medical sciences forum

MDPI

Proceeding Paper

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

Antia G. Pereira [1,2], Aurora Silva [1,3], Marta Barral-Martinez[1], Javier Echave [1], Franklin Chamorro [1], Sepidar Seyyedi Mansour [1], Lucia Cassani [1,2], Paz Otero [1,2], Jianbo Xiao [1,4], Fatima Barroso [3], Jesus Simal-Gandara [1,*] and Miguel A. Prieto [1,2,*]

1 Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, Ourense Campus, University of Vigo, E32004 Ourense, Spain; antia.gonzalez.pereira@uvigo.es (A.G.P.); mass@isep.ipp.pt (A.S.); marta.barral@uvigo.es (M.B.-M.); javier.echave@uvigo.es (J.E.); franklin.noel.chamorro@uvigo.es (F.C.); sepidar.seyyedi@uvigo.es (S.S.M.); luciavictoria.cassani@uvigo.es (L.C.); paz.otero@uvigo.es (P.O.); jianbo.xiao@uvigo.es (J.X.)

2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia, 5300-253 Bragança, Portugal

3 REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr António Bernardino de Almeida 431, 4200-072 Porto, Portugal; mfb@isep.ipp.pt

4 International Joint Research Laboratory of Intelligent Agriculture and Agri-products Processing, Jiangsu University, Zhenjiang, China; jianbo.xiao@uvigo.es

* Correspondence: jsimal@uvigo.es (J.S.-G.); mprieto@uvigo.es (M.A.P.)

† Presented at the 2nd International Electronic Conference on Antibiotics—Drugs for Superbugs: Antibiotic Discovery, Modes of Action And Mechanisms of Resistance, Online, 15–30 June 2022; Available online: https://eca2022.sciforum.net/.

Citation: Pereira, A.G.; Silva, A.; Barral-Martinez, M.; Echave, J.; Chamorro, F.; Mansour, S.S.; Cassani, L.; Otero, P.; Xiao, J.; Barroso, F.; et al. Antimicrobial Activity Screening of *Camellia japonica* Flowers (*var.* Conde de la Torre). *Med. Sci. Forum* **2022**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

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 [1]. This is in line with consumers 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 [2]. Among the bioactive molecules of camellias, it is worth highlighting phenolic compounds, anthocyanins, polysaccharides, polyphenols, polyunsaturated fatty acids and pigments [3]. 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 has been analyzed. Results from this work showed that the variety under study have 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 determinates the compounds responsible of 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 [4]. 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 microorganism [5]. 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 in 2050 bacterial infections will cause 10 million deaths worldwide [6]. Some studies even argue that infections associated with antimicrobial resistance are the first cause of death across the world surpassing those caused by cancer [7]. 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.* [8]. Therefore, research and development of a new generation of antimicrobials in order to alleviate the spread of antibiotic resistance has become essential.

Recently, consumer demand and environmental awareness have led to an increasing trend to discover new bioactive compounds for more natural sources. Plants can be considered a potential source of antimicrobial molecules since they are used in traditional remedies, cosmetic, and food products as preservatives [1]. Among plants, a possible alternative could be bioactive compounds presents in the flowers of *Camellia japonica* [2]. This specie 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, anti-inflammatory, and anticancer, among others. These bioactivities are due to the presence of phenolic compounds, anthocyanins, polysaccharides, polyphenols, polyunsaturated fatty acids and pigments [3]. 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 has been analyzed.

2. Material and Methods

2.1. Chemicals and Reagents

Dimethyl sulfoxide (DMSO), lactic acid and Mulher Hinton broth (MHB)were from Sigma-Aldrich Steinheim, Germany. The culture media Mulher 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, Minnesota 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 °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 °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 °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 measure according to the methodology described by Paz et al [10] with minor modifications. In this case, petri dishes containing Muller-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 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	*S. aureus*	10.84 ± 1.39
	S. epidermidis	14.02 ± 1.37
	B. cereus	5.04 ± 0.76
Negative	*P. aeruginosa*	10.36 ± 0.70
	S. enteritidis	7.98 ± 2.04
	E. coli	NI

NI—No inhibition detected.

Results revealed that *C. japonica (var.* Conde de la Torre*)* extract showed greater antimicrobial effect against *S. epidermidis.* In addition, *S. aureus* and *P. aeruginosa* resulted to be sensitive for *C. japonica* extract, although its activity against *B. cereus* and *S. enteritidis* was low. By contrast, *E. coli* was resistant to *C. japonica* extract (at 20 mg/mL) since no inhibition zones were observed. These results are similar to another one 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', 'Fanny Bolis' varieties have antimicrobial activity against 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 a potential antimicrobial agent. 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 determinates the compounds responsible of these bioactivities are needed.

Author Contributions: Conceptualization, A.G.-P., A.S., M.B.-M., J.E., F.C., S.S.M., L.C., P.O., J.X, F.B., J.S.-G. and M.A.P.; methodology, A.G.-P., A.S., M.B.-M., J.E., F.C., S.S.M., L.C., P.O., J.X. and F.B.; software, A.G.P., A.S. and F.B.; validation, F.B., L.C., J.S.-G. and M.A.P.; formal analysis, A.S., M.C. and L.C.; investigation, A.G.P., A.S. and F.B.; writing—original draft preparation, A.G.P. and L.C.; writing—review and editing, A.G.P. and L.C.; visualization, L.C., A.G.-P. and F.B.; supervision, L.C., A.S., F.B., J.S.-G. and M.A.P. All authors have read and agreed to the published version of the manuscript.

