



UNIVERSIDADE FEDERAL DE UBERLÂNDIA

Instituto de Ciências Biomédicas

Programa de Pós Graduação em Imunologia e Parasitologia Aplicadas



**CONTROLE DE BACTÉRIAS FITOPATOGÊNICAS POR CHALCONAS
CATECÓLICAS E SUA TOXICIDADE**

Daniela Silva Gonçalves

Uberlândia – MG

2024



UNIVERSIDADE FEDERAL DE UBERLÂNDIA

Instituto de Ciências Biomédicas

Programa de Pós Graduação em Imunologia e Parasitologia Aplicadas



CONTROLE DE BACTÉRIAS FITOPATOGÊNICAS POR CHALCONAS CATECÓLICAS E SUA TOXICIDADE

Dissertação apresentada ao Colegiado do
Programa de Pós Graduação em
Imunologia e Parasitologia Aplicadas
como requisito parcial para obtenção do
título de Mestre.

Discente: Daniela Silva Gonçalves

Orientador: Prof. Dr. Carlos Henrique
Gomes Martins

Uberlândia – MG

2024



UNIVERSIDADE FEDERAL DE UBERLÂNDIA

Instituto de Ciências Biomédicas

Programa de Pós Graduação em Imunologia e Parasitologia Aplicadas



Daniela Silva Gonçalves

Dissertação apresentada ao Colegiado do Programa de Pós Graduação em Imunologia e Parasitologia Aplicadas como requisito parcial para obtenção do título de Mestre.

Área de concentração: Bacteriologia (Epidemiologia das doenças infecciosas e crônico-degenerativas e Biotecnologia empregada no diagnóstico e controle de doenças).

Banca Examinadora

Presidente:

Prof. Dr. Carlos Henrique Gomes Martins, UFU.

Titulares:

Prof. Dr. Manuel Ricardo Costa Calhella, CIMO-IPB, Portugal.

Profa. Dra. Cristiane Yumi Koga Ito, UNESP

Suplente

Prof.^a. Dra. Ana Carolina Gomes Jardim, UFU.

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

G635
2024
Gonçalves, Daniela Silva, 1999-
CONTROLE DE BACTÉRIAS FITOPATOGÊNICAS POR CHALCONAS
CATECÓLICAS E SUA TOXICIDADE [recurso eletrônico] /
Daniela Silva Gonçalves. - 2024.

Orientador: Carlos Henrique Gomes Martins .
Dissertação (Mestrado) - Universidade Federal de
Uberlândia, Pós-graduação em Imunologia e Parasitologia
Aplicadas.

Modo de acesso: Internet.

Disponível em: <http://doi.org/10.14393/ufu.di.2024.578>

Inclui bibliografia.

1. Imunologia. I. , Carlos Henrique Gomes Martins,
1968-, (Orient.). II. Universidade Federal de
Uberlândia. Pós-graduação em Imunologia e Parasitologia
Aplicadas. III. Título.

CDU: 612.017

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:

Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074



ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Imunologia e Parasitologia Aplicadas				
Defesa de:	Dissertação de Mestrado Acadêmico nº 301 do PPGIPA				
Data:	Vinte e nove de agosto de dois mil e vinte e quatro	Hora de início:	9 h	Hora de encerramento:	11 h e 26 min
Matrícula do Discente:	12222IPA004				
Nome do Discente:	Daniela Silva Gonçalves				
Título do Trabalho:	Controle de bactérias fitopatogênicas por <i>Chalconas Catecólicas</i> e sua toxicidade				
Área de concentração:	Imunologia e Parasitologia Aplicadas				
Linha de pesquisa:	Biotecnologia empregada no diagnóstico e controle de doenças				
Projeto de Pesquisa de vinculação:	Ensaio Biológicos Antimicrobianos In Vitro e In Vivo de Produtos Naturais e Sintéticos				

Reuniu-se, em 29 de agosto de 2024, às 9 horas, por vídeo conferência web, a Banca Examinadora designada pelo Colegiado do Programa de Pós-graduação em Imunologia e Parasitologia Aplicadas, assim composta pelos titulares: Profa. Dra. Cristiane Yumi Koga Ito - Instituto de Ciência e Tecnologia - Campus de São José dos Campos - ICT/UNESP; Prof. Dr. Manuel Ricardo Costa Calhelha - Instituto Politécnico de Bragança (CIMO/IPB) - Portugal; Prof. Dr. Carlos Henrique Gomes Martins - PPGIPA/ICBIM/UFU (Presidente) e orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da mesa, Prof. Dr. Carlos Henrique Gomes Martins, apresentou a Comissão Examinadora e a candidato(a), agradeceu a presença do público, e concedeu à Discente a palavra para a exposição do seu trabalho. A duração da apresentação da Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir, o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

APROVADA

Esta defesa faz parte dos requisitos necessários à obtenção do título de Mestre.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata, que após lida e achada conforme, foi assinada pela Banca Examinadora.



Documento assinado eletronicamente por **Carlos Henrique Gomes Martins, Professor(a) do Magistério Superior**, em 29/08/2024, às 11:26, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Manuel Ricardo Costa Calhella, Usuário Externo**, em 29/08/2024, às 11:29, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Cristiane Yumi Koga Ito, Usuário Externo**, em 29/08/2024, às 11:30, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **5654905** e o código CRC **922851C4**.

Agradecimentos

Agradeço a Deus por ter me guiado até aqui e nunca me abandonado;

À minha família, meus pais Vani e Heliomar, meu irmão Ruan, por tudo que fizeram e fazem por mim;

Ao meu primo Richard, tia Eliane, comadre Tamara, por se fazerem presente e terem me apoiado quando mais precisei;

À minha namorada, Mariana Brentini Santiago, pelo companheirismo, pela paciência e por tudo que me ensina todos os dias;

Aos meus amigos, em nome de Amanda, Laura e Vitor, por todos os momentos, risos e choros compartilhados;

Ao meu orientador, Carlos Henrique Gomes Martins, por incentivar as ideias deste trabalho, pelas conversas e pelos conselhos muito além de profissionais, como também pessoais;

Ao grupo de pesquisa do Laboratório de Ensaaios Antimicrobianos (LEA), por todas as trocas e aprendizados ao longo desses últimos anos;

Ao Laboratório de Bacteriologia Vegetal (LABAC), em nome de Nilvanira Donizete Tebaldi, por ter aberto as portas do laboratório e aceitado essa colaboração, e Lara Caroline Borges Moreira Mota, por ter me ensinado desde a plantar os tomates até a fase de avaliação da doença.

E ao Laboratório de Antibióticos e Quimioterápicos em nome de Luis Octávio Regasini e Paulo de Tarso da Costa por toda ajuda na parte química.

Lista de abreviações

$\leq$	Less than or equal to
$\geq$	Greater than or equal to
$<$	Less than
$>$	Greater than
Y	Disease Intensity
t	Interval between evaluations
i	Number of evaluations
Σ	Summation notation
$\bar{x}$	Mean
°C	Celsius
μg	Microgram
μL	Microliter
^{13}C NMR	Nuclear Magnetic Resonance of Carbon-13
^1H NMR	Nuclear Magnetic Resonance of Hydrogen -1
ALSD	Almond Leaf Scorch Disease
AUDPC	Area Under the Disease Progress Curve
BHI	Brain Heart Infusion broth
BOD	Biochemical Oxygen Demand
-Br	Bromine
CFU	Colony Forming Units
-CH ₃	Methyl
-Cl	Chlorine
CLSI	Clinical and Laboratory Standards Institute
CoA	Coenzyme A
C.V.	Coefficient of variation
CVC	Citrus Variegated Chlorosis
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
EPS	Exopolysaccharide
-F	Fluorine
FAO	Food and Agriculture Organization
FIC	Fractional Inhibitory Concentration

FICI	Fractional Inhibitory Concentration Index
FtsZ	Filamentous temperature-sensitive protein Z
IUPAC	International Union of Pure and Applied Chemistry
ISPM	International Standards for Phytosanitary Measures
L02	Human normal liver cell line
LABAC	Laboratory of Vegetal Bacteriology
LC ₅₀	Lethal Concentration
LEA	Laboratory of Antimicrobial Testing
MBC	Minimum Bactericidal Concentration
mg	Milligram
MIC	Minimum Inhibitory Concentration
mL	Milliliter
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
Mt	Million tonnes
NA	Nutrient Agar
NB	Nutrient Broth
NGM	Nematode Growth Medium
-OH	Hydroxyl
OQDS	Olive Quick Decline Syndrome
PCYE	Phosphate Charcoal Yeast Extract
PD	Pierce's Disease of grapevine
PLS	Plum Leaf Scald
PPD	Phony Peach Disease
pv.	Pathovar
PW	Periwinkle Wilt
RC	Resistant to Copper
SC	Sensible to Copper
spp.	Species
subsp.	Subspecie
syn.	Synonym
TC	Tolerant to Copper
THC	Tetrahydroxchalcone
UFU	Federal University of Uberlândia
USD	United States Dollar

Lista de figuras

Capítulo II Figura 1: Structures of the catecholic chalcones **1-14** evaluated in this study.

Capítulo II Figura 2: Toxicity in *C. elegans in vivo* model of catecholic chalcones **1** (A); **2** (B); **3** (C); **4** (D).

Lista de tabelas

Capítulo II Tabela 1: Minimum Inhibitory and Bactericidal Concentrations (MIC/MBC) in $\mu\text{g/mL}$ of catecholic chalcones against phytopathogenic bacteria.

Capítulo II Tabela 2: Bacterial leaf spot control in tomato with catecholic chalcones.

Sumário

CAPÍTULO I	11
1. Bactérias fitopatogênicas	12
2. Manejo fitossanitário	15
3. Chalconas	16
Referências.....	18
CAPÍTULO II	22
CONTROL OF PHYTOPATHOGENIC BACTERIA BY CATECHOLIC CHALCONES AND THEIR TOXICITY	23
Introduction	25
Material and Methods.....	27
Results	32
Discussion	33
References	39
CAPÍTULO III	46
Considerações finais	46
CAPÍTULO IV	48
Contribuições extraordinárias	48

Resumo

Todos os anos bilhões de dólares são gastos na agricultura direcionados para o controle de pragas. Dentre elas, mais de 200 espécies são apenas de bactérias fitopatogênicas, incluindo: *Xanthomonas citri* subsp. *citri*; *X. euvesicatoria* pv. *perforans*; *X. vasicola* pv. *vasculorum*; *X. campestris* pv. *campestris*; *Pectobacterium carotovorum*; *Xylella fastidiosa*; *Ralstonia solanacearum*; *Pantoea ananatis*; *Pseudomonas fluorescens*; *Burkholderia cepacia*. O manejo fitossanitário se dá principalmente pela aplicação exacerbada de pesticidas à base de metais pesados e antibióticos que resultaram no surgimento de mecanismos de resistência, destacando a necessidade de busca por novas medidas alternativas como compostos bioativos naturais. As chalconas (1,3-difenilprop-2-en-1-ona), presentes naturalmente nas plantas, têm chamado atenção devido às suas propriedades terapêuticas e potencial antimicrobiano. Sua fácil sintetização química e modificações nas moléculas são de notável interesse aos pesquisadores e indústrias a fim de obter cada vez mais aplicações biológicas. Dessa forma, os objetivos deste presente estudo foram explorar a atividade antibacteriana contra fitopatógenos de chalconas catecólicas e sua toxicidade por meio de estudos *in vitro* e *in vivo*. As chalconas catecólicas apresentaram atividade bactericida em concentrações promissoras variando de 12.5 a 50 µg/mL e toxicidade em concentrações maiores do que aquelas com propriedade antibacteriana. Com base nos resultados obtidos, conclui-se que as chalconas catecólicas possuem atividade promissora frente a bactérias fitopatogênicas, incentivando mais estudos sobre seus mecanismos de ação e sugerindo sua possível aplicação segura como pesticida.

Palavras chave: atividade antibacteriana; bactérias fitopatogênicas, chalconas catecólicas; toxicidade.

Abstract

Every year billions of dollars are spent on agriculture in order to control pests. Among them, more than 200 species are only phytopathogenic bacteria, including: *Xanthomonas citri* subsp. *citri*; *X. euvesicatoria* pv. *perforans*; *X. vasicola* pv. *vasculorum*; *X. campestris* pv. *campestris*; *Pectobacterium carotovorum*; *Xylella fastidiosa*; *Ralstonia solanacearum*; *Pantoea ananatis*; *Pseudomonas fluorescens*; *Burkholderia cepacia*. Phytosanitary management occurs mainly through the excessive application of pesticides based on heavy metals and antibiotics that resulted in the emergence of resistance mechanisms, highlighting the need to search for new alternative measures such as natural bioactive compounds. Chalcones (1,3-diphenylprop-2-en-1-one), naturally present in plants, have attracted attention due to their therapeutic properties and antimicrobial potential. Its easy chemical synthesis and modifications in molecules are of notable interest to researchers and industries in order to obtain more biological applications. Therefore, the objectives of this present study were to explore the antibacterial activity against phytopathogens of catecholic chalcones and their toxicity through *in vitro* and *in vivo* studies. Catecholic chalcones showed bactericidal activity in promising concentrations varying from 12.5 to 50 µg/mL and toxicity at concentrations greater than those with antibacterial properties. Based on the results obtained, it is concluded that catecholic chalcones have promising activity against phytopathogenic bacteria, encouraging further studies on their mechanisms of action and suggesting their possible safe application as a pesticide.

Keywords: antibacterial activity; catecholic chalcones; phytopathogenic bacteria, toxicity.

1. Bactérias fitopatogênicas

Assim como os seres humanos, as plantas também estão constantemente suscetíveis e podem sofrer com infecções causadas por vários tipos de patógenos, como, por exemplo, bactérias, fungos, vírus, nematódeos e insetos (Saberri Riseh et al. 2022). Dentre eles, mais de 200 espécies são apenas de bactérias, em que se encontram os bastonetes Gram-negativos dos gêneros *Xanthomonas*, *Pectobacterium*, *Xylella*, *Ralstonia*, *Pantoea*, *Pseudomonas* e *Burkholderia*, responsáveis por perdas econômicas globais de mais de um bilhão de dólares todos os anos na produção agrícola (Vicente et al. 2022).

O gênero *Xanthomonas* pertence à família *Xanthomonadaceae*, ordem *Xanthomonadales*, classe *Gammaproteobacteria*, filo *Proteobacteria* (Schoch et al. 2020). Do grego, “*xanthos*”, que significa amarelo e do latim “*monas*”, que significa unidade, formam colônias amareladas e compreendem, atualmente, 34 espécies validamente descritas (Parte et al. 2020). São capazes de infectar no mínimo 124 monocotiledôneas e 268 dicotiledôneas, causando sintomas não-específicos como cancro, murcha, necrose, apodrecimento e manchas encharcadas (Catara et al. 2021; Feng et al. 2023).

