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ORIGINAL ARTICLE

Camellia japonica flower extracts suppress plant bacterial pathogens via biofilm disruption and virulence inhibition

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Abstract

Bacterial phytopathogens such as *Erwinia amylovora* and *Xanthomonas campestris* cause severe economic losses in fruit and vegetable crops worldwide. Their control is increasingly challenging due to limited availability of effective bactericides and the emergence of resistant strains. In this context, there is an urgent need to identify sustainable and environmentally friendly alternatives to conventional chemical control strategies. This study evaluates the antibacterial activity of hydromethanolic extracts from *Camellia japonica* cv. 'Lipstick' against these two major bacterial pathogens, exploring potential mechanisms of action and in planta efficacy. *Camellia japonica* L. is traditionally valued in Eastern medicine for its bioactive compounds, which have diverse pharmacological properties. This study evaluated the antibacterial potential of hydromethanolic leaf and flower extracts from *C. japonica* cv. 'Lipstick' against the phytopathogens *Erwinia amylovora* and *Xanthomonas campestris* pv. *campestris*, significant contributors to global agricultural losses. Gas chromatography–mass spectrometry revealed distinct phytochemical profiles, with D-fucose (33.6%), dihydroxyacetone (7.6%), and methoxy-phenyl-oxime (5.7%) predominating in the leaf extracts, whereas the flower extracts contained methoxy-phenyl-oxime (14.1%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4 H-pyran-4-one (6.3%) as major constituents. The flower extracts exhibited superior antibacterial activity, with minimum inhibitory concentrations (MICs) of 1000 $\mu g \cdot mL^{-1}$ against *X. campestris* and 1500 $\mu g \cdot mL^{-1}$ against *E. amylovora*; in contrast, the leaf extracts showed no activity at the tested concentrations. The flower extract altered bacterial membrane permeability, significantly inhibited biofilm formation (by up to 74%), disrupted mature biofilms, and reduced amylovoran production in *E. amylovora* by 2.57-fold. *In planta* assays on *Pyrus communis* showed a 71% reduction in fire blight severity. Scanning electron microscopy confirmed biofilm disruption and prevention of bacterial adhesion. These findings highlight the potential of *C. japonica* flower extracts as promising eco-friendly alternatives to conventional antibiotics for managing bacterial plant diseases, offering a potential solution for treating fire blight in pear orchards.

Keywords Camellia japonica cv. 'Lipstick' · Hydromethanolic flower extract · Erwinia amylovora (fire blight pathogen) · Xanthomonas campestris pv. campestris (black rot pathogen) · Biofilm inhibition and disruption · Amylovoran production and virulence suppression

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Introduction

Bacterial diseases represent a major constraint to global agricultural productivity, leading to substantial crop losses and threatening food security. Among these, fire blight of pome fruit caused by *Erwinia amylovora* and black rot of brassicas caused by *Xanthomonas campestris* pv. *campestris* are two of the most destructive bacterial plant diseases worldwide. These pathogens are capable of rapid spread and can severely affect yield and quality, resulting in major economic impact on fruit and vegetable production systems.

In recent years, considerable attention has been given to plant-derived bioactive compounds as alternatives to synthetic bactericides, with several botanical extracts showing promising activity against phytopathogenic bacteria [Recent refs 2022–2024]. However, their efficacy varies depending on plant species, tissue type, extraction method, and the target pathogen. *E. amylovora* is recognized as a quarantine pathogen responsible for fire blight, a devastating disease of apple and pear trees that can lead to complete crop failure in severe outbreaks. *X. campestris* pv. *campestris* causes black rot, one of the most important diseases affecting brassicas worldwide, resulting in significant yield losses in cabbage, cauliflower, and other cruciferous crops. The economic importance of these pathogens makes them ideal models for evaluating novel plant-derived antibacterial agents.

Camellia japonica L. is an evergreen woody tree or shrub belonging to the Theaceae family that is cultivated worldwide in various cultivars. The leaves, measuring 5–7 cm in length, are glossy, leathery, coriaceous, and serrate, with a glabrous stem of approximately 1 cm. The bracteoles and sepals are velutinous. The flowering period spans from October to mid-March, with individual blooms lasting three to four weeks. Although camellia flowers are typically non-fragrant, their leaves are known to contain distinctive flavoring phytochemicals.

The genus *Camellia*, including *C. japonica*, has been traditionally employed in Asian folk medicine (Japan, China, Vietnam, Korea, and India) because of its bioactive properties, associated with the presence of triterpenes, flavonoids, tannins, and fatty acids, predominantly in the leaves (Lee et al. 2017; Majumder et al. 2020; Piao et al. 2011; Teixeira and Sousa 2021). In Spain, *C. japonica* is cultivated primarily as an ornamental shrub in Galicia (northwestern Spain), which represents a significant global market for its production (Pereira et al. 2023).

The wide variety of *C. japonica* cultivars complicates the analysis of their chemical composition. Generally, the principal constituents include phenolic compounds, terpenoids, and fatty acids, accompanied by minor components such as pigments and biosugars (Meng et al. 2018; Pereira et al. 2022a). The concentrations of these compounds vary significantly depending on edaphoclimatic and physical factors, including geographic area, climatic conditions, soil characteristics, and exposure to environmental stresses such as drought, heavy rainfall, or ultraviolet radiation (Meng et al. 2018). Furthermore, the phytochemical profile varies between plant organs and tissues, highlighting the species' complex chemistry and warranting further investigation.

Several phytochemicals with characteristic flavoring properties, including vanillin, 5-ethylfurfural, 2,3-dihydro-3,5-dihydroxy-6-methyl-4 H-pyran-4-one (DDMP), phenylacetaldehyde, and 1-(4-hydroxy-3-methoxyphenyl) ethanone, have demonstrated antimicrobial properties (Lasekan 2013). However, despite reports on antimicrobial activity against human pathogens (Teixeira and Sousa 2021) and indications of antibiofilm activity (H.-A. Choi et al. 2017), the antimicrobial effects of *C. japonica* against phytopathogens remain largely unexplored.

To address this knowledge gap, this study investigated the antibacterial properties of hydromethanolic extracts from the leaves and flowers of *C. japonica* cv. 'Lipstick' against plant pathogenic bacteria responsible for significant global crop losses. We hypothesized that hydromethanolic extracts of *C. japonica* cv. 'Lipstick' would inhibit plant bacterial pathogens by interfering with biofilm formation and virulence traits. This study particularly focused on *Erwinia amylovora*), which affects pear orchards, and *Xanthomonas campestris* pv. *campestris*, which damages *Brassicaceae* crops. This research aims to provide growers with an eco-friendly alternative to conventional antibiotics, potentially mitigating the development of antibiotic resistance associated with their widespread use in controlling bacterial diseases.

In summary, the main contributions of this study are as follows: (i) evaluation of hydromethanolic extracts from *Camellia japonica* cv. 'Lipstick' flowers and leaves against two major bacterial phytopathogens, *E. amylovora* and *X. campestris*; (ii) investigation of possible antibacterial mechanisms, including interference with biofilm formation and virulence factor suppression; (iii) demonstration of the in planta efficacy of the floral extract in reducing disease severity under greenhouse conditions; (iv) characterization of phytochemical profiles to link chemical composition with bioactivity.

Materials and methods

Plant material, chemicals, and bacterial strains

Camellia japonica cv. 'Lipstick' samples were gathered in May 2021 from the Pazo de Santa Cruz de Rivadulla (Vedra, A Coruña, Spain). The samples were identified and

authenticated by Prof. Dr. B. Herrero-Villacorta from the Department of Agroforestry Sciences, ETSIIAA, University of Valladolid. Voucher specimens were deposited at the ETSIIAA herbarium. Separate composite samples were prepared from leaves and flowers collected from 25 samples.

Two-year-old *Pyrus communis* L. cv. 'San Pietro' plants for *in planta* experiments were obtained from Zerbini Garden (Ferrara, Italy).

Luria–Bertani (LB) agar and LB broth were procured from Liofilchem (Roseto degli Abruzzi, Teramo, Italy). Propidium iodide (PI; CAS 25535-16-4), cetylpyridinium chloride (CPC; CAS 6004-24-6), phosphate-buffered saline (PBS), monobasic potassium phosphate (CAS 7778-77-0), potassium phosphate dibasic (CAS 7758-11-4), ammonium sulfate (CAS 7783-20-2), glycerol (CAS 56–81-5), citric acid (CAS 77–92-9), magnesium sulfate (CAS 7487-88-9), sorbitol (CAS 50–70-4), and crystal violet solution (CAS 548-62-9) were sourced from Merck KGaA (Darmstadt, Germany). Glutaraldehyde (CAS 1121-89-7) and potassium phosphate (KPO_4; CAS 7778-77-0) were obtained from Thermo Fisher (Fisher Scientific Italia, Segrate, Italy).

The *E. amylovora* (EA) and *X. campestris* pv. *campestris* (Xcc) strains used in this study were provided by the Emilia-Romagna Phytosanitary Agency. The reference strains *E. amylovora* DSM 30,165 and *X. campestris* pv. *campestris* DSM 3586 from the Leibniz Institute DSMZ-German Collection of Microorganisms served as controls. All bacteria were cultured on LB agar (30 $g \cdot L^{-1}$) or in LB broth at 25/28°C. The inoculum concentrations were adjusted as described in the respective subsections below, following established protocols.

Preparation of leaf and flower extracts

The plant material (leaves or flowers) was dried and extracted with a methanol/water mixture (1:24 *v/v*). The extraction protocol involved heating at 50 °C for 30 min, followed by probe-type ultrasonication (model UIP1000 hdT; Hielscher Ultrasonics, Teltow, Germany) and centrifugation at 9000 × g for 15 min. The resulting supernatant was filtered through Whatman No. 1 paper and then freeze-dried to obtain a solid residue. The extraction yield differed significantly between plant parts: 51.9% for leaves and 6.8% for flowers.

