

Proceeding Paper

Antimicrobial Activity Screening of *Camellia japonica* Flowers (*var.* Conde de la Torre) †

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Abstract: The increased resistance of pathogenic microorganisms to a wide range of antibiotics has driven recent research efforts towards exploring and developing effective preservatives with better potential and new strategies to prevent this multi-resistance. Many of these studies have been on natural matrices such as plants. This is in line with consumer demand for more organic and natural products. A possible alternative could be using bioactive compounds from *Camellia japonica* flowers as bio-preservatives since they have been traditionally used in cosmetic products due to their biological properties. Among the bioactive molecules of camellias, it is worth highlighting phenolic compounds, anthocyanins, polysaccharides, polyphenols, polyunsaturated fatty acids, and pigments. However, to incorporate these bioactive molecules into products with antimicrobial purposes, it is necessary to conduct an extraction and purification of the target compounds. Thus, in this study, the antimicrobial activity of one variety of *C. japonica* flowers (*var.* Conde de la Torre), obtained by an easy and profitable extraction technique such as maceration, was analyzed. Results from this work showed that the variety under study has a significant antimicrobial activity in terms of inhibition zones against *Staphylococcus epidermidis* (14.02 mm), *Staphylococcus aureus* (10.84 mm), *Pseudomonas aeruginosa* (10.36 mm), *Salmonella enteritidis* (7.98 mm), and *Bacillus cereus* (5.05 mm). However, the *var.* Conde de la Torre of *C. japonica* did not show significant activity against *Escherichia coli*. In conclusion, Conde de la Torre can be used as a potential antimicrobial agent. However, more studies that determinate the compounds responsible for these bioactivities are needed.

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

1. Introduction

The inappropriate use of antibiotics together with inadequate infection control has led to the emergence of resistant strains that represent a public health problem and a risk to the global economy. Currently, the development of microbial resistance is considered one of the biggest public health problems [1]. In consequence, some of the currently available therapeutic options are ceasing to be useful against certain pathogenic microorganisms,

which escalates the morbidity and mortality associated with infectious diseases caused by microorganisms [2]. Antibiotic-resistant bacteria are estimated to be the cause of 700,000 deaths worldwide and 33,000 deaths in Europe each year. It is estimated that by 2050, bacterial infections will cause 10 million deaths worldwide [3]. Some studies even argue that infections associated with antimicrobial resistance are the greatest cause of death across the world surpassing those caused by cancer [4]. Some bacteria with multidrug resistance are *Enterococcus faecium* (*E. faecium*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter* spp. [5]. Therefore, research and development of a new generation of antimicrobials in order to alleviate the spread of antibiotic resistance have become essential.

Recently, consumer demand and environmental awareness have led to an increasing trend to discover new bioactive compounds from more natural sources. Plants can be considered a potential source of antimicrobial molecules since they are used in traditional remedies, cosmetics, and food products as preservatives [6]. Among plants, a possible alternative could be bioactive compounds present in the flowers of *Camellia japonica* [7]. This species could have several applications due to the numerous biological activities and bioactive compounds that have been described in *C. japonica* flowers. Some bioactivities that have been recognized include antioxidant, antimicrobial, antiinflammatory, and anticancer, among others. These bioactivities are due to the presence of phenolic compounds, anthocyanins, polysaccharides, polyphenols, polyunsaturated fatty acids, and pigments [8]. Despite these health-promoting activities, camellias are still considered an underexploited resource. Greater efforts are needed to achieve their chemical and bioactive characterization. In this work, the antimicrobial activity of one variety of *C. japonica* flowers (*var.* Conde de la Torre), obtained by an easy and profitable extraction technique such as maceration, was analyzed.

2. Material and Methods

2.1. Chemicals and Reagents

Dimethyl sulfoxide (DMSO), lactic acid, and Mueller–Hinton broth (MHB) were from Sigma-Aldrich, Steinheim, Germany. The culture media Mueller–Hinton Agar II was acquired from Biolife Milan, Italy.

Staphylococcus aureus (ATCC 25923), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (ATCC 10145), and *Salmonella enteritidis* (ATCC 13676) were provided by Selectrol, Buckingham, UK; *Escherichia coli* (NCTC 9001) and *Staphylococcus epidermidis* (NCTC 11047) were from Microbiologics, MN, USA.

