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Potencial biológico de ConA e ConM, extraídas das sementes de *Canavalia ensiformis* (L.) DC. e *Canavalia rosea* (Sw.) DC., frente a fungos do gênero *Candida*.

VICTOR JUNO ALENCAR FONSECA

Crato - CE
2021

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Relatório de Dissertação de Mestrado, apresentado ao Programa de Pós-Graduação em Química Biológica, da Universidade Regional do Cariri, como requisito parcial para obtenção de Título de Mestre em Química Biológica.

Orientadora: Profª. Dra. Maria Flaviana Bezerra Morais Braga.
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CEP: 63100-010	Cidade: CRATO	Estado: CEARÁ
Fone/Celular: (88) 9 9713 6365	E-mail victorjuno5@gmail.com	
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Orientador: MARIA FLAVIANA BEZERRA MORAIS BRAGA		
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Victor Juno Alencar Fonseca

Assinatura do Orientador:

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BANCA EXAMINADORA:

Maria Flaviana Bezerra Morais Braga / Orientadora

Professora Titular da Universidade Regional do Cariri - URCA

Henrique Douglas Melo Coutinho / Membro Titular

Professor Titular da Universidade Regional do Cariri - URCA

Jacqueline Cosmo Andrade Pinheiro / Membro Titular

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Alexandre Magno Rodrigues Teixeira / Membro Suplente

Professor Titular da Universidade Regional do Cariri - URCA

Fabíola Fernandes Galvão Rodrigues / Membro Suplente

Professora Titular do Centro Universitário Leão Sampaio

Dedido este trabalho aos meus pais, ***Antovania Venina Alencar Fonseca e José Otaviano da Fonseca Junior.***

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RESUMO

As lectinas são biomoléculas que atuam se ligando a carboidratos específicos, que podem estar presente na superfície dos micro-organismos, podendo causar danos à parede celular e impedindo sua adesão a células hospedeiras. Além disso, atuam potencializando a ação de antibióticos e antifúngicos aos quais os micro-organismos apresentam resistência. Lectinas, como ConA e ConM se ligam especificamente a carboidratos presentes na superfície de micro-organismos, e dessa forma desempenham atividades antimicrobianas. Este estudo busca analisar o potencial das lectinas ConA e ConM, extraídas de sementes de *Canavalia ensiformis* e *Canavalia rosea*, respectivamente, isoladas e combinadas em concentração subinibitória ao antifúngico fluconazol contra cepas padrão de *Candida albicans* e *Candida tropicalis*, bem como seu efeito sobre a transição morfológica das espécies. Um levantamento bibliográfico foi realizado no PubMed sobre as potencialidades de lectinas em geral contra bactérias, fungos e protozoários. As soluções de ConA e ConM foram microdiluidas em concentrações de 0,5 a 512 µg/mL. O teste da CFM foi realizado em placas de Petri contendo meio ASD, por subcultivo da microdiluição. No ensaio antifúngico da combinação/lectina fluconazol foram utilizadas concentrações subinibitórias de ConA e ConM e antifúngico com variando de 0,5 a 512 µg/mL. As lectinas foram testadas quanto a sua capacidade de inibir a transição morfológica das cepas de *Candida*, através do cultivo em câmara úmida. Os resultados da concentração inibitória mínima revelaram ausência de atividade antifúngica contra as cepas testadas (≥ 512 µg/mL). Entretanto, as lectinas modificaram a ação do fluconazol, reduzindo a IC_{50} do antifúngico, inibindo o crescimento de *C. albicans* desde as menores concentrações testadas. Embora ConA e ConM não tenham apresentado atividade antifúngica sozinhos, elas mostraram uma importante ação sinérgica com o antibiótico fluconazol, principalmente contra *C. albicans.* Além disso, apresentaram discreta inibição no desenvolvimento de hifas de forma dose dependente, chegando reduzir o comprimento de hifas de *C. tropicalis* em mais de 50% nas concentrações mais baixas (256 µg/mL). As lectinas podem ser usadas para modular a ação de drogas antimicrobianas, bem como afetar seus fatores de virulência, e dessa forma, representam uma alternativa a terapia vigente.

Palavras-chave: Proteínas vegetais. Patógenos oportunistas. Antifúngico.

ABSTRACT

Lectins are biomolecules that act by binding to specific carbohydrates, which may be present on the surface of microorganisms, causing damage to the cell wall and preventing its adhesion to host cells. In addition, they act by enhancing the action of antibiotics and antifungals to which microorganisms are resistant. Lectins such as ConA and ConM specifically bind to carbohydrates present on the surface of microorganisms, and thus perform antimicrobial activities. This study seeks to analyze the potential of the lectins ConA and ConM, extracted from *Canavalia ensiformis* and *Canavalia rosea* seeds, respectively, isolated and combined in a subinhibitory concentration to the antifungal fluconazole against standard strains of *Candida albicans* and *Candida tropicalis*, as well as their effect on the transition morphological aspects of the species. A bibliographic survey was carried out in PubMed on the potential of lectins in general against bacteria, fungi and protozoa. ConA and ConM solutions were microdiluted at concentrations from 0.5 to 512 µg/ml. The CFM test was performed in Petri dishes containing ASD medium, by microdilution subculture. In the fluconazole combination antifungal/lectin assay, subinhibitory concentrations of ConA and ConM and antifungal ranging from 0.5 to 512 µg/mL were used. Lectins were tested for their ability to inhibit the morphological transition of *Candida* strains, through cultivation in a humid chamber. The minimal inhibitory concentration results revealed the absence of antifungal activity against the strains tested (≥ 512 µg/mL). However, the lectins modified the action of fluconazole, reducing the IC50 of the antifungal, inhibiting the growth of *C. albicans* from the lowest concentrations tested. Although ConA and ConM did not show antifungal activity alone, they showed an important synergistic action with the antibiotic fluconazole, mainly against C. albicans. Furthermore, they presented a mild dose-dependent inhibition of hyphae development, reducing the length of *C. tropicalis* hyphae by more than 50% at the lowest concentrations (256 µg/mL). Lectins can be used to modulate the action of antimicrobial drugs, as well as affect their virulence factors, and thus, represent an alternative to current therapy.

Keywords: Vegetable proteins; Opportunistic pathogens; Antifungal.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

® Marca Registrada

ASD – Agar sabouraud dextrose

CA – *Candida albicans*

ConA – *Concanavalin A*

ConM – *Concanavalin M*

CC – Controle de crescimento

CFM – Concentração fungicida mínima

CI – Candidíase invasiva

CIM – Concentração inibitória mínima

CSD – Caldo sabouraud dextrose

CT – *Candida tropicalis*

DNA – Ácido desoxirribonucleico

DRC – Domínio de reconhecimento dos carboidratos

EB – Extrato bruto

EHEC – *Escherichia coli* enterohemorragica

ELISA – *Enzyme-Linked Immunosorbent Assay*

ELLA – Ensaio de lectina ligada a enzima

ERO's – Espécies reativas de oxigênio

FCZ – Fluconazol

GalNAc – N-acetil-galactosamina

GlcNAc – N-acetilglucosamina

HIV – Vírus da imunodeficiência humana

HSV – Herpes simplex vírus

IC_{50} – Concentração que inibe 50% do efeito

IFN – Interferon

Ig – Imunoglobulina

IL – Interleucina

INCQS – Instituto Nacional de Controle de Qualidade em Saúde

LMAC – Laboratório de Micologia Aplicada do Cariri

LPS – Lipopolissacarideo

MEV – Microscopia eletrônica de varredura

MIF – Fator inibitório de migração

MRSA – *Staphylococcys aureus* resistente a meticilina

MTZ – Metronidazol

NO – Óxido nítrico

PDA – *Potato Dextrose Agar*

PDB – *Potato Dextrose Broth*

PGN – Peptidoglicano

RNA – Ácido ribonucleico

SAP – Protease aspártica secretada

Th – Linfócitos T helper

TNF – Fator de necrose tumoral

tRNA – Ácido ribonucluico transportador

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INTRODUÇÃO GERAL

1.1. Objetivos e questionamentos

Com o passar dos anos, os diferentes fatores ligados a difusão e propagação da candidíase invasiva (CI) mudaram gradativamente em todo mundo. Os motivos dessa mudança estão ligados ao surgimento de várias espécies de *Candida* (GHAZI *et al.*, 2019). A incidência de candidemia varia entre 2 a 14 por 100.000 pessoas em todo mundo, e segundo pesquisas populacionais, pelo menos 10 a 20% dos infectados chegam a óbito. Dessa forma, as infecções sanguíneas causadas por fungos do gênero *Candida* representam a posição de quarta etiologia mais comum em países desenvolvidos (PAPPAS *et al.*, 2018).

