

Original Article



# In Vitro Antibacterial Activity of Medicinal Plant Essential Oils Against *Burkholderia gladioli* pv. *gladioli*

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## ABSTRACT

**Background:** Saffron (Zaeferan in Persian), known as Red Gold, is obtained from the dried stigma of the *Crocus sativus* L. (Iridacea) plant. It plays a critical role in non-oil exporting in the economy of Iran, and the livelihoods of many farmers and workers depend on its yields. *Burkholderia (Pseudomonas) gladioli* pv. *gladioli* causes severe reductions the saffron yield in Iran. According to responsible authorities of Iran, no pesticides are registered or recommended for saffron cultivations (except for two herbicides). Most essential oils are listed as generally recognized as safe substances and can exhibit a crucial role in exporting pesticides free saffron.

**Objectives:** The study aimed to identify the most effective antibacterial essential oils against *B. gladioli* pv. *gladioli*.

**Methods:** Essential oils were extracted using a clevenger apparatus from 25 medicinal plants belonging to the Lamiaceae, Compositae, and Apiaceae families, followed by an initial evaluation through disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Dimethyl sulfoxide (DMSO) and amikacin served as a negative and positive control, respectively.

**Results:** Out of 25 essential oils, only 4 of them exhibited antibacterial effects on this pathogen including *Satureja hortensis* (22.66 mm inhibition), *Thymus vulgaris* (20.33 mm), *Trachyspermum ammi* (17.66 mm), and *Sclerorhachis leptoclada* (10.66 mm). Their MICs were 1, 2, 4, and 32 µL/mL, respectively.

**Conclusion:** These findings highlight promising essential oils for controlling *B. gladioli*, supporting pesticide-free saffron production and safeguarding Iran's valuable crop yields.

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## Introduction

**C***rocus sativus* L. (Saffron, known as ‘Red Gold’) is a perennial autumn flowering plant that belongs to the Iridaceae family plants. The dried stigma of the saffron plant is the most expensive spice and has a critical role in non-oil exporting economy of Iran [1]. The genus *Crocus* (Iridaceae) currently includes 261 species worldwide [2], with 24 species recognized in Iran [3]. According to the agronomic, physiological and biological traits of *C. sativus*, it could exploit marginal land and can substitute applicable crops in low-input cropping systems for sustainable agriculture [4]. Two possible origin areas have been reported for saffron: The Mediterranean area (Greece) and the East, especially Turkey, Iran, and India [5]. Saffron is mainly cultivated in Iran, Afghanistan, India, Morocco, Italy, Spain, and Greece. Iran, with 90% of the global saffron production, is largest producer in the world with an average yield of 3.53 kg ha<sup>-1</sup> (108000 ha) in 2017 (Razavi Khorasan Province with 84738 ha is main area in Iran) [6].

The most important bacterial pathogens of saffron corms are *Burkholderia gladioli* pv. *gladioli* and *Bacillus croci* [7]. *B. gladioli* pv. *gladioli* cause soft rot of emerging leaves and shoots and spots on corms and leaves in saffron and reduced flowering by approximately 80% [8]. Pathovars of *B. gladioli* have various host plants like species of *Crocus* and *Iris*, and also cause grain rot and leaf-sheath browning in rice and soft rot in onion [9]. Phytopathogenic strains of *B. gladioli* were divided into two pathovars (*gladioli* and *alliicola*) based on plant host and nutrition differences [10]. Using biochemical and molecular methods for bacterial isolate identification, Karimi-Shari et al. reported the first account of *B. gladioli* in saffron corms from Khorasan Razavi fields, Iran [11].