Characterization procedures

Before extraction, infrared spectroscopy was employed to analyze the dried plant parts for the identification of functional groups. The spectra were recorded via a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 Fourier transform infrared spectrometer equipped with an in-built diamond attenuated total reflection (ATR) system. The spectra were recorded in the 4000–400 cm^{-1} range with a spectral resolution of 1 cm^{-1}, with 64 scans per interferogram.

For gas chromatography–mass spectrometry (GC–MS) analysis, the freeze-dried extracts were dissolved in HPLC-grade methanol to obtain 5 $mg \cdot mL^{-1}$ solutions, followed by additional filtration. GC−MS characterization was conducted by the Research Support Services (STI) at Universidad de Alicante (Alicante, Spain), utilizing a gas chromatograph model 7890 A coupled to a quadrupole mass spectrometer model 5975 C (both from Agilent Technologies). The chromatographic conditions included an injection volume of 1 µL, an injector temperature of 280 °C in splitless mode, and an initial oven temperature of 60 °C for 2 min, followed by a ramp of 10 $°C \cdot min^{-1}$ up to a final temperature of 300 °C, which was held for 15 min. The chromatographic column used for compound separation was an Agilent Technologies HP-5MS UI with dimensions of 30 m in length, a 0.250 mm diameter, and a 0.25 µm film. The mass spectrometer conditions included a temperature of 230 °C for the electron impact source and 150 °C for the quadrupole, with an ionization energy of 70 eV. The compounds were identified through library matching via the National Institute of Standards and Technology (NIST11) database.

Antibacterial activity assessment

- Bacterial strains used.

The bacterial strains *X. campestris* pv. *campestris* and strains *E. amylovora* were selected based on their well-documented agronomic relevance (Tables 1 and 2). These pathogens cause fire blight and black rot, respectively, two high-impact bacterial diseases in pome fruit and brassica crops. Their inclusion allows the evaluation of antibacterial efficacy against both woody and herbaceous host pathogens of major economic importance in the Mediterranean region and worldwide.

Minimum inhibitory concentration determination

The microdilution method was performed according to Akhlaghi et al. (2019) to determine the minimum inhibitory concentration (MIC) of each extract. *Erwinia amylovora* was grown in LB broth overnight at 28 °C and 160 rpm in a shaking incubator (MaxQ 4000; Thermo Scientific Italia, Milano, Italy). *Camellia japonica* extracts (leaf or flower) were serially diluted in LB broth to achieve concentrations ranging from 62.5 to 1500 $\mu g \cdot mL^{-1}$, with a final volume of 200 µL per well in 96-well plates (Costar Corning, Corning, New York, USA). We selected LB medium because it allows robust and reproducible growth of both *E. amylovora*

Table 1 *Xanthomonas Campestris* pv. *Campestris* isolates and the source of isolation

BACTERIA	Sample ID	Host	Source/ Province of Isolation	Year of Isolation
Xanthomonas campestris pv. campestris	10,863	BRASSICA seeds	Bologna	2011
Xanthomonas campestris pv. campestris	11,043	BRASSICA seeds	Bologna	2011
Xanthomonas campestris pv. campestris	15,616	BRASSICA seeds	Forlì-Cesena	2011
Xanthomonas campestris pv. campestris	15,619	BRASSICA seeds	Forlì-Cesena	2012
Xanthomonas campestris pv. campestris	15,622	BRASSICA seeds	Forlì-Cesena	2012
Xanthomonas campestris pv. campestris	30,788	BRASSICA seeds	Ravenna	2014
Xanthomonas campestris pv. campestris	3586	BRASSICA seeds	DSMZ	1995

and *X. campestris* strains in our experimental system and is routinely used in many antimicrobial screening assays for these pathogens. Each well was inoculated with 10 µL of the overnight bacterial culture adjusted to 10^4 CFU·mL^{-1}. The OD_{600} of bacterial suspensions was previously correlated to a plate count curve, allowing us to standardize the inoculum concentration. Extracts were dissolved in the smallest possible volume of ethanol and then diluted in water. A solvent control at the equivalent ethanol concentration was included, and no effect on bacterial growth was observed. Streptomycin was included as a positive control, and its MIC values were used for comparison. The plates were incubated statically at 25 °C for 48 h. The same methodology was applied to Xcc. All experiments were performed in triplicate and repeated three times.

Membrane permeability assay

Bacterial suspensions of EA were cultured in LB broth at 25 °C for 24 h and adjusted to 10^5 CFU·mL^{-1}. The bacterial suspensions were treated with *C. japonica* flower extract at concentrations of 1×MIC and 1/2×MIC on the basis of its superior inhibitory effects compared with those of the leaf extract. The samples were incubated for 5, 60, 120, or 180 min, followed by centrifugation at 10,000 rpm for 5 min in darkness. The fluorescence intensity was measured via a microplate reader (Tecan-Fluoroscan; Tecan Italia, Cernusco sul Naviglio, Milano, Italy). Untreated EA cells served as negative controls, whereas EA cells treated with 10% bleach were used as positive controls. This protocol was replicated for Xcc. All experiments were performed in triplicate and repeated three times.

Amylovoran production assay

Amylovoran serves as a critical virulence determinant in the context of EA, where pathogenicity hinges on the expression of the type III secretion system (T3SS) and the synthesis of this exopolysaccharide. The assessment of amylovoran production utilized the cetylpyrimidinium chloride (CPC) assay, following the protocol outlined by Bellemann et al. (1994).

Initially, EA overnight cultures were centrifuged at 4 °C, and pellets were washed with PBS before being diluted 1:100 in modified Burkholderia minimal agar (MBMA) medium, which contained 3 g of KH_2PO_4, 7 g of K_2HPO_4, 1 g of $[NH_4]_2SO_4$, 2 mL of glycerol, 0.5 g of citric acid, and 0.03 g of $MgSO_4$, supplemented with 1% sorbitol and flower extract at the MIC. The amylovoran content was determined by adding 50 µL of a 50 mg·mL^{-1} CPC solution to each milliliter of MBMA culture supernatant and incubating for 10 min. The control group consisted of untreated EA cells. Turbidity was measured at 600 nm (OD_{600}) via a

Table 2 *Erwinia Amylovora* isolates and the source of isolation

BACTERIA	Sample ID	Host	Province of Isolation	Year of Isolation
Erwinia amylovora	49,536	Apple tree	Forlì-Cesena	2018
Erwinia amylovora	49,540	Apple tree	Reggio Emilia	2018
Erwinia amylovora	50,163	Apple tree	Ravenna	2018
Erwinia amylovora	50,340	Apple tree	Ravenna	2018
Erwinia amylovora	50,535	Pear tree	Piacenza	2018
Erwinia amylovora	51,198	Pear tree	Ravenna	2018
Erwinia amylovora	51,201	Pear tree	Ravenna	2018
Erwinia amylovora	51,431	Pear tree	Piacenza	2018
Erwinia amylovora	51,657	Pear tree	Ravenna	2018
Erwinia amylovora	52,140	Apple tree	Bologna	2018
Erwinia amylovora	52,458	Pear tree	Ferrara	2018
Erwinia amylovora	30,165	Pear tree	DSMZ	Before 22 August 1990

Table 3 Main absorption bands of the FTIR spectra of the *C. japonica* plant parts

Leaf	Flower	Assignment
3366	3339	Bonded O–H stretching (cellulose, hemicellulose, lignin)
	3243	OH absorption band in oximes
2916	2917	$–CH_2$ asymmetric stretching of alkyls (cutin, wax, pectin)
2849	2849	$–CH_2$ symmetric stretching (cutin and wax); $CH_2–(C_6)–$ bending (cellulose); C–H aldehyde group
1732	1732	C=O stretching of alkyl ester/C=O in pyranones
1625	1625	symmetrical C=C stretch/C=O stretch/C=N in oximes
	1594	asymmetrical C=C stretch (at 1580 cm^{-1} in dihydroxyacetone)
1517	1520	aromatic ring stretching vibration (i.e., in acetosyringone)
1464	1463	CH_2 scissoring
	1441	C–H deformation; C=C stretching (furan ring); $O–CH_3$ stretching
1376	1369	typical of acetosyringone
	1313	CH in-plane bending in cellulose; O–H deformation in oximes
1244	1245	aromatic C–O stretching (in cyclic ethers); C–O–C stretching
	1202	symmetric C–H wagging
1163	1150	C–O–C asymmetric stretching in cellulose; C–C in plane; C–O features
1102	1100	C–O–C stretching in the pyranose ring skeletal
1073		C–O in alcohols
1028	1027	$O–CH_3$ in phenolics; C–O stretching; O–H deformation
	919	N–OH in oximes (i.e., methoxyphenyloxime)
	867	present in the dihydroxyacetone spectra
	817	C–H out-of-plane from guaiacyl units in lignin derivatives
	777	typical of phenyl ketones (i.e., acetosyringone)
719	718	CH_2 rocking

spectrophotometer. All experiments were performed in triplicate and repeated three times.

Antibiofilm activity evaluation

Biofilm formation was assessed using crystal violet staining according to Wilson et al. (2017). Initially, EA suspensions (10^6 CFU·mL^{-1}) were cultured in LB broth containing *C. japonica* flower extract at nonlethal concentrations in 96-well U-bottom microplates for 72 h at 25 °C. Following incubation, the growth media, *C. japonica* extracts, and planktonic cells were meticulously removed, and the wells were rinsed with sterile deionized water. The wells were stained with 1% crystal violet solution for 30 min at room temperature. The dye solution was subsequently discarded the dye solution. To enhance crystal violet solubility, decoloring solutions consisting of 90−95% ethanol (200 µL) were introduced into each well and incubated for 15 min at room temperature. The contents of the 96-well plate were then transferred to a fresh and clean microplate, and biofilm formation was quantified by measuring the absorbance at 570 nm using a microplate reader (Tecan-Sunrise; Tecan Italia, Cernusco sul Naviglio, Milano, Italy); OD_{570} values are reported in Supplementary materials (Tables S3 to S6). The same protocol was applied for Xcc. Analyses were conducted on the basis of data obtained from three independent experiments, each conducted in triplicate for each bacterium.