Cada vez mais a resistência dos micro-organismos aos antimicrobianos vem desafiando profissionais de saúde em todo o mundo, mostrando ser um desafio à saude publica e uma grande ameaça global. Essa situação se agrava principalmente quando se trata de agentes antifúngicos, classe que contém um número limitado de medicamentos com diferentes componentes fúngicos como alvo (MAUBON *et al.*, 2014). Dessa forma, pesquisadores em todo mundo buscam formas, atraves de produtos de origem natural, de combater esses micro-organismos multiresistentes, seja por ação direta de um único composto ou em combinação com um fármaco comercial.

O Brasil tornou-se um país com fortes tradiçoes no uso de plantas medicinais e medicamentos fitoterápicos. Isso ocorre devido o país ter a maior cobertura vegetal do planeta, composto por seis ricos biomas, com mais de 120 mil espécies de plancas (LIMA-SARAIVA *et al.*, 2015). Os compostos naturais extraídos de plantas representam fontes valiosas para manutenção da saúde humana e é a partir desses compostos naturais que muitas vezes surgem novos medicamentos (ATANASOV *et al.*, 2015). Diversas substâncias são produzidas pelas plantas em resposta a estímulos da natureza, sejam, físicos, químicos ou biológicos, de modo a conferir protenção contra predadores herbívoros e/ou patógenos (PINTO; RIBEIRO; OLIVEIRA, 2011).

De acordo com Hasan; Ozeki; Kabir (2014), as plantas são mais propensas a infecções microbianas em comparação aos animais. Isso ocorre devido à falta de um sistema imunológico bem desenvolvido, além de não poderem se mover. Então, para garantir a perpetuação da espécie, os órgãos reprodutivos, como corpos de frutas e tubérculos, são

desenvolvidos para armazenar uma série de moléculas auto protetoras contra os micróbios invasores. Dentre as substâncias produzidas por plantas, muitas vezes chamadas de metabólitos secundários, estão as lectinas, que participam da defesa contra micro-organismos e na sinalização de danos causados à superfície celular e/ou intracelular das plantas (LANNOO; VAN DAMME, 2014).

As lectinas são proteínas que desempenham um papel muito importante na natureza, podem ser secretadas em resposta a um agente invasor, como as bactérias (ZHANG *et al.*, 2018), e devido a seu reconhecimento de carboidratos podem se ligar a superfície desses micro-organismos e promover diversas atividades antimicrobianas (LIU *et al.*, 2019). As leguminosas produzem sementes ricas em lectinas bioativas, proteínas que a auxiliam na sua defesa, sendo o principal representante dessas lectinas a ConA, extraída de *Canavalia ensiformis* (VAN DAMME *et al.*, 1998).

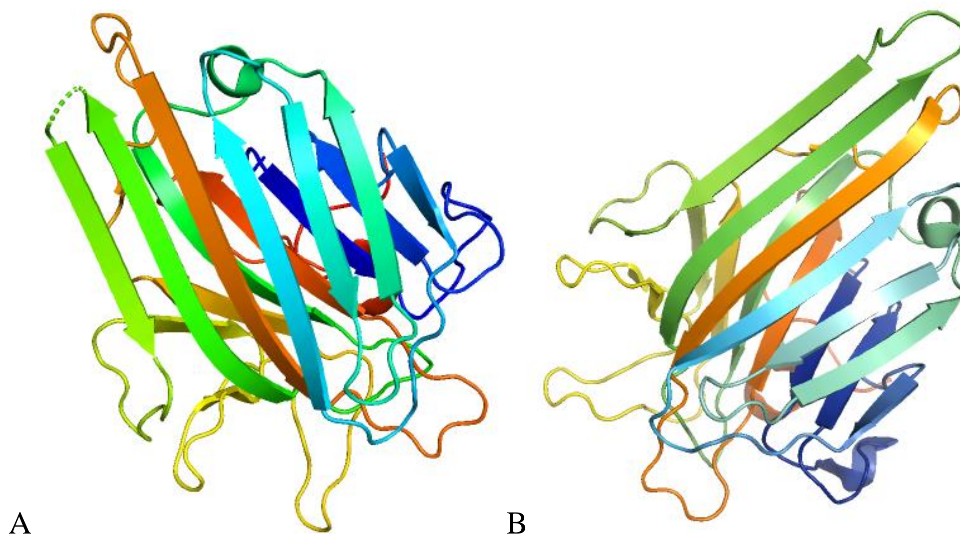
As lectinas do gênero *Canavalia* apresentam uma diversidade de efeitos contra micro-organismos. A leguminosa *Canavalia ensiformis* produz uma lectina específica de manose (ConA) com notável atividade antibiofilme contra *E. coli* enterohemorrágica (EHEC) e *Listeria monocytogenes* (JIN; LEE; HONG, 2019). ConA também foi eficaz em modular a atividade da gentamicina contra *Staphylococcus aureus* e *E. coli*, reduzindo a MIC do antibiótico em 80% e 37,5% respectivamente (SANTOS *et al.*, 2020). Essa proteína ainda foi capaz de estimular as capacidades fagocíticas de células mononucleares do sangue periférico, além de aumentar a produção de citocinas como IFNg, IL-6, TNF-α, IL-4, IL-2 e IL-10, que na infecção por *Leishmania amazonensis*, encontravam-se diminuídas (THOMAZELLI *et al.*, 2018). A lectina Conbr, extraída de *Canavalia brasiliensis* inibiu o crescimento de diversas espécies de *Candida* (GOMES *et al.*, 2012; KLAFKE *et al.*, 2013).

Dessa forma, a multivalência de lectinas pertencentes ao gênero *Canavalia* as tornam promissores agentes antimicrobianos. Uma possível atividade antifúngica contra espécies de *Candida*, bem como uma possível interação sinérgica com antibióticos deve ser analisada. É devido essas atividades biológicas que o presente estudo busca analisar o potencial das lectinas ConA e ConM, extraídas de sementes de *Canavalia ensiformis* (L.) DC. e *Canavalia rosea* (Sw.) DC. respectivamente, contra cepas padrão de *Candida albicans* e *Candida tropicalis*, bem como sua atividade moduladora com antifúngicos e inibição da morfogênese, importante fator de virulência das espécies de *Candida*.