Two partial genomes of *B. gladioli* strains have been submitted in GenBank (13B 16S ribosomal RNA gene, Accession number: OP183349 and 13A 16S-23S ribosomal RNA, Accession number: OP218019) in 2022. This bacterium in Iran causes severely reduce saffron yields each year. Infected plants showed early leaf yellowing and drying. On the corms, decaying signs on the sheaths while some newly formed sprouts had tissue burnings and browning were observed. Furthermore, a ring shape red brown discoloration on the root germination zone of some infected saffron corms were observed that gradually decayed and extended to the deep corm. According to responsible authorities (plant protection organization) of Iran, no pesticides are registered or recommended in any level for saffron cultivations (except for two herbicides) [12]. Considering Iran’s substantial saf-

fron exports, which require pesticide-free products, developing alternative control methods is critical. Most essential oils are classified as generally recognized as safe. They consist of volatile secondary metabolites extracted from various plant parts and possess bioactive properties such as anti-inflammatory, antibacterial, preservative, and decontaminant effects. Some components like carvacrol, resorcylic acid, eugenol, cinnamaldehyde, trans-cinnamaldehyde, vanillin, and thymol mainly have antibacterial activity [13].

Among the plant families, Lamiaceae plants have most and effective spices with high level of essential oils. *Satureja hortensis* L. (Summer savory; Marze in Persian) and *Thymus vulgaris* L. belong to the Lamiaceae family. *S. hortensis* is an aromatic annual plant with cosmopolitan distribution that is cultivated across the world; it is traditionally used as a natural food preservative, spice, anti-inflammatory, antifungal and antibacterial agent [14]. *T. vulgaris* (Thyme; Avishan in Persian), is an evergreen perennial, woody plant and indigenous to the Mediterranean region, parts of Asia and Northern Africa. *T. vulgaris* is cultivated around the world and people have used it as a culinary and flavoring agent as well as an herbal medicine. *T. vulgaris* has antibiotic, antiseptic, and antifungal activities [15]. *Sclerorhachis leptoclada* Rech. (Compositae) (Mastar in Persian) is an aromatic perennial plant used as an herbal medicine (aerial parts); it is useful for blood purification, increasing lactation, headache, treating digestive disorders and body pains [16]. *Trachyspermum ammi* (L.) Sprague (Apiaceae) (Ajwain; Zeniyan in Persian) is an aromatic annual herb that originated in India, Middle East, Iran, Egypt and Afghanistan and is also cultivated in many countries as medicinal, culinary and for the food, flavoring and spices industries.

*T. ammi* has some biological activity consisting of antioxidant, antimicrobial, and antiviral properties [17]. *T. ammi* exhibits diverse biological activities, including antioxidant, antimicrobial, and antiviral properties. Its methanolic and ethanolic extracts and essential oil demonstrated maximum inhibitory activity against *Staphylococcus aureus* [18, 19]. According to the literature, there is one study concerning antibacterial effects of essential oils against *B. gladioli*, which examined the antibacterial effect of clover on *Tremella fuciformis* Berk. (1856) (Fungi: Tremellaceae) as the host of pathogen [20]. Other studies have focused on other species of *Burkholderia*, such as *Burkholderia cepacia* complex [21-23], and *B. glumae* [24]. This study aimed to screen essential oils from 25 medicinal plants for their potent antibacterial activity against *B. gladioli*, a bacterial pathogen that reduces saffron yields in Iran, promoting pesticide-free crop protection.

## Materials and Methods

### Plants materials

All plants have been collected from South and Razavi Khorasan, Iran, during spring and summer of 2022 (Table 1). Plants species were identified by a Botanist (Khorasan Razavi Agricultural & Natural resources Research Center) and dried for 2-3 weeks at 4 °C in shade. Plant

materials were ground into a fine powder by an electric grinder and preserved in brown and isolated glass and stored in 4 °C until use.

### Essential oil extraction

To extract essential oils from plant material, the clevenger apparatus type was used via the hydro-distillation method, following the standard procedure of the Euro-

