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Antimicrobial Activity Screening of *Camellia japonica* Flowers (var. Carolyn Tuttle) for Potential Drug Development†

Antia G. Pereira[1], Aurora Silva[2,1], Clara Grosso[2], Javier Echave[1], Franklin Chamorro[1], Sepidar Mansour[1], Pauline Donn[1], María Fraga-Corral[1], Fatima Barroso[2] and Miguel A. Prieto[1]

1. Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, E32004 Ourense, Spain; (A.G.P.) antia.gonzalez.pereira@uvigo.es; (J.E.) javier.echave@uvigo.es; (F.C.) franklin.noel.chamorro@uvigo.es; (P.D) donn.pauline@uvigo.es; (S.S.M) sepidar.seyyedi@uvigo.es, (A.PV) pau_barci@hotmail.es (L.C) luciavictoria.cassani@uvigo.es, (M.A.P) mprieto@uvigo.es
2. REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr António Bernardino de Almeida 431, 4249-015 Porto, Portugal (A.S.) mass@isep.ipp.pt, (C.G) claragrosso@graq.isep.ipp.pt, (M.F:B) mfb@isep.ipp.pt

* Correspondence: mfb@isep.ipp.pt, mprieto@uvigo.es

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Abstract: The escalating global problem of antibiotic resistance among pathogenic microorganisms necessitates the exploration of effective alternatives to combat multi-resistance. Consumer demand for organic products has stimulated research on natural-origin matrices, such as plants, to develop antimicrobial additives. *Camellia japonica* flowers have demonstrated remarkable biological properties, making them a potential source of bioactive molecules for use as bio-preservatives. This study evaluated the antimicrobial activity of *C. japonica* flowers (var. Carolyn Tuttle) against food-related microorganisms using an agar diffusion assay. Extracts were obtained via a conventional and cost-effective maceration method (50 °C, 1 h) using 60% methanol as the solvent. Results revealed significant antimicrobial activity of *C. japonica* flowers (var. Carolyn Tuttle) against *Staphylococcus aureus* (10.29 mm), *Pseudomonas aeruginosa* (9.24 mm), and *Salmonella enteritidis* (6.95 mm). However, it did not exhibit activity against *Escherichia coli*, *Staphylococcus epidermidis*, and *Bacillus cereus*, unlike other varieties of *C. japonica* that displayed activity against these microorganisms. In conclusion, *C. japonica* flowers (var. Carolyn Tuttle) demonstrated potential as antimicrobial agents with promising applications in the food and pharmaceutical industries. This research contributes to developing natural and organic additives to combat antimicrobial resistance and meet consumer demands for safer and more sustainable products.

Keywords: *Camellia japonica*; flowers; bioactive compounds; antimicrobial

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1. Introduction

The increasing worldwide challenge posed by antibiotic resistance among pathogenic microorganisms has encouraged the search for viable alternatives to address multi-resistance issues [1]. In response to growing consumer preferences for organic products, there has been a surge in research exploring natural-origin matrices, particularly plants, as potential sources of antimicrobial additives [2]. Among these natural resources is *Camellia japonica*. *C. japonica* is a flowering evergreen shrub native to East Asia, particularly Japan and Korea. This remarkable plant species has captured the attention of researchers for its multifaceted applications, which extends beyond its ornamental uses [3].

C. japonica flowers have demonstrated remarkable biological properties, making them a potential source of bioactive molecules for use as bio-preservatives [4]. They have been traditionally used in various cosmetic and skincare products due to their potential skin-enhancing qualities [5]. However, beyond their cosmetic utility, *C. japonica* flowers possess inherent qualities that make them an interesting raw material for scientific investigations.

This study pursues to assess the antimicrobial activity of *C. japonica* flowers extracts, particularly from 'Carolyn Tuttle' variety, against a range of food-related microorganisms using an agar diffusion assay. By harnessing the natural antimicrobial properties of *C. japonica* flowers, we hope to contribute to the development of sustainable and eco-friendly solutions to combat the pressing issue of antibiotic resistance, thereby safeguarding food safety and quality.