1.2. Estratégias de pesquisa

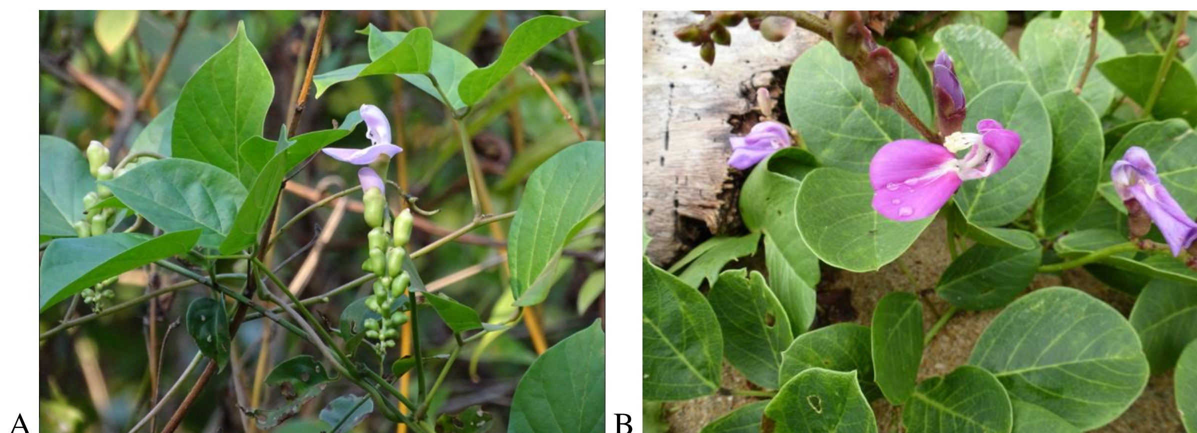
Diversos estudos vêm sendo desenvolvidos e direcionados à descoberta de novos agentes antimicrobianos provenientes de extratos vegetais e outros produtos naturais, com o objetivo de descobrir compostos com atividade comparada à dos tradicionalmente utilizados, porém, com menor toxicidade, mais eficazes contra a resistência de micro-organismos patogênicos e com menor impacto ambiental (BONA *et al.*, 2014)). Lectinas são moléculas biologicamente versáteis com notáveis efeitos antimicrobianos. As lectinas de *Canavalia ensiformis* e *Canavalia rosea* (Figura 1) têm potencial para desempenhar atividade antifúngica devido a sua afinidade a glicose e manose, substâncias presentes na parede celular dos fungos (ARAÚJO-FILHO *et al.*, 2010). Além disso, a lectina de *Cavanalia brasiliensis* foi ativa contra *C. parapsilosis* (KLAFKE *et al.*, 2013), tornando as lectinas do gênero *Canavalia* promissores alvos de estudo (Figuras 2).

Figura 1: Estrutura tridimensional das lectinas Concanavalin A (ConA) e Concanavalin M (ConM).



A: Concanavalin A (ConA). Fonte: Park et al. (2017); B: Concanavalin M (ConM). Fonte: Moreno et al. (2007).

Figura 2: Imagens representativas das plantas *Canavalia ensiformis (L.) DC e Canavalia rosea (SW.) DC.*



A: *Canavalia ensiformis (L.) DC.* Fonte: Rujuta Vinod - www.inaturalist.org; B: *Canavalia rosea (SW.) DC.* Fonte: Carel Jongkind - www.inaturalist.org.

Na avaliação da atividade antifúngica de produtos naturais, diferentes métodos podem ser utilizados, sendo os seguintes os mais conhecidos: método de difusão em ágar por poço, que vai avaliar a formação de um halo de inibição ao redor do poço, à medida que o produto se difunde pelo meio e inibe o crescimento fúngico, disco-difusão, que diferente do primeiro somente pela ausência de poços, onde no lugar serão utilizados discos absorventes embebecidos no produto (CLSI 2009), e métodos de microdiluição, os quais são realizados em caldo, e consistem na realização de diluições seriadas do produto ao longo de uma placa de 96 poços (JAVADPOUR *et al.*, 1996).

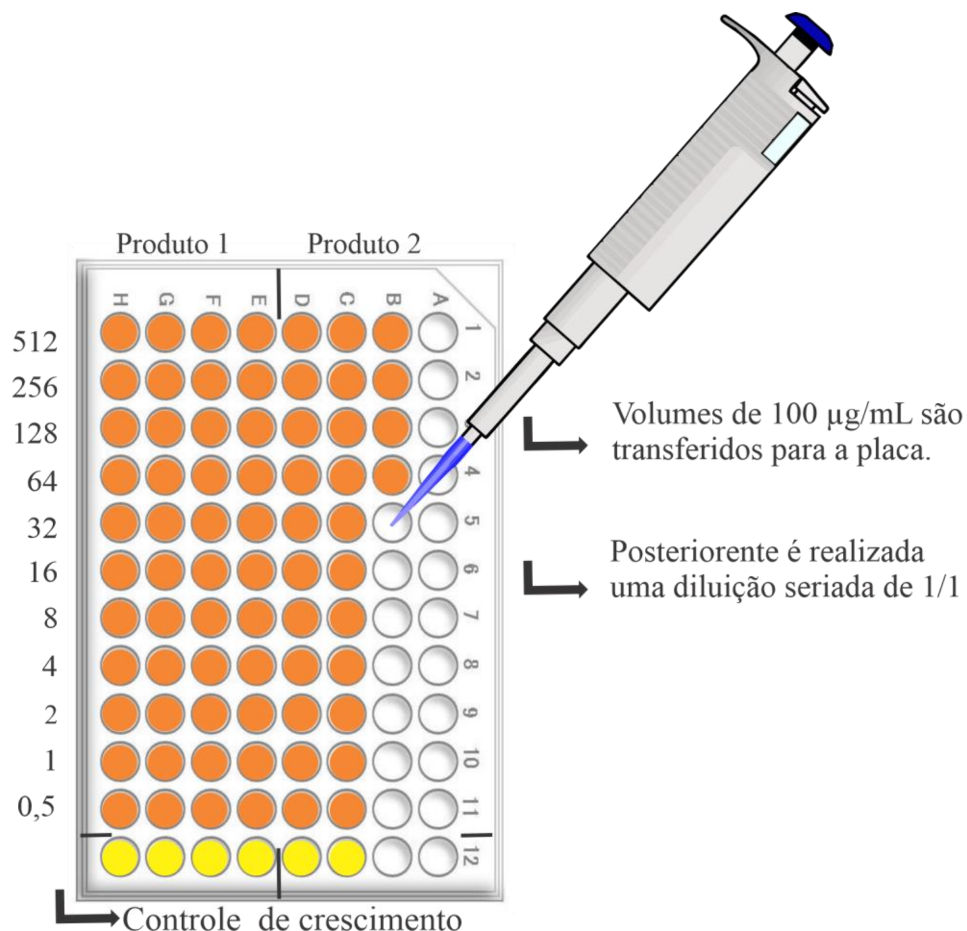
Em estudo realizado por de Bona *et al*. (2014), as metodologias supracitadas foram testadas e comparadas, obtendo resultados melhores para o método de microdiluição em comparação com disco-difusão. De acordo com o autor sugere que a difusão do produto pelo meio pode ocorrer de forma incompleta, principalmente em compostos menos polares, que se difundem mais lentamente no meio de cultura, evidenciando assim uma limitação da técnica. Dessa forma, para a realização do presente trabalho, foi escolhido o método de microdiluição em caldo (Figura 1), devido a sua ampla aceitação por parte de pesquisadores em todo o mundo, e devido a possibilidade de realizar a leitura dos resultados em aparelho de espectrofotometria – ELISA, de modo a obter a curva de inibição dos produtos testados.

Foi realizado o método de microdiluição em caldo utilizando placas de 96 poços, conforme descrito por (JAVADPOUR *et al.*, 1996). Para os testes quantitativos, as placas

foram levadas para leitura em aparelho de espectrofotometria de ELISA (Termoplate®), com comprimento de onda de 630 nm (MORAIS-BRAGA *et al.*, 2016). Os resultados forneceram a concentração inibitória mínima (CIM) dos produtos testados, bem como a IC_{50}. Os testes foram realizados em quadruplicata.

Para verificar se a atividade apresentada foi fungicida ou fungistática, foi realizada a metodologia da Concentração Fungicida Mínima (CFM), uma técnica simples e rápida que verifica se a concentração inibitória mínima (CIM) foi capaz de afetar a viabilidade da célula fúngica. No caso de as células permanecerem viáveis após o tratamento com o produto, então as colônias serão capazes de crescer na placa (ERNST *et al.*, 2000).