**Table 1.** Selected medical plants

Family Plant	Scientific Name	Common Name	Persian Name	Parts of Plant
Lamiaceae	<i>Hyssopus officinalis</i>	Hyssop	Zoofa	Flowers, leaves
	<i>Lavandula angustifolia</i>	English lavender	Ostoghodus	Flowers, leaves
	<i>Melissa officinalis</i>	Lemon balm	Badranjbooye	Leaves, stem
	<i>Mentha piperita</i> L.	Peppermint	Nana felfeli	Leaves, stem
	<i>Mentha spicata</i>	Mint	Nana mamooli	Leaves, stem
	<i>Origanum majorana</i>	Marjoram	Marzanjoosh	Leaves
	<i>Rosmarinus officinalis</i>	Rosemary	Eklile koohi	Leaves
	<i>Salvia officinalis</i>	Common sage	Maryam goli	Flowers, leaves
	<i>S. hortensis</i>	Summer savory	Marzeh	leaves
	<i>T. vulgaris</i>	Thyme	Avishan baghi	leaves
Compositae	<i>Anthemis pseudocotula</i>	Chamomile	Babone shirazi	Flowers
	<i>Artemisia scoparia</i>	Mugwort	Dermane jarooyi	Leaves
	<i>Artemisia sieberi</i>	Mugwort	Dermane koohi	Leaves
	<i>Cichorium intybus</i>	Chicory	Kasni	Leaves
	<i>S. leptoclada</i>	-	Mastar	Flowers, leaves
	<i>Achillea millefolium</i>	Yarrow	Boomadaran	Flowers, leaves
Apiaceae	<i>Anethum graveolens</i>	Dill	Shivid	Seeds
	<i>Apium graveolens</i>	Celery	Karafs	Seeds
	<i>Carum carvi</i>	Caraway	Zire siah	Seeds
	<i>Cuminum cyminum</i>	Cumin	Zire sabz	Seeds
	<i>Daucus carota</i>	Wild carrot	Havij vahshi	Seeds
	<i>Foeniculum vulgare</i>	Fennel	Raziane	Seeds
	<i>Heracleum persicum</i>	Persian hogweed	Golpar	Seeds
	<i>Pimpinella anisum</i>	Anise	Anison	Seeds
	<i>Trachyspermum copticum</i>	Ajwain	Zeniyan	Seeds

pean pharmacopoeia (European pharmacopoeia, 2004, France). Fifty grams of powdered plant material and 500 mL of distilled water were added to a Pyrex extraction flask. After 3 h, essential oils were extracted from the aqueous layer and sodium sulfate anhydrous ( $\text{Na}_2\text{SO}_4$ ) was used to dehydrate the essential oils. Dehydrated essential oils were stored in brown and isolated glasses at 4 °C until use.

### Microorganisms

*B. gladioli* pv. *gladioli* (strain code: 150) was obtained from the culture collection of Khorasan Razavi Agricultural & Natural Resources Research Center, Iran. Strain 150 was isolated from infected saffron corm that collected from Khorasan Razavi saffron Field. This strain was cultured on nutrient agar (NA) and nutrient broth (NB) at 28 °C.

### The disk diffusion assay

The bacterial isolate was cultured in NB at 28 °C for 48 h. After incubation, a suspension with a concentration of  $10^8$  CFU/mL was prepared using sterile distilled water. Subsequently, 100 µL of the suspension was evenly spread on the surface of NA using a sterilized glass inoculating loop. The antibacterial activity was evaluated using a modified disk diffusion assay [14]. The bacterial suspension was allowed to absorb on the NA for 10 min at room temperature. Five microliters of each essential oil was added to a paper disk (5 mm diameter- Whatman No.1) and placed in the center of the NA surface. Amikacin 30 µg disk and dimethyl sulfoxide 10% (DMSO) were used as positive and negative controls, respectively [25]. All treatments (3 replicates) were incubated at 26 °C for 48 h. The inhibition zone (mm) was measured using a caliper.

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

For treatments with visible halos from disk diffusion assay, MIC was assessed via the modified microdilution protocol of Mihajilov-Krstev et al. [26]. Strain 150 was

cultured in NB at 28 °C for 48 h. 1000 µL sterilized NB plus 10 µL DMSO were added to a sterile microtube (2 mL). Then, 32 µL of each essential oils were added to the microtube to obtain 32 µL/mL at first microtube and two-fold dilution was used to make 16, 8, 4, 2, 1, 0.5, 0.25 and 0.12 µL/mL concentrations. A total of 10 µL/mL of bacterial suspension ( $10^6$  CFU/mL) was inoculated to each microtube and incubated on a shaker oven (300 rpm, 28 °C) for 48 h. After confirming bacterial growth (turbidity) in the control, 200 µL from each microtube was transferred to 96-well microplates, and MIC values were determined using an ELISA reader (model: ELx800; wavelength: 630 nm). One thousand microliters of sterilized NB plus 10 µL DMSO was considered as the negative control and 1000 µL NB plus 10 µL DMSO inoculated with 10 µL/mL of bacterial suspension ( $10^6$  CFU/mL) was used as the positive control. To determine MBC, NA plates were streaked from each treatment showing no growth at the lowest essential oil concentration.