Mature biofilm disruption characterization by scanning electron microscopy

The ability of the flower extract to disrupt mature biofilms was assessed by scanning electron microscopy (SEM). Biofilm formation was carried out in 6-well polypropylene plates fitted with cell strainer inserts (BD Italia, Milano, Italy), which served as a support for bacterial adhesion. Bacterial suspensions of EA and Xcc, adjusted to a final concentration of 10^6 CFU·mL^{-1} in LB medium, were added to the wells and incubated for 72 h at 28 °C to allow mature biofilm development.

Following this period, the medium was gently removed and replaced with fresh LB medium containing the flower extract at the desired concentration. The incubation was then continued for an additional 24 h under the same conditions to allow for biofilm disruption.

At the end of the treatment, the inserts were carefully removed and rinsed twice with sterile phosphate-buffered saline (PBS) to eliminate nonadherent cells. The samples were then fixed with 2.5% glutaraldehyde prepared in 0.1 M potassium phosphate (KPO_4) buffer (pH 7.2) for 2 h at room temperature. After fixation, the samples were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%) and dried using a critical point dryer. The samples were subsequently mounted on aluminum stubs and sputter-coated with a gold/palladium alloy to increase the conductivity. SEM imaging was performed using a Zeiss EVO40

Table 4 Antibacterial activity of *Camellia Japonica* extracts reported in the literature

Plant Organ	Solvent	Pathogen	Dose	References
Leaves (5 cultivars)	Ethanol	*Escherichia coli*	Full inhibition not attained at 50,000 µg·mL^{-1}; IZ = 9.1–14.1 mm	(Buyun et al. 2021)
New leaves, stems, and stems–leaves	Methanol	*Bacillus cereus* *Malassezia pachydermatis* *Staphylococcus aureus*	Full inhibition not attained; IZ = 8.90–17.76 mm	(M.-H. Choi et al. 2013)
Leaves	Methanol Water Butanol Chloroform *n*–Hexane Ethylacetate	*E. coli* *Salmonella typhimurium* *S. aureus* *Listeria monocytogenes*	MICs = 625–2500 µg·mL^{-1} (methanol and butanol), 2500 µg·mL^{-1} (water)	(Hahn 2005)
Petals	Methanol (basic, acidic, and neutral fractions)	*S. typhimurium* *E. coli* *L. monocytogenes* *S. aureus*	Full inhibition not attained; IZ = 13–19 mm	(Kim et al. 2001)
Young leaves, mature leaves, flower buds, flowers, bark, branches, and seeds	Methanol	*Bacillus subtilis* *Streptomyces fradiae* *S. aureus* *E. coli* *Pseudomonas aeruginosa* *Enterobacter* spp. *S. typhimurium*	MIC = 1000–15,000 µg·mL^{-1} for young leaves	(S.-Y. Lee et al. 2005)
Flowers	Water: methanol	*Staphylococcus epidermidis* *S. aureus* *P. aeruginosa* *Salmonella enteritidis* *B. cereus* *E. coli*	IZ = 5.05–14.02 mm, no inhibition against *E. coli*	(Pereira et al. 2022b)
Leaves	Ethanol and methanol (fermented)	*S.epidermidis* *B. subtilis* *Klebsiella pneumoniae* *E. coli*	IZ = 3.3–4.2 mm, depending on the pathogen, at a dose of 16,000 µg	(Moon and Kim 2018)

IZ: inhibition zone, *MIC*: minimum inhibitory concentration

scanning electron microscope (Carl Zeiss Microscopy, Cambridge, UK) operated at an accelerating voltage (EHT) of 15.00 kV. High-resolution micrographs were acquired to assess the biofilm architecture and structural alterations induced by the extract.

In planta protection test against *E. amylovora*

Greenhouse trials were performed on *P. communis* cv 'San Pietro' plants. Throughout the experiments, the plants were kept under controlled conditions at 25 °C and 70% relative humidity, with approximately 12 h of daily sunlight exposure.

Erwinia amylovora strains were cultured overnight in 5 mL of LB broth at 28 °C for 24 h. Following centrifugation, the bacterial pellets were resuspended in a PBS solution to achieve a bacterial concentration of 10^7 CFU·mL^{-1}.

In the control group, a 50 µL aliquot of the EA suspension was introduced into shoots (15–20 cm) using a scissor and a syringe. In the treatment group, the shoots received 50 µL of the EA suspension. Seven days postinoculation, when symptoms appeared, *C. japonica* flower extract at its MIC (1500 µg·mL^{-1}) was sprayed onto each infected shoot. Disease symptoms on the shoots were evaluated 14 and 28 days after the initial EA inoculation. We used standard horticultural soil as substrate. Four plants were included in total, one per treatment (one plant per negative control, solvent control, positive control, one plant per type of extract - treatment). The spray volume was standardized at 10 mL per shoot. A vehicle-treated infected control was included.

For each shoot, both the total shoot length and the length of the lesions were measured. Fire blight susceptibility was quantified via the disease index (DI): DI = (length of blighted shoot/total shoot length) × 100. The experiment was conducted in triplicate to ensure the robustness and reproducibility of the results.

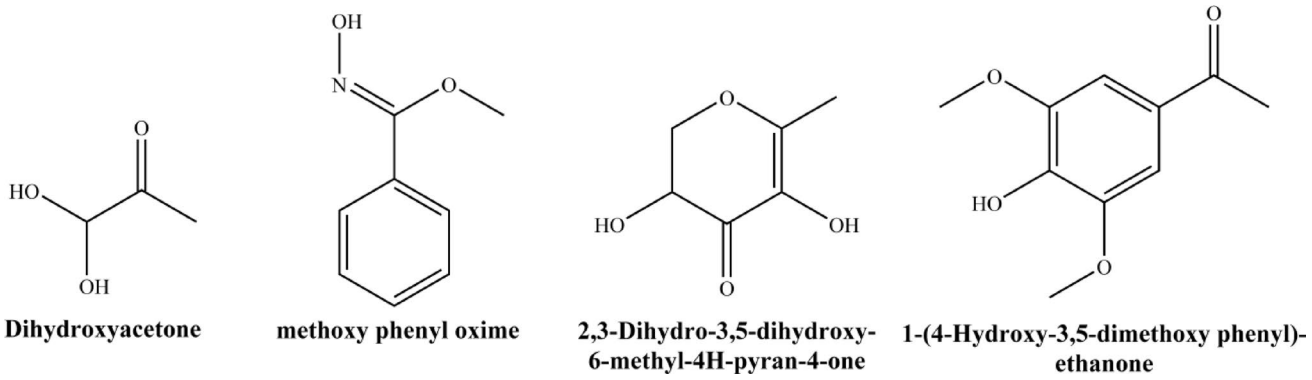


Fig. 1 Flavoring compounds identified by GC−MS in *C. japonica* leaves extract

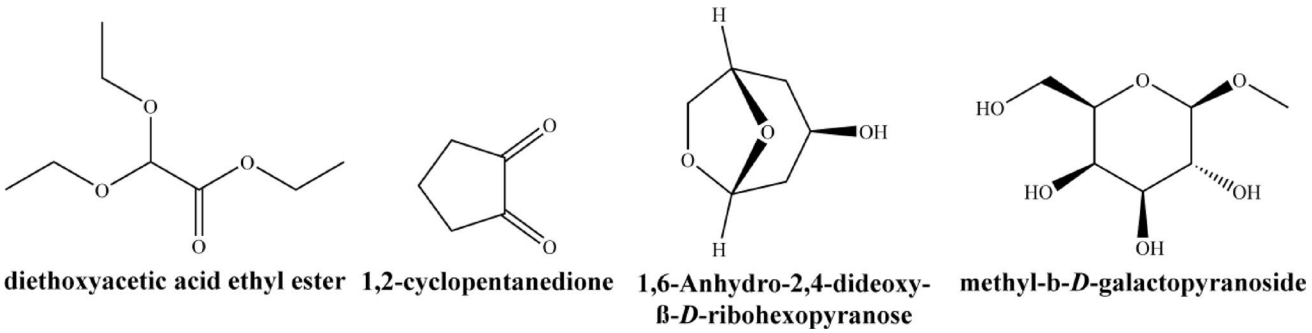


Fig. 2 Some of the phytoconstituents identified by GC−MS in *C. japonica* flower extract

Statistical analysis

All experiments were conducted with at least three independent biological replicates and two technical replicates. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine significant differences between treatments at $p<0.05$. Statistical analyses were performed using GraphPad Prism (version 9.1) and results are expressed as means ± standard deviation (SD). Where appropriate, non-parametric tests were applied to validate data consistency. Details of sample sizes (n) and statistical tests are provided in the figure legends.The Shapiro–Wilks test and Bartlett test were employed to assess the statistical assumptions of normality and homoscedasticity, respectively. Following these evaluations, comparisons were conducted through two-way ANOVA, followed by Dunnett's post hoc test.

Results

Functional groups identified by infrared spectroscopy

The FTIR spectrum of *C. japonica* leaves was less complex than that of the flowers, with 14 distinct bands compared with 22 in the flowers (3, Fig. S1). The most significant peaks, located at 2916–2849, 1732, 1625, 1245, and 1027 cm^{-1}, indicate functional groups associated with cutin/wax/pectin, pyranones, oximes, cyclic ethers/saponins, and methoxyphenolics, respectively. The increased presence of methoxy-phenyl-oxime (MPO) in flowers, as determined by GC–MS (detailed in the following section), is reflected in the flower spectrum by the presence of additional bands attributable to MPO.

