

Antimicrobial Activity of *Jasminum grandiflorum* Absolute *in vitro* and *in situ* Study against Phytopathogenic Bacteria

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Traditional medicine has long used jasmine oil to treat a wide range of conditions. Jasmine oil offers numerous health benefits, from stress relief to improved digestion. The culinary industry is one of the many potential applications for jasmine essential oil. The aim of this study was to evaluate the antibacterial properties of *Jasminum grandiflorum* absolute essential oil (JGEO). To this end, its antibacterial activity was evaluated in the vapour phase on fruit and vegetable models and *in vitro* using the disc diffusion method and minimum inhibitory concentration (MIC). The results showed that *Agrobacterium radiobacter* was the most susceptible to JGEO. This was confirmed by both approaches. JGEO had potent antibacterial activity against *A. radiobacter* and *Priestia megaterium* after inoculation on fruit and vegetable models in *in situ* tests. Our results, together with previous studies, show that JGEO has strong antibacterial properties. This makes it a viable option for use as a preservative against phytopathogenic bacteria in fruit and vegetable models.

Keywords: antimicrobial agents, *in vitro* and *in situ* activity, fruits, vegetables, phytopathogenic bacteria

1 Introduction

Aromatic products made from jasmine are derived from the fresh flowers of a large number of species of jasmine (*Jasminum* sp.) of the Oleaceae family. There are more than 2,000 species. *Jasminum officinale* L., *J. grandiflorum* L., *J. floribundum* R. Br. ex Fresen, *J. humile* L., *J. odoratissimum* L., *J. paniculatum* Roxb. and *J. sambac* (L.) Ait. are the most important species in the perfumery, cosmetology, food aromatization and medical fields (Jirovetz et al., 2006). Jasmine varieties originated in India. They are now popular throughout the Mediterranean regions of Europe, Asia and Africa, as well as in the Comoros, India and China. The main producers of jasmine perfumes are Egypt, Morocco, Algeria, Italy and France (Jirovetz et al., 2007). Benzyl acetate, linalool, benzyl alcohol, indole, cis-jasmone, geraniol, methyl anthranilate, α -terpineol, cis-3-hexenyl benzoate, eugenol, nerol, farnesol, *p*-cresol, benzoic acid, benzaldehyde, nerolidol, isophytol, phytol, and phytyl acetate are among the more than 100 constituents

that have been identified in various jasmine samples, particularly concretes, absolutes and other extracts (Bauer et al., 2008; Husain et al., 1988; Makeri & Salihu, 2023; Ramachandra Rao & Rout, 2002; Rao & Rout, 2003; Tamogami et al., 2001). Traces or small amounts of certain volatile N-heterocycles and fatty acid esters have also been reported (Jirovetz et al., 2007). Although jasmine oil is not harmful or irritating, it can cause sensitization, which is why some people have an allergic reaction to it [Frosch]. It is not recommended to use during pregnancy because it stimulates lactation. As jasmine oil is supposed to be relaxing, using too much of it could interfere with concentration. Surprisingly, however, it has also been shown to have energizing effects. Aphrodisiac, antidepressant, antibacterial, cicatricial, expectorant, galactagogue, parturient, sedative and uterotonic are among the medicinal properties of jasmine (Jirovetz et al., 2007).

The negative perception of artificial food additives has been exacerbated by the growing health consciousness

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of consumers. To ensure the removal of pathogens from a given food matrix, a significant number of safe and natural essential oils (EOs) began to be used in the food industry under the guise of 'organic' or 'green' strategies. These effective and safe EOs were aimed at replacing conventional additives (Falleh et al., 2020; Jemaa et al., 2018). Consequently, the European Commission (EC) and the FDA in the United States have approved and classified many EOs and EO components as generally recognized as safe (GRAS) for use as food flavourings and/or preservatives (Jemaa et al., 2018). The future use of essential oils as natural antioxidants and antimicrobials is very promising. Investigating these bioactive mixtures and providing sufficient scientific evidence increases the possibility of creating potent, environmentally friendly and safer food preservatives that can become suitable replacements for artificial chemicals (Bibow & Oleszek, 2024). Because they occur naturally in food and have few known "side effects" on human health, they are considered safe to eat (Ronis et al., 2018).

The aim of our work was to determine the antimicrobial effect of *Jasminum grandiflorum* absolute essential oil (JGEO) on selected species of phytopathogenic bacteria under *in vitro* and *in situ* conditions.