### Results

The antibacterial activities of essential oils derived from 25 medicinal plants were evaluated against *B. gladioli* (strain code: 150). Among the tested essential oils, 4 exhibited significant in vitro antibacterial effects. These oils included *S. hortensis* and *T. vulgaris* from the Lamiaceae family, *T. ammi* from the Apiaceae family, and *S. leptoclada* from the Compositae family. The diameters of the inhibition zones produced by these effective essential oils are summarized in Table 2. The negative control, 10% DMSO, did not produce any inhibition zones in the disk diffusion assay. *S. hortensis* essential oil generated the largest inhibition zone with an average diameter of 22.66 mm, comparable to the positive control Amikacin (30 µg) ( $P < 0.05$ ), which produced an average inhibition zone of 22.00 mm. The inhibition zone of *T. vulgaris* (20.33 mm) was comparable to that of *S. hortensis*, while *T. ammi* (17.66 mm) and *S. leptoclada* (10.66 mm) exhibited weaker effects. Spectrophotometric turbidity measurements indicated that absorption coefficients ranging from 0.70 to 1.00 corresponded

**Table 2.** Inhibition zone diameters of essential oils

Rep\EOs Inhibition Zone (mm)	<i>S. hortensis</i>	<i>T. vulgaris</i>	<i>T. ammi</i>	<i>S. leptoclada</i>	DMSO 10% Control (-)	Amikacin 30 µg Control (+)
1	24	20	18	10	nd	22
2	21	21	18	13	nd	23
3	23	20	17	9	nd	21

EOs: Essential oils; nd: Not determined.

**PBR**

**PBR**

**Table 3.** MIC of essential oils

Rep\EOs (µL/mL)	32	16	8	4	2	1	0.5	0.25	0.12	Control (-)	Control (+)	
<i>S. hortensis</i> (Lamiaceae)	1	0.20	0.10	0.08	0.05	0.07	0.07	0.90	0.87	0.88	0.04	1.08
	2	0.18	0.10	0.07	0.07	0.06	0.08	0.82	0.98	0.89	0.03	0.87
	3	0.21	0.07	0.08	0.08	0.06	0.07	0.84	0.97	0.98	0.04	0.94
<i>T. vulgaris</i> (Lamiaceae)	1	0.14	0.13	0.06	0.06	0.08	0.68	0.76	0.56	0.63	0.05	0.83
	2	0.11	0.09	0.05	0.07	0.09	0.46	0.61	0.53	0.51	0.04	1.02
	3	0.12	0.12	0.07	0.06	0.07	0.87	0.77	0.84	0.78	0.07	0.92
<i>T. ammi</i> (Apiaceae)	1	0.11	0.06	0.06	0.05	0.63	0.76	0.90	0.81	0.71	0.05	0.69
	2	0.10	0.07	0.05	0.05	0.60	0.92	0.85	0.79	0.75	0.04	0.80
	3	0.09	0.07	0.06	0.06	0.79	0.88	0.85	0.94	0.95	0.04	0.93
<i>S. leptoclada</i> (Compositae)	1	0.10.0	0.76	0.81	0.90	0.89	0.86	0.94	0.88	0.94	0.03	0.99
	2	0.21.0	0.77	0.97	0.97	0.97	0.91	0.97	0.96	0.89	0.05	1.01
	3	0.16.0	0.73	0.72	0.72	0.98	0.89	0.83	0.9	0.95	0.05	0.95

EOs: Essential oil.

Note: Control (-): 10% DMSO; Control (+): 30 µg/mL amikacin.

to full bacterial growth (positive control: Nutrient broth (NB)+DMSO+bacteria), while values between 0.03 and 0.10 indicated inhibited growth (negative control: NB+DMSO) after 48 h at 28 °C. The MIC assay results confirmed the disk diffusion findings (Table 3).