Phytochemical profiling by GC–MS

The main constituents identified in the hydromethanolic leaf extract of *C. japonica* were (Fig. 1, Fig. S2, Table S1): *D*-fucose (33.6%); dihydroxyacetone (DHA 7.6%); methoxy-phenyl-oxime (MPO, 5.7%); 2,3-dihydro-3,5-dihydroxy-6-methyl-4 H-pyran-4-one or hydroxydihydromaltol (DDMP, 5.3%); ethyl 3,4-dihydroxybenzoate (4.9%); 5-hydroxymethylfurfural (4.5%); *n*-hexadecanoic acid (2.9%); 4-hydroxy-3,5-dimethoxy-benzaldehyde (2.5%); dihydro-4-hydroxy-2(3 H)-furanone (2.4%); squalene (1.9%); and 1-(4-hydroxy-3,5-dimethoxyphenyl)-ethanone or acetosyringone (1.3%).

The phytochemical profile of the *C. japonica* flower extract (Fig. 2, Fig. S3, Table S2) revealed that MPO (14.1%) and DDMP (6.3%) were the major constituents

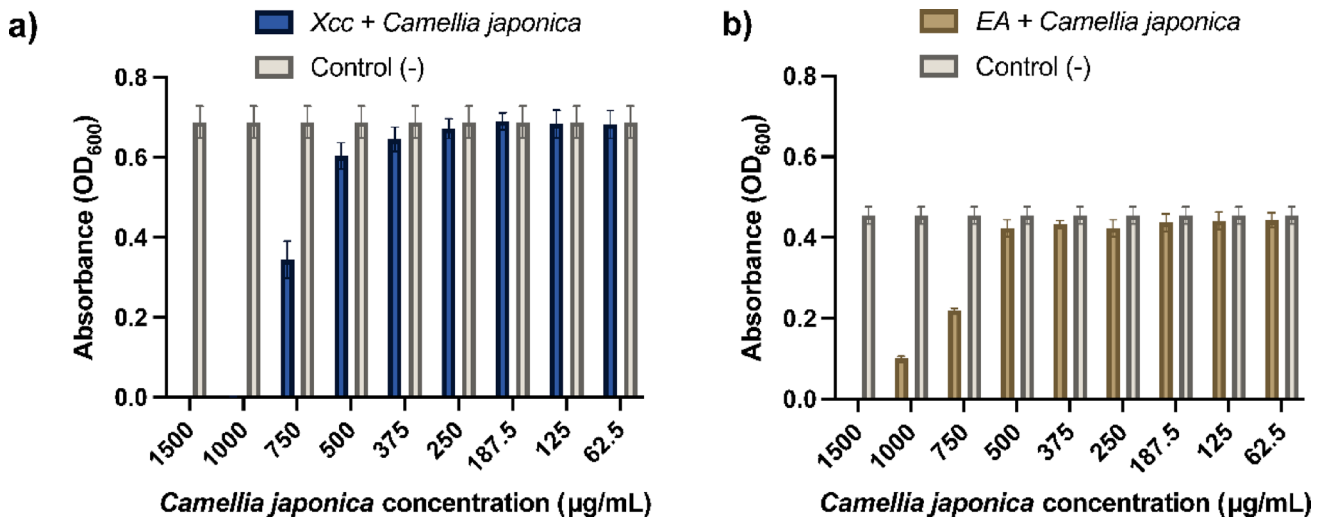


Fig. 3 Antibacterial activity of *C. japonica* flower extract against **a** Xcc and **b** EA

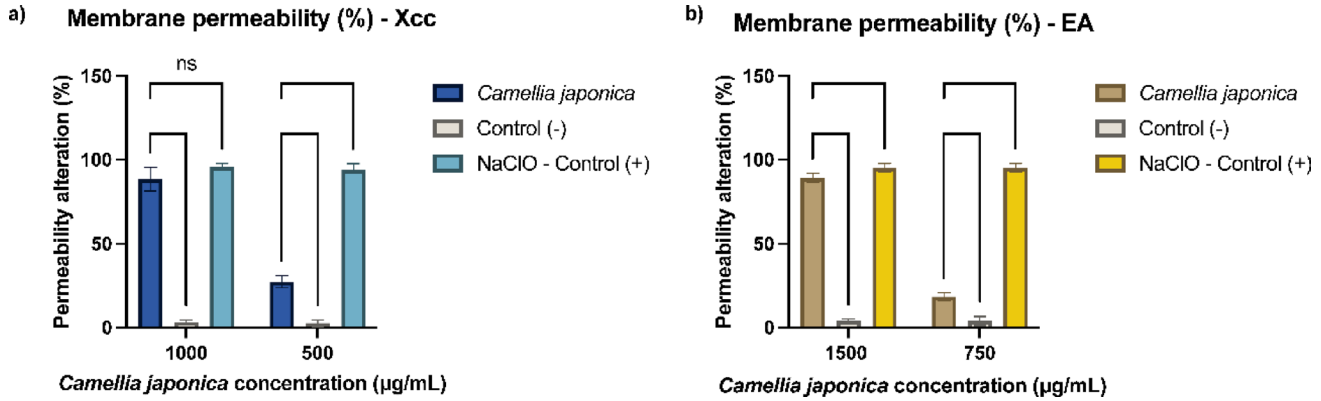


Fig. 4 Effects of *C. japonica* flower extract on **a** Xcc and **b** EA membrane permeability at t = 60 min. Blue and brown columns represent bacterial suspensions treated with the correspondent concentration of *C. japonica* (1500, 1000, 750, or 500 µg·mL^{-1}); light blue and yellow columns represent bacterial suspensions treated with NaClO 7% as positive control, while the light brown columns represent bacterial suspensions as negative control; ns, * and **** stand for not significant, $p < 0.05$, and $p < 0.0001$, respectively

shared with the leaf extract. Additionally, the flower extract contains diethoxyacetic acid ethyl ester (6.6%), nonanoic acid (5.9%), 1,2-cyclopentanedione (4.2%), eicosane (3.9%), 1,6-anhydro-2,4-dideoxy-*β*-*D*-ribohexopyranose (3.5%), 5-hydroxymethylfurfural (3.1%), methyl-*β*-*D*-glucopyranoside (2.7%), and methyl *β*-*D*-galactopyranoside (2.2%).

Antimicrobial activity

In vitro antibacterial activity

The antimicrobial activity of the *C. japonica* extracts was evaluated against Xcc and EA, and their effects on bacterial growth, membrane permeability, biofilm formation, and amylovoran production (specific to EA) were examined. Growth inhibition studies (Fig. 3) demonstrated that the flower extract exhibited greater bactericidal and bacteriostatic activity against the tested Xcc strains at 1000 µg·mL^{-1}, with consistent effects across strains, showing a moderate activity compared with conventional bactericides (streptomycin). For EA, both the MIC and minimum bactericidal concentration (MBC) were 1500 µg·mL^{-1}. The leaf extract exhibited no inhibitory activity against either bacterial species at the maximum tested concentration of 1500 µg·mL^{-1} (Figs. S4–S5).

- Membrane permeability alteration.

For the membrane permeability analysis (Fig. 4), subminimal inhibitory concentrations (MIC/2) and the MIC concentration of *C. japonica* flower extract were administered to bacterial suspensions of Xcc and EA. At 60 min (Fig. 4), the MIC/2 concentration led to 25% and 17% increases in membrane permeability in Xcc and EA, respectively. The effects of the MIC concentration were comparable to those

observed with the positive control, in which bacteria were treated with hypochlorous acid.

Initial alterations in membrane permeability were already detectable at 30 min posttreatment (data not shown) and remained statistically consistent throughout the 180-minute experimental period. The maximum permeability alteration occurred at 60 min and was maintained for the remainder of the assay duration, indicating a sustained disruptive effect of the extract on bacterial membranes.

Antibiofilm activity

To investigate the potential antibiofilm effects of *C. japonica* flower extract and visualize the biofilm removal process, biofilm formation (Fig. 5a, b) and removal (Fig. 5c, d) on abiotic surfaces, such as a plastic cell strainer, were examined. In vitro experiments using crystal violet staining demonstrated that the flower extract inhibited biofilm formation and led to its removal from inert surfaces. However, it demonstrated differential efficacy between bacterial species, reducing biofilm formation by 74% for Xcc at 1000 $\mu g \cdot mL^{-1}$ (Fig. 5a) and by 58% for EA at 1500 $\mu g \cdot mL^{-1}$ (Fig. 5b). Similarly, in the removal assay, the extract effectively reduced mature biofilms by 57% for Xcc (Fig. 5c) and by 36% for EA (Fig. 5d).

Biofilm disruption was subsequently examined via SEM. In the case of Xcc (Fig. 6), exposure to the flower extract resulted in a 50% reduction in planktonic cells, as assessed by optical density measurements of the culture supernatant. SEM images also revealed visible signs of cellular stress, including membrane folding, compared with those of the controls. Untreated Xcc cells formed well-defined, three-dimensional biofilms with abundant, densely packed, multilayered cellular clusters. An extracellular matrix was abundantly produced during biofilm maturation, as observed in Fig. 6a-d, where matrix foils and thin fibers connecting and covering cells were evident. Bacterial cells were clustered in localized groups within the interspaces of the cell strainers, where they multiplied and appeared suspended in air spaces, likely protected by a drying extracellular polymeric substance (EPS) during sample fixation. In contrast, after treatment with the flower extract (Fig. 6e-h), Xcc bacteria were primarily observed as isolated, planktonic cells.

