria was tested. All the test tubes were incubated for 24 hours at the temperature 37 °C. Then, the turbidity of inoculated bacteria was examined. The last growth inhibition tube was recorded as the inhibitory concentration of the extract. Subsequently, the tube in which the bacteria had not grown was used to determine MBC by surface culture method. To this end, 100 ml of the tubes that had shown no bacterial growth was spread on Muller Hinton agar medium. After incubating for 24 hours, the plates were cultured for the presence of microbial growth control. The tube containing the lowest extract concentration in which no bacterial growth was observed was considered as the MBC extract (35, 36).

Disk Diffusion Method

In this method, the antibacterial properties of the *M. officinalis* L. extract was evaluated based on agar diffusion bioassay (37). To this end, a suspension equivalent to 0.5 of McFarland (1.5×10^8 CFU/ml) was prepared, and then 0.1 ml of the bacterial suspension was cultured on Müller Hinton agar medium (Muller Hinton Agar, Merck) to be completely spread on the medium by L-shaped glass bar. To inoculate the extract, 30 microliters of each *M. officinalis* L. extract with a concentration of 5 mg/ml were injected into each sterile Whatman filter paper discs. Then, the discs were placed on a sterile mesh plate for one hour to completely absorb the extract. The antibiotic discs of gentamicin and ampicillin at a concentration of 4 mg/ml. was used as a positive control, and sterile distilled water disk as well as dimethyl sulfoxide (DMSO) disk were used as negative control. All the plates were incubated for 24 hours at 37 ° C and the antibacterial activity was performed based on measuring the diameter of the non-growth range around the discs in millimeters. Subsequently, it was compared with the control groups. All the disc placements were repeated three times (37, 38).

Well Diffusion Method

In this method, first a turbidity equivalent to 0.5 McFarland was prepared from the tested bacteria and then cultured in Müller Hinton agar medium. Subsequently, on the Müller Hinton, the wells with a diameter of 5mm and intervals of 2.5mm from each other and 2.4 mm from the edge of the plate were created by sterile Pasteur pipette. In each of the wells, 30 micro litter of different dilutions of the studied extracts were filled. Ampicillin and Gentamicin antibiotics with a concentration of 4 mg/ml were used as positive controls and DMSO (Di Methyl Sulfa Oxide) and distilled water were used as negative controls. All the cultures were incubated at 37 ° C for 24 h. Bacterial cultures were then measured by a caliper for the formation or non-formation of a growth range in millimeters and their mean was recorded (39).

Statistical Analysis

This experiment was carried out as a completely randomized block with three replications. A combined analysis of variance was performed using SAS (ver. 9.2, 2010). The means of treatments were compared by One-Way ANOVA and the protected least significant

difference (LSD) procedure at P<0.05.

Results and Discussion

Chemical composition

The chemical components of the plant extracts identified by GC–MS have been presented in Table 2. GC–MS analysis identified 9 major components (for ~77-85% of the total), i.e. viz., Citronellal (), Z-Citral, E-Citral, Caryophylleneoxide, Caryophyllene, Linalool, carvacrol, α-Pinene and Geraniol, respectively. The results indicated that our extracts

Table 2: Chemical compositions of extract in control and foliar spraying of *M. officinalis*L.plants.