2. Material and methods

2.1. Chemicals and reagents

Dimethyl sulfoxide (DMSO), lactic acid, and Mulher Hinton broth (MHB) were procured from Sigma-Aldrich located in Steinheim, Germany. The culture medium, Mulher Hinton Agar II, was obtained from Biolife in Milan, Italy. Strains of *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (ATCC 10145), and *Salmonella enteritidis* (ATCC 13676) were generously supplied by Selectrol in Buckingham, UK. Additionally, *Escherichia coli* (NCTC 9001) and *Staphylococcus epidermidis* (NCTC 11047) were sourced from Microbiologics in Minnesota, USA.

2.2. Raw material and extraction protocol

C. japonica petals (var. Carolyn Tuttle) underwent botanical identification through official germplasm banks and reference materials. They were gathered in Northwestern Spain (coordinates 42.431° N, 8.6444° W) in January 2020. Following collection, the samples were subjected to lyophilization using a LyoAlfa10/15 freeze dryer from Telstar, ThermoFisher Scientific, based in Waltham, MA, USA. After lyophilization, the samples were finely pulverized using a blender and subsequently stored at a temperature of -20°C until the extraction process. Extraction procedure was the same as previously reported in the literature [4].

2.3. Agar diffusion assay

The evaluation of flower extract antimicrobial activity encompassed Gram-positive bacterial strains, including *S. aureus* (ATCC 25923), *S. epidermidis* (NCTC 11047), and *B. cereus* (ATCC 14579), as well as Gram-negative strains, such as *P. aeruginosa* (ATCC 10145), *S. enteritidis* (ATCC 13676), and *E. coli* (NCTC 9001). Samples were dissolved in DMSO to reach a final concentration of 20 mg/mL and subsequently sterilized by filtration using a 0.2 μm syringe filter. To ensure consistency, the initial colony forming units were normalized to a 0.5 McFarland scale, as determined by measuring turbidity at 600 nm [6].

The assessment of antimicrobial activity followed the methodology established by Paz et al. [7]. The diameters of the inhibition zones were determined using a digital caliper rule. Experimental data were collected in triplicate and are presented as the mean ± standard deviation (SD).

3. Discussion

Table 1 illustrates the antimicrobial activity of *C. japonica* (var. Carolyn Tuttle) against a range of Gram-positive and Gram-negative bacteria, as evaluated through the agar diffusion test. These particular microorganisms were chosen due to their prevalence in food-related contexts, and in the case of *S. aureus* and *S. epidermidis*, their propensity to origin opportunistic infections.

Table 1. Average diameter of inhibition zone ± standard deviation (mm).

Microorganism	Inhibition Zone

Gram-negative	*P. aeruginosa*	9.24±0.46
	S. enteritidis	6.95±1.00
	E. coli	Nd
Gram-positive	*S. aureus*	10.29 ±0.46
	S. epidermidis	Nd
	B. cereus	Nd

Nd: not detected.

Results revealed that *C. japonica* var. Carolyn Tuttle extract showed greater antimicrobial effect against S. aureus, with an inhibition zone of 10.29 mm. Additionally, *P. aeruginosa* and *S. enteritidis* were also sensitive to this *C. japonica* flower extract. By contrast, *E. coli, S. epidermidis* and *B. cereus* were resistant to *C. japonica* var. Carolyn Tuttle as no inhibition zone were observed against any of these strains. In previous articles, it has been observed that the flowers of *C. japonica* also exhibited antimicrobial activity against some of these strains, as well as other not considered in this study [8,9]. These variations in activity could be attributed to the use of different solvents, as demonstrated in prior research [9–11]. However, it is worth noting that more comprehensive studies are essential to elucidate the specific compounds responsible for each bioactivity. This will enable a better understanding of the variations among different varieties, linking them to their chemical characterization. Hence, future investigations should prioritize the exploration of potential synergistic and/or antagonistic interactions among the constituents of *C. japonica*. This approach will facilitate a deeper comprehension of the reported bioactivities in flower extracts, ultimately paving the way for their potential industrial applications.

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