Figura 3: Teste de microdiluição em caldo.



Fonte: Autor, 2021.

Para verificar se as lectinas podem modular a ação do fluconazol, um dos antifúngicos mais utilizados na terapia contra fungos, foi realizada a metodologia de Coutinho *et al.*, (2008), que consiste em uma técnica de microdiluição em caldo, utilizando concentrações subinibitória do produto juntamente com o antibiótico. A terapia combinada é o tratamento mais comumente recomendado contra infecções microbianas em unidades de terapia intensiva, uma vez que nem todos os patógenos são sensíveis a monoterapia (JOUNG *et al.*, 2016). O uso de produtos naturais e antibióticos sintéticos combinados tem aumentado para o controle estratégico de micro-organismos resistentes. Essa técnica tem sido usada com eficiência, e foi capaz de relevar atividade sinérgica de lectinas com antibacterianos, como ampicilina, carbanicilina, cefotaxima, cefalexina, cefuroxima (DA SILVA *et al.*, 2019), gentamicina (SILVA *et al.*, 2019) e norfloxacina (SEPTAMA; PANICHAYUPAKARANANT, 2016). Já com antifúngicos, a lectina Apul, de *Alpinia purpurata*, foi capaz de modular a atividade do fluconazol (FERREIRA *et al.*, 2018), provando a eficiência dessa metodologia.

Para verificar a ação do produto natural sobre um dos fatores de virulência fúngica, a formação de hifas, serão montadas câmaras úmidas estéreis, nas quais serão inoculadas as espécies fúngicas em contato com as lectinas e analisadas após um período de incubação de 24 h (37 ºC) (MENDES, 2011; SIDRIM; ROCHA, 2010). O método foi escolhido por se adequar aos micro-organismos *Candida albicans* e *Candida tropicalis*, que mediante as condições adequadas (depleção e presença de soro), são capazes de formar hifas verdadeiras (SHARMA, J. *et al.*, 2019). Através deste teste é possível obter resultados gráficos da transição morfológica dessas linhagens, bem como o efeito dos produtos sobre a morfologia.

1.3. Estrututa da dissertação

A dissertação apresenta quatro capítulos, onde serão abordadas as características das lectinas de modo geral, como afinidade a carboidratos, grupo do qual provem a lectina e as bioatividades apresentadas por elas, bem como os mecanismos de ação propostos na literatura e pelo autor do presente trabalho.

A fundamentação teórica, no capítulo I, abordará brevemente as atividades desempenhadas por lectinas, seja de origem animal ou vegetal, e suas afinidades. Também apresentada a caracterização e patogenia de fungos do gênero *Candida*, como *Candida albicans* e *Candida tropicalis*, explorando seus mecanismos de resistência e diversos fatores

de virulência, tornando evidente a urgência de pesquisas com novos produtos antifúngicos que mitiguem o avanço da candidíase.

O artigo de revisão no capítulo II, que foi submetido a revista *International Journal of Biological Macromolecules*, aborda de forma detalhada sobre os mecanismos de ação das atividades antimicrobianas de lectinas, com foco em lectinas com atividade antibacteriana, antifúngica e antiprotozoária. Os resultados são expressos na forma de relatos de literatura, obtidos através do banco de dados do pubmed, com uma janela periódica de 20 anos. Em sequência, as lectinas com atividade antibiofilme são relatadas, demonstrando a importância dessas proteínas em inibição fatores de virulência sem afetar a viabilidade celular, dificultando assim a formação de resistência a antimicrobianos. Outras atividades menos relatadas, mas igualmente importantes, são as capacidades aglutinantes, que podem dificultar a adesão dos micro-organismos a uma superfície, a modulação com antibióticos sintéticos, elevando sua eficácia, e os danos diretos causados a estrutura celular dos micro-organismos, levando a morte deles.

Os capítulos I e II se complementam, onde na fundamentação teórica o foco está nos micro-organismos de interesse para o trabalho, como as espécies do gênero *Candida*, evidenciando seus mecanismos de resistência a fim de revelar possíveis alvos farmacológicos. No capítulo II o foco é nas lectinas, mais precisamente nas múltiplas potencialidades expressas por elas frente a micro-organimos eucariotos e procariotos, evidenciando as formas de interação dessas proteínas de forma a impedir ou controlar o crescimento de agentes infecciosos.

O manuscrito no capítulo III, que foi submetido a revista *Biochimie*, apresenta os dados dos ensaios antimicrobianos e modulação das lectinas ConA e ConM com antibióticos sintéticos, frente as cepas de *C. albicans* e *C. tropicalis*. Nesse capítulo, estão apresentadas todas as etapas concluídas e as discussões pertinentes dos mecanismos de ação e possíveis causas para presença ou ausência de atividade antifúngica para os produtos testados.

Por fim, são apresentadas as conclusões do trabalho no capítulo IV, dando um panorama do material desenvolvido, destacando pontos como fatores a serem considerados no direcionamento de pesquisas futuras, explorando faltas na literatura e formas de saná-las, complementando as contribuições cientificas dos trabalhos apresentados.

CAPÍTULO I: FUNDAMENTAÇÃO TEÓRICA

1.1. Lectinas

Primeiramente descobertas como glicoproteínas, as lectinas se tornaram alvo de pesquisas ao longo do tempo por serem proteínas ligantes de carboidratos, como monossacarídeos e oligossacarídeos. Essas proteínas podem ser encontradas em diferentes organismos, desde micro-organismos a plantas e animais. Apesar de sua ampla distribuição, atualmente são extraídas, principalmente, de plantas (SHARON, 2007; SLIFKIN; DOYLE, 1990).

Devido a capacidade de ligação a carboidratos, os quais estão presentes nas membranas de diversos organismos, as lectinas estão entre as biomoléculas que atuam na defesa contra micro-organismos e sinalização de danos na superfície celular. Estas proteínas apresentam ao menos um domínio não catalítico que age de forma seletiva, reconhecendo e se ligando a carboidratos de forma reversível (LANNOO; VAN DAMME, 2014). De acordo com sua especificidade a carboidratos, as lectinas foram divididas primeiramente em ligantes de galactose, N-acetilglucosamina (GlcNAc), N-acetil-galactosamina (GalNAc), manose, glicose, maltose, L-fucose, e ácido siálico, sendo posteriormente dada uma nova classificação de acordo com suas características e quantidade de domínios ligantes, como merolectinas, hololectinas, quimimerolectinas e superlectinas (HASHIM; JAYAPALAN; LEE, 2017).

As plantas proveem as lectinas mais pesquisadas atualmente, comprovando a heterogeneidade de suas estruturas, principalmente no que diz respeito ao sítio de ligação a carboidratos, o qual é responsável pelas diversas atividades biológicas, tais como antimicrobiano, inseticida, mitogênico, antitumoral e anti-inflamatório (OLIVEIRA, 2018). Isso se evidencia com os resultados de várias pesquisas que revelam que lectinas derivadas de algas e plantas conseguem inibir o crescimento celular e a formação de biofilme por bactérias e leveduras clinicamente relevantes. Além disso, lectinas conhecidas como ConA, extraídas da leguminosa *Canavalia ensiformis*, conseguem agir em etapas iniciais de formação do biofilme por *Streptococcus mutans*, apresentando uma atividade superior de aderência a células-alvo dentre várias outras lectinas de plantas que apresentam especificidade diversa a carboidratos, o que a torna um agente antibiofilme bacteriano (ISLAM *et al.*, 2009; TEIXEIRA *et al.*, 2006; VASCONCELOS *et al.*, 2014).