Compounds (%)	Foliar application (mg/l)									
	Control	W	SA50	SA100	SA150	SA200	Ch50	Ch100	Ch150	Ch200
Citronellal	2.41 d	2.48 d	2.55 d	2.59 d	5.60 c	7.62 b	2.57 d	2.62 d	5.79 c	9.23 a
Z-Citral	16.57 c	16.55 d	16.70 c	17.82 b	18.56 b	13.63 e	16.68 c	18.55 b	19.67 a	15.27 d
E-Citral	26.43 a	26.33 a	26.41 a	26.61 a	18.84 b	18.66 b	26.36 a	26.75 a	16.87 d	17.75 c
Caryophyllene oxide	8.47 bc	8.88 ab	8.86 ab	9.11 ab	9.32 a	8.13 c	8.95 ab	8.93 ab	9.33 a	9.16 a
Caryophyllene	5.12 c	5.26 bc	5.22 bc	5.34 bc	5.63 b	6.84 a	5.22 bc	5.30 bc	6.74 a	5.51 bc
Linalool	0.56 c	0.66 c	0.72 c	0.72 c	1.12 b	1.67 a	0.67 c	0.67 c	0.70 c	0.92 bc
Carvacrol	4.97 c	5.03 c	5.00 c	5.01 c	5.05 c	5.16 c	4.95 c	5.11 c	6.21 b	7.64 a
α-Pinene	0.13 e	0.15 de	0.15 de	0.14 e	0.32 a	0.23 b	0.16 cde	0.17 cd	0.23 b	0.19 c
Geraniol	13.75 d	13.90 d	13.81 d	14.92 c	16.02 b	19.22 a	13.72 d	13.72 d	15.11 c	15.39 bc
Total	78.41	79.24	79.42	82.26	80.46	81.16	79.28	81.82	80.65	81.06

C: control, W: Distilled water, SA: Salicylic Acid, Ch.: Chitosan.

The rows with different letters mean statistically different according to LSD ($p \leq 0.05$) test.

Compounds with percentages less than 0.1 are not shown in the table

Table 3: The diameters of inhibition zones (mm) of *M. officinalis.* L extracts against the bacterial strains.

Foliar application (mg/l)	Bacterial strain				
	S.aurous	***B.subtilis***	***E.coli***	***S.enterica***	***P.mirabilis***
SA50	13.67ef	12.33ef	11.67 h	10.33 f	8.67fg
SA100	14ef	13.67cde	14.33 f	12.67 e	9fg
SA150	15.67 d	15 c	15ef	14 e	10.33 e
SA200	17.33 c	15 c	16.33 d	15.67 d	11.67 d
Ch50	10.67 hi	11.67 f	13 g	13.67 e	9.33 f
Ch100	11.67gh	13.33 de	16 de	14 e	10.67 e
Ch150	13fg	14.33 cd	20.33 c	15.33 d	11.67 d
Ch200	15 de	14.33 cd	23.67 b	17.67 c	13.33 c
W	12gh	13def	12.33gh	13.33 e	8.33fg
Control	10.67 hi	11.67 f	11.67 h	10.67 f	8 g
DMSO	10i	9 g	9i	9 g	8 g
Amp	23.33 b	19.67 b	24.67 b	26.33 b	16.67b
Gen	26.33 a	22 a	28.67 a	27.67 a	19 a

C: control, W: Distilled water, SA: Salicylic Acid, Ch.: Chitosan

The columns with different letters mean statistically different according to LSD ($p \leq 0.05$) test.

were characterized by the presence of three dominating constituents in monoterpenoid family type aldehyds, and an important fraction includes Citral, Z-Citral and geranial, respectively.

The One-Way ANOVA revealed that distinct concentrations of the foliar application of SA and Ch. had significant effects on the major constitutes of *M. officinalis* L. extracts ($P<0.01$). The amount of some chemical compounds, namely citronellal, caryophyllene, linalool, carvacrol and geraniol significantly increased by the foliar application with high concentrations of SA and Ch. compared with the control and other treatments. Some compounds in the extracts such as Z-citral, E-Citral, caryophylleneoxide and α-Pinenewere decreased under high foliar application of SA, and compared with the lower concentration might be attributed to stress conditions in *M. officinalis* L. plants (Table 2).

Antibacterial Activity

The antibacterial activities of *M. officinalis* L. plants extracts with regard to diameters of inhibition zones, MIC and MBC have been presented in Tables 3 and 4, respectively.