O ciclo de vida de *Xanthomonas* spp. constitui basicamente de três fases: fora da planta hospedeira, na superfície da planta (epifítica) e dentro da planta (endofítica). Fora da planta as bactérias podem estar presentes em lesões de folhas caídas ou livres pelo solo, em menor escala. Ao serem transportadas pelo vento, pela chuva ou por insetos, as células chegam até a superfície hospedeira onde lidam com condições adversas como radiação, patógenos predadores e competidores, limitação de nutriente e umidade, enquanto tentam adentrar o tecido foliar através de aberturas naturais (estômatos e hidatódios) ou feridas, por intermédio de um único flagelo e pili. Uma vez dentro da planta, as bactérias se multiplicam a ponto de reemergirem à superfície para serem transportadas novamente para novos hospedeiros (An et al. 2020; Alvarez-Martinez et al. 2021).

De forma geral, os fatores de virulência são imprescindíveis para o processo de adaptação aos ambientes e infecção, sendo eles a formação de biofilme, enzimas extracelulares, fitotoxinas e fatores de motilidade (Feng et al. 2023). A goma xanthana é o principal exopolissacarídeo (EPS) presente na matriz do biofilme de *Xanthomonas* spp., conferindo aderência, proteção em condições adversas e a reações defensivas do hospedeiro, além de ser comercialmente conhecida nos setores alimentícios, cosméticos e farmacêuticos como agente espessante e estabilizante (An et al. 2020; Berezina et al. 2024).

O gênero *Pectobacterium* pertence ao mesmo filo e classe, porém à ordem *Enterobacterales* e família *Pectobacteriaceae* (Schoch et al. 2020). Como o próprio sufixo já

diz, “pecto-“ é relativo à pectina, ou seja, trata-se de uma bactéria pectinolítica, com 22 espécies descritas (Parte et al. 2020). Na parede celular das plantas, a lamela média é responsável pela junção das células, constituída de pectinas, polímeros complexos que possuem ácido D-galacturônico, e que podem ser degradadas por enzimas como a pectato liase, pectina liase, pectato hidrolase e poligalacturonases. Ao secretar essas enzimas, *Pectobacterium* spp. causam uma maceração que se espalha pelo tecido da planta, levando à podridão de caules e frutos principalmente, e, conseqüentemente, morte da planta (Lorrai and Ferrari 2021; Rafique et al. 2021). A transmissão se dá via armazenamento, irrigação e persistência no solo (Oulghazi et al. 2021). Ao entrar nos tubérculos das plantas através de lenticelas ou feridas, por meio de flagelos peritríquios, as bactérias podem se multiplicar e atingir outros tecidos ou entrar em período de latência, sem apresentar sintomas, o que torna as formas de controle ainda mais complicadas (Maciag et al. 2024).

Xylella spp., do grego “xylon”, que significa pedaço de madeira, e do latim o sufixo diminutivo “ella”, faz referência à sua pequena morfologia que varia de 0.1-0.5 μm x 1.0-5.0 μm (Parte et al. 2020). Compartilha do mesmo grupo taxonômico de *Xanthomonas* spp., é um gênero relativamente pequeno, constituído de duas espécies: *Xylella fastidiosa*, mais observada, e *Xylella taiwanensis* (Schoch et al. 2020). São bactérias não flageladas, com motilidade mediada por pili, que colonizam o sistema de vasos das plantas, especificamente o xilema, transmitidas através do inseto vetor conhecido como Cigarrinha, da família Cicadellidae, ao se alimentar da seiva bruta. Devido à multiplicação e formação de biofilme dentro dos vasos, ocorre a interrupção do fornecimento de água e sais minerais até as partes mais extremas do hospedeiro provocando os sintomas de murchamento, seca e necrose marginal das folhas, galhos atrofiados e murchos, frutos secos, duros e de tamanho reduzido que podem resultar na morte da planta (Landa et al. 2022; Trkulja et al. 2022).

A cepa *X. fastidiosa* 9a5c teve seu genoma completamente sequenciado no Brasil, publicado na literatura nos anos 2000, a partir de uma cultura patogênica coletada de galhos de laranja doce Valência com Clorose Variegada dos Citros (CVC) em Macaúbal, São Paulo (Simpson et al. 2000). Endêmica no continente americano surgiu pela primeira vez no continente europeu em 2013, na Itália, responsável pela Síndrome do Declínio Rápido da Oliveira (OQDS), com previsão de custo de até 5.2 bilhões de euros para os próximos 50 anos caso as árvores não sejam substituídas (Castro et al. 2021). Atualmente é capaz de infectar mais de 690 espécies de plantas, de 88 famílias diferentes (EFSA et al. 2023b). Por sua vez, *Xylella taiwanensis* foi reclassificada como nova espécie em 2016, afeta peras da espécie

Pyrus pyrifolia causando queimadura foliar, restrita, por enquanto, à ilha de Taiwan (Su et al. 2016; EPPO 2024).

O gênero *Ralstonia* spp., em homenagem a Erika Ralston, pertence à família *Burkholderiaceae*, ordem *Burkholderiales* e classe *Betaproteobacteria*, com 11 espécies validamente publicadas (Parte et al. 2020; Schoch et al. 2020). Sua principal espécie representante é *R. solanacearum*, encontrada na África, América, Ásia, Europa e Oceania, capaz de infectar aproximadamente 450 espécies de plantas de 54 famílias botânicas, incluindo a família *Solanaceae* (Bhatt et al. 2024; EPPO 2024).

É um fitopatógeno transmitido pelo solo, mas que também pode se disseminar pela água e sementes. Ao alcançar um hospedeiro suscetível, através dos seus flagelos, pili e fímbrias, inicia o processo de aderência e colonização nas raízes, de preferência laterais, por meio de aberturas naturais ou feridas. Posteriormente, passando pelo córtex, as bactérias invadem os vasos do xilema, onde se multiplicam, formam biofilme e bloqueiam o transporte de água e sais minerais para as extensões da planta, o que leva ao surgimento dos sintomas de murcha. Durante a fase de infecção, a população de células é tão densa que pode chegar a 10^8 UFC/g de tecido da planta. Após a morte do hospedeiro, as bactérias voltam para o meio ambiente onde podem continuar viáveis por anos no solo, na água, em resíduos de plantas e ervas daninhas assintomáticas, que funcionam como fonte de inóculo (Mansfield et al. 2012; Wang et al. 2023; Bhatt et al. 2024).

Pantoea spp., pertence à família *Erwiniaceae*, ordem *Enterobacterales* e classe *Gammaproteobacteria* (Schoch et al. 2020). Do grego “pantoios”, significa de todos os tipos ou fontes, é um gênero presente em diversos nichos ecológicos e geográficos, contendo 20 espécies reconhecidas (Parte et al. 2020). Essas bactérias possuem flagelos peritríquios, são encontradas no solo, na água e em diferentes hospedeiros como plantas, animais, insetos e seres humanos, seu ciclo de vida é variável, podendo ser epifítico, endofítico, saprofítico, sobrevivendo em restos de colheita (Lv et al. 2022).

A transmissão pode ocorrer através de sementes ou por vetores que se alimentam da seiva bruta como tripses *Frankliniella fusca*, besouros *Diabrotica virgifera virgifera* e *Chaetocnema pulicaria*, este último conhecido como besouro-pulga do milho, sendo o milho uma das plantas infectadas por *Pantoea* spp. (EFSA et al. 2023a; Shetty et al. 2023). Uma vez dentro do xilema da planta, ocorre a formação de biofilme, a interrupção da passagem de água e nutrientes para as extremidades e o aparecimento dos sintomas de descoloração, mancha ou apodrecimento de diferentes órgãos do hospedeiro (Baltenneck et al. 2021; Azizi et al. 2023).

Com relação aos gêneros *Pseudomonas* spp. e *Burkholderia* spp., as respectivas espécies *P. aeruginosa* e *B. cepacia* são muito estudadas pelo homem devido à importância clínica. Por outro lado, estão presentes no solo e na água e podem ser patogênicas também para as plantas e os animais. *Pseudomonas* spp. pertence à família *Pseudomonadaceae*, ordem *Pseudomonadales* e classe *Gammaproteobacteria*, sendo mais de 60 patovares patogênicas para as plantas responsáveis por sintomas como cancro, galha e mancha foliar (Schoch et al. 2020; Silverio et al. 2022; Sakata and Ishiga 2023). O ciclo de vida se inicia na superfície da folha, ao adentrar por estômatos ou feridas, através dos flagelos e pili, as bactérias se multiplicam no apoplasto e leva ao aparecimento de clorose e necrose (Sakata and Ishiga 2023). Já *Burkholderia* spp. pertence à família *Burkholderiaceae*, ordem *Burkholderiales* e classe *Betaproteobacteria* (Schoch et al. 2020). Recebeu esse nome em homenagem a W. H. Burkholder por ter descoberto o agente etiológico da podridão do bulbo da cebola (Parte et al. 2020).

Em 2012, Mansfield e colaboradores publicaram uma lista de grande relevância ainda hoje com as 10 bactérias mais importantes científica e economicamente. Dentre elas, quatro foram utilizadas neste trabalho: *Ralstonia solanacearum*; *Xanthomonas campestris* patovares; *Xylella fastidiosa* e *Pectobacterium carotovorum*. No panorama atual, frente a vários cenários como aumento da população mundial, globalização e mudanças climáticas, já é previsto a expansão geográfica dos patógenos, além do aumento na severidade das doenças, colocando cada vez mais em risco a segurança alimentar (Catara et al. 2021).

2. Manejo fitossanitário

Até abril de 2024, foram adotadas 46 Normas Internacionais para Medidas Fitossanitárias (ou International Standards for Phytosanitary Measures – ISPMs, em inglês), 33 Protocolos de Diagnóstico e 46 Tratamentos Fitossanitários. Essas normas são adotadas pela Comissão de Medidas Fitossanitárias, que é o órgão regulador da Convenção Internacional de Proteção dos Vegetais. As normas internacionais ajudam a proteger a agricultura sustentável e melhorar a segurança alimentar global; proteger o meio ambiente, florestas e biodiversidade; e facilitar o desenvolvimento econômico e comercial (IPPC 2024).

De forma geral, o sistema para manter a ausência de patógenos em uma produção, seja antes ou durante a estação de cultivo, envolve medidas: preventivas, como material de propagação livre de pragas e eliminação de outros hospedeiros; de exclusão, como barreiras físicas, telas, controles de equipamentos, maquinário, plantas, solo e meios de crescimento; e

de controle de pragas, como métodos culturais, tratamentos e cultivares resistentes (ISPM10 2016).

Uma vez identificado o patógeno, o processo de erradicação inclui três principais atividades: investigar completamente sua distribuição; evitar sua propagação e tratar quando encontrado. Entre as medidas de tratamento e controle encontram-se: destruição do hospedeiro; desinfestação de equipamentos e instalações; esterilizantes de solo; descanso periódico da terra, sem hospedeiros; uso de cultivares que suprimem ou eliminem populações de pragas; armadilhas, iscas ou outros métodos de controle físico; liberação inundativa de agentes de controle biológico; uso de inseto estéril; biopesticida ou pesticida químico (ISPM9 2017).

Infelizmente, existe uma grande dependência dos pesticidas químicos na agricultura (Pandit et al. 2022). De acordo com a Organização das Nações Unidas para Alimentação e Agricultura, os pesticidas são qualquer substância ou mistura de substâncias destinadas a prevenir, destruir e controlar pragas, ou ainda regular o processo de crescimento das plantas, podendo ser aplicados até mesmo depois da colheita para proteger a mercadoria da deterioração durante armazenamento e transporte (Guerrero Ramirez et al. 2023).

O uso contínuo e em concentrações cada vez mais elevadas dos agroquímicos geram uma série de desvantagens como bioacumulação, toxicidade, desequilíbrio da microbiota do solo, transporte pelo vento, solo e água, atingindo e provocando efeitos adversos também em animais e humanos (Pandit et al. 2022). Além disso, a utilização desses produtos contendo metais pesados, como o cobre, e antibióticos, como estreptomicina, resultaram em mecanismos de resistência tanto aos metais quanto aos antibióticos, o que torna necessária a implementação de novas táticas de manejo para doenças bacterianas, sendo uma das alternativas o uso de compostos antimicrobianos naturais, por exemplo (Sharma et al. 2023).

3. Chalconas

Desde a antiguidade, os produtos naturais são fonte de descobertas para novos medicamentos. Os flavonoides são metabólitos secundários que proveem cor, sabor e fragrância aos frutos, flores e sementes. Além de promoverem o crescimento das plantas, proteção contra fitopatógenos e fatores ambientais como radiação UV, calor e frio, possuem também propriedades terapêuticas como antioxidante, anti-inflamatória, antimicrobiana, antiviral e anticâncer, por exemplo (Dan and Dai 2020; Dias et al. 2021).

Na natureza, uma etapa importante para a biossíntese dos flavonoides é a catalisação do processo de condensação de três moléculas de malonil-CoA e uma de *p*-cumaroil-CoA,

realizada pela enzima chalcona sintase, para a produção da chalcona naringenina [4',2',4',6'-tetrahidróxichalcona] (Liu et al. 2021). As chalconas, do grego “chalcos”, significa bronze, formam o esqueleto base dos flavonoides, constituídas por dois anéis aromáticos (A e B) ligados por um sistema α,β -carbonil insaturado de três carbonos, nomeadas pela União Internacional de Química Pura e Aplicada (IUPAC) como 1,3-difenilprop-2-en-1-ona. São moléculas pequenas, de baixo peso molar que variam entre 300–600 g/mol, coloração amarelada, encontradas nas formas *cis* e *trans*, porém mais termodinamicamente estáveis na forma *trans* (Rudrapal et al. 2021; Dziagwa-Becker et al. 2024; Narwal et al. 2024).

As chalconas têm se tornado moléculas de grande interesse farmacêutico devido sua fácil sintetização e alteração de substituintes nas moléculas, o que garante a obtenção de uma diversidade de compostos ativos e maior versatilidade de atividades biológicas como antibacteriana, antiviral, antiparasitária, antidiabética, antitumoral, anti-Alzheimer, anti-Parkinson, antioxidante, anti-inflamatória e antinociceptiva (Rajendran et al. 2022; da Silva et al. 2023). As chalconas podem ser sintetizadas quimicamente pelos seguintes métodos: assistido por micro-ondas; irradiação por ultrassom; reações de acoplamento como Suzuki-Miyaura, Heck, Sonogashira; reação de Witting; olefinação de Julia-Kocienski; acilação de Friedel-Crafts; Rearranjo de Photo-Fries; síntese One-Pot e o mais comum, a condensação de Claisen-Schmidt na presença de ácido ou base (Dan and Dai 2020; Rajendran et al. 2022). Entre as possíveis modificações que podem ser realizadas nas moléculas estão: substituição dos anéis A e B por heterocíclicos; substituição dos hidrogênios por grupos funcionais como hidroxilas, metilas, halogênios, aminas, catecol constituído de um benzeno com duas hidroxilas laterais (3,4-diOH) e fusão dos anéis às modificações anteriores (Thapa et al. 2021).