A semente da leguminosa *C. ensiformis* possui a lectina ConA, sendo a primeira lectina cristalizada pelo pesquisador James B. Sumner, em 1919, através de técnicas de purificação de proteínas (SUMNER, 1919). Para que ocorra a complexação da lectina ao carboidrato, são necessários cátions divalentes em sítio ligante a metal, onde em cada subunidade estão presentes íons Ca^{2+} e Mn^{2+}, responsáveis por sua atividade. Os aminoácidos envolvidos nessas ligações apresentam-se altamente conservados (VAN DAMME *et al.*, 1998).

A interação da lectina com carboidratos presentes na superfície de células bacterianas e fúngicas é o que confere sua ação antimicrobiana, alterando a estrutura das membranas e aumentando sua permeabilidade, provocando inibição do desenvolvimento desses micro-organismos ou até mesmo sua morte (GAIDAMASHVILI; VAN STADEN, 2002). Essa interação ocorre entre porções apolares dos carboidratos com resíduos de aminoácidos aromáticos, como a tirosina e a fenilalanina, sendo reforçadas por interações de Van der Waals entre as hidroxilas presentes nos carboidratos com as cadeias laterais do domínio de reconhecimento dos carboidratos (DRC). Dentre os aminoácidos presentes no DRC de lectinas de leguminosas, estão: Tyr12 Asn14, Leu99, Tyr100, Asp208 e Arg228, onde podem ser encontrados regiões de "loop" responsáveis pela especificidade a monossacarídeos como glicose, manose ou galactose (LORIS *et al.*, 1998).

Fungos, assim como artrópodes, apresentam uma proteção de quitina em sua parede, a qual é constituída de carboidratos como β-(1,4)-poli-N-acetil-D-glucosamina, sendo um dos polímeros mais abundantes do planeta, ficando atrás apenas da celulose. É a ligação de lectinas vegetais a esse carboidrato que permite a ação inibitória de fungos que apresentem quitina em sua parede celular (ZIATABAR *et al.*, 2018).

1.2. Gênero *Candida*

Candida é um tipo de levedura de brotamento que pertence à família *Saccharomycetaceae*, no filo *Ascomycota*. As cepas mais comuns que causam infecções são *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis* e *C. lusitaniae*. Essas leveduras podem causar infecções nosocomiais da corrente sanguínea, e é *C. albicans* a espécie mais frequentemente isolada de infecções humanas (ALI R; SABAH; AHMED, 2018; SHAHABUDIN; AZMI, 2020).

Fungos desse gênero causam candidíase, e embora existam quase 200 espécies de *Candida*, apenas uma pequena quantidade deles é patogênico. A candidíase pode ocorrer de diversas formas conforme o local: Bucal, onde é denominado como sapinho, e que se caracteriza por manchas brancas formadas no lado interno da boca, topo da língua e ao redor dos lábios, causando inflamação; Cutânea, que ocorre em áreas da pele com menos ventilação e baixa umidade, como virilha, e forma manchas vermelhas úmidas; Vaginal, que pode causar coceira e dor na vagina, com secreção espessa e de odor característico, e que causa irritação durante a relação sexual; Profunda, quando os fungos invadem o corpo e se espalham pela corrente sanguínea por todo o seu interior. Esta forma de candidíase ocorre principalmente em pessoas com sistema imunológico criticamente debilitado (QADIR; ASIF, 2019).

As infecções causadas por fungos ocupam lugar entre as enfermidades mais difíceis de tratar nos seres humanos, e estas ocorrem quase que exclusivamente com o micro-organismo na forma de levedura, mas é a capacidade de assumir várias formas, conhecido como dimorfismo, que é decisiva no desenvolvimento de doenças nos seres humanos. Uma característica dos fungos dimórficos é que, na natureza, eles se apresentam na forma hifal, que produz esporos que se dispersão no ambiente, entram no organismo penetrando barreiras e alcançando tecidos distantes através de espaços cheios de ar, como pulmões e seios da face, para finalmente se metamorfosear em levedura (KÖHLER; CASADEVALL; PERFECT, 2015).

Vários fatores podem influenciar a presença ou ausência de doenças associadas a esses micro-organismos. Em resposta ao estresse do ambiente em que estão inseridas, como pH, temperatura e ausência de nitrogênio, as espécies de *Cadida* podem alterar sua forma, indo de levedura em brotamento (blastoconídios, blastosporos), pseudo-hifas (células alongadas com prolongamentos filamentosos), hifas verdadeiras e clamidósporos (ALI R; SABAH; AHMED, 2018; CHEN *et al.*, 2020; SHAHABUDIN; AZMI, 2020).

As espécies de *Candida spp.* geralmente causam doenças em pessoas acometidas por diversos processos patológicos, fisiológicos ou traumáticos, que podem facilitar a sua colonização no organismo do hospedeiro (BAROT *et al.*, 2015). Além disso, a incidência de candidemia e candidíase associada a esses patógenos apresentam significativa morbimortalidade (LOCKHART, 2014). Muitos dos fungos leveduriformes pertencem ao gênero *Candida* e estão presentes na microbiota humana, vivendo de forma comensal. Quando ocorrem mudanças no seu ambiente, como baixa na atividade do sistema

imunológico, algumas dessas espécies de fungo podem se tornar patógenos oportunistas, se proliferando no hospedeiro e causando doenças (DEORUKHKAR; SAINI; MATHEW, 2014).

Esses fungos podem ser encontrados em membranas mucosas de mamíferos, especialmente as do trato gastrointestinal e geniturinário. Geralmente estão presentes na forma leveduriforme, como células unicelulares que se reproduzem por brotamento, apenas esperando uma oportunidade para se proliferar e provocar infecções superficiais e sistêmicas (MICELI; DÍAZ; LEE, 2011).

1.3. *Candida albicans*

Candida albicans faz parte da microbiota humana como uma levedura polimórfica diploide, presente nas superfícies mucosas, trato gastrointestinal, sistema respiratório e geniturinário. Na maior parte do tempo é inofensivo, vivendo de forma comensal. Entretanto, em indivíduos imunocomprometidos, pode transformar-se em um micro-organismo oportunista, causando doenças que podem levar a óbito (KASHEM *et al.*, 2015). É a principal causa de candidíase na maioria dos ambientes clínicos (ARMSTRONG-JAMES *et al.*, 2017). Diversos fatores físicos, químicos e biológicos podem ser decisivos no seu crescimento e patogenicidade, tais como temperatura, pH, danos a membrana, metabólitos e concentrações de íons (CHEN *et al.*, 2020).

Uma de suas principais característica é a capacidade de alterar sua morfologia, passando da forma leveduriforme (dispersiva) para a filamentosa (invasiva). Essa mudança pode ser induzida por sinais do ambiente, tais como: presença de soro de mamífero; temperatura corporal; densidade celular; alterações de pH e estado de inanição (KADOSH; LOPEZ-RIBOT, 2013; PRISTOV; GHANNOUM, 2019). Além disso, *C. albicans* apresenta outros mecanismos de patogenicidade que atuam desde o primeiro contato com o hospedeito até a invasão de seus tecidos, como a produção de adesinas e Saps (CHEN, H. *et al.*, 2020).

Para que *Candida albicans* possa invadir os tecidos do hospedeiro, é necessário realizar processo de adesão, através de proteínas chamadas adesinas, que estão presentes na superfície celular das leveduras. Essas proteínas são mediadores moleculares da adesão, presentes na superficie celular, permitindo a ligação do micro-organismo às celulas do hospedeiro (MARTIN; KAVANAGH; VELASCO-TORRIJOS, 2020). Esse contato inicial do

fungo com a célula do hospedeiro favorece o dimorfismo característico da espécie de *Candida,* provocando um crescimento direcionado conhecido como tigmotropismo. Nesse momento, outro grupo de proteínas de superfície são expressas, chamadas invasinas, que vão promover mecanismos de adsorção do fungo por meio de endocitose induzida. A partir desse ponto, a fixação da levedura de *C. albicans* em superficie biótica e a plasticidade fenotípica favorece a formação de biofilme, um acúmulo organizado de células que se sobrepõem camada sob camada. Além disso, o fungo se adapta ao microambiente em resposta ao estresse, flexibilidade metabólica, absorção de diferentes compostos e regulação do pH (MAYER; WILSON; HUBE, 2013).