The One-Way ANOVA indicated that distinct concentrations of the foliar application of SA and Ch. had remarkable impacts on the antibacterial activity of the extracts ($P<0.01$). In the present study, the antimicrobial capacity of the extracts from all the treatments against bacteria was determined. In all the treatments, the diameters of inhibition zones were dependent to concentration of salicylic acid and chitosan foliar application. Significant inhibitory effects were observed against all the bacteria at concentrations more than 100 mg/l SA and 50 mg/l[1]Chtreatmentscompared to the control. The antibacterial activities of conventional antibiotics such as Amp and Gen against all the bacteria were stronger than Ch., and SA treatment and the antibacterial activity of Gen was greater than Amp. The comparison the diameters of inhibition zones indicated that Gram-negative bacteria were more resistant to the antimicrobial effect in *M. officinalis* L. plants extracts compared to the Gram-positive bacteria. Furthermore, the antimicrobial activity of the Ch. treatments was more effective against Gram-negative bacteria than Gram-positive bacteria. The results showed that *Proteus mirabilis* had the highest resistance with the inhibition zone diameter ranging from8.67 to 11.67 mm and 9.33 to 13.33 mm against the Ch. and SA *M. officinalis* L. extracts, respectively. Moreover, the most resistant bacteria were identified as *S. enterica, B. cereus, E. coli* and *S. aurous* (Table 3).

In all the bacteria, higher foliar application with Ch. and SA increased the antimicrobial susceptibility. The MIC

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of the ethanolic extract of *M. officinalis.* ondifferent bacterial strains.

Foliar application (mg/l)	MIC (ppm)					MBC (ppm)				
	S.aurous	***B.subtilis***	***E.coli***	***S.enterica***	***P.mirabilis***	***S.aurous***	***B.subtilis***	***E.coli***	***S.enterica***	***P.mirabilis***
SA50	156.5	313.5	650	1250	1250	313.5	650	1250	1250	1250
SA100	156.5	156.5	313.5	650	650	313.5	313.5	650	1250	1250
SA150	78.25	78.25	156.5	313.5	650	156.5	156.5	156.5	650	1250
SA200	78.25	78.25	78.25	156.5	313.5	156.5	156.5	156.5	313.5	650
Ch50	313.5	650	650	650	650	650	1250	1250	1250	1250
Ch100	156.5	313.5	313.5	313.5	313.5	313.5	650	650	1250	1250
Ch150	78.25	156.5	78.25	156.5	313.5	156.5	313.5	156.5	313.5	650
Ch200	78.25	78.25	78.25	156.5	156.5	156.5	156.5	156.5	313.5	313.5
W	313.5	650	650	1250	1250	650	1250	1250	1250	1250
Control	313.5	650	650	1250	1250	650	1250	1250	1250	1250

C: control, W: Distilled water, SA: Salicylic Acid, Ch.: Chitosan
MIC: Minimal Inhibitory Concentration, MBC: Minimal Bactericidal Concentration

values ranged from 78.25to 1250 ppm while MBC values ranged from 156.5 to 1250 ppm in foliar application treatments. The extract from *M. officinalis* L. foliar application with Ch. (200 mg/l) was found to be prominently active against the bacteria at the concentrations 78.25-313.5 ppm (MIC and MBC). The extracts obtained from SA200, Ch100 and Ch150 treatments showed moderate antibacterial activities against the bacteria (MIC and MBC). MIC and MBC increased with increasing concentrations of SA and Ch. in all the treatments. In SA200 and Ch200treatment groups, the highest inhibitory effect was observed in *S. aurous*, *B. subtilis*, *E. coli* (MIC= 78.25 ppm, MBC=156.5 ppm) and the lowest inhibitory impact was observed in S. *enterica* (MIC= 156.5 ppm, MBC=313.5 ppm) and *P. mirabilis*. (MIC= 313.5 and 156.5, MBC= 650 and 313.5 ppm, respectively). Thus, *S. aurous*, *B. subtilis* and *E. coli* exhibited the highest degree of antimicrobial susceptibility and *S. enterica* and *P. mirabilis*. showed the least degree of the same susceptibility (Table 4).