The flower extract also led to significant EA biofilm disruption (Fig. 7e-h). The treated EA cells largely failed to fill the cell strainer voids or colonize the filaments, with only a few bacterial aggregates observed on isolated portions. The extract inhibited the maturation of multilayered EA biofilms. This suggests potential interference with quorum sensing (QS) systems and post-dissemination adhesion processes. Importantly, biofilm maturation is a progressive and dynamic process that extends beyond initial surface adhesion. Even in established biofilms, cell–cell communication (QS), matrix production, dissemination and structural

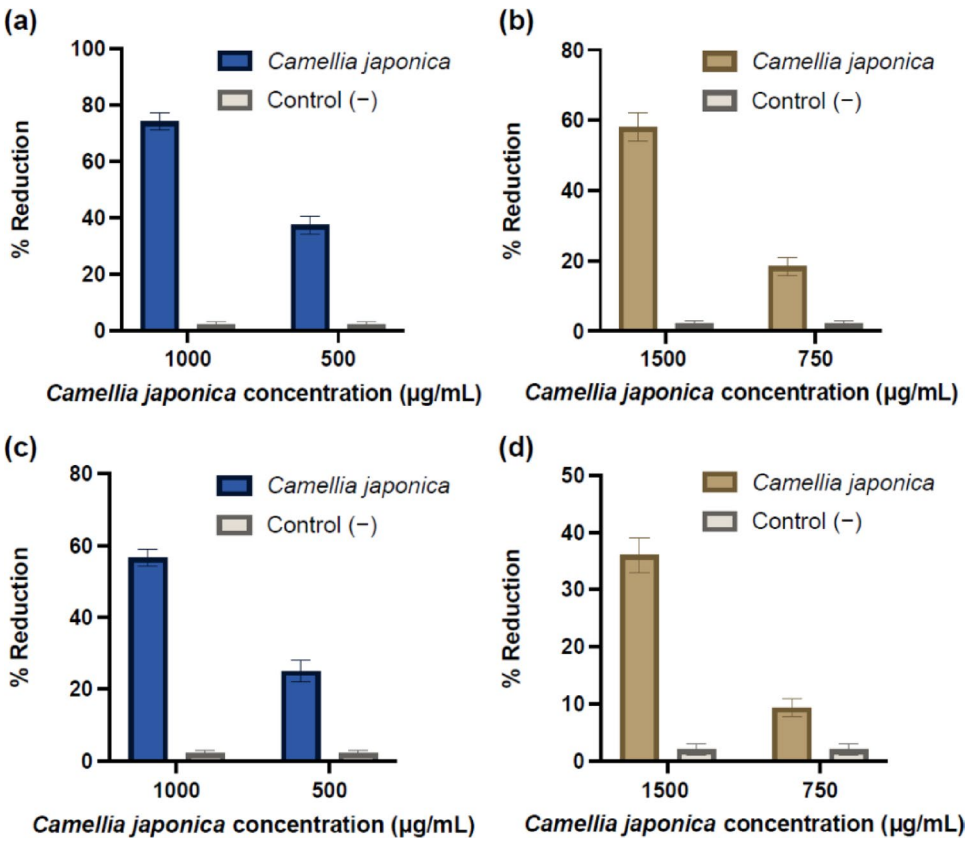


Fig. 5 Effect of *C. japonica* flower extract on biofilm formation (% reduction) of **a** Xcc and **b** EA; and on biofilm removal (% reduction) of **c** Xcc and **d** EA

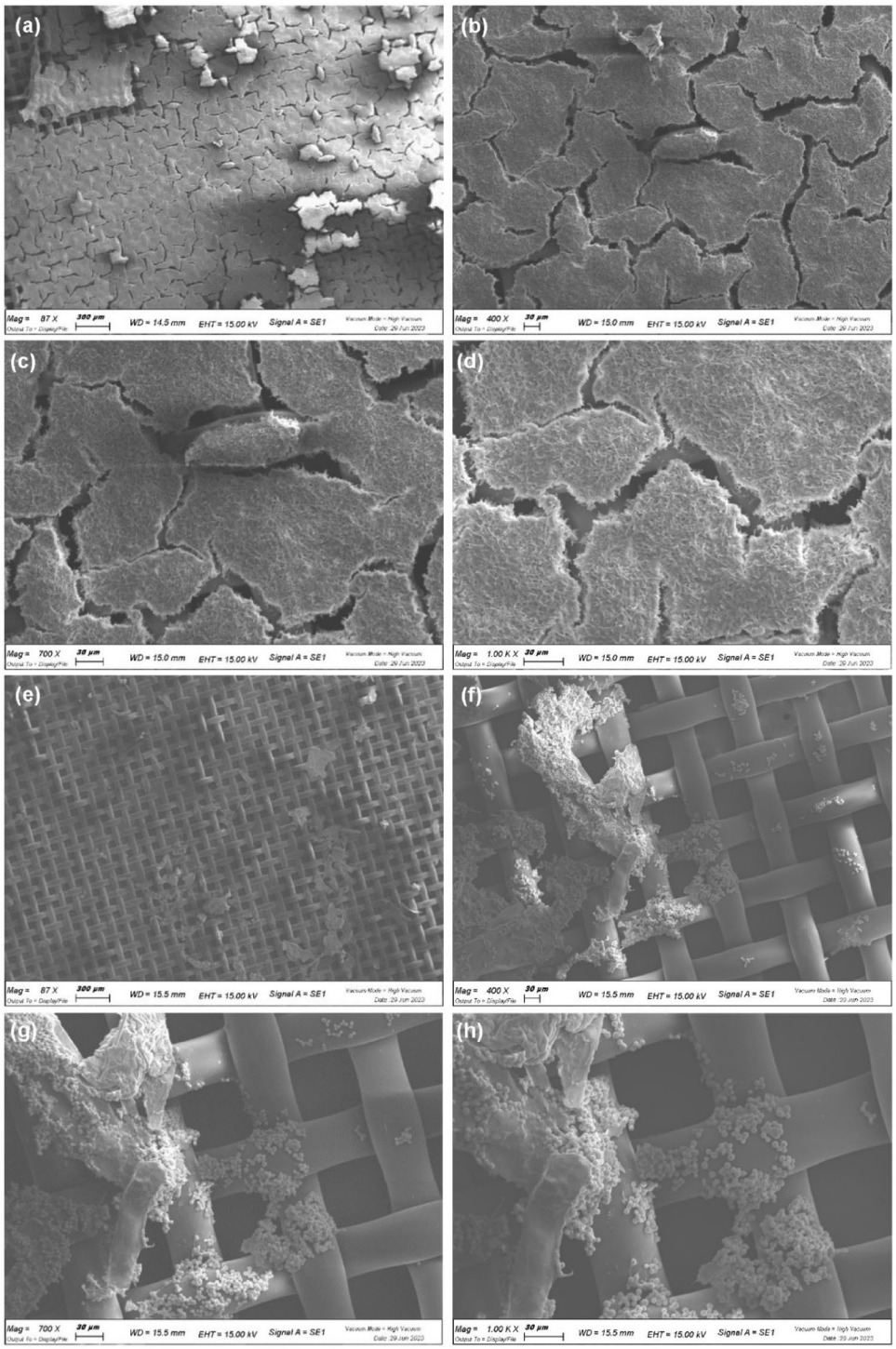


Fig. 6 SEM micrographs of the effects of *C. japonica* flower extract on Xcc biofilm maturation on an abiotic surface: (**a-d**) control samples; (**e-h**) *C. japonica*-treated samples

remodeling remain active. EA cells treated with the MIC/2 concentration of the flower extract exhibited poor adherence and failed to form complex three-dimensional structures. In particular, in the control group (Fig. 7a-d), dense and multilayered biofilm structures were evident. At lower magnification (Fig. 7a, b), the surface appeared extensively colonized by bacterial cells, forming a thick biofilm network with filamentous structures bridging the interstitial gaps between the mesh fibers. Higher magnification images (Fig. 7c, d) further revealed mature biofilm morphology, with embedded bacterial aggregates and an EPS matrix clearly observable. These structures are indicative of advanced biofilm development, with cells adhering tightly to the substratum and to each other, suggesting a robust and well-established biofilm phenotype. In contrast, samples treated with *C. japonica* flower extract (Fig. 7e-h) presented markedly altered surface