1.4. *Candida tropicalis*

Candida tropicalis é classificada entre a segunda e a terceira espécie de *Candida* não-*albicans* mais frequentemente isolada em pacientes com infecção por *Candida* (SARDI *et al*., 2013). Segundo dados epidemiológicos, *C. tropicalis* esta relacionada a infecções do trato urinário e até mesmo malignidade hematológica (NEGRI *et al.*, 2012). Apesar de haver tratamentos eficazes, a fungemia causada por essa espécie apresenta altas taxas de mortalidade, sendo frequentemente encontrada em pacientes em unidades de terapia intensiva (SILVA *et al.*, 2012).

Diversos fatores de virulência podem ser identificados para *C. tropicalis*, por exemplo: desenvolvimento de hifas, produção de proteinases, fosfolipases, lipases e atividade hemolítica (JIANG *et al.*, 2016), adesão a células epiteliais e endoteliais bucais e troca fenotípica, além de ser um forte produtor de biofilme (PRISTOV; GHANNOUM, 2019). Esse micro-organismo também é considerado um forte produtor de biofilme, apresentando alta aderência às células epiteliais e endoteliais (ZUZA-ALVES; SILVA-ROCHA; CHAVES, 2017). Sua capacidade de alterar sua morfologia, conhecida como dimorfismo (mudança da forma leveduriforme para pseudohifa e hifa) figura como um dos seus principais fatores de virulência. Além disso, a expressão do gene EFG1 (*Enhanced Filamentous Growth*) tem papel em diversos processos como a formação de biofilme e regulação de transições morfológicas (MANCERA *et al.*, 2015).

Candida tropicalis ainda é capaz de formar tubos germinativos, embora alguns processos ligados ao seu dimorfismo permaneçam incertos (CHEN *et al.*, 2014). Apesar de

sua relevância como patógeno humano, o seu potencial enquanto agente infeccioso ainda é pouco conhecido (MORALEZ *et al.*, 2014).

1.5. Resistência

Dentre as opções terapêuticas disponíveis, os azoles (fluconazol, voriconazol, posaconazol e isavuconazole) e os polienos (anfotericina B) atuam modificando estruturas na membrana da célula, como ácidos nucleicos e proteínas, causando interferência no processo de síntese de DNA, RNA e proteínas fúngicas, enquanto as equinocandinas (caspofungina, anidulafungina e micafungina) atuam diretamente na parede celular (PERLIN; SHOR; ZHAO, 2015).

A resistência a compostos azólicos é mais comum, com relatos para várias espécies de *Candida*, como *C. tropicalis* e *C. albicans*. Somente para o fluconazol, uma taxa de resistência entre 10% e 25% tem sido observada, além de apresentarem resistência cruzada a outros compostos dessa classe de antifúngicos (MORAIS-BRAGA, 2016). Além disso, *C. tropicalis* revela grande resistência a compostos azólicos, com alta resistência especificamente a fluconazol (ZUZA-ALVES; SILVA-ROCHA; CHAVES, 2017). Já para as equinocandinas, a taxa de resistência ainda é baixa (< 2-3%), embora já tenham sido relatados o aumento de incidência em algumas espécies de *Candida* (PERLIN; SHOR; ZHAO, 2015).

A exposição a agentes antifúngicos resulta no desenvolvimento da resistência por parte desses micro-organismos, mas não ocorre da mesma forma para todos. Para *C. albicans*, o uso antifúngico a longo prazo, seguido de infecções recorrentes, como candidíase mucocutânea crônica ou candidíase orofaríngea recorrente em pacientes com infecção não controlada pelo vírus da imunodeficiência humana, aumentam as chances de uma resistência adquirida. Para várias espécies de *Candida* não-*albicans*, como a *Candida krusei*, existe uma menor susceptibilidade a diversas classes de antifúngicos, enquanto outras, como *Candida glabrata*, desenvolvem resistência logo após a exposição a agentes antifúngicos (ARENDRUP; PATTERSON, 2017).

1.6. Virulência

Os fatores de virulência são importantes para o estabelecimento do micro-organismo em ambientes hostis. A expressão de enzimas hidrolíticas extracelulares, como as fosfolipases, proteinases e hemolisinas, facilitam sua adesão e fuga do sistema imunológico, provocando danos ao tecido do hospedeiro. Ambas as espécies de *C. albicans* e não-*albicans*, produzem essas biomoléculas (CHIN *et al.*, 2013; MATTEI *et al.*, 2013). Quando presentes em um biofilme, as células fúngicas apresentam fenótipo alterado, conferindo uma resistência extraordinária a muitos antifúngicos, dificultando a erradicação do processo infeccioso (NAVES *et al.*, 2013).

Um dos principais fatores de virulência nas espécies de *Candida* inclui uma grande família de proteases aspárticas segregadas (Saps), que auxiliam na aquisição de nutrientes importantes para o crescimento do fungo, além de inativar componentes do sistema complemento. As Saps também permitem a fuga e sobrevivência das células fúngicas após interação com fagócitos do sistema imune (BOCHENSKA *et al.*, 2016; KOZIK *et al.*, 2015). A família do gene Sap em *C. albicans* contém pelo menos 10 isoenzimas que vão de Sap1 a Sap10. A expressão de Sap1-3 é predominante em candidíase na mucosa e pele, enquanto Sap4-6 pode ser significativo em infecções sistêmicas (ALI R; SABAH; AHMED, 2018). Outro grande fator é a produção fosfolipases, que participam do processo de penetração na célula do hospedeiro ao catalisar a hidrólise de fosfolipídios de membrana (SUDBERY, 2011).

A variação fenotípica, conhecida como *switching*, é reversível e é favorecida pela mudança no nicho do fungo, provocando mudanças no comportamento celular como aparência da colônia, metabolismo, atributos bioquímicos e moleculares que favorecem sua adaptação, se tornando cada vez mais virulenta durante a infecção (PRISTOV; GHANNOUM, 2019).

Os patógenos fúngicos oportunistas, como *C. albicans*, apresentam glicoproteínas extracelulares que mediam a adesão a superfícies bióticas e abióticas e a agregação célula-célula. Essas proteínas são críticas para o comensalismo e virulência do fungo, e suas atividades incluem a fixação e invasão de células endoteliais e epiteliais, morfogênese e formação de biofilmes no tecido hospedeiro e em cateters de uso hospitalar (HO *et al.*, 2019).

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CAPÍTULO II - A review on the antimicrobial properties of lectins

Link: https://www.elsevier.com/journals/international-journal-of-biological-macromolecules/0141-8130/guide-for-authors

Victor Juno Alencar Fonseca[1]; Ana Lays Braga[1]; Jaime Ribeiro Filho[2]; Claudener Souza Teixeira[3]; Gabriel C. A. da Hora[4]; Maria Flaviana Bezerra Morais-Braga[1,*]

[1] Laboratório de Micologia Aplicada do Cariri – LMAC, Universidade Regional do Cariri – URCA, Crato, CE Brazil[1,2,4]; victorjunobiomedico@gmail.com[1]; Ana.lays@hotmail.com[2]; flavianamoraisb@yahoo.com.br [4]

[2] Laboratório de Investigação em Genética e Hematologia Translacional, Instituto Gonçalo Moniz (IGM), Fundação Oswaldo Cruz (Fiocruz), Salvador[3]; jaimeribeirofilho@gmail.com[3]

[3] Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal do Cariri, Crato, CE, Brazil. claudener@gmail.com.