In this study, the impacts of the foliar application of SA and Ch. were investigated on alcoholic extract components and antibacterial activity of *M. officinalis* L. in the flowering stage. The results of the present study showed that different concentrations of the foliar application of SA and Ch. Had remarkable effects on the main components in the extract of *M. officinalis* L. Many studies have been conducted on the variation in the chemical composition of essential oils and extracts from foliar application with Ch. and SA33 (40-43).

In the present study, 9 main combinations, including citronellal, Z-Citral, E-Citral, caryophyllene oxide, caryophyllene, linalool, carvacrol, α-Pinene and geraniol were identified in *M. officinalis*. In the study conducted by Saeb and Gholamrezaee (44), the major components were geraniol, caryophyllene oxide, geranyl acetate, geranyl, caryophyllen, carvacrol and linalool (44). Uyanik and Gurbuz (45) reported that the major constituents were citral (25.22% and 21.20%), caryophyllene oxide (21.95% and 18.44%) and Z-Citral (19.08% and 16.03%) in leaves and flowers (45).

Ch. and SA lead increase the chemical composition of the extracts. This result has also been reported in other studies (39-48). Ch. is influential in stimulating the generation of secondary metabolites such as alkaloids, flavonoids and paranoid (49). Ch. is effective on increasing the chlorophyll content and photosynthesis of the plants. Moreover, it is an irritant element in the plant by developing chloroplast (50). It has been reported that the phenolic compounds and flavonoids in the Pune plant treated with Ch. are increased (48). Vasconsuelo et al. (46) indicated that the use of Ch. in Rubiaincreases the production of anthraquinone (46). It has also been stated that the application of Ch. in cell suspension cultures resulting from cultivation of Citrus grandis increases linalool and limonene in this plant (47). SA can alter the secondary metabolites and its pathway through its impacts on plastid and chlorophyll level (41). Idreeset al. (51) reported that any improvement in the content of essential oil by the foliar use of SA might be caused by the increase in cycle growth, nutrients uptake or alterations in leaf oil gland population and monoterpenes biosynthesis (51). SA has been referred to as a significant signaling element which is effective on the establishment of the local and systemic disease resistance response of plants following the pathogen attack (43).

In this study, the antibacterial activities of *M. officinalis* L. plants extract in terms of diameters of inhibition zones, MIC and MBC were investigated. The results showed that the antibacterial activities of *M. officinalis* L. extracts increased at more than 100 mg/lSA and 50 mg/l Ch foliar application in all the bacteria compared to the control. The application of 200 mg/l Ch was competitive with Amp commercial antibiotic. The extract of all the treatments had antimicrobial properties against all the bacteria, namely *S. aurous*, *B. subtilis*, *E. coli*, *S. enterica* and *P. mirabilis*. *S. aurous* was the most susceptible bacteria to the extract. Anicicet al. (52) found similar results in their investigation of the antimicrobial activity of *M. officinalis* L. (52). In general, Gram-negative bacteria exhibited a higher degree of resistance than Gram-positive bacteria. This may be attributed to lipopolysaccharides in the outer membrane of Gram-negative bacteria, which intrinsically resists external factors, including antibiotics (53). The results showed that **t**he impacts of chitosan on Gam-negative bacteria and salicylic acid on gram-positive bacteria were greater. The biological activities are often attributed to major compounds in the extracts (54). There were several compounds in extract

of *M. officinalis* L. with antibacterial activity such as caryophyllene oxide, linalool, carvacrol and geraniol. The cell wall is a site for action of the extract compounds. There were several mechanisms of action for the extracts. An extract and its components are hydrophobic. They divide the lipids of the bacterial cell membrane and mitochondrial, disrupting their structure and causing them to be more permeable. Subsequently, the leakage of ions and other cell contents may take place (54). Moreover, chemical components are capable of acting on cell proteins embedded in the cytoplasmic membrane (55). It has been indicated that enzymes such as ATPase's are located in the cytoplasmic membrane and are bordered by lipid molecules (54).