Na literatura, já foi descrito que oliveiras, quando infectadas com *X. fastidiosa*, apresentaram um aumento na quantidade de flavonoides comprovando que esses compostos possuem papel crucial na defesa das plantas (Novelli et al. 2019). Resultados promissores estes que incentivam a busca por novas pesquisas a fim de avaliar se metabólitos precursores, como as chalconas, também exibem esse perfil de atividade e, assim, propiciar novas estratégias de controle não-tóxicas frente a bactérias fitopatogênicas.

Referências

- Alvarez-Martinez, C.E., Sgro, G.G., Araujo, G.G., Paiva, M.R.N., Matsuyama, B.Y., Guzzo, C.R., Andrade, M.O. and Farah, C.S. (2021) Secrete or perish: The role of secretion systems in *Xanthomonas* biology. *Comput Struct Biotechnol J* **19**, 279-302. 10.1016/j.csbj.2020.12.020.
- An, S.Q., Potnis, N., Dow, M., Vorholter, F.J., He, Y.Q., Becker, A., Teper, D., Li, Y., Wang, N., Bleris, L. and Tang, J.L. (2020) Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen *Xanthomonas*. *FEMS Microbiol Rev* **44**, 1-32. 10.1093/femsre/fuz024.
- Azizi, M.M.F., Ismail, S.I., Ina-Salwany, M.Y., Hata, E.M. and Zulperi, D. (2023) The emergence of *Pantoea* species as a future threat to global rice production. *Journal of Plant Protection Research*. 10.24425/jppr.2020.133958.
- Baltenneck, J., Reverchon, S. and Hommais, F. (2021) Quorum Sensing Regulation in Phytopathogenic Bacteria. *Microorganisms* **9**. 10.3390/microorganisms9020239.
- Berezina, O.V., Rykov, S.V., Schwarz, W.H. and Liebl, W. (2024) Xanthan: enzymatic degradation and novel perspectives of applications. *Appl Microbiol Biotechnol* **108**, 227. 10.1007/s00253-024-13016-6.
- Bhatt, S., Faridi, N., Raj, S.M.P., Agarwal, A. and Punetha, M. (2024) Recent advances in immuno-based methods for the detection of *Ralstonia solanacearum*. *J Microbiol Methods* **217-218**, 106889. 10.1016/j.mimet.2024.106889.
- Castro, C., DiSalvo, B. and Roper, M.C. (2021) *Xylella fastidiosa*: A reemerging plant pathogen that threatens crops globally. *PLoS Pathog* **17**, e1009813. 10.1371/journal.ppat.1009813.
- Catara, V., Cubero, J., Pothier, J.F., Bosis, E., Bragard, C., Dermic, E., Holeva, M.C., Jacques, M.A., Petter, F., Pruvost, O., Robene, I., Studholme, D.J., Tavares, F., Vicente, J.G., Koebnik, R. and Costa, J. (2021) Trends in Molecular Diagnosis and Diversity Studies for Phytosanitary Regulated *Xanthomonas*. *Microorganisms* **9**. 10.3390/microorganisms9040862.
- da Silva, L., Donato, I.A., Goncalves, C.A.C., Scherf, J.R., Dos Santos, H.S., Mori, E., Coutinho, H.D.M. and da Cunha, F.A.B. (2023) Antibacterial potential of chalcones and its derivatives against *Staphylococcus aureus*. *3 Biotech* **13**, 1. 10.1007/s13205-022-03398-7.
- Dan, W. and Dai, J. (2020) Recent developments of chalcones as potential antibacterial agents in medicinal chemistry. *Eur J Med Chem* **187**, 111980. 10.1016/j.ejmech.2019.111980.
- Dias, M.C., Pinto, D. and Silva, A.M.S. (2021) Plant Flavonoids: Chemical Characteristics and Biological Activity. *Molecules* **26**. 10.3390/molecules26175377.
- Dziagwa-Becker, M., Oleszek, M., Zielinska, S. and Oleszek, W. (2024) Chalcones-Features, Identification Techniques, Attributes, and Application in Agriculture. *Molecules* **29**. 10.3390/molecules29102247.
- EFSA, Bragard, C., Baptista, P., Chatzivassiliou, E., Di Serio, F., Gonthier, P., Jaques Miret, J.A., Justesen, A.F., MacLeod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., Parnell, S., Potting, R., Stefani, E., Thulke, H.H., Van der Werf, W., Civera, A.V., Yuen, J., Zappala, L., Migheli, Q., Vloutoglou, I., Maiorano, A., Streissl, F. and Reignault, P.L. (2023a) Pest categorisation of *Pantoea ananatis*. *EFSA J* **21**, e07849. 10.2903/j.efsa.2023.7849.
- EFSA, Gibin, D., Pasinato, L. and Delbianco, A. (2023b) Update of the *Xylella* spp. host plant database – systematic literature search up to 31 December 2022 **21**. 10.2903/j.efsa.2023.8061.
- EPPO (2024) EPPO Global Database <https://gd.eppo.int> 2024, 30 jun. .

- Feng, Y.M., Long, Z.Q., Xiang, H.M., Ran, J.N., Zhou, X. and Yang, S. (2023) Research on Diffusible Signal Factor-Mediated Quorum Sensing in *Xanthomonas*: A Mini-Review. *Molecules* **28**. 10.3390/molecules28020876.
- Guerrero Ramirez, J.R., Ibarra Munoz, L.A., Balagurusamy, N., Frias Ramirez, J.E., Alfaro Hernandez, L. and Carrillo Campos, J. (2023) Microbiology and Biochemistry of Pesticides Biodegradation. *Int J Mol Sci* **24**. 10.3390/ijms242115969.
- IPPC (2024) Adopted Standards <https://www.ippc.int/en/core-activities/standards-setting/ispm> 2024, 30 jun
- ISPM9 (2017) *Guidelines for pest eradication programmes* Rome IPPC, FAO. .
- ISPM10 (2016) *Requirements for the establishment of pest free places of production and pest free production sites* Rome IPPC, FAO.
- Landa, B.B., Saponari, M., Feitosa-Junior, O.R., Giampetruzzi, A., Vieira, F.J.D., Mor, E. and Robatzek, S. (2022) *Xylella fastidiosa*'s relationships: the bacterium, the host plants, and the plant microbiome. *New Phytol* **234**, 1598-1605. 10.1111/nph.18089.
- Liu, W., Feng, Y., Yu, S., Fan, Z., Li, X., Li, J. and Yin, H. (2021) The Flavonoid Biosynthesis Network in Plants. *Int J Mol Sci* **22**. 10.3390/ijms222312824.
- Lorrai, R. and Ferrari, S. (2021) Host Cell Wall Damage during Pathogen Infection: Mechanisms of Perception and Role in Plant-Pathogen Interactions. *Plants (Basel)* **10**. 10.3390/plants10020399.
- Ly, L., Luo, J., Ahmed, T., Zaki, H.E.M., Tian, Y., Shahid, M.S., Chen, J. and Li, B. (2022) Beneficial Effect and Potential Risk of *Pantoea* on Rice Production. *Plants (Basel)* **11**. 10.3390/plants11192608.
- Maciag, T., Koziel, E., Otulak-Koziel, K., Jafra, S. and Czajkowski, R. (2024) Looking for Resistance to Soft Rot Disease of Potatoes Facing Environmental Hypoxia. *Int J Mol Sci* **25**. 10.3390/ijms25073757.
- Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G. and Foster, G.D. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol Plant Pathol* **13**, 614-629. 10.1111/j.1364-3703.2012.00804.x.
- Narwal, S., Devi, B., Dhanda, T., Kumar, S. and Tahlan, S. (2024) Exploring Chalcone Derivatives: Synthesis and Their Therapeutic Potential. *Journal of Molecular Structure* **1303**. 10.1016/j.molstruc.2024.137554.
- Novelli, S., Gismondi, A., Di Marco, G., Canuti, L., Nanni, V. and Canini, A. (2019) Plant defense factors involved in *Olea europaea* resistance against *Xylella fastidiosa* infection. *J Plant Res* **132**, 439-455. 10.1007/s10265-019-01108-8.
- Oulghazi, S., Sarfraz, S., Zaczek-Moczydlowska, M.A., Khayi, S., Ed-Dra, A., Lekbach, Y., Campbell, K., Novungayo Moleleki, L., O'Hanlon, R. and Faure, D. (2021) *Pectobacterium brasiliense*: Genomics, Host Range and Disease Management. *Microorganisms* **9**. 10.3390/microorganisms9010106.
- Pandit, M.A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Rawat, C.D., Mishra, V. and Kaur, J. (2022) Major Biological Control Strategies for Plant Pathogens. *Pathogens* **11**. 10.3390/pathogens11020273.
- Parte, A.C., Sarda Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C. and Goker, M. (2020) List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* **70**, 5607-5612. 10.1099/ijsem.0.004332.
- Rafique, N., Bashir, S., Khan, M.Z., Hayat, I., Orts, W. and Wong, D.W.S. (2021) Metabolic engineering of *Bacillus subtilis* with an endopolygalacturonase gene isolated from *Pectobacterium carotovorum*; a plant pathogenic bacterial strain. *PLoS One* **16**, e0256562. 10.1371/journal.pone.0256562.

Rajendran, G., Bhanu, D., Aruchamy, B., Ramani, P., Pandurangan, N., Bobba, K.N., Oh, E.J., Chung, H.Y., Gangadaran, P. and Ahn, B.C. (2022) Chalcone: A Promising Bioactive Scaffold in Medicinal Chemistry. *Pharmaceuticals (Basel)* **15**. 10.3390/ph15101250.

Rudrapal, M., Khan, J., Dukhyil, A.A.B., Alarousy, R., Attah, E.I., Sharma, T., Khairnar, S.J. and Bendale, A.R. (2021) Chalcone Scaffolds, Bioprecursors of Flavonoids: Chemistry, Bioactivities, and Pharmacokinetics. *Molecules* **26**. 10.3390/molecules26237177.

Saberi Riseh, R., Gholizadeh Vazvani, M., Ebrahimi-Zarandi, M. and Skorik, Y.A. (2022) Alginate-Induced Disease Resistance in Plants. *Polymers (Basel)* **14**. 10.3390/polym14040661.

Sakata, N. and Ishiga, Y. (2023) Prevention of Stomatal Entry as a Strategy for Plant Disease Control against Foliar Pathogenic *Pseudomonas* Species. *Plants (Basel)* **12**. 10.3390/plants12030590.

Schoch, C.L., Ciufu, S., Domrachev, M., Hottot, C.L., Kannan, S., Khovanskaya, R., Leipe, D., McVeigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J.P., Sun, L., Turner, S. and Karsch-Mizrachi, I. (2020) NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database (Oxford)* **2020**. 10.1093/database/baaa062.

Sharma, A., Gupta, A.K. and Devi, B. (2023) Current trends in management of bacterial pathogens infecting plants. *Antonie Van Leeuwenhoek* **116**, 303-326. 10.1007/s10482-023-01809-0.

Shetty, S., Kamble, A. and Singh, H. (2023) Insights into the Potential Role of Plasmids in the Versatility of the Genus *Pantoea*. *Mol Biotechnol.* 10.1007/s12033-023-00960-3.

Silverio, M.P., Kraychete, G.B., Rosado, A.S. and Bonelli, R.R. (2022) *Pseudomonas fluorescens* Complex and Its Intrinsic, Adaptive, and Acquired Antimicrobial Resistance Mechanisms in Pristine and Human-Impacted Sites. *Antibiotics (Basel)* **11**. 10.3390/antibiotics11080985.

Simpson, A.J., Reinach, F.C., Arruda, P., Abreu, F.A., Acencio, M., Alvarenga, R., Alves, L.M., Araya, J.E., Baia, G.S., Baptista, C.S., Barros, M.H., Bonaccorsi, E.D., Bordin, S., Bove, J.M., Briones, M.R., Bueno, M.R., Camargo, A.A., Camargo, L.E., Carraro, D.M., Carrer, H., Colauto, N.B., Colombo, C., Costa, F.F., Costa, M.C., Costa-Neto, C.M., Coutinho, L.L., Cristofani, M., Dias-Neto, E., Docena, C., El-Dorry, H., Facincani, A.P., Ferreira, A.J., Ferreira, V.C., Ferro, J.A., Fraga, J.S., Franca, S.C., Franco, M.C., Frohme, M., Furlan, L.R., Garnier, M., Goldman, G.H., Goldman, M.H., Gomes, S.L., Gruber, A., Ho, P.L., Hoheisel, J.D., Junqueira, M.L., Kemper, E.L., Kitajima, J.P., Krieger, J.E., Kuramae, E.E., Laigret, F., Lambais, M.R., Leite, L.C., Lemos, E.G., Lemos, M.V., Lopes, S.A., Lopes, C.R., Machado, J.A., Machado, M.A., Madeira, A.M., Madeira, H.M., Marino, C.L., Marques, M.V., Martins, E.A., Martins, E.M., Matsukuma, A.Y., Menck, C.F., Miracca, E.C., Miyaki, C.Y., Monteriro-Vitarello, C.B., Moon, D.H., Nagai, M.A., Nascimento, A.L., Netto, L.E., Nhani, A., Jr., Nobrega, F.G., Nunes, L.R., Oliveira, M.A., de Oliveira, M.C., de Oliveira, R.C., Palmieri, D.A., Paris, A., Peixoto, B.R., Pereira, G.A., Pereira, H.A., Jr., Pesquero, J.B., Quaggio, R.B., Roberto, P.G., Rodrigues, V., de, M.R.A.J., de Rosa, V.E., Jr., de Sa, R.G., Santelli, R.V., Sawasaki, H.E., da Silva, A.C., da Silva, A.M., da Silva, F.R., da Silva, W.A., Jr., da Silveira, J.F., Silvestri, M.L., Siqueira, W.J., de Souza, A.A., de Souza, A.P., Terenzi, M.F., Truffi, D., Tsai, S.M., Tsuhako, M.H., Vallada, H., Van Sluys, M.A., Verjovski-Almeida, S., Vettore, A.L., Zago, M.A., Zatz, M., Meidanis, J. and Setubal, J.C. (2000) The genome sequence of the plant pathogen *Xylella fastidiosa*. The *Xylella fastidiosa* Consortium of the Organization for Nucleotide Sequencing and Analysis. *Nature* **406**, 151-159. 10.1038/35018003.