[4] Department of Chemistry, University of Utah, Salt Lake City, Utah 84112-0850, USA. gabriel.costa.hora@gmail.com

***Autor for correspondence:** Universidade Regional do Cariri—URCA, Centro de Ciências Biológicas e da Saúde—CCBS, Departamento de Ciências Biológicas - DCBio, Laboratório de Micologia Aplicada do Cariri - LMAC. Av. Cel. Antonio Luiz, 1161, Pimenta, CEP: 63105-000 Crato, CE, Brasil.
flavianamoraisb@yahoo.com.br

ABSTRACT

Lectins are biologically versatile biomolecules with remarkable antimicrobial effects, notably against bacteria, fungi and protozoa, in addition to modulating host immunity. For this, the lectins bind to carbohydrates on the surface of the pathogen, which can cause damage to the cell wall and prevent the attachment of microorganisms to host cells. Thus, this study intends to review the biological activities of lectins, with an emphasis on antimicrobial activity. Lectins of plant stood out for its antimicrobial effects, demonstrating that they act against a variety of strains, where *in vitro* were able to inhibit their development and affect their morphology. *In vivo*, they modulated host immunity, signaling and activating defense cells. Some of these lectins were capable to modulate the action of antibiotics, indicating their

potential to minimize the antibiotic resistance. The results suggest that lectins have antimicrobial activity with potential to be used in drug development.

Keywords: Lectins, antibacterial, antifungal, antiprotozoal, review.

1. Introduction

Lectins are proteins with a variety of biological functions, among which antimicrobial defense has been highlighted in the literature. These naturally occurring molecules are commonly secreted in response to invasive agents such as bacteria [1], binding to carbohydrates on the surface of both pathogens and leukocytes, which results in direct and indirect antimicrobial effects, respectively [2]. Since these proteins are produced by a great variety of living organisms, they are often categorized according to their natural source as animal lectins, plant lectins, fungal lectins, or bacterial lectins. Alternatively, lectins are classified according to their binding affinity to specific carbohydrates (ex. Galactose-binding lectin, fucose-binding lectin, N-glycan-binding lectin) [3]. In this context, lectin-carbohydrate binding is governed by attractive forces, which include hydrogen bonds, hydrophobic and electrostatic interactions [4]. In addition, the binding of some lectins to their specific carbohydrates requires the participation of certain ions, such as Ca^{2+} and Mg^{2+} [5].

Lectins also play important roles in the innate immunity of multicellular organisms, which can be due to their similarity with the epitopes of some glycans found on the cell surface of pathogens. Such similarity favors the activation of signaling pathways in a variety of cells, through the activation of surface receptors [6]. Especially in animals and plants, lectin-triggered signaling contributes to cell immobilization, as well as to cytotoxic mechanisms associated with growth inhibition and cell death [7,8].

Studies have identified a series of mechanisms through which lectins combat infectious agents. Research conducted by Zhang et al. [9] demonstrated that a lectin extracted from the fish species *Misgurnus anguillicaudatus* had significant agglutinating activity against Gram-negative bacteria, possibly due to its interaction with lipopolysaccharides (LPS) present in the outer membrane of these microorganisms. This mechanism seems to be common to most lectins and possibly explains how these molecules inhibit the formation of biofilms and bacterial aggregates [10]. On the other hand, evidence has indicated that the

antifungal effects of lectins result from the interaction with chitin (a biopolymer found on the surface of these microorganisms), which may in incomplete development of spores [11]. Additionally, lectins have immunomodulatory activities, regulating the polarization of CD4+ lymphocytes, including Th1, Th2, and Th17 [12] and thus stimulating the production of cytokines such as IFN- γ, IL-6, TNF-α, IL-4, IL-2, and IL-10 [13], which contributes to the elimination of a variety of microbes and parasites.

Thus, this study intends to review the biological activities of lectins, with an emphasis on the advances and perspectives of targeted antimicrobial research.

2. Methodology

PubMed was consulted as a scientific literature database, and the general information did not specify a periodic window. As for applications and researches related to lectins, the period established was from 2000 to 2021. The following keywords were used in the search engines: "Lectin Antiprotozoal Activity", "Lectin Antifungal Activity", "Lectin Antibacterial Activity", totaling 1770 articles, of which, through a detailed analysis, 88 articles were selected, which was not included review articles, nor those that addressed lectins, but did not bring results regarding the intended biological activities. Cellular organisms were also prioritized, excluding viruses from the inclusion criteria. Both *in vivo* and *in vitro* studies were included, with or without description of mechanisms of action.

3. Antimicrobial activities of lectins

A summary of the antibacterial, antifungal, and antiparasitic activities of lectins is shown in Tables 1, 2, and 3, respectively. A total of 27 articles investigated the effects of lections on bacterial and fungal growth. In addition, the present search demonstrated the ability of lections to modulate the activity of antimicrobial drugs (6 articles) and inhibit biofilm formation (12 articles), which significantly contributes to their antimicrobial effects, especially against bacteria.

With regard to the mechanisms underlying the antimicrobial activities of lectins, the search carried out in the present study found that lectins are capable of causing direct damage to bacterial, fungal, and protozoan cells (5 articles), in addition to acting as agglutinating

agents (8 articles) and inhibiting spore germination (5 articles). Here, we discuss both the antimicrobial effects and potential mechanisms of actions of lectins of both plant and animal origin, emphasizing their potential use in antimicrobial drug development.

At the end of each session, figures with the suggested bioactivity performed by the lectins were added. In section 3.1, Figure 5 shows the mechanisms of agglutination, anti-adherent, anti-biofilm and drug synergism. In section 3.2, Figure 7 shows how the lectin prevents the adhesion of spores to a surface, as well as inhibiting the entry of extracellular carbohydrate into the fungal cell, interfering with spore germination. In section 3.3, Figure 8 shows the immunomodulatory role of lectins against protozoa.

3.1. Lectins with antibacterial activity

3.1.1. Lectins with immunomodulatory activity

Studies have demonstrated that lectins have a remarkable immunomodulatory activity, and therefore can stimulate the defense system against invading agents. In this context, C-type lectins are present in several organisms, where they play important roles in innate immunity. Accordingly, previous research demonstrated that OppCTL, a Ca^{2+}-dependent lectin extracted from the fish *Oplegnathus punctatus*, has its expression significantly increased (especially in the liver), during infectious processes. This lectin was found to present agglutinating activity in the presence of bacterial cell wall components such as lipopolysaccharide (LPS) and peptidoglycan (PGN), corroborating its increased production in response to invasive pathogens [2]. Similar findings were reported for PcLec6, another Ca^{2+}-dependent lectin extracted from the crustacean *Procambarus clarkii*. In addition to presenting high affinity to LPS and PGN, this lectin showed agglutinating activity in the presence of *Staphylococcus aureus* and *Vibrio alginolyticus*. Furthermore, the inoculation of the lectin into the crustacean facilitated the clearance of the bacterium *V. alginolyticus*, indicating a possible immune role in the crustacean [1].

Li et al. [14], investigating the antibacterial activity of LvCTL3, a recombinant lectin extracted from *Litopenaeus vannamei*, against Gram-negative and Gram-positive bacteria, showed agglutinating activity against *V. alginolyticus, Vibrio parahaemolyticus*, and *Bacillus*

subtilis in the presence of Ca^{2+}. In addition, this lectin was capable of reducing the mortality of shrimps infected with *V. parahaemolyticus* when compared to the vehicle-treated group. Importantly, LvCTL3 was detected in all shrimp tissues, especially in hemocytes and gills, suggesting that this lectin may be involved in pathogen recognition and immune response. These findings are corroborated by the study of Sun et al. [15], who demonstrated that Fc-hsL, a lectin extracted from the shrimp *Fenneropenaeus chinensis*, had agglutinating activity and inhibited the growth of both Gram-positive (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Micrococcus luteus*, and S. *aureus)* and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae)* bacteria. Importantly, during infection, FC-hsL was also detected in the hepatopancreas, one of the most important organs of the humoral response of shrimps.