2 Material and Methods

2.1 Essential Oil

Hanus s.r.o. (Nitra, Slovakia) provided the essential oil (JGEO) of *Jasminum grandiflorum* used in this study. The EO was extracted from the fresh flowers of *J. grandiflorum* grown in India. The JGEO was stored at 4 °C in the dark for analysis. Benzyl acetate, benzyl benzoate, linalool, phytol, isophytol, geranyl linalool and eugenol were the main constituents of the JGEO.

2.2 Microorganisms Used for the Study

Agrobacterium radiobacter CCM 2,926, *Pectobacterium carotovorum* CCM 1,008, *Priestia (Bacillus) megaterium* CCM 2007, *Pseudomonas syringae* CCM 2868 and *Xanthomonas arboricola* CCM 1,441 were among the bacterial strains used in the study to evaluate the antibacterial properties of the EO. The Czech Collection of Microorganisms (CCM), located in Brno, Czech Republic, provided bacterial samples. Mueller-Hinton Broth (MHB, Oxoid, Basingstoke, UK) was used to incubate bacterial cultures for 24 hours at 37 °C prior to testing. The optical density of the inoculum was adjusted to the 0.5 McFarland standard on the day of testing (Kačániová et al., 2023).

2.3 Disc Diffusion Method

The disk diffusion method was employed, using the same microbial strains as in the previous experiment, to assess microbial susceptibility to the EO. Mueller-Hinton Agar was used to culture the bacterial inoculum (MHA, Oxoid, Basingstoke, UK). A solution of JGEO absolute was used to soak tiny discs, each measuring 6 mm in diameter. The bacterial cultures were kept at 37 °C for 24 hours. The zone of inhibition was measured to determine the EO's antibacterial efficacy; mild inhibition was defined as 0–5 mm, moderate inhibition as 5–10 mm, and strong inhibition as greater than 10 mm. Controls were established using clear blank discs and conventional antibiotics (gentamicin; Oxoid, Basingstoke, UK).

2.4 Minimal Inhibition Concentration

As previously mentioned, established procedures were used to calculate the minimum inhibitory concentration (MIC) values (MIC₅₀ and MIC₉₀). First, 150 µL of microbial inoculum was added to each well of a 96-well microtitre plate. Different concentrations of JGEO were then added to Mueller-Hinton Broth (MHB), ranging from 10 mg.mL⁻¹ to 0.00488 mg.mL⁻¹. MHB with the inoculum and MHB with EO at the appropriate doses were added to provide negative and positive controls. A spectrophotometer (Glomax, Promega Inc., Madison, WI, USA) was then used to quantify bacterial growth and calculate optical density at 570 nm. The lowest doses of JGEO that inhibited 50% and 90% of microbial growth were determined as the MIC₅₀ and MIC₉₀ values, respectively. Three separate runs of this experiment were performed.

2.5 Antimicrobial Activity on Vapour Phase

Using a variety of bacterial strains on apricot, kiwi, beetroot, and potato substrates, the antibacterial properties of JGEO in the vapour phase were assessed (Kačániová et al., 2024a). After being chopped into 0.5 cm pieces, these substrates were carefully cleaned, dried, and put in 60 mm Petri dishes to be infected with the microbial strains. Filter sheets soaked in ethyl acetate served as controls while ECEO was dissolved in ethyl acetate at different concentrations (500, 250, 125, and 62.5 µg.mL⁻¹). Following that, the Petri dishes were sealed and cultured for seven days at 37 °C. The ImageJ software was used to estimate the density of the microbial colonies (Kačániová et al., 2024b), and the growth in the vapour phase was assessed using conventional microbiological methods for bacteria.

2.6 Statistical Method

The experimental evaluations were carried out in triplicate, and the mean values and matching standard deviations (SD) are shown as the outcomes. ANOVA

calculator version 6.0 was used to perform statistical analyses, such as one-way ANOVA and Tukey's HSD test at a significance level of $p \leq 0.05$.

3 Results and Discussion

3.1 In vitro Antimicrobial Activity

Five bacterial phytopathogenic strains were used in the disc diffusion method to evaluate the antibacterial properties of JCEO absolute. The results showed that JGEO had the strongest antibacterial activity against the following bacteria: *Agrobacterium radiobacter* (14.67 mm), *Pectobacterium carotovorum* (12.33 mm), *Priestia (Bacillus) megaterium* (9.33 mm), *Pseudomonas syringae* (8.67 mm), and *Xanthomonas arboricola* (7.33 mm). Furthermore, it was shown that JGEO was less efficient against germs than antibiotics. However, gentamicin showed the strongest antibiotic resistance against *A. radiobacter* and *P. syringae* (Table 1). In a different study the *E. coli* MTCC443 strain was shown to be somewhat susceptible to natural jasmine oil, its synthetic mixtures and its individual components at a concentration of 2.5 μL (the lowest concentration tested) per disc, based on the results of the initial screening using the disc diffusion method (Rath et al., 2008). Research has documented the antimicrobial effect of essential against fungi and bacteria (Ahmed et al., 2016; Thaweboon et al., 2018).