2.2. Sampling and Extraction Procedure

C. japonica flowers (*var.* Conde de la Torre) were collected in Galicia (NW Spain) in the winter season of 2020. Samples were lyophilized (LyoAlfa10/15, Telstar, Thermo Fisher Scientific, Waltham, MA, USA), pulverized into a fine powder by a blender, and stored at $-20\text{ }^{\circ}\text{C}$ until extraction.

Flower samples were subjected to heat-assisted extraction (HAE). To this aim, 0.8 g of sample was placed into a dark amber flask with 20 mL of solvent (aqueous methanol 60% *v/v*), and the mixture was stirred at 150 rpm using Thermo Scientific™ Cimarec™ Micro Stirrers in a thermostatic bath at $50\text{ }^{\circ}\text{C}$ for 1 h. The resulting crude extract was centrifuged to remove the remaining plant material residues. Supernatant was collected and lyophilized. Freeze-dried extracts were then stored at $-20\text{ }^{\circ}\text{C}$ until the assays.

2.3. Antibacterial Test

The antimicrobial activity of flower extracts was assessed against the following Gram-positive bacterial strains: *S. aureus* (ATCC 25923), *S. epidermidis* (NCTC 11047), and *B. cereus* (ATCC 14579); and the Gram-negative strains: *P. aeruginosa* (ATCC 10145), *S. enteritidis* (ATCC 13676), and *E. coli* (NCTC 9001).

Samples were dissolved in DMSO to the final concentration of 20 mg/mL and sterilized by filtration using 0.2 µm syringe filter. The initial number of colony-forming units was normalized (0.5 McFarland scale) by measuring the turbidity at 600 nm [9].

The antimicrobial activity was measured according to the methodology described by Paz [10] with minor modifications. In this case, petri dishes containing Mueller–Hinton agar were divided into four quadrants. Then, 50 µL of each studied microorganism was seeded and spread with sterile swabs. Next, 15 µL of sample was placed in one quadrant, 15 µL of DMSO and 15 µL lactic acid 40% (*v/v*) were also placed as negative and positive control, respectively.

Petri dishes were incubated at 37 °C for 24 h and the inhibition zone diameters were determined with a digital caliper rule.

The experimental data were conducted by triplicate and expressed as the mean ± standard deviation (SD).

3. Results and Conclusions

Table 1 presents the antimicrobial activity of *C. japonica* (*var.* Conde de la Torre) against several Gram-positive and Gram-negative bacteria in terms of a plate diffusion test. Microorganisms were selected because they are the most common food-related microorganisms and, in the case of *S. aureus* and *S. epidermidis*, they cause opportunistic infections.

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

Gram	Microorganism	Inhibition Zone
Positive	<i>S. aureus</i>	10.84 ± 1.39
	<i>S. epidermidis</i>	14.02 ± 1.37
	<i>B. cereus</i>	5.04 ± 0.76
Negative	<i>P. aeruginosa</i>	10.36 ± 0.70
	<i>S. enteritidis</i>	7.98 ± 2.04
	<i>E. coli</i>	NI

NI—no inhibition detected.

Results revealed that the *C. japonica* (*var.* Conde de la Torre) extract showed the greatest antimicrobial effect against *S. epidermidis*. In addition, *S. aureus* and *P. aeruginosa* resulted in being sensitive to the *C. japonica* extract, although its activity against *B. cereus* and *S. enteritidis* was low. By contrast, *E. coli* was resistant to the *C. japonica* extract (at 20 mg/mL) since no inhibition zones were observed. These results are similar to another study that analyzed methanolic extracts of *C. japonica* flowers. In this study, the extract produced an inhibitory zone of 14 to 19 mm (diameter) in a disk assay against the pathogens *Salmonella typhimurium* DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* on agar plates [11]. Other studies showed that *C. japonica* ‘Kramer’s Supreme’, ‘C.M. Wilson’, ‘La Pace’, ‘Mrs. Lyman Clarke’, ‘Benikarako’, and ‘Fanny Bolis’ varieties have antimicrobial activity against the clinical cefuroxime-resistant *Enterobacter cloacae* strain [12]. However, currently, there are still very few studies on the bioactivities of this plant.

4. Conclusions

In conclusion, *C. japonica* (*var.* Conde de la Torre) extracts can be used as potential antimicrobial agents. This is because the maceration technique allows the extraction of compounds with potential antimicrobial activity as several selected bacteria strains were sensitive to the extracts studied. Therefore, this study provides scientific evidence for the potential of *C. japonica* extracts in the development of new products with antimicrobial properties. However, more studies that determinate the compounds responsible for these bioactivities are needed.

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Conflicts of Interest: The authors declare no conflict of interest.

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