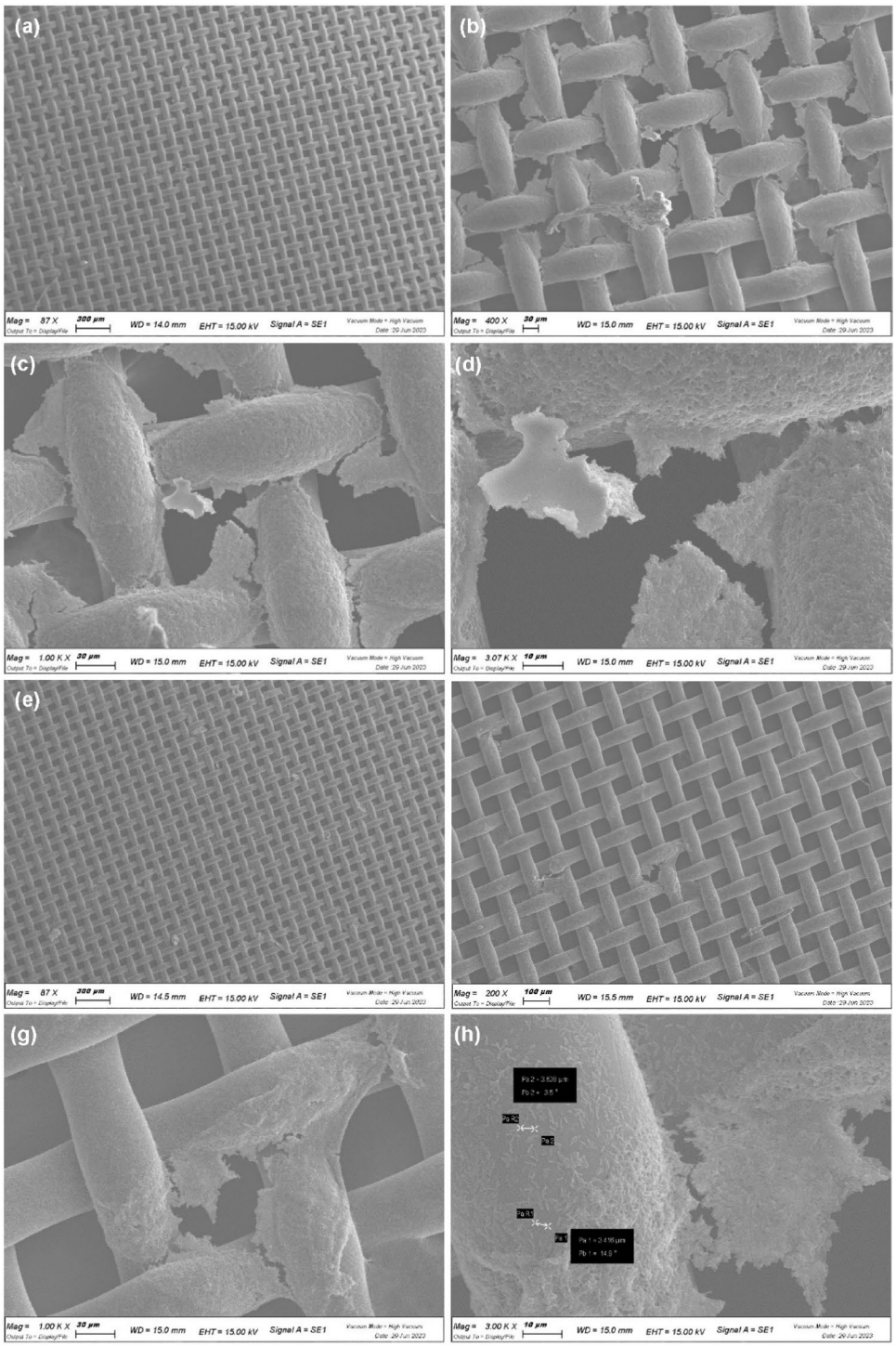


Fig. 7 SEM micrographs of the effects of *C. japonica* flower extract on EA biofilm maturation on an abiotic surface: **a-d** control samples; **e-h***C. japonica*-treated samples

colonization. At lower magnifications (Fig. 7e, f), the mesh appeared largely devoid of bacterial coverage, suggesting significant inhibition of biofilm maturation. Higher magnification images (Fig. 7g, h) revealed disrupted and scattered bacterial residues, with no evident EPS production or mature biofilm architecture.

- Amylovoran production.

At MIC/2 (750 $\mu g \cdot mL^{-1}$), treatment with the *C. japonica* hydromethanolic flower extract resulted in a 2.57-fold reduction in amylovoran production by *E. amylovora*, as indicated by OD_{600} measurements (Fig. 8).

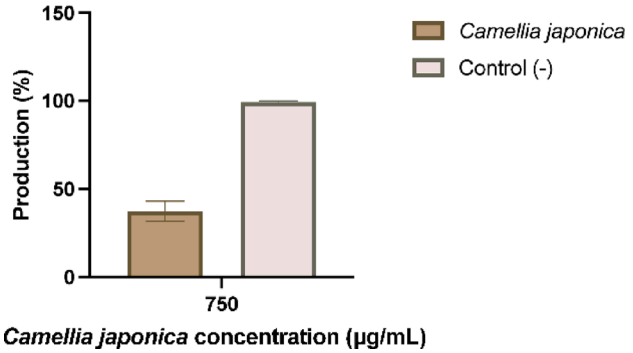


Fig. 8 Effect of *C. japonica* flower extract on amylovoran production

In planta antibacterial activity

To investigate the effectiveness of *C. japonica* flower extract against EA, an initial study was undertaken to evaluate the antibacterial effects within a plant model. Considering that EA is responsible for fire blight in apple and pear trees, the antibacterial properties of *C. japonica* flower extracts were tested on pear trees artificially inoculated with the bacterium. The disease begins at the tips of young shoots and quickly advances into older twig parts. The affected twigs initially appear water-soaked and then darken to brown or black. As infected shoots wilt, they bend at the growth point, forming a shape similar to a shepherd's crook or an inverted 'J'. Blighted leaves may remain on dead branches throughout summer but fall off during humid conditions. In warm and humid weather, infected shoots might exude creamy white bacterial droplets.

As illustrated in Fig. 9, the control group experienced complete defoliation within 10 days post infection (Fig. 9c-d). Conversely, the extract-treated group presented a notable reduction in infection, although some wilting symptoms were still evident after treatment. The extract demonstrated high effectiveness, achieving a 71% reduction in the wilting area. Pear tree branches in the control group (Fig. 9) exhibited worsening fire blight symptoms over 10 days postinoculation, with noticeable bacterial exudate indicating severe disease progression. In contrast, branches treated with *C. japonica* flower extract presented a significant decrease in disease severity. Seven days posttreatment (Fig. 10b), only a few necrotic areas were observed on some leaves, indicating partial treatment efficacy.

Discussion

The results of this study confirm that Camellia japonica flower extracts exhibit potent antibacterial activity against two major bacterial phytopathogens, supporting and extending recent findings on the antimicrobial potential of plant-derived compounds. These results are particularly relevant given the increasing need for sustainable plant protection strategies in the context of reduced pesticide use and growing concerns about antimicrobial resistance.

Phytochemical profile

In the literature, vitamin E, *n*-eicosane, lupeol, neophytadiene, *n*-octacosane, 6,9-pentadecadien-1-ol, *α*-linolenic acid, *n*-hexadecanoic acid, and *trans*-squalene have been documented as key components in *C. japonica* leaf extracts (Yoon et al. 2017). For flower extracts, Pereira et al. (2022a) highlighted gallic acid, hydroxybenzoic acid, quercetin, kaempferol, sexangularetin, camellianoside, and epicatechin as primary phytochemicals, with minor quantities of flavonoid-glucopyranosides. However, our GC−MS results

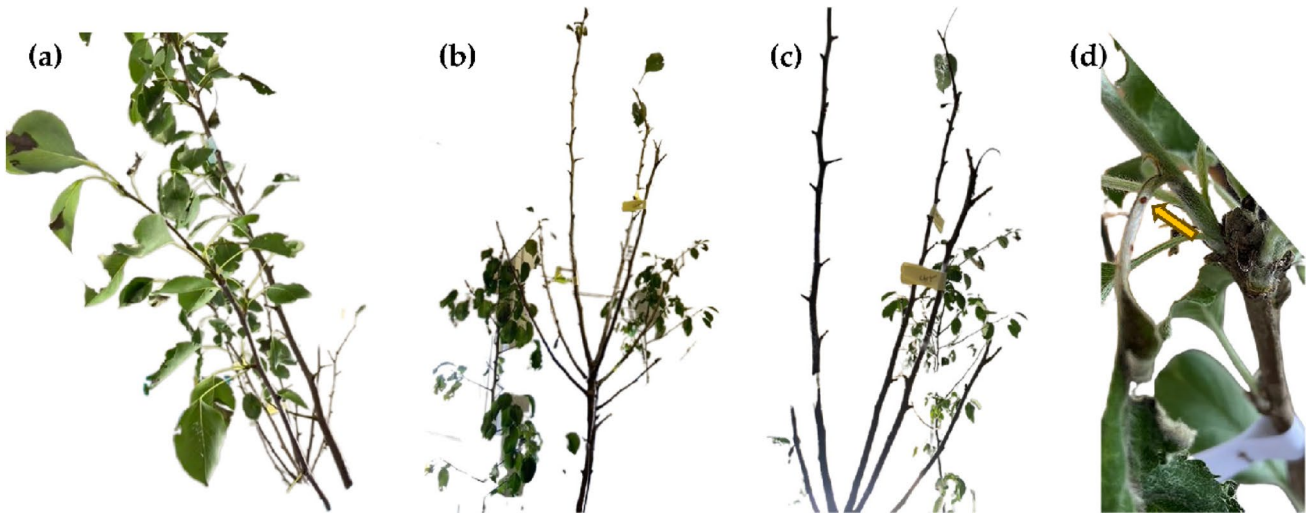


Fig. 9 *In planta* assay with EA: positive control. **a** A control pear tree branch preinoculation, **b** a control pear tree branch 7 days postinoculation, **c** a control pear tree branch 10 days after inoculation, and **d** a control pear tree branch 10 days after inoculation with a focus on single branches

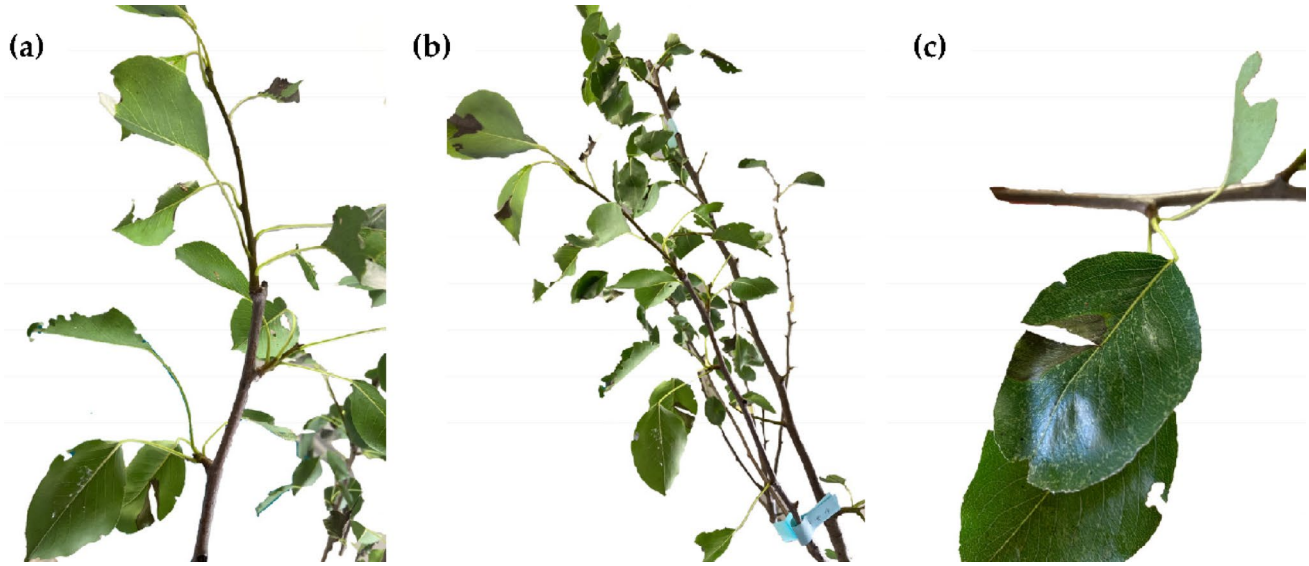


Fig. 10 *In planta* assay depicting the effect of the *C. japonica* flower extract on EA. **a** A pear tree branch 2 days postinoculation/before treatment, **b** a pear tree branch 7 days after treatment/14 days postinoculation, and **c** a pear branch 7 days after treatment with a focus on individual leaves

only coincide in the presence of *n*-hexadecanoic acid and squalene (for leaves) and eicosane and glucopyranosides (for flowers). Disparities in composition may be correlated with our specific extraction procedure, the method of characterization employed, or the potential existence of distinct chemotypes.