The MIC values for the 4 essential oils ranged from 1 µL/mL for *S. hortensis*, 2 µL/mL for *T. vulgaris*, 4 µL/mL for *T. ammi*, and 32 µL/mL for *S. leptoclada*. Slight increases in absorption coefficients were observed at higher essential oils concentrations (16 and 32 µL/mL for *S. hortensis*, *T. ammi*, and *T. vulgaris*, and 64 µL/mL for *S. leptoclada*), likely due to concentration-dependent effects.

The MBC results indicated that *T. vulgaris* and *S. hortensis* could inhibit *B. gladioli* at 2 µL/mL, with *S. hortensis* exhibiting a stronger inhibitory effect (Table 4). The largest discrepancy between MIC and MBC was observed for *T. ammi*, which inhibited bacterial growth at 4 µL/mL but required 16 µL/mL to exert bactericidal activity.

**Table 4.** Minimum bacterial concentration of essential oils

Medicinal Plant	<i>S. hortensis</i>	<i>T. vulgaris</i>	<i>T. ammi</i>	<i>S. leptoclada</i>
MIC (µL/mL)	2	2	16	64

## Discussion

The antibacterial activity of plant essential oils is primarily attributed to their diverse bioactive constituents, particularly phenolic compounds and monoterpenes. Compounds such as carvacrol, thymol, borneol, limonene, linalool, terpinen-4-ol, and (E)-nerolidol have been widely reported to exhibit strong antimicrobial properties. These constituents exert their effects through multiple mechanisms, including disruption of bacterial cell membranes, increased membrane permeability, and interference with essential cellular processes, ultimately resulting in the inhibition of bacterial growth or cell death.

Previous studies have demonstrated notable antibacterial activity for carvacrol and thymol [23], borneol and monoterpenes such as limonene and linalool [26], terpinen-4-ol [27], and (E)-nerolidol [28]. Thymol has been identified as the predominant compound in the essential oils of *T. vulgaris* and *T. ammi* [29, 30], which may account for their strong antibacterial activity observed in the present study. Similarly, terpinen-4-ol and

(E)-nerolidol are among the major constituents reported in *S. leptoclada* essential oil [31]. In addition, carvacrol, a compound well known for its potent antimicrobial properties, has been identified as a major constituent of *S. hortensis*. The pronounced inhibitory activity of *S. hortensis* essential oil against *B. gladioli* may therefore be attributed to the presence of this phenolic compound.

The results of the MIC and MBC assays further confirmed the antibacterial potential of these essential oils. *S. hortensis* and *T. vulgaris* exhibited the lowest MIC and MBC values, indicating strong antibacterial activity against *B. gladioli*. In contrast, *S. leptoclada* showed comparatively weaker activity, which may be related to differences in the concentration or composition of its active constituents.

The variability in antibacterial activity among the tested essential oils may be attributed to differences in their chemical profiles, which are influenced by plant species, geographical origin, harvest stage, and extraction methods [32, 33].

Overall, the findings of this study demonstrate that certain medicinal plant essential oils, particularly *S. hortensis* and *T. vulgaris*, possess considerable antibacterial activity against *B. gladioli*. These results suggest that such essential oils may serve as potential natural antibacterial agents for the management of bacterial diseases in plants; however, further investigations under greenhouse and field conditions are required to confirm their practical applicability.

## Conclusion

This study identified 4 essential oils with significant antibacterial activity against *B. gladioli*, with *S. hortensis* demonstrating the strongest inhibitory effect comparable to the antibiotic Amikacin. MIC and MBC assays confirmed the potent bacteriostatic and bactericidal properties of these oils. These findings highlight the potential of *S. hortensis*, *T. vulgaris*, *T. ammi*, and *S. leptoclada* essential oils as natural, eco-friendly alternatives for controlling *B. gladioli* infections in saffron cultivation. Further research should focus on formulation development and field trials to evaluate practical applications.

## Ethical Considerations

### Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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## Authors' contributions

Methodology and supervision: Mahmoud Reza Karimi-Shahri; Data collection: Arash Honarmand, Samaneh Rahamouz-Haghighi, and Elaheh Taheri; Data analysis: Mahmoud Reza Karimi-Shahri; Investigation and writing: all authors.

## Conflict of interest

The authors declared no conflict of interest.

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