Conclusion

The result showed that SA and Ch both had high potentials in increasing the chemical composition and antibacterial activity of the essential oil in *M. officinalis* L. The result indicated that different concentrations of Ch. and SA had significant impacts on the chemical components of *M. officinalis* L. extract. All the tested bacteria were sensitive to the extracts of *M. officinalis* L. and antimicrobial activities of the extracts against the tested bacteria depending on the dose. The highest and lowest diameters of inhibition zones of the plant extracts were observed in *S. aurous* and *P. mirabilis* respectively. Among the studied bacteria, *S. aureus* and *E. coli* were the most sensitive bacteria. It was also observed that Ch. treatments were more effective on Gram-negative bacteria, while SA treatments had a greater impact on Gram-positive bacteria.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Feng W, Sheng X. Essential oils to control Alter aria alternate in vitro and in vivo. Food Control. 2007;18(9):1126-30.
2. Cowan MM. Plant products as antimicrobial agents. Clin.Microbiol. rev. 1999;12(4):564-482.
3. Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–an overview. Complement. Med Res. 2009;16(2):79-90.
4. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharma. 2013;6(12):1451-74.
5. Ertürk O. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants.Biol. 2006;61:275-8.
6. Nolkemper S, Reichling J, Stintzing FC, Carle R, Schnitzler P. Antiviral effect of aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 in vitro. Planta med. 2006;72:1378-82.
7. Koytchev R, Alken RG, Dundarov S. Balm mint extract (Lo-701) for topical treatment of recurring herpes labialis. Phytomed. 1999;6:225-30.
8. Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N, Oishi I, Inami R, Yamane M, Nakamura M, Murata H, Nakanishi T. Anti-HIV-1 activity of herbs in labiatae.Biol. Pharm. Bull. 1998;21:829-33.
9. Canadanović-Brunet J, Ćetković G, Đilas S, Tumbas V, Bogdanović V, Mandić A, Markov S, Cvetkovic D, Canadanovic V. Radical scavenging, antibacterial and antiproliferative activities of Melissa officinalis L. Extracts. J. Med Food. 2008;11:133-43.
10. Drozd J, Anuszewska E. The effect of the Melissa officinalis L. extract on immune response in mice. Acta. pol. pharm. 2003;60:467-70.
11. Soulimani R, Fleurentin J, Mortier F, Misslin R, Derrieu G, Pelt JM. Neurotropic action of the hydroalcoholic extract of Melissa officinalis L. in the mouse. Planta Med. 1991;57:105-9.
12. Bolkent S, Yanardag R, Karabulut-Bulan O, Yesilyaprak B. Protective role of Melissa officinalis L. extract on liver of hyperlipidemic rats: a morphological and biochemical study. J. ethnopharmacol. 2005;99:391-8.
13. Khayyal MT, El-Ghazaly MA, Kenawy SA, Seif-El-Nasr M, Mahran LG, Kafafi YA, Okpanyi SN. Antiulcerogenic effect of some gastrointestinally acting plant extracs and their combination. Arzneimittelforschung. 2001;51:545-53.
14. 1Betoni JEC, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes JA. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem. Inst. Memórias do Instituto Oswaldo Cruz. 2006;101:387-90.
15. Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr. J.Biotechnol. 2007;6:2886-96.
16. Stefanovic O, Stanojevic D, Comic Lj. Synergistic, antibacterial activity of Salvia officinalis L. and Cichoriumintybus extracts and antibiotics. Acta Pol Pharm. 2012;457-63.
17. Hăncianu M, Aprotosoaie AC, Gille E, Poiată A, Tuchiluş C, Spac A, Stănescu U. Chemical composition and in vitro antimicrobial activity of essential oil of Melissa officinalis L. from Romania. Revista medico-chirurgicala a Societatii de Medici siNaturalisti din Iasi. 2008;112(3):843-7.
18. Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of Melissa officinalis L. (Lamiaceae) essential oil. J. Agric. food chem. 2004;52:2485-9.
19. Birdane YO, Buyukokuroglu ME, Birdane FM, Cemek M, Yavuz H. Anti-inflammatory and antinociceptive effects of Melissa officinalis L. in rodents. Rev. Méd. Vét. 2007;158(02):75-81.
20. Stanojevic D, Comic LJ, Stefanovic O, Sukdolak SS. In vitro synergistic antibacterial activity of Melissa officinalis L. and some preservatives. Span. J. Agric. Res. 2010;8(1):109-15.
21. Sharopov FS, Wink M, Khalifaev DR, Zhang H, Dosoky NS, Setzer WN. Composition and bioactivity of the essential oil of Melissa officinalis L. growing wild in Tajikistan. Int. J. Tradit. Nat Med. 2013;2(2):86-96.