Su, C.C., Deng, W.L., Jan, F.J., Chang, C.J., Huang, H., Shih, H.T. and Chen, J. (2016) *Xylella taiwanensis* sp. nov., causing pear leaf scorch disease. *Int J Syst Evol Microbiol* **66**, 4766-4771. 10.1099/ijsem.0.001426.

Thapa, P., Upadhyay, S.P., Suo, W.Z., Singh, V., Gurung, P., Lee, E.S., Sharma, R. and Sharma, M. (2021) Chalcone and its analogs: Therapeutic and diagnostic applications in Alzheimer's disease. *Bioorg Chem* **108**, 104681. 10.1016/j.bioorg.2021.104681.

Trkulja, V., Tomic, A., Ilicic, R., Nozinic, M. and Milovanovic, T.P. (2022) *Xylella fastidiosa* in Europe: From the Introduction to the Current Status. *Plant Pathol J* **38**, 551-571. 10.5423/PPJ.RW.09.2022.0127.

Vicente, T.F.L., Felix, C., Felix, R., Valentao, P. and Lemos, M.F.L. (2022) Seaweed as a Natural Source against Phytopathogenic Bacteria. *Mar Drugs* **21**. 10.3390/md21010023.

Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J. and Zhang, Z. (2023) *Ralstonia solanacearum* - A soil borne hidden enemy of plants: Research development in management strategies, their action mechanism and challenges. *Front Plant Sci* **14**, 1141902. 10.3389/fpls.2023.1141902.

CAPÍTULO II

Control of phytopathogenic bacteria by catecholic chalcones and their toxicity

Manuscrito a ser submetido para publicação no periódico Journal of Applied Microbiology

Fator de impacto: 3.2

Qualis periódico: A3 – Ciências Biológicas III

CONTROL OF PHYTOPATHOGENIC BACTERIA BY CATECHOLIC CHALCONES AND THEIR TOXICITY

Daniela Silva Gonçalves¹, Nagela Bernadelli Sousa Silva¹, Lara Caroline Borges Moreira Mota², Lucas Couto Duarte², Nilvanira Donizete Tebaldi², Paulo de Tarso da Costa³, Luis Octávio Regasini³, Carlos Henrique Gomes Martins¹.

¹Federal University of Uberlândia, Institute of Biomedical Sciences, Uberlândia, MG, Brazil.

²Federal University of Uberlândia, Institute of Agricultural Science Uberlândia, MG, Brazil.

³São Paulo State University, Institute of Biosciences, Humanities and Exact Sciences, São José do Rio Preto, MG, Brazil.

Corresponding author: Carlos H. G. Martins – carlos.martins2@ufu.br

Av. Pará, 1720 Block 2B Room 221

Campus Umuarama – Mailbox 592

Uberlândia - MG – 38.405-320

Tel.: + 55 34 3225-8670

Running headline: Catecholic chalcones against phytopathogenic bacteria

Abstract

Aims: The aims of this study were to evaluate the effect *in vitro* and *in vivo* of 14 catecholic chalcones against phytopathogenic bacteria and their *in vivo* toxicity.

Methods and Results: Antibacterial testing was conducted using Minimum Inhibitory and Bactericidal Concentration (MIC/MBC) assays. The Fractional Inhibitory Concentration Index (FICI) was calculated. Toxicity study was performed using the *in vivo* model of *Caenorhabditis elegans*. Bacterial leaf spot control in tomato was evaluated. Catecholic chalcones **1** (3-Br), **2** (2-CH₃), **3** (3-OH) and **4** (4-OH) presented promising MIC/MBC values varying from 12.5 to 50 µg/mL, with bactericidal effect. Toxicity assay showed Lethal Concentration (LC₅₀) values of **1**, **2**, **3** and **4** higher than concentrations that have antibacterial properties. Catecholic chalcones and streptomycin did not show synergistic activity. Catecholic chalcones **2** and **3** were able to control the severity of the disease caused by *Xanthomonas euvesicatoria* pv. *perforans* in tomato at concentrations of 25 and 50 µg/mL, respectively.

Conclusion: These results revealed the promising agrochemical potential of catecholic chalcones and their possible safety application as pesticide.

Impact Statement: One of the main threats to food security in the world is plant diseases, caused by more than 200 species of phyto bacteria, which generate costs of billions of dollars every year with pesticides, in addition to toxic residues and resistance mechanisms. Chalcones are natural compounds, precursors in the biosynthesis of flavonoids, with biological applications such as herbicides, fungicides, nematocides, insecticides and bactericides.

Keywords: antibacterial activity; bacterial leaf spot; catecholic chalcones; phytopathogenic bacteria; toxicity.

Introduction

According to Food and Agriculture Organization (FAO 2009a), food production will need to almost double to feed a world population of 9.1 billion people in 2050. Consequently, the challenges to attend this demand will also increase. Monoculture over large areas facilitates the spread of pathogens, as well globalized trade facilitates their dissemination (Straub et al. 2021). That way, plant diseases are a constant threat to food security and cause significant impacts to the world economy (Kulabhusan et al. 2022).

Among phytopathogens, more than 200 species are phyto bacteria, some of the most relevant aggressive genera are *Xanthomonas*, *Pectobacterium*, *Xylella*, *Ralstonia*, *Pantoea*, *Pseudomonas* and *Burkholderia*, all Gram-negative bacillus (Vicente et al. 2022). *Xanthomonas citri* subsp. *citri* is the cause of citrus canker; *X. euvesicatoria* pv. *perforans* causes bacterial spot in tomato and pepper; *X. vasicola* pv. *vasculorum* causes bacterial leaf streak in maize and gumming disease in sugarcane; *X. campestris* pv. *campestris* causes black rot in crucifers (An et al. 2020). *Pectobacterium carotovorum* is responsible for soft rot in several crops, including carrot, cabbage, onion, radish, green pepper and, mainly, potato (Baltenneck et al. 2021). *Xylella fastidiosa* is associated with Citrus Variegated Chlorosis - CVC, Pierce's Disease of grapevine - PD, Olive Quick Decline Syndrome - OQDS, Almond Leaf Scorch Disease - ALS D; Phony Peach Disease - PPD and Plum Leaf Scald - PLS (Greco et al. 2021). *Ralstonia solanacearum* infects a variety of solanaceas causing bacterial wilt and brown rot, beyond the Moko disease of banana (Paudel et al. 2020). *Pantoea ananatis* affects onion causing center rot, rice causing bacterial leaf blight and grain discoloration, maize causing leaf spot disease, eucalyptus causing blight/dieback and many other host plants (EFSA et al. 2023). *Pseudomonas fluorescens* is found in different environmental niches and, sometimes, is an opportunistic pathogen that can cause medullary necrosis in tomato and also affect crucifers (Silverio et al. 2022). Likewise, *Burkholderia cepacia* is widespread in environment and, opportunely, causes soft rot in onion (Elshafie and Camele 2021).

In 2022, 3.7 million tonnes (Mt) of pesticides were used in agriculture and 6.9 Mt were exported with a total value of USD 48.8 billion (FAO 2024b). Besides pollution and toxic residues, the stress caused by the indiscriminate use of pesticides, principally those copper-based and streptomycin, results in antibacterial resistance through mechanisms including gene mutation induction, activation of efflux pumps and inhibition of outer membrane pores (Qiu et al. 2022). Inevitably it is necessary to search for new and effective control strategies for these microorganisms (Braga et al. 2022).

Flavonoids, secondary metabolite widely found in plants, provide pigments, fragrance, taste, protection, growth and development to flowers, seeds and fruits (Roy et al. 2022). In nature, from one molecule of *p*-coumaroyl-CoA and three molecules of malonyl-CoA, through the action of chalcone synthase, narigenin chalcone (4,2,4,6-tetrahydroxychalcone [THC] [chalcone]) is generated, first key intermediate product in the flavonoids metabolic pathway providing their fundamental structure (Liu et al. 2021).

Chalcones, precursors in the biosynthesis of flavonoids, are composed of two aromatic rings (A and B) joined by a three carbon unsaturated α,β -carbonyl system, while hydrogens can be conveniently altered by functional chemical groups, like -OH, -CH₃, -F, -Cl, -Br, catechol constituted of a benzene with two lateral hydroxyls (3,4-diOH), in order to obtain a variety of biological applications (Ouyang et al. 2021), such as: herbicides (Chotsaeng et al. 2019), fungicides (Zhou et al. 2022), antiviral (Zhang et al. 2024), nematocides (Cao et al. 2023), insecticides (Ruiz Hidalgo et al. 2019).

The antibacterial potential of chalcones and their derivatives is due to their ability to bind to various receptors and inhibit crucial processes like DNA replication by inhibition of DNA gyrase and FtsZ protein, essential in cell division; efflux pump inhibitor; type II fatty acid biosynthetic pathway; protein tyrosine phosphatases and virulence factors (Dhaliwal et al. 2022). Therefore, the objectives of this present study were to explore the antibacterial and

synergistic activity of catecholic chalcones against phytopathogenic bacteria, the capacity of bacterial leaf spot control in tomato and their toxicity.

Material and Methods

Catecholic chalcones

Catecholic chalcones were synthesized in acidic medium by Claisen-Schmidt aldol condensation between 3,4-dihydroxybenzaldehyde and the corresponding acetophenones, employing ethanol as a solvent (Vásquez-Martínez et al. 2019). The structure of the chalcones was confirmed through experiments of nuclear magnetic resonance of hydrogen (^1H NMR) and carbon-13 (^{13}C NMR). The molecules of the 14 catecholic chalcones evaluated in this study are presented in **Figure 1** with the following nomenclature, respectively: **1** (E)-1-(3-bromophenyl)-3-(3,4-dihydroxyphenyl)prop-2-en-1-one; **2** (E)-3-(3,4-dihydroxyphenyl)-1-(o-tolyl)prop-2-en-1-one; **3** (E)-3-(3,4-dihydroxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one; **4** (E)-3-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one; **5** (E)-3-(3,4-dihydroxyphenyl)-1-phenylprop-2-en-1-one; **6** (E)-3-(3,4-dihydroxyphenyl)-1-(furan-2-yl)prop-2-en-1-one; **7** (E)-3-(3,4-dihydroxyphenyl)-1-(3-methoxyphenyl)prop-2-en-1-one; **8** (E)-3-(3,4-dihydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one; **9** (E)-3-(3,4-dihydroxyphenyl)-1-(4-fluorophenyl)prop-2-en-1-one; **10** (E)-3-(3,4-dihydroxyphenyl)-1-(p-tolyl)prop-2-en-1-one; **11** (E)-1-(3,4-dichlorophenyl)-3-(3,4-dihydroxyphenyl)prop-2-en-1-one; **12** (E)-3-(3,4-dihydroxyphenyl)-1-(naphthalen-2-yl)prop-2-en-1-one; **13** (E)-1-(4-bromophenyl)-3-(3,4-dihydroxyphenyl)prop-2-en-1-one; **14** (E)-3-(3,4-dihydroxyphenyl)-1-(4-ethylphenyl)prop-2-en-1-one.

Bacterial strains used in the assays

The strain *X. citri* subsp. *citri* (resistant to copper-RC) isolated in Bella Vista (Corrientes, Argentina); *X. citri* subsp. *citri* (tolerant to copper-TC) isolated in Paraná (Brazil); *X. citri* subsp. *citri* (sensible to copper-SC) was isolated in Paranavaí (Paraná, Brazil); *X. fastidiosa* (9a5c) collected from CVC affected Valencia sweet orange twigs in Macaubal (São Paulo, Brazil), all of them are part of the culture collection of the Laboratory of Antimicrobial Testing (LEA) of the Federal University of Uberlândia (UFU). The strain *X. euvesicatoria* pv. *perforans* was isolated from tomato in Uberlândia (Minas Gerais, Brazil); *X. vasicola* pv. *vasculorum* isolated from corn in Palotina (Paraná, Brazil); *X. campestris* pv. *campestris* isolated from cabbage in Uberlândia (Minas Gerais, Brazil); *P. carotovorum* isolated from potato in Perdizes (Minas Gerais, Brazil); *R. solanacearum* isolated from potato in Perdizes (Minas Gerais, Brazil); *P. ananatis* isolated from corn in Planaltina de Goiás (Goiás, Brazil); *P. fluorescens* isolated from tomato in Uberlândia (Minas Gerais, Brazil) and *B. cepacia* isolated from onion in Santa Juliana (Minas Gerais, Brazil), all of them cordially provided by the Laboratory of Vegetal Bacteriology (LABAC) of the Federal University of Uberlândia (UFU). All strains were maintained under cryopreservation at -20°C in Nutriente Broth –NB (FirstLab, Pinhais, Paraná, Brazil) except *X. fastidiosa* maintained in Periwinkle Wilt – PW – Broth (in house).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration capable of inhibiting the bacterial growth. MIC values of catecholic chalcones were determined by microdilution broth methodology in 96-well microplates as recommended by the Clinical and Laboratory Standards Institute (CLSI 2018) adapted for culture medium and incubation time

of phytopathogenic bacteria. The samples were solubilized in 5% of dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and diluted in NB or PW to the concentration of 1600 µg/mL. Microplates were prepared with NB or PW in order to the samples achieve final concentrations ranging from 0.195 µg/mL to 400 µg/mL. The inoculums were standardized on McFarland 0.5 scale, checked in densitometer (Densimat, Biomérieux, Grassina, Florence, Italy) and adjusted to a final concentration of 5×10^5 CFU/mL in well. Streptomycin (Sigma-Aldrich) was used as positive control in concentrations ranging from 0.0115 µg/mL to 5.9 µg/mL. Microplates were incubated in biochemical oxygen demand – BOD (Cientec, São Francisco, Belo Horizonte, Brazil) at 28°C, *Xanthomonas* spp. for 72 hours, *P. carotovorum*, *R. solanacearum*, *P. ananatis*, *P. fluorescens* and *B. cepacia* for 48 hours and *X. fastidiosa* for seven days. After incubation period, 30 µL of a 0.02% aqueous resazurin (Sigma-Aldrich) solution was added to each well and incubated again in BOD at 28°C for 12 hours, except *X. fastidiosa* for 24 hours. Resazurin blue color represents the absence of bacterial growth while resofurin pink color represents active metabolism of bacteria (Sarker et al. 2007). All assays were performed in triplicate.

The Minimum Bactericidal Concentration (MBC) is the lowest concentration at which there is no bacterial growth. MBC values of catecholic chalcones were determined using the same MIC microplates, after incubation and before resazurin, by removing an aliquot of 10 µL from each well and seeding on Nutrient Agar – NA (Difco Labs, Detroit, MI, USA) for most of strains or Phosphate Charcoal Yeast Extract - PCYE Agar (in house) for *X. fastidiosa*. Agar plates were incubated in BOD at 28°C, *Xanthomonas* spp. for 72 hours, *P. carotovorum*, *R. solanacearum*, *P. ananatis*, *P. fluorescens* and *B. cepacia* for 48 hours and *X. fastidiosa* for seven days. All assays were performed in triplicate.