Funding: Authors are grateful to Ibero-American Program on Science and Technology (CYTED—AQUA-CIBUS, P317RT0003), to the Bio Based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019) that supports the work of P. Otero, and M. Barral-Martínez. The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutrition and Food Security, has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295).

Acknowledgments: The research leading to these results was supported by MICINN supporting the Ramón y Cajal grant for M.A. Prieto (RYC-2017-22891) and Jianbo Xiao (RYC-2020-030365-I); by Xunta de Galicia for supporting the program EXCELENCIA-ED431F 2020/12, the post-doctoral grant of L. Cassani (ED481B-2021/152), and the pre-doctoral grant of A.G. Pereira (ED481A-2019/0228). The authors thank the program BENEFICIOS DO CONSUMO DAS ESPECIES TINTO-RERA-(CO-0019-2021) that supports the work of F. Chamorro. The research leading to these results was supported by the European Union through the EcoChestnut Project (Erasmus+ KA202) that supports the work of J. Echave.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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

References

1. Chassagne, F.; Samarakoon, T.; Porras, G.; Lyles, J.T.; Dettweiler, M.; Marquez, L.; Salam, A.M.; Shabih, S.; Farrokhi, D.R.; Quave, C.L. A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. *Front. Pharmacol.* **2021**, *11*. https://doi.org/10.3389/fphar.2020.586548.
2. Teixeira, A.M.; Sousa, C. A review on the biological activity of camellia species. *Molecules* **2021**, *26*, 2178. Available online: /pmc/articles/PMC8069326/ (accessed on 10 March 2022)
3. Pereira, A.G.; Garcia-Perez, P.; Cassani, L.; Chamorro, F.; Cao, H.; Barba, F.J.; Simal-Gandara, J.; Prieto, M.A. Camellia japonica: A phytochemical perspective and current applications facing its industrial exploitation. *Food Chem. X* **2022**, *13*, 100258, https://doi.org/10.1016/j.fochx.2022.100258.
4. León-Buitimea, A.; Garza-Cárdenas, C.R.; Garza-Cervantes, J.A.; Lerma-Escalera, J.A.; Morones-Ramírez, J.R. The Demand for New Antibiotics: Antimicrobial Peptides, Nanoparticles, and Combinatorial Therapies as Future Strategies in Antibacterial Agent Design. Vol. 11, Frontiers in Microbiology. *Front. Microbiol.* **2020**, 1669. https://doi.org/10.3389/fmicb.2020.01669.
5. World Health Organization. Antibiotic resistance [Internet]. Antibiotic Resistance. 2020. Available online: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (accessed on 27 April 2022).
6. Ratia, C.; Soengas, R.G.; Soto, S.M. Gold-Derived Molecules as New Antimicrobial Agents. *Front. Microbiol.* **2022**, *13*, 772. https://doi.org/10.3389/fmicb.2022.846959.
7. Murray, C.J.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **2022**, *399*, 629–655. Available online: http://www.thelancet.com/article/S0140673621027240/fulltext (accessed on 27 April 2022).
8. Mulani, M.S.; Kamble, E.; Kumkar, S.N.; Tawre, M.S.; Pardesi, K.R. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Front. Microbiol.* **2019**, *10*, 539, https://doi.org/10.3389/fmicb.2019.00539

9. Silva, A.; Rodrigues, C.; Garcia-Oliveira, P.; Lourenço-Lopes, C.; Silva, S.A.; Garcia-Perez, P.; Carvalho, A.P.; Domingues, V.F.; Barroso, M.F.; Delerue-Matos, C.; et al. Screening of Bioactive Properties in Brown Algae from the Northwest Iberian Peninsula. *Foods* **2021**, *10*, 1915, https://doi.org/10.3390/foods10081915.
10. Paz, M.; Gúllon, P.; Barroso, M.F.; Carvalho, A.P.; Domingues, V.; Gomes, A.M.; Becker, H.; Longhinotti, E.; Delerue-Matos, C. Brazilian fruit pulps as functional foods and additives: Evaluation of bioactive compounds. *Food Chem.* **2015**, *172*, 462–468, https://doi.org/10.1016/j.foodchem.2014.09.102.
11. Kim, K.Y.; Davidson, P.M.; Chung, H.J. Antibacterial Activity in Extracts of Camellia japonica L. Petals and Its Application to a Model Food System. *J. Food Prot.* **2001**, *64*, 1255–1260. Available online: https://pubmed.ncbi.nlm.nih.gov/11510672/ (accessed on 9 November 2020)
12. Kharchenko, I.; Tkachenko, H.; Buyun, L.; Kurhaluk, N.; Góralczyk, A.; Maryniuk, M.; Tomin, W.; Osadowski, Z. Evaluation of the In Vitro Antimicrobial Activity of Ethanolic Extracts Derived from Leaves Of Camellia Japonica Cultivars (Theaceae) Against Enterobacter Cloacae Strain. **2019**, 333–347. https://doi.org/10.15414/agrobiodiversity.2019.2585-8246.333-347.