22. Khan MMA, Samiullah SHA, Afridi MMRK. Yield and quality of fennel (Foeniculumvulgare Mill.) in relation to basal and foliar application of nitrogen and phosphorus. J. Plant Nutr. 1992;15(11):2505-15.
23. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food Chem. Toxicol. 2008;46(2):446-475.
24. Gharib FA. Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. Int. J. Agric. Biol. 2006;8(4):485-92.
25. Ali MB, Hahn EJ, Paek KY. Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in Panax ginseng bioreactor root suspension cultures. Mol. 2007;12(3):607-21.
26. Prapagdee B, Kotchadat K, Kumsopa A, Visarathanonth N. The role of chitosan in protection of soybean from sudden death syndrome caused by Fusariumsolani f. sp. glycines. Bioresour. Technol. 2007;98(7):1353-8.
27. Boonlertnirun S, Boonraung C, Suvanasara R. Application of chitosan in rice production. J. Met. Mater. Miner. 2017;18(2):47-52.
28. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. Environ. Exp. Bot. 2010;68(1):14-25.
29. Pastirova A, Repcak M, Eliasova A. Salicylic acid induces changes of coumarin metabolites in Matricariachamomilla L. J. Plant Sci. 2004;167(4):819-24.
30. Roller S, Covill N. The antimicrobial properties of chitosan in mayonnaise and mayonnaise-based shrimp salads. J. Food Prot. 2000;63(2):202-9.
31. EmamiBistagani Z, Siadat SA, Bakhshandeh AM, GhasemiPirbalouti, A. Effect of Drought Stress and Chitosan Elucidator on Photosynthetic Pigments, Proline, Soluble Sugars and Lipid Peroxidation of Membrane in Thyme (Thymus deanensisCelak.) in Shahrekord Climatic Conditions. J. Agric. Environ. sci. 2017;1:13-20.
32. Nourafcan H, Mahboubi A. The effect of salicylic acid foliar spraying on morphophysiological characteristics of common mallow and Moldavian balm. Agroecol. J. 2017;13(3): 25-33.
33. Das K, Tiwari RKS, ShrivastavaDK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J. Med. plant res. 2010;4(2):104-11.
34. Dąbrowski Ł. Evaluation of a Simplified Method for GC/MS Qualitative Analysis of Polycyclic Aromatic Hydrocarbons, Polychlorinated Biphenyls, and Organic Pesticides Using PARADISe Computer Program. Mol. 2020;25(16):3727.
35. CLSI. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2015. Available from: https://clsi.org/media/1632/m07a10_sample.pdf.
36. Scorzoni L, Benaducci T, Almeida AMF, Silva, DHS, Bolzani, VS, Mendes-Giannini, M.J.S. Comparative study of disk diffusion and microdilution methods for evaluation of antifungal activity of natural compounds against medical yeasts Candida spp and Cryptococcus sp. J. Basic Appl. Pharm. Sci. 2007;28(1):25-34.
37. Andrews JM. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 2001;48:5-16.
38. Baner AW, Kirby WMM, Sherries JC, Truck M. Antibiotic susceptibility testing by a stand-ardized single disc method. Am J ClinPathol. 1991;45:493-6.