Broth microdilution was used to determine minimum inhibitory concentrations, known as MIC_{50} and MIC_{90} .

The aim of the study was to have a better understanding of the antibacterial properties of JGEO. The study showed: JGEO had a significant effect on the suppression of phytopathogenic bacterial species (Table 2). In particular, *B. megaterium* and *P. syringae* showed the lowest MIC_{50} (0.462 and 0.462 $\text{mg}\cdot\text{mL}^{-1}$) and MIC_{90} (0.489 and 0.482 $\text{mg}\cdot\text{mL}^{-1}$), respectively. In different study jasmine oil showed antibacterial activity against *S. mutans*, *L. casei*, *E. coli* and all strains of *Candida* species with inhibition zones of 9 to 26 mm and MIC values of 0.19–1.56% v/v. The results of the current study provide scientific evidence that jasmine oil can be used as a natural antibacterial agent for the control of bacteria in the oral cavity (Thaweboon et al., 2018).

3.2 Antimicrobial Activity in Vapour Phase

In the following study, fruit and vegetables were used as food models for an *in situ* assessment of antibacterial activity. In addition, a variety of microorganisms were used *in vitro*. Table 3 shows the *in situ* data on the antibacterial activity of the fruit model. According to a comparison between the JGEO vapour phase and the *in vitro* experiment, the phytopathogenic bacteria of the apricot model were generally less effectively suppressed by the lowest concentration. The results showed that the most effective JGEO dose (62.5 $\mu\text{g}\cdot\text{L}^{-1}$) for inhibiting the growth of *A. radiobacter* in the apricot model was 87.70%. It was found that the lowest doses (62.5 $\mu\text{g}\cdot\text{L}^{-1}$) of JGEO tested were successful in inhibiting the growth of *P. carotovorum* and *X. arboricola* (86.22% and 86.34%, respectively) in the apricot model (Table 3).

Table 1 Antimicrobial activity with disc diffusion method

Bacteria	Inhibition zone (mm)	
	EO	ATB
<i>Agrobacterium radiobacter</i> CCM 2,926	14.67 \pm 0.58 ^a	30.67 \pm 0.58 ^a
<i>Pectobacterium carotovorum</i> CCM 1,008	12.33 \pm 0.58 ^b	30.33 \pm 0.58 ^a
<i>Priestia (Bacillus) megaterium</i> CCM 2,007	9.33 \pm 0.58 ^c	30.33 \pm 0.58 ^a
<i>Pseudomonas syringae</i> CCM 2,868	8.67 \pm 0.58 ^c	30.67 \pm 0.58 ^a
<i>Xanthomonas arboricola</i> CCM 1,441	7.33 \pm 0.58 ^d	30.33 \pm 0.58 ^a

Data are the mean (\pm SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$)

Table 2 Minimal inhibition concentration

Bacteria	MIC_{50}	MIC_{90}
<i>Agrobacterium radiobacter</i> CCM 2926	0.466 \pm 0.01 ^a	0.488 \pm 0.01 ^a
<i>Pectobacterium carotovorum</i> CCM 1008	0.575 \pm 0.01 ^c	0.595 \pm 0.00 ^c
<i>Priestia (Bacillus) megaterium</i> CCM 2007	0.462 \pm 0.03 ^a	0.489 \pm 0.0066 ^a
<i>Pseudomonas syringae</i> CCM 2868	0.462 \pm 0.00 ^a	0.482 \pm 0.00 ^a
<i>Xanthomonas arboricola</i> CCM 1441	0.551 \pm 0.022 ^b	0.581 \pm 0.01 ^b

Data are the mean (\pm SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$)