Checkerboard Assay

The checkerboard assays were performed to investigate the synergistic effect of the association between catecholic chalcones with promising MIC results of catecholic chalcones **1** (3-Br), **2** (2-CH₃), **3** (3-OH), **4** (4-OH) and streptomycin according to the protocol described by Chaturvedi et al. (2008) adapted for culture medium and incubation time of phytopathogenic bacteria. Microplates of 96-well were prepared and incubated under the same MIC conditions. Concentrations were combined in order to samples ranged from 0.39 µg/mL to 400 µg/mL, while streptomycin ranged from 0.09 µg/mL to 5.9 µg/mL against 5×10^5 CFU/mL in well. Resazurin was also added to interpret the results.

The Fractional Inhibitory Concentration (FIC_A) was calculated as the MIC of sample in combination/MIC of sample alone and FIC_B was calculated as the MIC of streptomycin in the combination/MIC of streptomycin alone. The Fractional Inhibitory Concentration Index (FICI) was determined as the following summation ($\sum FIC$) = FIC_A + FIC_B. The interpretations of the results were: synergistic if FICI ≤ 0.5; indifferent (no antagonism) if 0.5 < FICI < 4.0 and antagonistic if FICI ≥ 4.0 (Chaturvedi et al. 2008). All assays were performed in triplicate.

Toxicity assessment in *Caenorhabditis elegans*

Toxicity of catecholic chalcones was evaluated using the *in vivo* model of *C. elegans*, according to Andrade et al. (2020). *C. elegans* AU37 mutant strain was grown in Nematode Growth Medium – NGM (in house) agar plates seeded with *Escherichia coli* OP50 and incubated at 16 °C for 72 h in BOD (Marconi, Piracicaba, São Paulo, Brazil). After incubation period, larvae and eggs were removed from the NGM plates with M9 buffer and centrifuged to eliminate *E. coli*. Worms were killed by a treatment with sodium hypochlorite solution. The remaining eggs were added in plates containing only NGM agar and incubated at 16 °C for 24 h in BOD. Larvae in L1 and L2 stages were obtained after incubation, washed with M9

buffer, transferred to NGM plates seeded with *E. coli* OP50 and incubated at 16 °C for 24 h in BOD.

After synchronization, larvae in L4 stage were removed from the NGM plates with M9 buffer and centrifuged to eliminate *E. coli*. Next, 96-well microplates were prepared with a solution consisting of 60% M9 buffer, 40% Brain Heart Infusion broth (BHI, Kasvi, Pinhais, Paraná, Brazil), 5 mg/mL cholesterol (Sigma-Aldrich), 20 mg/mL streptomycin (Sigma-Aldrich), 20 mg/mL ampicillin (Sigma-Aldrich) and 10 mg/mL kanamycin (Sigma-Aldrich), samples ranged from 31.25 µg/mL to 1000 µg/mL and 20 µL containing from 10 to 20 L4 stage larvae was added to each well. Microplates were incubated at 25 °C for 72 h in BOD.

Every 24 hours larvae were counted under an inverted microscope. Larvae with movement were considered alive and static were considered dead. Lethal Concentration (LC₅₀) was determined as the lowest concentration able to kill 50% or more of larvae. All assays were performed in triplicate.

Bacterial leaf spot control in tomato

Plants of tomato Santa Clara (Agristar, Santo Antonio de Poses, São Paulo, Brazil) were grown in green house, in 500 mL pots containing a mixture of soil, sand, humus and vermiculite (4:1:1:1). Thirty eight days after sowing, for assessing preventive control, the plants with three to four-leaf stage were sprayed until runoff with catecholic chalcones solubilized sterile ultrapure water at MIC values against *X. euvesicatoria* pv. *perforans*, the cause of bacterial leaf spot in tomato. Commercial copper containing 53.8% of copper hydroxide (Kocide WDG Bioactive, Mitsui & Co, Bela Vista, São Paulo, Brazil) at concentration of 2 mg/mL was used as positive control and water as negative control. After 3 days, the plants were sprayed with 1×10^7 CFU/mL until runoff. Similarly, the plants used for assessing the curative control were first inoculated with bacterial suspension and, after 3 days,

were sprayed with the treatment solutions. Plants were kept in moist chamber for 24 hours before and after inoculation. Both experiments were performed at the same time.

Disease severity was evaluated at 3, 6, 9, 12, 15 and 18 days after inoculation using a diagrammatic scale according to Mello (1997). The Area Under the Disease Progress Curve (AUDPC) was calculated using the following formula: $AUDPC = \sum [(Y_i + Y_{i+1})/2] (t_{i+1} - t_i)$, in which “Y” is disease intensity, “t” is the interval between evaluations (3 days) and “i” is the number of evaluations made over time (Campbell and Madden 1990).

Statistical analysis

The data obtained from AUPDC in the bacterial leaf spot control in tomato essay were subjected to analysis of variance and the means were compared by Scott-Knott test with a $P \leq 0.05$ using the R Core Team software.

Results

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC values varied from 12.5 to >400 µg/mL. Sample 1 (3-Br) presented the greatest result against *X. citri* subsp. *citri* – RC and *X. citri* subsp. *citri* – TC at concentration of 12.5 µg/mL. MBC values were the same as MIC for all samples and strains. Streptomycin, as positive control, varied from 0.09 to 0.73 µg/mL. According to Kalli et al. (2021), phytochemicals compounds with MIC values ≤ 25 µg/mL are considered active; between 25 and 100 µg/mL are moderately active and MIC values ≥ 100 µg/mL are inactive. That way, catecholic chalcones with active and moderately active were chosen for the next tests. All samples were inactive against *P. ananatis*, *P. fluorescens* and *B. cepacia* (MIC and MBC

results of 200, 400 and >400 µg/mL). **Table 1** demonstrates the results with MIC and MBC values ≤ 100 µg/mL.

Checkerboard Assay

FICI values varied from 1.0 to 2.5, which represents that the combination of promising catecholic chalcones **1** (3-Br), **2** (2-CH₃), **3** (3-OH) and **4** (4-OH) with streptomycin against *Xanthomonas* spp. and *P. carotovorum* did not evidence any synergistic effects.

Toxicity assessment in *Caenorhabditis elegans*

The lowest concentration able to kill 50% or more of larvae (LC₅₀) of sample **1** (**Figure 2A**) was determined at 31.25 µg/mL, **2** (**Figure 2B**) and **4** (**Figure 2D**) at 125 µg/mL in 72 hours; **3** (**Figure 2C**) at 250 µg/mL in 48 hours.

Bacterial leaf spot control in tomato

For the Area Under the Disease Progress Curve (AUDPC) means, there was no significant difference between preventive ($\bar{x} = 19.6875$) and curative ($\bar{x} = 21.2500$) controls. In analysis of the treatments, as demonstrated in **Table 2**, catecholic chalcones **2** ($\bar{x} = 19.5000$) and **3** ($\bar{x} = 17.2500$) significantly reduced the disease severity at concentrations of 25 and 50 µg/mL, respectively, when compared to the other treatments. Positive and negative control showed no statistical difference, with $P \leq 0.05$.

Discussion

Based on literature, catecholic chalcones **1**, **2**, **3**, **4**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13** and **14** were never evaluated before against phytopathogenic bacteria, except for its basic structure (**5**). Guo et al. (2020) quantified the percentage of inhibition of some phyto-bacteria by

chalcone derivatives containing malonate at a concentration of 50 µg/mL, the compound **3c** inhibited 53.5% of *Xanthomonas axonopodis* pv. *citri* (synonymous *X. citri* subsp. *citri*); **3e** inhibited 50.2% of *Xanthomonas oryzae* pv. *oryzae* and **3j** inhibited 59.2% of *R. solanacearum*. Hu et al. (2023) explored the antibacterial activity of chalcone derivatives containing pyrazole oxime ethers through the turbidity method at a concentration of 50 µg/mL, the compound **H8** inhibited 62.1% of *X. axonopodis* pv. *citri*; **H17** inhibited 68.7% of *X. oryzae* pv. *oryzae* and **H22** inhibited 46.7% of *Pseudomonas syringae* pv. *actinidiae*. In this present study, MIC and MBC values were equal, which means that the samples inhibited 100% of bacterial growth, demonstrating a bactericidal effect. *X. citri* subsp. *citri* – RC was inhibited by catecholic chalcone **1** (3-Br) at 12.5 µg/mL, by **2** (2-CH₃), **3** (3-OH) and **4** (4-OH) at 50 µg/mL; *X. citri* subsp. *citri* – TC inhibited by **1** at 12.5 µg/mL, **2** and **3** at 25 µg/mL and **4** at 50 µg/mL; *X. euvesicatoria* pv. *perforans* inhibited by **1** and **2** at 25 µg/mL and **3** at 50 µg/mL; *X. vasicola* pv. *vasculorum* inhibited by **3** at 25 µg/mL, **2** and **4** at 50 µg/mL; *X. campestris* pv. *campestris* inhibited by **2** at 50 µg/mL and **3** at 25 µg/mL; *P. carotovorum* inhibited by **1** at 25 µg/mL.

Zhang et al. (2024) measured the optical density resulted by the bioactivity of chalcone derivatives containing triazole-pyridine against phytopathogenic bacteria at a concentration of 100 µg/mL, the compound **N3** inhibited 86.2% of *P. syringae* pv. *actinidiae*; **N23** inhibited 50% of *R. solanacearum* and 80.4% of *X. axonopodis* pv. *citri*; **N26** inhibited 75.9% of *Xanthomonas campestris* pv. *musacearum*, 80.2% of *Pectobacterium carotovorum* subsp. *brasiliense*, 84.2% of *Acidovorax citrulli* and 88% of *X. oryzae* pv. *oryzae*. In this work, at 100 µg/mL, *X. citri* subsp. *citri* – RC and *X. citri* subsp. *citri* – TC was inhibited by catecholic chalcone **5**, **6** and **7**; *X. citri* subsp. *citri* – SC inhibited by **3**; *X. euvesicatoria* pv. *perforans* inhibited by **4**, **5**, **6** and **7**; *X. vasicola* pv. *vasculorum* inhibited by **1**, **5** and **6**; *X.*

campestris pv. *campestris* inhibited by **1**, **4**, **5** and **6**; *P. carotovorum* inhibited by **3**; *X. fastidiosa* inhibited by **1**, **2** and **3** and *R. solanacearum* inhibited by **3**.

Vásquez-Martínez et al. (2019) observed by microdilution broth methodology that the presence of catechol group in two molecules of chalcone was necessary to exercise antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Catecholic chalcones derivatives synthesized in this research obtained the greatest results when added to 3-Br (**1**), 2-CH₃ (**2**), 3-OH (**3**) or 4-OH (**4**). Catecholic chalcone (**5**) with no other alterations was still better than 4-F (**9**), 4-CH₃ (**10**), 3,4-diCl (**11**), 2-naphthalene (**12**), 4-Br (**13**) and 4-ethyl (**14**). In other words, the position of changes in the molecules also matters, as demonstrated by Lagu et al. (2020), when using fluorinated chalcones, nitro or chloro substituents at *ortho* or *meta* position showed valuable antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*.

Drugs in combinations are used for two main reasons, (i) to prevent emergence of resistance during a treatment and (ii) to improve the activity of one or both agents (Brennan-Krohn and Kirby 2019). Vásquez-Martínez et al. (2019) investigated with checkerboard method the interaction of catecholic chalcones with methicillin against MRSA that showed an important role in potentiation capacity of the β -lactam antibiotic. In agriculture, the common association of heavy metals, as copper, and antibiotics, as streptomycin, has already led to co-selection and co-resistance genes in plants (Miller et al. 2022). Furthermore, the exposure of workers to copper can induce gastrointestinal, neurological, hematological, cardiological, renal effects and eventually mortality (Alengebawy et al. 2021). Taking into account works already published in literature, chalcones were never tested before in association against phytopathogenic bacteria. As demonstrated in this study, catecholic chalcones and streptomycin did not show synergistic activity.

When the introduction of a new product is proposed, it is necessary to know its toxicity profile, risks and safety limits. *Caenorhabditis elegans* is a nematode with a transparent body and more than 65% conserved human diseases-associated genes, very useful in drug screening and toxicity examination of chemical compounds (Yue et al. 2021). In toxicity essay with *C. elegans*, catecholic chalcones **2** (2-CH₃) and **3** (3-OH) presented LC₅₀ of 125 and 250 µg/mL, respectively, higher than MIC/MBC values of 25 and 50 µg/mL; **4** (4-OH) presented LC₅₀ of 125 µg/mL higher than MIC/MBC value of 50 µg/mL; **1** (3-Br), with a bromine atom in its radical *meta* positioned, presented the lowest value of LC₅₀ (31.25 µg/mL) and the lowest value of MIC/MBC (12.5 µg/mL), proving they are safe for application at concentrations with antibacterial properties. It has already been observed that chalcones with halogen groups like -Br, -F, -Cl, etc. have been found to exhibit significant cytotoxic potencies and selective toxicity for tumors (Ouyang et al. 2021). Fluorinated chalcones bearing an indole ring attached to the olefinic carbon showed no cytotoxicity on human normal liver cell line - L02 (Lagu et al. 2020). In another toxicity model, *Galleria mellonella* larvae showed that *C*-isoprenylated retrochalcones was not lethal at 100 µg/mL after 80 hours of treatment (Ozanique et al. 2024).

Zhang et al. (2024) investigated the protective and curative effective of chalcone containing triazole-pyridine against *P. carotovorum* subsp. *brasiliense* for harvested potatoes, the compound **N26** demonstrated protection at high concentrations of 200 and 100 mg/L, but poor curative activity for potatoes infected already. As regards to bacterial leaf spot, bacteria enter via stomata or wounds and colonize the apoplast, followed by cell death and tissue necrosis, while symptoms are small, irregular and water-soaked lesions (Vancheva et al. 2021). In this study, catecholic chalcones presented no difference between preventive and curative treatment against *X. euvesicatoria* pv. *perforans* in tomato. At a lower concentration of 25 µg/mL and 50 µg/mL, samples **2** (2-CH₃) and **3** (3-OH), respectively, were able to reduce

the severity of the bacterial leaf spot more than commercial copper at a higher concentration of 2 mg/mL.