Three principal components identified in our extracts (DHA, MPO, and DDMP) are known to participate in Maillard reactions (Petersen et al. 2004; Yu et al. 2013; Ledl et al. 1976). This suggests that the ultrasonication-mediated extraction procedure, even at 50 °C, might facilitate conditions conducive to some Maillard-type reactions or preserve these preexisting aroma-active compounds, similar to the effects observed in heat-based preservative treatments (Dursun et al. 2016). The distinctive tea-like aroma of *C. japonica*, similar to that of black tea (*Camellia sinensis* Kuntze), is likely attributable to a blend of DHA, MPO, DDMP, acetyl syringone, pentanal, and furanics. Among these, DHA, DDMP, and acetyl syringone contribute significantly to the flavor profile. In contrast, MPO, pentanal, and furanics (when present exclusively) result in a scent reminiscent of old books (Strlič et al. 2009).

With respect to the phytoconstituents identified in the leaf extract, fucose is a hexose previously detected in the roots of *Hemidesmus indicus* (L.) R.Br. (Das and Devaraj 2006). DHA, also known as glycerone, is a colorless 3-carbon sugar identified, for example, in *Agave americana* L. and *Pittosporum tobira* W.T.Aiton. It imparts a sweet, cooling-type flavor and exhibits antifungal properties (Stopiglia et al. 2010). DHA has been approved by the Food and Drug Administration (FDA) for applications in food, medicine, and pharmaceuticals (Bricotte et al. 2023). It is commonly sourced from plants such as sugar beet and sugarcane, as well as through the fermentation of glycerin.

Methoxyphenyl-oxime (methyl N-hydroxybenzimidate) is a plant oxime with a black tea flavor that is also found in *Digitalis purpurea* L. and *Momordica charantia* L. and functions as an antibacterial agent (Sa et al. 2017; Chipps et al. 2012). In plants, oximes serve as intermediate metabolites in growth and developmental biosynthetic pathways (Sørensen et al. 2018). The introduction of oxime groups into natural compounds enhances their biological activities, including antiviral, insecticidal, fungicidal, and bactericidal properties (Li et al. 2016; da Souza et al. 2016).

2,3-Dihydro-3,5-dihydroxy-6-methyl-4 H-pyran-4-one (DDMP) is a saponin that contributes to the toasty caramel aroma released in wine during aging with toasted wood. DDMP has been previously identified in several plant species, including *Acalypha indica* L., *Ammannia baccifera* L., *Borassus flabellifer* L., *Cocculus hirsutes* (L.) Diels, *Cucumis sativus* L., *Euphorbia serrata* L., *Lepidium sativum* L., *Leucas aspera* (Willd.) Link, *Litchi chinensis* Sonn., *Marsilea quadrifolia* L., *Punica granatum* L., *Sambucus nigra* L., and *Rumex vesicarius L.* (Suman et al. 2013; Sánchez-Hernández et al. 2022, 2023a, c). It has demonstrated antifungal effects (Teoh and Mashitah 2016) and has proven effective against various plant pathogens, such as *Diaporthe amygdali* (Delacr.) Udayanga, Crous & K.D. Hyde, *Diplodia seriata* De Notaris, *Phytophthora megasperma* Drechsler, *Verticillium dahliae* Klebahn, as well as against bacteria such as *E. amylovora* and *Xylophilus ampelinus*. The inhibition values ranged from 375 to 750 $\mu g \cdot mL^{-1}$ (Sánchez-Hernández et al. 2022, 2023a).

5-hydroxymethylfurfural (5-HMF), which is found, for instance, in *Citrus jabara* Hort. ex Y. Tanaka, *Ginkgo biloba* L., *Lycium chinense* Mill., *Punica granatum* L. var. *nana*, *Prunus mume* (Siebold) Siebold & Zucc., *Taxus baccata* L., and *Tussilago farfara* L. (Uchida et al. 2020; Yamada et al. 2010; Sánchez-Hernández et al. 2022, 2023a, b, c, d), emits the aroma of chamomile flowers and possesses antibacterial properties (Nafea et al. 2011). It has demonstrated activity against plant pathogens, including *D. seriata*, *E. amylovora*, and *X. ampelinus*, with MIC values in the 1000–1500 $\mu g \cdot mL^{-1}$ range (Sánchez-Hernández et al. 2022). Higher efficacies have also been reported for other pathogens, such as *Klebsiella* sp., where Kaur et al. (2018) reported MICs in the 40–160 $\mu g \cdot mL^{-1}$ range.

4,4-Dimethyl-5-methylene-2-allylamino-2-thiazoline has been reported as a product of the pyrolysis of *Manihot esculenta* Crantz rhizome (Praserttaweeporn et al. 2022). Although its specific antimicrobial activity is undocumented, as a thiazole derivative, it may possess such properties, a known characteristic of this chemical class.

1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone is a phenolic compound with a faint vanilla flavor. It has been reported as one of the phenolic constituents present in wood vinegar from *Litchi chinensis* Sonn. and *Solanum khasianum* C.B. Clarke leaf and root extracts, identified as a non-steroidal bioactive compound with antimicrobial activity (Yang et al. 2016; Chirumamilla et al. 2022).

Diethoxyacetic acid ethyl ester has been identified as a phytocomponent in the ethyl acetate extract of *Strychnos innocua* Delile root bark (Ibrahim et al. 2021). It exhibited antibacterial and antifungal activities, inhibiting the growth of *Staphylococcus aureus* Rosenbach and *Candida albicans* (C.P. Robin) Berkhout.

1,2-Cyclopentanedione has been identified in ethanolic leaf and fruit extracts of *Trichosanthes dioica* Roxb. (Kavitha 2021) and *Euphorbia inarticulata* Schweinf., which have antibacterial properties (Al Abboud et al. 2023).

1,6-Anhydro-2,4-dideoxy-*β*-*D*-ribohexopyranose has been found in the methanolic extracts of *Momordica cymbalaria* Fenzl (Gopu et al. 2021) and is the main constituent of the ethanolic extract of *Dichrostachys cinera* (L.), which exhibits antibacterial activity against *S. aureus*, *Escherichia coli* , and *Pseudomonas aeruginosa*, with MIC values in the 12.5–25 $mg \cdot mL^{-1}$ range (Yassin et al. 2022).

Methyl *β*-*D*-galactopyranoside has been identified in alcoholic extracts of young and mature leaves of khat leaves (Kofat, Gaifi, and Gahasha cultivars) (Alsanosy et al. 2020), and some of its derivatives have demonstrated in vitro activity against *Bacillus cereus* Frankland & Frankland, *Bacillus subtilis*, *E. coli*, *Salmonella typhimurium*, and *P. aeruginosa* (Ahmmed et al. 2022).

Ethyl 3,4-dihydroxybenzoate, identified as a phenolic compound, has demonstrated efflux pump inhibitory activity against drug-resistant *E. coli*, potentially potentiating antibiotic activity (Lu et al. 2022).

4-Hydroxy-3,5-dimethoxy-benzaldehyde (syringaldehyde), another phenolic compound found, for example, in the roots of *Vepris uguenensis* Engl., is also well known for its antimicrobial activity (Ibrahim et al. 2012).

Finally, dihydro-4-hydroxy-2(3 H)-furanone (or 3-hydroxybutyrolactone) does not have reported antimicrobial activity but is a precursor in the synthesis of various pharmaceuticals.

Antimicrobial activity of C. japonica extracts

This study represents the first investigation of the antimicrobial efficacy of *C. japonica* against agriculturally significant phytopathogens. While previous research has documented activity against human pathogens, including various *Bacillus*, *Staphylococcus*, and *Enterobacter* species (Table 4), the potential application of *C. japonica* extracts in plant disease management has remained unexplored until now.

Our findings demonstrate that *C. japonica* flower extracts exhibit potent bactericidal and bacteriostatic effects against both Xcc and EA, with MIC values of 1000 and 1500 $\mu g \cdot mL^{-1}$, respectively. Although direct comparisons are challenging due to differences in methodology (most studies employ the disc diffusion assay) and the pathogens under study, these concentrations fall within the lower range of previously reported values for human pathogens (625–15,000 $\mu g \cdot mL^{-1}$) (S.-Y. Lee et al. 2005; Hahn 2005), indicating relatively high antimicrobial potency. Notably, the flower extract significantly outperformed the leaf extract, which showed no activity at the tested concentrations, in contrast with earlier studies that suggested superior activity in young leaf hydroalcoholic extracts (S.-Y. Lee et al. 2005; Hahn 2005).