When the lowest dose ($62.5 \mu\text{g.L}^{-1}$) was compared with the other bacteria studied, a significant degree of inhibition was observed. In the kiwi model, JGEO was most successful in inhibiting *A. radiobacter* (77.13%) at also at the lowest concentration. Food spoilage is caused by a variety of bacteria. These may be the food's natural microflora or processing conditions such as those encountered during harvesting, production, storage and transport (Mafe et al., 2024). In some situations, the microflora has no significant effect on food safety or quality, but in others, it can have a variety of effects, such as causing food fermentation, spoilage, or foodborne illness (Lorenzo et al., 2018). While food fermentation is a desirable food transformation, food spoilage, foodborne infections and poisoning can cause significant financial loss, for example, if a particular batch of food is found to be associated with an outbreak of disease or has a short shelf life, necessitating the recall and destruction of the entire batch from the market (Skowron et al., 2022) due to poverty, fermentation is one of the most widely used preservation methods. It not only allows extending the shelf life of food, but also brings other benefits, including inhibiting the growth of pathogenic microorganisms, improving the organoleptic properties and product digestibility, and can be a valuable source of functional microorganisms. Today, there is a great interest in functional strains, which, in addition to typical probiotic strains, can participate in the treatment of numerous diseases, disorders of the digestive system, but also mental diseases, or stimulate our immune system. Hence, fermented foods and beverages are not only a part of the traditional diet, e.g., in Africa but also play a role in the nutrition of people around the world. The fermentation process for some products occurs

spontaneously, without the use of well-defined starter cultures, under poorly controlled or uncontrolled conditions. Therefore, while this affordable technology has many advantages, it can also pose a potential health risk. The use of poor-quality ingredients, inadequate hygiene conditions in the manufacturing processes, the lack of standards for safety and hygiene controls lead to the failure food safety systems implementation, especially in low- and middle-income countries or for small-scale products (at household level, in villages and scale cottage industries. The use of biopreservation is increasing due to customer demand, fewer negative effects, fewer environmental disadvantages, etc. A change in lifestyle has forced people to rely on processed food, fast food and other foods (Muthuvelu et al., 2023). Therefore, this element increases the possibility of microbes contributing to food spoilage. Improving the knowledge and understanding of biological preservation is of considerable importance as a means of combating the complexity of microbial interactions with food (Carrascosa et al., 2021).

The next step was to investigate the effect of JGEO on the vegetable models (Table 4). Potatoes and beetroot were chosen as the model food products. JGEO efficacy was most evident (97.45%) for *P. megaterium* at the lowest concentration ($62.5 \mu\text{g.L}^{-1}$) on the beetroot model. For *P. syringae*, JGEO efficacy (97.74%) was mainly observed also at the lowest concentration ($62.5 \mu\text{g.L}^{-1}$) in the potato model. Jasmine essential oil is widely used in the food industry to flavour and prevents food from spoiling. The process by which food deteriorates to the point where it is unfit for human consumption or loses some of its edible qualities is known as food spoilage. Food spoilage is caused by many external

Table 3 *In situ* analysis of the antimicrobial activity (%) of the vapour phase of JGEO on fruit model

Food model	Microorganisms	Concentration of JGEO ($\mu\text{g.L}^{-1}$)			
Apricot		62.5	125	250	500
Bacteria	<i>Agrobacterium radiobacter</i>	87.70 \pm 1.94 ^a	65.26 \pm 1.72 ^a	42.97 \pm 1.62 ^a	34.70 \pm 3.12 ^a
	<i>Pectobacterium carotovorum</i>	86.22 \pm 2.15 ^a	67.28 \pm 0.44 ^a	45.74 \pm 1.87 ^a	33.86 \pm 1.22 ^a
	<i>Priestia (Bacillus) megaterium</i>	85.96 \pm 2.61 ^a	66.48 \pm 0.70 ^a	44.84 \pm 0.81 ^a	35.67 \pm 1.11 ^a
	<i>Pseudomonas syringae</i>	84.86 \pm 2.44 ^a	65.37 \pm 0.70 ^a	45.99 \pm 1.77 ^a	35.71 \pm 0.06 ^a
	<i>Xanthomonas arboricola</i>	86.34 \pm 2.69 ^a	65.26 \pm 3.73 ^a	47.11 \pm 1.34 ^a	36.06 \pm 2.13 ^a
Kiwi		62.5	125	250	500
Bacteria	<i>Agrobacterium radiobacter</i>	77.13 \pm 2.29 ^a	65.00 \pm 2.67 ^a	55.36 \pm 2.42 ^a	44.71 \pm 1.00 ^a
	<i>Pectobacterium carotovorum</i>	77.03 \pm 0.36 ^a	66.71 \pm 1.22 ^a	56.44 \pm 1.63 ^a	46.07 \pm 2.78 ^a
	<i>Priestia (Bacillus) megaterium</i>	74.95 \pm 3.77 ^a	67.88 \pm 1.82 ^a	54.63 \pm 2.02 ^a	42.70 \pm 0.96 ^a
	<i>Pseudomonas syringae</i>	74.36 \pm 3.91 ^a	64.66 \pm 3.32 ^a	56.81 \pm 1.05 ^a	45.74 \pm 2.10 ^a
	<i>Xanthomonas arboricola</i>	75.50 \pm 4.23 ^a	67.21 \pm 1.61 ^a	56.03 \pm 3.20 ^a	45.11 \pm 2.41 ^a