It can be concluded that catecholic chalcones derivatives demonstrated antibacterial activity against phytopathogenic bacteria, more precisely **1**, **2**, **3**, and **4** added to 3-Br, 2-CH₃, 3-OH and 4-OH, respectively. Their use is safe, since the toxic concentration was greater than the concentration of bactericidal action. Catecholic chalcones **2** and **3** proved to be effective in bacterial leaf spot control in tomato caused by *Xanthomonas euvesicatoria* pv. *perforans*. These results revealed the promising agrochemical potential of catecholic chalcones evaluated in this study and suggesting their possible application as pesticide. More studies are necessary to evaluate the control of other plant diseases and mechanisms of action

Author contributions

Daniela S. Gonçalves (Conceptualization, Investigation, Methodology, Writing, Review & Editing); Nagela B. S. Silva (Methodology); Lara C. B. M. Mota (Methodology); Lucas C. Duarte (Methodology); Nilvanira D. Tebaldi (Methodology, Review & Editing); Paulo T. Costa (Methodology & Writing); Luis O. Regasini (Methodology, Review & Editing); Carlos H. G. Martins (Supervision, Funding Acquisition, Conceptualization, Investigation, Methodology, Writing, Review & Editing).

Conflict of interest

No conflict of interest declared.

Funding

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES finance code 001, scholarship granted), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grant 307974/2019-7).

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

- Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R. and Wang, M.Q. (2021) Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics* **9**. 10.3390/toxics9030042.
- An, S.Q., Potnis, N., Dow, M., Vorholter, F.J., He, Y.Q., Becker, A., Teper, D., Li, Y., Wang, N., Bleris, L. and Tang, J.L. (2020) Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen *Xanthomonas*. *FEMS Microbiol Rev* **44**, 1-32. 10.1093/femsre/fuz024.
- Andrade, G., Orlando, H.C.S., Scorzoni, L., Pedroso, R.S., Abrao, F., Carvalho, M.T.M., Veneziani, R.C.S., Ambrosio, S.R., Bastos, J.K., Mendes-Giannini, M.J.S., Martins, C.H.G. and Pires, R.H. (2020) Brazilian Copaifera Species: Antifungal Activity against Clinically Relevant Candida Species, Cellular Target, and In Vivo Toxicity. *J Fungi (Basel)* **6**. 10.3390/jof6030153.
- Baltenneck, J., Reverchon, S. and Hommais, F. (2021) Quorum Sensing Regulation in Phytopathogenic Bacteria. *Microorganisms* **9**. 10.3390/microorganisms9020239.
- Braga, G.U.L., Silva-Junior, G.J., Brancini, G.T.P., Hallsworth, J.E. and Wainwright, M. (2022) Photoantimicrobials in agriculture. *J Photochem Photobiol B* **235**, 112548. 10.1016/j.jphotobiol.2022.112548.
- Brennan-Krohn, T. and Kirby, J.E. (2019) When One Drug Is Not Enough: Context, Methodology, and Future Prospects in Antibacterial Synergy Testing. *Clin Lab Med* **39**, 345-358. 10.1016/j.cll.2019.04.002.
- Campbell, C.L. and Madden, L.V. (1990) Introduction to plant disease epidemiology. Wiley, 532.
- Cao, X., Qiu, D., Zhang, R., Li, Z. and Xu, X. (2023) Synthesis, nematicidal evaluation, and SAR study of benzofuran derivatives containing 2-carbonyl thiophene. *Chinese Chemical Letters* **34**. 10.1016/j.cclet.2022.107800.
- Chaturvedi, V., Ramani, R., Ghannoum, M.A., Killian, S.B., Holliday, N., Knapp, C., Ostrosky-Zeichner, L., Messer, S.A., Pfaller, M.A., Iqbal, N.J., Arthington-Skaggs, B.A., Vazquez, J.A., Sein, T., Rex, J.H. and Walsh, T.J. (2008) Multilaboratory testing of antifungal combinations against a quality control isolate of *Candida krusei*. *Antimicrob Agents Chemother* **52**, 1500-1502. 10.1128/AAC.00574-07.
- Chotsaeng, N., Laosinwattana, C. and Charoenying, P. (2019) Herbicidal Activity of Flavokawains and Related trans-Chalcones against *Amaranthus tricolor* L. and *Echinochloa crus-galli* (L.) Beauv. *ACS Omega* **4**, 20748-20755. 10.1021/acsomega.9b03144.
- CLSI (2018) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* 11th ed. M7-A9 Wayne, PA, USA Clinical and Laboratory Standards Institute.
- Dhaliwal, J.S., Moshawih, S., Goh, K.W., Loy, M.J., Hossain, M.S., Hermansyah, A., Kotra, V., Kifli, N., Goh, H.P., Dhaliwal, S.K.S., Yassin, H. and Ming, L.C. (2022) Pharmacotherapeutics Applications and Chemistry of Chalcone Derivatives. *Molecules* **27**. 10.3390/molecules27207062.
- EFSA, Bragard, C., Baptista, P., Chatzivassiliou, E., Di Serio, F., Gonthier, P., Jaques Miret, J.A., Justesen, A.F., MacLeod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., Parnell, S., Potting, R., Stefani, E., Thulke, H.H., Van der Werf, W., Civera, A.V., Yuen, J., Zappala, L., Migheli, Q., Vloutoglou, I., Maiorano, A., Streissl, F. and Reignault, P.L. (2023) Pest categorisation of *Pantoea ananatis*. *EFSA J* **21**, e07849. 10.2903/j.efsa.2023.7849.
- Elshafie, H.S. and Camele, I. (2021) An Overview of Metabolic Activity, Beneficial and Pathogenic Aspects of *Burkholderia* Spp. *Metabolites* **11**. 10.3390/metabo11050321.
- FAO (2009a) High Level Expert Forum - How to Feed the World in 2050 Rome, Italy Food and Agriculture Organization of United Nations.

FAO (2024b) Pesticides use and trade, 1990–2022. Rome, Italy Food and Agriculture Organization of United Nations. 10.4060/cd1486en.

Greco, D., Aprile, A., De Bellis, L. and Luvisi, A. (2021) Diseases Caused by *Xylella fastidiosa* in *Prunus* Genus: An Overview of the Research on an Increasingly Widespread Pathogen. *Front Plant Sci* **12**, 712452. 10.3389/fpls.2021.712452.

Guo, T., Xia, R., Liu, T., Peng, F., Tang, X., Zhou, Q., Luo, H. and Xue, W. (2020) Synthesis, Biological Activity and Action Mechanism Study of Novel Chalcone Derivatives Containing Malonate. *Chem Biodivers* **17**, e2000025. 10.1002/cbdv.202000025.

Hu, D., Zhang, N., Zhou, Q., Zhou, Y., Gong, C., Zhang, Y. and Xue, W. (2023) Synthesis and biological activities of novel chalcone derivatives containing pyrazole oxime ethers. *Fitoterapia* **166**, 105458. 10.1016/j.fitote.2023.105458.

Kalli, S., Araya-Cloutier, C., Hageman, J. and Vincken, J.P. (2021) Insights into the molecular properties underlying antibacterial activity of prenylated (iso)flavonoids against MRSA. *Sci Rep* **11**, 14180. 10.1038/s41598-021-92964-9.

Kulabhusan, P.K., Tripathi, A. and Kant, K. (2022) Gold Nanoparticles and Plant Pathogens: An Overview and Prospective for Biosensing in Forestry. *Sensors (Basel)* **22**. 10.3390/s22031259.

Lagu, S.B., Yejella, R.P., Bhandare, R.R. and Shaik, A.B. (2020) Design, Synthesis, and Antibacterial and Antifungal Activities of Novel Trifluoromethyl and Trifluoromethoxy Substituted Chalcone Derivatives. *Pharmaceuticals (Basel)* **13**. 10.3390/ph13110375.

Liu, W., Feng, Y., Yu, S., Fan, Z., Li, X., Li, J. and Yin, H. (2021) The Flavonoid Biosynthesis Network in Plants. *Int J Mol Sci* **22**. 10.3390/ijms222312824.

Mello, S.C.T., A.; Lopes, C.A. (1997) Escala diagramática para avaliação da mancha-bacteriana do tomateiro. *Fitopatologia Brasileira* **22**, 447-448.

Miller, S.A., Ferreira, J.P. and LeJeune, J.T. (2022) Antimicrobial Use and Resistance in Plant Agriculture: A One Health Perspective. *Agriculture* **12**. 10.3390/agriculture12020289.

Ouyang, Y., Li, J., Chen, X., Fu, X., Sun, S. and Wu, Q. (2021) Chalcone Derivatives: Role in Anticancer Therapy. *Biomolecules* **11**. 10.3390/biom11060894.

Ozanique, P.R., Helena, A.L., Menezes, R.P., Goncalves, D.S., Santiago, M.B., Dilarri, G., Sardi, J.C.O., Ferreira, H., Martins, C.H.G. and Regasini, L.O. (2024) Synthesis, Antibacterial Effects, and Toxicity of Licochalcone C. *Pharmaceuticals (Basel)* **17**. 10.3390/ph17050634.

Paudel, S., Dobhal, S., Alvarez, A.M. and Arif, M. (2020) Taxonomy and Phylogenetic Research on *Ralstonia solanacearum* Species Complex: A Complex Pathogen with Extraordinary Economic Consequences. *Pathogens* **9**. 10.3390/pathogens9110886.

Qiu, D., Ke, M., Zhang, Q., Zhang, F., Lu, T., Sun, L. and Qian, H. (2022) Response of microbial antibiotic resistance to pesticides: An emerging health threat. *Sci Total Environ* **850**, 158057. 10.1016/j.scitotenv.2022.158057.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B.S., Babalghith, A.O., Alshahrani, M.Y., Islam, S. and Islam, M.R. (2022) Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. *Biomed Res Int* **2022**, 5445291. 10.1155/2022/5445291.

Ruiz Hidalgo, J., Santillán, M., Parellada, E.A., Khyaliya, P., Neske, A. and Ameta, K.L. (2019) Synthetic bis- and mono-chalcones with insecticide effects on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *International Journal of Pest Management* **66**, 116-121. 10.1080/09670874.2019.1575487.

Sarker, S.D., Nahar, L. and Kumarasamy, Y. (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in

the in vitro antibacterial screening of phytochemicals. *Methods* **42**, 321-324. 10.1016/j.ymeth.2007.01.006.

Silverio, M.P., Kraychete, G.B., Rosado, A.S. and Bonelli, R.R. (2022) *Pseudomonas fluorescens* Complex and Its Intrinsic, Adaptive, and Acquired Antimicrobial Resistance Mechanisms in Pristine and Human-Impacted Sites. *Antibiotics (Basel)* **11**. 10.3390/antibiotics11080985.

Straub, C., Colombi, E. and McCann, H.C. (2021) Population Genomics of Bacterial Plant Pathogens. *Phytopathology* **111**, 23-31. 10.1094/PHYTO-09-20-0412-RVW

Vancheva, T., Bogatzevska, N., Moncheva, P., Mitrev, S., Verniere, C. and Koebnik, R. (2021) Molecular Epidemiology of *Xanthomonas euvesicatoria* Strains from the Balkan Peninsula Revealed by a New Multiple-Locus Variable-Number Tandem-Repeat Analysis Scheme. *Microorganisms* **9**. 10.3390/microorganisms9030536.

Vásquez-Martínez, Y., Osorio, M., San Martín, D., Carvajal, M., Vergara, A., Sanchez, E., Raimondi, M., Zacchino, S., Mascayano, C., Torrent, C., Cabezas, F., Mejias, S., Montoya, M. and Cortez-San Martín, M. (2019) Antimicrobial, Anti-Inflammatory and Antioxidant Activities of Polyoxygenated Chalcones. *Journal of the Brazilian Chemical Society* **2**, 286-304. 10.21577/0103-5053.20180177.

Vicente, T.F.L., Felix, C., Felix, R., Valentao, P. and Lemos, M.F.L. (2022) Seaweed as a Natural Source against Phytopathogenic Bacteria. *Mar Drugs* **21**. 10.3390/md21010023.

Yue, Y., Li, S., Shen, P. and Park, Y. (2021) *Caenorhabditis elegans* as a model for obesity research. *Curr Res Food Sci* **4**, 692-697. 10.1016/j.crfs.2021.09.008

Zhang, N., Zeng, W., Sun, Z., Zhou, Q., Meng, K., Hu, Y., Qin, Y. and Xue, W. (2024) Design, synthesis, and bioactivity studies of chalcone derivatives containing [1,2,4]-triazole-[4,3-a]-pyridine. *Fitoterapia* **172**, 105739. 10.1016/j.fitote.2023.105739.

Zhou, Q., Tang, X., Chen, S., Zhan, W., Hu, D., Zhou, R., Sun, N., Wu, Y. and Xue, W. (2022) Design, Synthesis, and Antifungal Activity of Novel Chalcone Derivatives Containing a Piperazine Fragment. *J Agric Food Chem* **70**, 1029-1036. 10.1021/acs.jafc.1c05933.

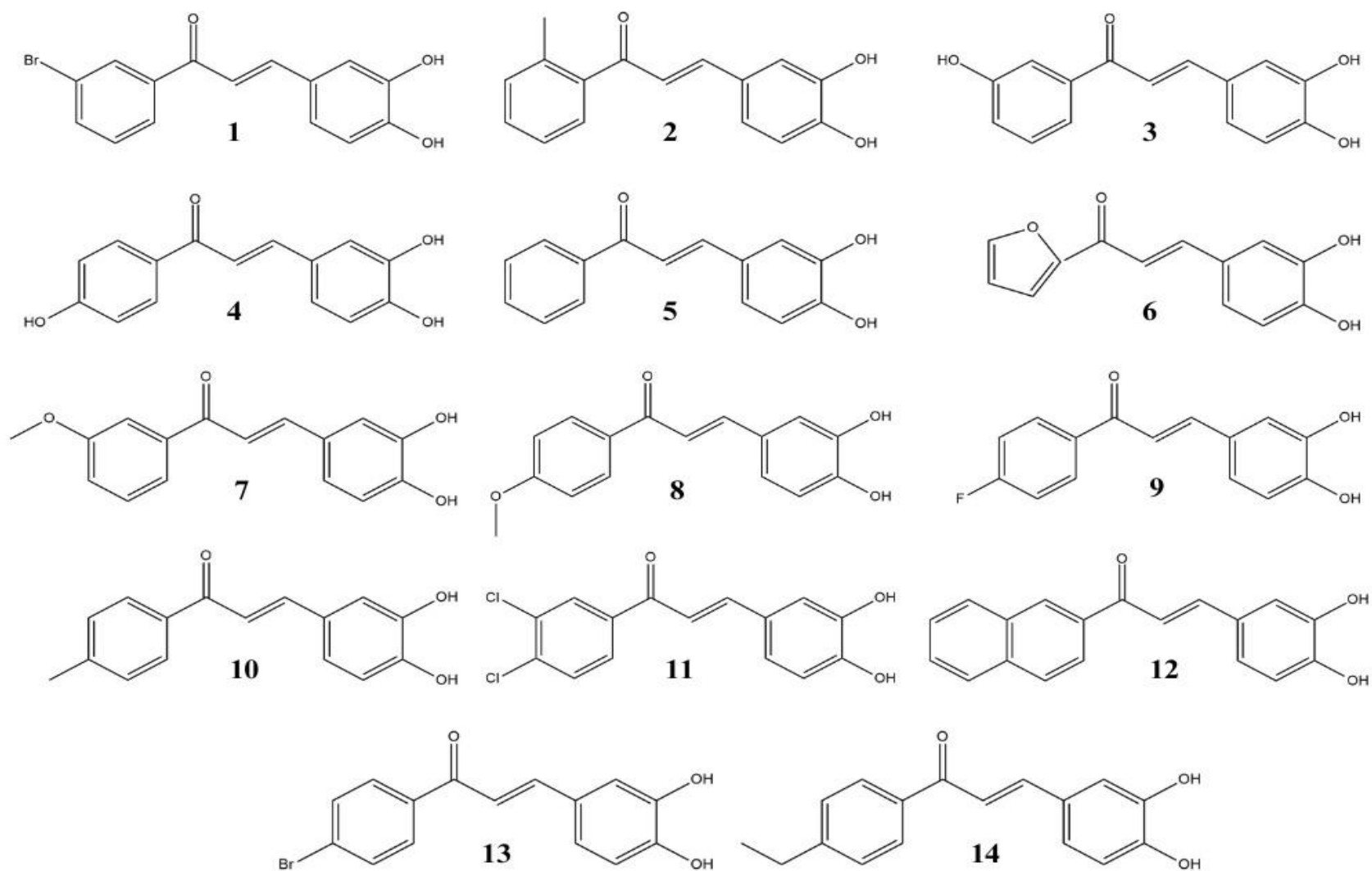


Figure 1. Structures of the catecholic chalcones **1-14** evaluated in this study.