39. Platikanov S, Evstatieva L, Nikolov S. Dinamic of acumulation of the flavonoids in Astragalushamosus L. Proceeding of the 7th Symposium on Flora of Southeastern Serbia and Neighbouring Regions. Dimitrovgrad. 2002;214-6.
40. Bittelli M, Flury M, Campbell GS, Nichols EJ. Reduction of transpiration through foliar application of chitosan. Agric. For. Meteorol. 2001;107(3):167-75.
41. RowshanV, Khoi MK, Javidnia K. Effects of salicylic acid on quality and quantity of essential oil components in Salvia macrosiphon. J. Biol. Environ. Sci. 2010;4(11):77-82.
42. Farouk S, Amany AR. Improving growth and yield of cowpea by foliar application of chitosan under water stress. Egypt. J. Biol. 2012:14(1):14-26.
43. Ghasemi-Pirbalouti A, Rahimmalek M, Elikaei-Nejhad L, Hamedi B. Essential oil compositions of summer savory under foliar application of jasmonic acid and salicylic acid. J. Essent. Oil Res. 2014;26(5):342-7.
44. Saeb K, Gholamrezaee S. Variation of essential oil composition of Melissa officinalis L. leaves during different stages of plant growth. Asian Pac. J. Trop. Biomed. 2012;2(2):547-9.
45. Uyanik M, Gurbuz B. Chemical diversity in essential oil compositions of leaf, herb and flower in lemon balm (Melissa officinalis L.) J. Agric. Nat. Sci. 2014;1(2):210-4.
46. Vasconsuelo A, Giulietti AM, Boland R. Signal transduction events mediating chitosan stimulation of anthraquinone synthesis in Rubiatinctorum. J. Plant Sci. 2004;166(2):405-13.
47. Putalun W, Luealon W, De-Eknamkul W, Tanaka H, Shoyama Y. Improvement of artemisinin production by chitosan in hairy root cultures of Artemisia annua L. Biotechnol. Lett. 2007;29(7):1143-6.
48. Yin H, Frette XC, Christensen LP, Grevsen K. Chitosan oligosaccharides promote the content of polyphenols in Greek oregano (Origanumvulgare ssp. hirtum). J. Agric. Food Chem. 2011;60(1):136-43.
49. Namdeo AG. Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn. Rev. 2007;1(1):69-79.
50. Limpanavech P, Chaiyasuta S, Vongpromek R, Pichyangkura R, Khunwasi C, Chadchawan S, Lotrakul P, Bunjongrat R, Chaidee A, Bangyeekhun T. Chitosan effects on floral production, gene expression, and anatomical changes in the Dendrobium orchid. Sci. Hortic. 2008;116(1):65-72.
51. Idrees M, Khan MMA, Aftab T, Naeem M, Hashmi N. Salicylic acid-induced physiological and biochemical changes in lemongrass varieties under water stress. J. Plant Interact. 2010;5(4):293-303.
52. Anicic NV, Dimitrijevic S, Ristic MS, Petrović SS, Petrović SD, Antimicrobial activity of essential oil of Melissa officinalis L, Lamiaceae. Hem. ind. 2005;59(9-10):243-7.
53. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercuscoccifera L. and Juniperusphoenicea L. fruit extracts. Food Chem. 2007;105(3):1126-34.
54. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. food microbiol. 2004;94(3):223-53.
55. Knobloch K, Pauli A, Iberl B, Weigand H, Weis N. Antibacterial and antifungal properties of essential oil components. J. Essent. Oil Res. 1989;1(3):119-28.