The antimicrobial activity observed in this study is likely attributable to the significant contributions of two principal components identified via GC–MS, MPO and DDMP, which potentially act synergistically. MPO exerts its antibacterial effects through multiple mechanisms centered on its oxime group ($R_1R_2C{=}N{-}OH$). First, the nitrogen and oxygen atoms within the oxime group can form hydrogen bonds with bacterial enzymes, particularly *β*-ketoacyl-(acyl-carrier-protein) synthase III (FabH), a key enzyme in fatty acid biosynthesis. FabH inhibition disrupts membrane lipid synthesis, ultimately leading to cell death (Luo et al. 2012). Second, certain oxime derivatives enhance antibiotic efficacy by blocking the *β*-lactamase enzymes responsible for antibiotic resistance in Gram-negative bacteria (Odžak et al. 2013). Third, oximes generate reactive oxygen species

(ROS) that exacerbate oxidative stress and damage bacterial DNA and proteins (Schepetkin et al. 2021). The methylphenyl moiety of MPO further enhances its antimicrobial activity by increasing its lipophilicity, which facilitates the penetration of bacterial membranes and interactions with hydrophobic membrane regions, thereby destabilizing the membrane structure (Barghouthi et al. 2017).

DDMP contributes to antimicrobial effects through complementary mechanisms. This compound generates reactive oxygen species that induce DNA strand breakage and mutagenesis in bacterial cells, as demonstrated by Hiramoto et al. (1997) in *S. typhimurium*. Furthermore, DDMP inhibits tyrosinase activity, which may prevent enzymatic browning and tissue weakening in infected plant hosts, providing an additional protective effect beyond direct antimicrobial action (Xu et al. 2016).

The impact of the extract on EA virulence factors represents a particularly significant finding. Amylovoran, the primary exopolysaccharide virulence factor (Zhao and Qi 2011; Zhao et al. 2009), showed a 2.57-fold reduction in production following treatment. This suppression likely contributed to the 71% reduction in fire blight severity observed in our *in planta* experiments. The amylovoran biosynthetic pathway, encoded by the 12-gene *ams* operon (*amsA* to *amsL*) (Bernhard et al. 1993; Bugert and Geider 2006), presents multiple potential targets for phytochemical intervention. While specific molecular targets remain to be elucidated, recent evidence suggests that phytochemicals can modulate bacterial gene expression, potentially affecting virulence factor production (Hirooka et al. 2007).

Scanning electron microscopy observations of biofilm disruption provide further mechanistic insights. The complete prevention of mature biofilm formation at MIC suggests interference with QS systems and bacterial adhesion processes. The flower extract not only prevented initial biofilm establishment but also disrupted existing biofilms, reducing the mature biofilm mass by 57% for Xcc and 36% for EA. This dual action (inhibiting biofilm formation and disrupting established biofilms) enhances the potential practical application of these extracts in agriculture, where both prophylactic and therapeutic strategies are valuable.

The broad-spectrum activity against both tested phytopathogens, combined with the multiple mechanisms of action, suggest that *C. japonica* flower extracts could provide effective control of various Gram-negative plant pathogens. The natural origin of these compounds, coupled with their demonstrated efficacy at relatively low concentrations, makes them promising alternatives to conventional antibiotics in sustainable agriculture.

The results of this study confirm that *Camellia japonica* flower extracts exhibit potent antibacterial activity against two major bacterial phytopathogens, supporting and extending recent findings on the antimicrobial potential of plant-derived compounds. These results are particularly relevant given the increasing need for sustainable plant protection strategies in the context of reduced pesticide use and growing concerns over antimicrobial resistance. Fire blight and black rot remain persistent challenges for growers due to the limited availability of effective bactericides and regulatory restrictions on antibiotic use in agriculture. The activity of *C. japonica* extracts against both pathogens supports the idea that natural products may help bridge this gap, providing alternative or complementary solutions for disease control. This is especially relevant in light of increasing regulatory pressure to reduce chemical inputs and the emergence of resistant pathogen strains.

Practical applications, limitations, and future research directions

Previous studies on *Camellia* spp. have mainly addressed the antimicrobial or antifungal activity of leaf or seed extracts, most often from *C. sinensis* or *C. japonica*, and only rarely considered bacterial plant pathogens (Anita et al. 2014). Moreover, the majority of these reports focused on growth inhibition and did not explore specific virulence-associated traits. Our work expands this literature in several ways. First, we characterize a hydromethanolic flower extract from *C. japonica* cv. 'Lipstick', an ornamental cultivar that has been scarcely investigated as a source of antimicrobial compounds. Second, we demonstrate activity against two major bacterial phytopathogens, *E. amylovora* and *X. campestris* pv. *campestris*, which are responsible for fire blight and black rot, respectively. Third, beyond reducing bacterial growth, the extract significantly interferes with biofilm formation, amylovoran production and membrane integrity, pointing to a broader antivirulence effect. Taken together, these features indicate that *C. japonica* flower extracts complement previously described *Camellia*-derived products and support their potential use in integrated management strategies against bacterial plant diseases.

In the current study, the analysis was performed on bacterial isolates provided by the Phytosanitary Service of the Emilia-Romagna Region, a reliable and accredited source; therefore, we did not perform further molecular identifications (i.e., MLSA/MLST, species-specific PCR, or MALDI-TOF).

The demonstrated efficacy of *C. japonica* flower extracts against major bacterial phytopathogens presents significant opportunities for sustainable crop protection. Although the extraction yield from flowers (6.8%) was considerably lower than that from leaves (51.9%), the superior antimicrobial activity of the flower extracts compensates for this difference. Although the leaf extract showed a higher

extraction yield, the GC–MS analysis indicates it is rich in primary metabolites such as D-fucose and dihydroxyacetone, which are not known to exert antibacterial activity at the tested concentrations. In contrast, the flower extract contained higher relative levels of methoxy-phenyl-oxime and DDMP, compounds with documented or suspected antimicrobial properties. The higher yield of the leaf extract likely reflects the abundance of non-active components that dilute any bioactive molecules, leading to no detectable activity. This finding has important implications for the commercial viability of *C. japonica* cultivation, particularly in regions such as Galicia, Spain, where the plant is already grown extensively as an ornamental species. Dual-purpose cultivation (ornamental value and bioactive extract production) could increase the economic sustainability of *C. japonica* farming.

The field application of these extracts faces several practical considerations. The hydromethanolic extraction process employed in this study, while effective for research purposes, may require optimization for large-scale production. Alternative extraction methods, such as aqueous extraction or the use of food-grade solvents, should be explored to increase the environmental sustainability and regulatory acceptability of the final product. Further investigations into the stability of active compounds under field conditions (UV radiation, temperature, precipitation) are crucial for developing optimal formulations.

Several limitations of the current study warrant consideration. First, the research focused on a single cultivar ('Lipstick'), and given the reported chemical variability among *C. japonica* varieties, broader screening of cultivars is necessary to identify those with optimal antimicrobial profiles. Second, while our *in planta* experiments demonstrated efficacy against fire blight under controlled greenhouse conditions, field trials under diverse environmental conditions are essential to validate its practical effectiveness. The influence of edaphoclimatic factors on both the phytochemical composition of *C. japonica* and the efficacy of extracts requires systematic investigation.

While this study identified multiple mechanisms of action (membrane permeabilization, biofilm inhibition, and virulence factor reduction), deeper molecular characterization is needed. Identifying the specific bacterial targets of key compounds such as DDMP and MPO would facilitate the development of more potent formulations and help predict potential resistance mechanisms. Transcriptomic and proteomic analyses of treated bacteria could elucidate the global cellular responses to *C. japonica* extracts and identify novel therapeutic targets.

Future research should address several key areas. The synergistic effects between *C. japonica* extracts and other natural or synthetic antimicrobials should be explored to increase their efficacy and potentially reduce the required concentrations. The development of controlled-release formulations could extend the duration of protection while minimizing the application frequency. Investigation of the effects of the extract on beneficial soil microbiota and plant growth-promoting rhizobacteria is crucial to ensure that widespread application does not disrupt beneficial microbial communities.

The potential for bacterial resistance development, while likely lower than that of conventional antibiotics due to the multitarget nature of the extract, requires monitoring through serial passage experiments. Additionally, comprehensive toxicological studies, including studies on the effects on nontarget organisms, soil health, and potential accumulation in food crops, are necessary before commercial deployment. Economic analyses comparing the cost-effectiveness of *C. japonica* extracts with that of conventional bactericides could guide adoption decisions by growers and inform policy development for sustainable agriculture.

Conclusion

This study establishes *Camellia japonica* cv. 'Lipstick' flower extracts as effective antibacterial agents against economically important phytopathogens. The hydromethanolic flower extracts demonstrated potent activity, with MIC values of 1000 $\mu g \cdot mL^{-1}$ against *Xanthomonas campestris* pv. *campestris* and 1500 $\mu g \cdot mL^{-1}$ against *Erwinia amylovora*; the leaf extracts showed no activity at the tested concentrations. Compared with those of the leaf extracts, the superior efficacy of the flower extracts correlated with higher concentrations of bioactive compounds, particularly methoxy-phenyl-oxime (14.1%) and DDMP (6.3%).

The antimicrobial mechanism involved multiple targets, as evidenced by complete membrane permeabilization at MIC concentrations, 74% and 58% reductions in biofilm formation for *X. campestris* and *E. amylovora*, respectively, and a 2.57-fold decrease in amylovoran production for *E. amylovora*. These multifaceted effects resulted in significant *in planta* disease control, as evidenced by a 71% reduction in fire blight severity on infected pear branches treated with the flower extract at 1500 $\mu g \cdot mL^{-1}$. The comprehensive antimicrobial activity of *C. japonica*, combined with its natural origin and relatively low effective concentrations, makes *C. japonica* flower extracts promising ecological alternatives to conventional bactericides.

These findings open new avenues for the sustainable management of bacterial diseases, particularly fire blight in pome fruit orchards and black rot in cruciferous crops. However, translating these findings to field applications necessitates optimizing extraction processes, developing

stable formulations, and conducting comprehensive field trials, also in synergy with other antimicrobial compounds. The dual ornamental and antimicrobial value of *C. japonica* presents an opportunity for integrated agricultural systems that combine aesthetic landscaping with the production of natural crop protection agents, contributing to the development of more sustainable and environmentally conscious agricultural practices.

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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