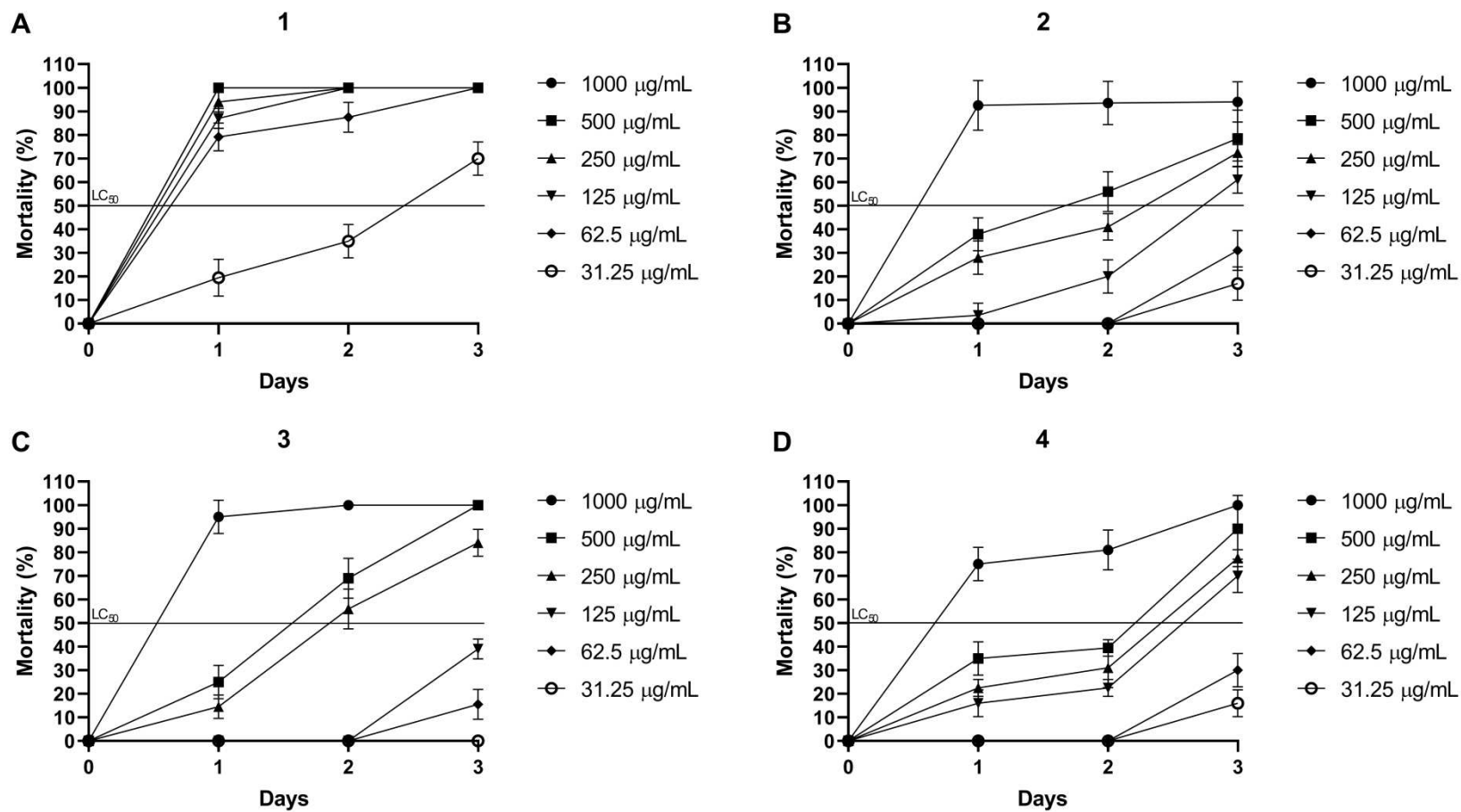


Figure 2. Toxicity in *C. elegans* in vivo model of catecholic chalcones **1** (A); **2** (B); **3** (C); **4** (D).

Table 1. Minimum Inhibitory and Bactericidal Concentrations (MIC/MBC) in µg/mL of catecholic chalcones against phytopathogenic bacteria.

Bacteria	Catecholic chalcones							Positive control
	1	2	3	4	5	6	7	Streptomycin
<i>Xanthomonas citri</i> subsp. <i>citri</i> – Resistant to copper	12.5/12.5	50/50	50/50	50/50	100/100	100/100	100/100	0.09/0.09
<i>Xanthomonas citri</i> subsp. <i>citri</i> – Tolerant to copper	12.5/12.5	25/25	25/25	50/50	100/100	100/100	100/100	0.73/0.73
<i>Xanthomonas citri</i> subsp. <i>citri</i> – Sensible to copper	-	-	100/100	-	-	-	-	0.09/0.09
<i>Xanthomonas euvesicatoria</i> pv. <i>perforans</i>	25/25	25/25	50/50	100/100	100/100	100/100	100/100	0.73/0.73
<i>Xanthomonas vasicola</i> pv. <i>vasculorum</i>	100/100	50/50	25/25	50/50	100/100	100/100	-	0.36/0.36
<i>Xanthomonas campestris</i> pv. <i>campestris</i>	100/100	50/50	25/25	100/100	100/100	100/100	-	0.36/0.36
<i>Pectobacterium carotovorum</i>	25/25	-	100/100	-	-	-	-	0.73/0.73
<i>Xylella fastidiosa</i> 9a5c	100/100	100/100	100/100	-	-	-	-	0.36/0.36
<i>Ralstonia solanacearum</i>	-	-	100/100	-	-	-	-	0.73/0.73

-: inactive results of 200, 400 or >400 µg/mL; **bold**: active and moderately active results chosen for next tests, according to Kalil et al. (2021).

Table 2. Bacterial leaf spot control in tomato with catecholic chalcones.

Treatment	Preventive	Curative	Mean
1 - 25 µg/mL	20.2500	22.5000	21.3750 b
2 - 25 µg/mL	19.5000	19.5000	19.5000 a
3 - 50 µg/mL	16.5000	18.0000	17.2500 a
4 - 100 µg/mL	19.1250	24.0000	21.5625 b
Kocide-2 mg/mL	19.5000	21.7500	20.6250 b
Water	23.2500	21.7500	22.5000 b
Total	19.6875 A	21.2500 A	20.4687
C.V. (%)	13.68%		

C.V. - Coefficient of variation. Means followed by different lowercase letters in the row and uppercase letters in the column are significantly different according to the Scott-Knott test ($P < 0.05$).

CAPÍTULO III

Considerações finais

Com base na análise dos dados obtidos e nas discussões realizadas ao longo deste trabalho, é possível destacar a relevância e o impacto promissor das chalconas catecólicas no controle de bactérias fitopatogênicas. As chalconas catecólicas apresentaram atividade bactericida *in vitro* em baixas concentrações frente principalmente ao gênero *Xanthomonas* spp. *In vivo*, as chalconas catecólicas conseguiram controlar a severidade da mancha foliar bacteriana no tomateiro melhor do que o cobre comercial e em concentrações menores. Das chalconas catecólicas avaliadas neste estudo, destacam-se aquelas com os grupos funcionais 3-Br, 2-CH₃, 3-OH e 4-OH. O ensaio de toxicidade *in vivo*, por sua vez, demonstrou o perfil de baixo risco das chalconas catecólicas nas concentrações bactericidas.

Esses resultados incentivam pesquisas futuras a explorar os produtos naturais e seus potenciais biológicos, bem como a contínua avaliação das chalconas catecólicas no controle de outras doenças e seus impactos a longo prazo no meio ambiente e na saúde humana. A presente dissertação contribui para o avanço do conhecimento sobre o uso de chalconas catecólicas no controle de bactérias fitopatogênicas, oferecendo uma alternativa promissora e sustentável aos pesticidas convencionais. Espera-se que as descobertas aqui apresentadas estimulem aplicações práticas no campo da agricultura, promovendo uma produção agrícola mais segura e eficiente.

CAPÍTULO IV

Contribuições extraordinárias



Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Exploring the composition and properties of *Centella asiatica* metabolites and investigating their impact on BSA glycation, LDL oxidation and α -amylase inhibition

Ana Luiza Silva Borges^{a,1}, Vinícius Prado Bittar^{a,1}, Allisson Benatti Justino^a, Maria Sol Peña Carrillo^a, Renner Francisco Mateus Duarte^a, Nagela Bernadelli Sousa Silva^b, Daniela Silva Gonçalves^b, Diego Godina Prado^c, Iasmin Aparecida Cunha Araújo^d, Mário Machado Martins^e, Larissa Campos Motta^f, Carlos Henrique Gomes Martins^b, Françoise Vasconcelos Botelho^a, Neide Maria Silva^d, Alberto de Oliveira^c, Wanderson Romão^g, Foued Salmen Espíndola^{a,*,2}

^a Laboratory of Biochemistry and Molecular Biology in Institute of Biotechnology, Federal University of Uberlândia, Uberlândia, MG 38400-902, Brazil

^b Laboratory of Antimicrobial Testing, Institute of Biomedical Sciences, University of Uberlândia, Campus Umuarama, Uberlândia, MG 38405-320, Brazil

^c Nucleus of Research in Natural Products (NuPeN), Federal University of Uberlândia, Uberlândia, MG 38400-902, Brazil

^d Laboratory of Immunoparasitology, Institute for Biomedical Sciences, Federal University of Uberlândia, Uberlândia, MG 38400-902, Brazil

^e Laboratory of Nanobiotechnology "Dr. Luis Ricardo Goulart Filho", in Institute of Biotechnology, Federal University of Uberlândia, Uberlândia, MG 38400-902, Brazil

^f Laboratory of Petroleum and Forensics, of the Center of Competence in Petroleum Chemistry – NCQP, Federal University of Espírito Santo (UFES), Vitória, ES 29075-910, Brazil

^g Federal Institute of Education, Science, and Technology of Espírito Santo, Vila Velha, 29106-010, Brazil

ARTICLE INFO

Keywords:

Centella asiatica
glycation
LDL
antioxidant

ABSTRACT

Centella asiatica (L.) Urb. is a small herbaceous plant belonging to the Apiaceae family that is rich in triterpenes, such as asiaticoside and madecassoside. *Centella asiatica* finds broad application in promoting wound healing, addressing skin disorders, and boosting both memory and cognitive function. Given its extensive therapeutic potential, this study aimed not only to investigate the *Centella asiatica* ethanolic extract but also to analyze the biological properties of its organic fractions, such as antioxidant antiglycation capacity, which are little explored. We also identified the main bioactive compounds through spectrometry analysis. The ethanolic extract (EE) was obtained through a static maceration for seven days, while organic fractions (HF: hexane fraction; DF: dichloromethane fraction; EAF: ethyl acetate fraction; BF: n-butanol fraction and HMF: hydromethanolic fraction) were obtained via liquid-liquid fractionation. The concentration of phenolic compounds, flavonoids, and tannins in each sample was quantified. Additionally, the antiglycation (BSA/FRU, BSA/MGO, and ARG/MGO models) and antioxidant (FRAP, ORAC, and DPPH) properties, as well as the ability to inhibit LDL oxidation and hepatic tissue peroxidation were evaluated. The inhibition of enzyme activity was also analyzed (α -amylase, α -glucosidase, acetylcholinesterase, and butyrylcholinesterase). We also evaluated the antimicrobial and cytotoxicity against RAW 264.7 macrophages. The main compounds present in the most bioactive fractions were elucidated through ESI FT-ICR MS and HPLC-ESI-MS/MS analysis. In the assessment of antioxidant capacity (FRAP, ORAC, and DPPH), the EAF and BF fractions exhibited notable results, and as they are the phenolic compounds richest fractions, they also inhibited LDL oxidation, protected the hepatic tissue from peroxidation

Abbreviations: HPLC-ESI, High-performance liquid chromatography-electrospray ionization source; FRAP, Ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ORAC, Oxygen radical absorbance capacity; AGEs, Advanced glycation end products; MGO, Methylglyoxal; FRU, Fructose; BSA, Bovine serum albumin; LDL, Low-density lipoprotein; ARG, Arginine; PBS, Phosphate buffer saline; OHT, Oxidized hepatic tissue; HT, Hepatic tissue; MIC, Minimum Inhibitory Concentration; AChE, Acetylcholinesterase; BChE, Butyrylcholinesterase; ROS, Reactive oxygen species; IC50, Inhibition concentration; CC50, cytotoxicity concentration; TBARS, thiobarbituric acid reactive substance; MDA, malondialdehyde; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

* Correspondence to: Universidade Federal de Uberlândia, Instituto de Biotecnologia, Rua Acre s/n, Bloco 2E, Uberlândia, MG 38400-319, Brazil.

E-mail address: foued@ufu.br (F.S. Espíndola).

¹ Equal contributions authors: & ALSB and VPB

² <https://orcid.org/0000-0002-6937-1411>

<https://doi.org/10.1016/j.jpba.2024.116143>

Received 21 December 2023; Received in revised form 26 March 2024; Accepted 31 March 2024

Available online 12 April 2024

0731-7085/© 2024 Published by Elsevier B.V.



Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Bioactive compounds from the leaves of *Maytenus ilicifolia* Mart. ex Reissek: Inhibition of LDL oxidation, glycation, lipid peroxidation, target enzymes, and microbial growth

Vinicius Prado Bittar^{a,1}, Ana Luiza Silva Borges^{a,1}, Allisson Benatti Justino^a, Maria Sol Peña Carrillo^a, Renner Francisco Mateus Duarte^a, Nagela Bernadelli Sousa Silva^e, Daniela Silva Gonçalves^e, Diego Godina Prado^b, Iasmin Aparecida Cunha Araújo^d, Mário Machado Martins^c, Carlos Henrique Gomes Martins^e, Françoise Vasconcelos Botelho^a, Neide Maria Silva^d, Alberto de Oliveira^b, Foued Salmen Espíndola^{a,*}

^a Laboratory of Biochemistry and Molecular Biology in Institute of Biotechnology, Federal University of Uberlândia, Uberlândia, MG, 38400-902, Brazil

^b Nucleus of Research in Natural Products (NuPPeN), Federal University of Uberlândia, Uberlândia, MG, 38400-902, Brazil

^c Laboratory of Nanobiotechnology "Dr. Luiz Ricardo Goulart Filho", Institute of Biotechnology, Federal University of Uberlândia, Uberlândia, MG, 38400-902, Brazil

^d Laboratory of Immunopathology, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, MG, 38400-902, Brazil

^e Laboratory of Antimicrobial Testing, Institute of Biomedical Sciences, University of Uberlândia, Campus Umuarama, Uberlândia, MG, 38405-320, Brazil

ARTICLE INFO

Keywords:

Antioxidant
Phytochemical analysis
Lipid peroxidation
Antiglyciant

ABSTRACT

Ethnopharmacological relevance: *Maytenus ilicifolia* Mart. ex Reissek, a medicinal plant used for treating gastritis, ulcers, and gastric disorders, possesses therapeutic properties attributed to diverse leaf compounds—terpenoids, alkaloids, flavonoids, phenols, and tannins, reflecting the ethnopharmacological knowledge of traditional users. **Aims of the study:** We aimed to assess the antioxidant and antiglycant capacities of *Maytenus ilicifolia*'s ethanolic extract and organic fractions, identify bioactive compounds through HPLC-MS/MS analysis, and conduct phytochemical assessments. We also assessed their potential to inhibit digestive and cholinesterase enzymes, mitigate oxidation of human LDL and rat hepatic tissue, and examine their antimicrobial and cytotoxic properties.

Materials and methods: Organic fractions (hexane - HF-Mi, dichloromethane - DMF-Mi, ethyl acetate - EAF-Mi, n-butanol - BF-Mi, and hydromethanolic - HMF-Mi) were obtained via liquid-liquid partitioning. Antioxidant (DPPH, FRAP, ORAC) and antiglycant (BSA/FRU, BSA/MGO, ARG/MGO/LDL/MGO models) capacities were tested. Phytochemical analysis employed HPLC-MS/MS. We also studied the inhibitory effects on α -amylase, acetylcholinesterase, butyrylcholinesterase, human LDL and rat hepatic tissue oxidation, antimicrobial activity, and cytotoxicity against RAW 264.7 macrophages.

Results: HPLC-ESI-MS/MS identified antioxidant compounds such as catechin, quercetin, and kaempferol derivatives. Ethanolic extract (EE-Mi) and organic fractions demonstrated robust antioxidant and antiglycant activity. EAF-Mi and BF-Mi inhibited α -amylase (2.42 μ g/mL and 7.95 μ g/mL) compared to acarbose (0.144 μ g/mL). Most organic fractions exhibited ~50% inhibition of acetylcholinesterase and butyrylcholinesterase, rivaling galantamine and rivastigmine. EAF-Mi, BF-Mi, and EE-Mi excelled in inhibiting lipid peroxidation. All fractions, except HMF-Mi, effectively countered LDL oxidation, evidenced by the area under the curve. These fractions protected LDL against lipid peroxidation.

Conclusion: This study unveils *Maytenus ilicifolia*'s ethanolic extract and organic fractions properties. Through rigorous analysis, we identify bioactive compounds and highlight their antioxidant, antiglycant, enzyme inhibition, and protective properties against oxidative damage. These findings underline its significance in modern pharmacology and its potential applications in healthcare.

* Corresponding author. Universidade Federal de Uberlândia, Instituto de Biotecnologia, Rua Acre s/n, Bloco 2E, 38400-319, Uberlândia, MG, Brazil.

E-mail address: foued@ufu.br (F.S. Espíndola).

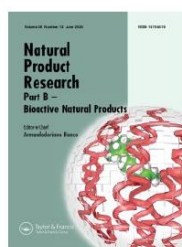
¹ Equal contributions authors: VPB and ALSB.

<https://doi.org/10.1016/j.jep.2023.117315>

Received 20 July 2023; Received in revised form 28 September 2023; Accepted 11 October 2023

Available online 17 October 2023

0378-8741/© 2023 Elsevier B.V. All rights reserved.



Natural Product Research
Formerly Natural Product Letters



ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/gnpl20

Chemical composition and anti-*Xanthomonas citri* activities of essential oils from *Schinus molle* L. fresh and dry leaves and of its major constituent spathulenol

Isac R. R. da Silva, Cassia C. Fernandes, Daniela S. Gonçalves, Carlos H. G. Martins & Mayker L. D. Miranda

To cite this article: Isac R. R. da Silva, Cassia C. Fernandes, Daniela S. Gonçalves, Carlos H. G. Martins & Mayker L. D. Miranda (22 Aug 2023): Chemical composition and anti-*Xanthomonas citri* activities of essential oils from *Schinus molle* L. fresh and dry leaves and of its major constituent spathulenol, Natural Product Research, DOI: [10.1080/14786419.2023.2249584](https://doi.org/10.1080/14786419.2023.2249584)

To link to this article: <https://doi.org/10.1080/14786419.2023.2249584>



View supplementary material [↗](#)



Published online: 22 Aug 2023.



Submit your article to this journal [↗](#)



Article views: 115



View related articles [↗](#)



View Crossmark data [↗](#)

Full Terms & Conditions of access and use can be found at
<https://www.tandfonline.com/action/journalInformation?journalCode=gnpl20>

scientific reports



OPEN Exploring the antifungal, antibiofilm and antienzymatic potential of Rottlerin in an in vitro and in vivo approach

Nagela Bernadelli Sousa Silva¹, Ralciane Paula Menezes², Daniela Silva Gonçalves¹, Mariana Brentini Santiago¹, Noemi Chagas Conejo¹, Sara Lemes Souza¹, Anna Livia Oliveira Santos¹, Robinson Sabino da Silva³, Salvador Boccaletti Ramos⁴, Eloisa Amália Vieira Ferro⁵ & Carlos Henrique Gomes Martins^{1✉}

Candida species have been responsible for a high number of invasive infections worldwide. In this sense, Rottlerin has demonstrated a wide range of pharmacological activities. Therefore, this study aimed to evaluate the antifungal, antibiofilm and antivirulence activity of Rottlerin in vitro against *Candida* spp. and its toxicity and antifungal activity in vivo. Rottlerin showed antifungal activity against all yeasts evaluated, presenting Minimum Inhibitory and Fungicidal Concentration (MIC and MFC) values of 7.81 to >1000 µg/mL. Furthermore, it was able to significantly inhibit biofilm production, presenting Biofilm Inhibitory Concentration (MICB₅₀) values that ranged from 15.62 to 250 µg/mL and inhibition of the cell viability of the biofilm by 50% (IC₅₀) from 2.24 to 12.76 µg/mL. There was a considerable reduction in all hydrolytic enzymes evaluated, with emphasis on hemolysin where Rottlerin showed a reduction of up to 20%. In the scanning electron microscopy (SEM) analysis, Rottlerin was able to completely inhibit filamentation by *C. albicans*. Regarding in vivo tests, Rottlerin did not demonstrate toxicity at the therapeutic concentrations demonstrated here and was able to increase the survival of *C. elegans* larvae infected. The results herein presented are innovative and pioneering in terms of Rottlerin's multipotentiality against these fungal infections.

Keywords Rottlerin, Antifungal activity, Antibiofilm, Hydrolytic enzymes, *Caenorhabditis elegans*

Infections caused by yeasts of the genus *Candida* have increased significantly over the years, being identified as the fourth leading cause of healthcare-related bloodstream infections in the United States and the seventh cause in Brazil, impacting morbidity and mortality rates^{1,2}. In association with that, there is the fact that resistant isolates are increasingly linked to outbreaks, especially by *C. parapsilosis* and *C. auris*^{3,4}. The isolation of these species is worrying due to the fact that they are less susceptible to the antifungals of choice for the treatment of fungal Healthcare-associated Infections (HAI)⁵. These infections are influenced by several factors, with the production of biofilm and hydrolytic enzymes being the main ones⁶. The biofilm is a form of protection against the cells of the host's immune system and antifungal agents and the hydrolytic enzymes act in the invasion of host tissues, evasion of the immune system and in the maintenance of the viability and multiplication of yeast in the host^{7–9}.

Despite the four classes of antifungals available on the market for the treatment of these invasive fungal infections, mortality remains high and the toxicity of these medications continues to be a relevant problem. Additionally, the mechanism of action of these antifungals is limited, demonstrating the urgency of discovering a new antifungal agent¹⁰.

In this sense, the molecule called Rottlerin, a red polyphenol powder produced from the fruits of *Mallotus philippinensis* Muell. Arg (Euphorbiaceae), stands out as an important source of relevant bioactive molecules,

¹Laboratory of Antimicrobial Testing, Institute of Biomedical Sciences (ICBIM), Federal University of Uberlândia (UFU), Av. Pará, 1720 - Umuarama, Uberlândia 38405-320, Brazil. ²Technical School of Health (ESTES), Federal University of Uberlândia (UFU), Uberlândia, Brazil. ³Innovation Center in Salivary Diagnostic and Nanotheranostics, Institute of Biomedical Sciences (ICBIM), Federal University of Uberlândia (UFU), Uberlândia, Brazil. ⁴Department of Engineering and Exact Sciences, Faculty of Agricultural and Veterinary Sciences - Jaboticabal (FCAV), São Paulo State University (UNESP), Jaboticabal, Brazil. ⁵Laboratory of Immunophysiology of Reproduction, Institute of Biomedical Sciences (ICBIM), Federal University of Uberlândia, Uberlândia, Brazil. ✉email: carlos.martins2@ufu.br



Article

Synthesis, Antibacterial Effects, and Toxicity of Licochalcone C

Patrick Rômulo Ozanique ¹, Alvaro Luiz Helena ¹, Ralciane de Paula Menezes ², Daniela Silva Gonçalves ², Mariana Brentini Santiago ², Guilherme Dilarri ³, Janaína de Cássia Orlandi Sardi ⁴, Henrique Ferreira ³, Carlos Henrique Gomes Martins ² and Luis Octávio Regasini ^{1,*}

¹ Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil; patrick.ozanique@unesp.br (P.R.O.); alvaro.helena@unesp.br (A.L.H.)

² Department Microbiology, Institute of Biomedical Sciences, Federal University of Uberlândia (UFU), Umuarama 38405-320, MG, Brazil; ralciane@ufu.br (R.d.P.M.); dani_sg_1999@outlook.com (D.S.G.); mari.brentini@hotmail.com (M.B.S.); carlos.martins2@ufu.br (C.H.G.M.)

³ Department of Biochemistry and Microbiology, Institute of Biosciences, São Paulo State University (Unesp), Rio Claro 13506-900, SP, Brazil; gui_dila@hotmail.com (G.D.); henrique.ferreira@unesp.br (H.F.)

⁴ Dental Research Division, Guarulhos University, Guarulhos 07023-070, SP, Brazil; janasardi@gmail.com

* Correspondence: luis.regasini@unesp.br; Tel.: +55-17-32212362

Abstract: Drug-resistant bacteria constitute a big barrier against current pharmacotherapy. Efforts are urgent to discover antibacterial drugs with novel chemical and biological features. Our work aimed at the synthesis, evaluation of antibacterial effects, and toxicity of licochalcone C (LCC), a naturally occurring chalcone. The synthetic route included six steps, affording a 10% overall yield. LCC showed effects against Gram-positive bacteria (MIC = 6.2–50.0 µg/mL), *Mycobacterium* species (MIC = 36.2–125 µg/mL), and *Helicobacter pylori* (MIC = 25 µg/mL). LCC inhibited the biofilm formation of MSSA and MRSA, demonstrating MBIC₅₀ values of 6.25 µg/mL for both strains. The investigations by fluorescence microscopy, using PI and SYTO9 as fluorophores, indicated that LCC was able to disrupt the *S. aureus* membrane, similarly to nisin. Systemic toxicity assays using *Galleria mellonella* larvae showed that LCC was not lethal at 100 µg/mL after 80 h treatment. These data suggest new uses for LCC as a compound with potential applications in antibacterial drug discovery and medical device coating.

Keywords: licochalcone; chalcone; *Glycyrrhiza*; membrane; biofilm; antibacterial; drug-resistant; *Galleria mellonella*



Citation: Ozanique, P.R.; Helena, A.L.; Menezes, R.d.P.; Gonçalves, D.S.; Santiago, M.B.; Dilarri, G.; Sardi, J.d.C.O.; Ferreira, H.; Martins, C.H.G.; Regasini, L.O. Synthesis, Antibacterial Effects, and Toxicity of Licochalcone C. *Pharmaceuticals* **2024**, *17*, 634. <https://doi.org/10.3390/ph17050634>

Academic Editor: Cheng-Wei Tom Chang

Received: 30 March 2024

Revised: 11 May 2024

Accepted: 12 May 2024

Published: 14 May 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Antimicrobial resistance (AMR) is a challenge to human health, resulting in alarming numbers of deaths around the world. According to predictive statistical models performed by AMR collaborators, there was an estimate of 4.95 million (3.62–6.57) deaths associated with bacterial AMR in 2019 [1]. The discovery of new effective agents against multidrug-resistant bacteria is an urgent topic in global public health. The World Health Organization (WHO) indicated a human pathogen priority list, which included *Pseudomonas aeruginosa* (critical), *Staphylococcus aureus* and *Helicobacter pylori* (high), and *Streptococcus pneumoniae* (medium) [2]. Additionally, bacterial biofilms are barriers against current pharmacotherapy, which are constituted by cellular communities able to adhere to abiotic and biological surfaces, blocking the antibacterial drug penetration and diffusion into their complex extracellular matrix [3].

Natural products from plants are successful sources of antimicrobial drugs [4,5]. Out of 162 new antibacterial agents approved between 1981 and 2019, 55% are natural products and their derivatives, highlighting their relevance to modern drug discovery [6]. Among the promising bioactive natural products are retrochalcones, which are rare plant compounds and restricted to *Glycyrrhiza* genus. This taxon is constituted by several medicinal plants