Data are the mean (\pm SD) of 3 samples. Different letters in each column (for each fruit model: apricot and kiwi) refer to significant differences (Tukey, $p \leq 0.05$)

Table 4 *In situ* analysis of the antimicrobial activity (%) of the vapour phase of JGEO on vegetable model

Food model	Microorganisms	Concentration of JGEO ($\mu\text{g}\cdot\text{L}^{-1}$)			
Beetroot		62.5	125	250	500
Bacteria	<i>Agrobacterium radiobacter</i>	96.30 \pm 2.12 ^a	84.52 \pm 2.76 ^a	74.89 \pm 3.66 ^a	66.51 \pm 2.38 ^a
	<i>Pectobacterium carotovorum</i>	95.53 \pm 2.22 ^a	87.64 \pm 1.05 ^a	74.48 \pm 1.78 ^a	63.71 \pm 1.71 ^a
	<i>Priestia</i> (Bacillus) <i>megaterium</i>	97.45 \pm 1.15 ^a	86.66 \pm 1.72 ^a	76.32 \pm 1.66 ^a	66.07 \pm 2.33 ^a
	<i>Pseudomonas syringae</i>	97.00 \pm 1.66 ^a	84.52 \pm 3.17 ^a	76.55 \pm 1.92 ^a	63.86 \pm 1.76 ^a
	<i>Xanthomonas arboricola</i>	97.10 \pm 1.53 ^a	86.56 \pm 2.73 ^a	73.75 \pm 1.58 ^a	67.60 \pm 1.49 ^a
Potato		62.5	125	250	500
Bacteria	<i>Agrobacterium radiobacter</i>	96.74 \pm 1.17 ^a	86.99 \pm 2.18 ^a	73.97 \pm 2.06 ^a	66.76 \pm 1.23 ^a
	<i>Pectobacterium carotovorum</i>	96.96 \pm 3.21 ^a	84.15 \pm 3.22 ^a	75.53 \pm 3.77 ^a	65.92 \pm 2.81 ^a
	<i>Priestia</i> (Bacillus) <i>megaterium</i>	93.81 \pm 4.29 ^a	84.81 \pm 2.59 ^a	74.56 \pm 1.74 ^a	64.93 \pm 2.56 ^a
	<i>Pseudomonas syringae</i>	97.74 \pm 1.00 ^a	85.60 \pm 2.11 ^a	77.69 \pm 1.02 ^a	63.56 \pm 1.73 ^a
	<i>Xanthomonas arboricola</i>	93.79 \pm 2.01 ^a	85.82 \pm 2.58 ^a	73.92 \pm 2.78 ^a	68.12 \pm 0.46 ^a

Data are the mean (\pm SD) of 3 samples. Different letters in each column (for each vegetable model: beetroot and potato) refer to significant differences (Tukey, $p \leq 0.05$)

factors. It happens when food is harvested as a result of oxidation (atmospheric oxygen), enzyme attack and microbes. Jasmine oil is used in foods and beverages for its antibacterial, antioxidant and flavoring properties (Butt, 2017). One of the alternative techniques is to use EOs in the vapour phase. They could be used on large areas or on things that do not come into direct contact with surfaces. The vapour phase of EOs has been shown to have antibacterial activity in a number of studies (Goñi et al., 2009; Nedorostova et al., 2009; Khumalo et al., 2017; Songsamoe et al., 2017).

4 Conclusions

Food needs to be kept safe and undamaged using the perfect food preservative. Whole EOs or their components can help prevent food from spoiling. Different types of plants differ in the strength of the EOs they contain or the naturally occurring antibacterial compounds they contain. A combination of EOs or their constituents from different species can help achieve the preservation goal. Natural chemicals, extracts, or EOs can be used. The mechanism of action, and synergistic and antagonistic effects of the agent must be taken into account as the ideal agent should provide total protection against food spoilage. Among its many other uses, jasmine EO has been shown to be effective against a wide range of gram-positive and gram-negative bacteria and fungi. Jasmine EO is suitable for use in food preservation due to this property.

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