Lectins with immunomodulatory activity participate in the immune response of invertebrate organisms. Studies have shown that they can opsonize invasive bacteria or foreign particles, increasing their phagocytosis. The expression of HSL lectins in the sea cucumber *Holothuria scabra*, was found to increase in response to artificial bacterial infection, reducing the number of colony-forming units from the first day of testing, reaching complete inhibition at the fifth day of inoculation. This lectin also inhibited bacterial growth *in vitro* in comparison with the control ampicillin [16].

A lectin extracted from the mussel *Mytilus trossulus* (MTL), which agglutinated in the presence of *Vibrio proteolyticus*, had its activity inhibited in the presence of D-galactose. Following the challenge with *V. proteolyticus*, the expression of MTL was significantly upregulated, suggesting that this lectin may also be involved in the immune response of mollusks to infections by pathogenic microorganisms in aquatic environments [17]. Accordingly, CGL, another mussel lectin extracted from *Crenomytilus grayanus* (Figure 1), also inhibited the growth of Gram-positive and Gram-negative bacteria, strongly binding to *E. coli*, which was inhibited in the presence of D-galactose, indicating that mussel lectins may have similar antibacterial properties [18]. According to He et al. [19], PmCTL-1, a C-type lectin extracted from the oyster *Pinctada fucata* showed strong activity against Gram-positive bacteria such as *M. luteus, S. aureus* and *B. subtilis*.

Some animals produce venom as a defense mechanism, such as snakes. In relation to these animals, lectins with antibacterial activity can be isolated from their venom. About snake lectins, the fraction P8-I, extracted from the venom of *Bothriopsis oligolepis* showed

antibacterial activity against *Salmonella choleraesuis*, *M. luteus*, and *S. aureus*, the latter being the most sensitive to the protein, which had inhibitory concentrations comparable to those obtained with the positive control ampicillin [20]. |On the other hand, lectin BIL extracted from the snake venom *Bothrops leucurus* showed antibacterial activity against *S. aureus, Enterococcus faecalis*, and *B. subtilis.* However, a bactericidal effect was achieved only at high concentrations [21].

Two lectin fractions extracted from the skin of the wild frog *Bufo arenarum* (LBP1 and LBP2) were tested *in vitro* against microorganisms naturally present in the skin of the animal. The results showed that both fractions likewise suppressed the growth of bacteria in a solid medium. Additionally, when the fractions were removed, the bacteria grew back, indicating that the lectins present in the fraction present a bacteriostatic activity [22].

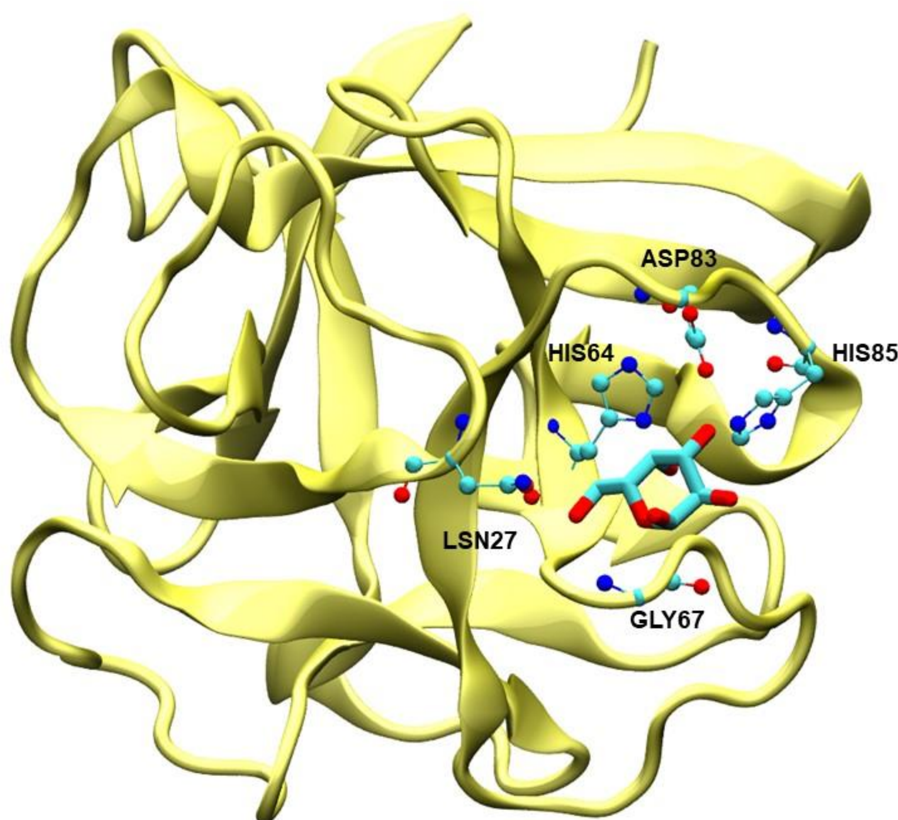

Figure 1: Image of the *Crenomytilus grayanus* lectin (cartoon) crystal structure forming a complex with D-galactose (stick). The residues closest to the galactose are shown in ball-and-stick representation. Oxygens are colored in red, carbons in cyan, and nitrogen atoms in blue. PDB ID: 5F8W [23].

A lectin extracted from the fish *Misgurnus anguillicaudatus* (MaCTL) showed strong agglutinating activity in human and hare erythrocytes. However, this activity was only observed in the presence of Ca^{2+}, corroborating the evidence that C-type lectins have a Ca^{2+}-dependent activity. In this context, Zhang et al. [9] demonstrated that this lectin induced agglutination in the presence of the six different bacteria (*B. subtilis, M. luteus, S. aureus, A. hydrophila, E. coli,* and *Vibrio anguillarum*). However, this effect was achieved exclusively in the presence of Ca^{2+}. The lectin also inhibited the growth of 4 of the 6 bacteria *(A. hydrophila, E. coli, V. anguillarum*, and *S. aureus*), demonstrating significant activity against Gram-negative strains, which can be explained by the strong affinity of lectins to LPS. This type of bonding can cause damage to the membrane, leading to the generation of pores, which results in cell death [9].

While some lectins specifically recognize a particular type of sugar, others can recognize monosaccharides, disaccharides, oligosaccharides, and polysaccharides, although the affinity for each type of sugar may vary significantly. This is the case of PeRoL, a lectin extracted from the roots of *Portulaca elatior*, which can bind to sugars such as trehalose mannose, glucose, galactose and N-acetylglycosamine. Importantly, antimicrobial studies demonstrated its bacteriostatic activity against *E. faecalis, P. aeruginosa,* and *S. aureus.* With regard to the mechanism of action, bacteriostatic agents usually inhibit one of the following vital processes: cell wall synthesis, membrane functions, and proteins and nucleic acid synthesis [24].

Another non-selective lectin can be extracted from the serum of the larvae of the aquatic insect *Stenopsyche kodaikanalensis.* Studies demonstrated that this lectin agglutinated in the presence of lactose, galactose, laminarin, and fetuin. With regard to the biological activity, antimicrobial tests demonstrated its agglutinating and bacteriolytic activities against activity *B. subtilis* and *Bacillus flexus*, respectively [25]. The ability of lectins to agglutinate bacteria results in microbial mass concentration, which may require less biocide to achieve the effects on the cells [26].

A lectin extracted from the mycelium of *Aspergillus gorakhpurensis* was found to specifically bind to mucin and other complex carbohydrates such as N-acetyl-D-galactosamine, chondroitin-6-sulfate, fetine, N-glycolil neuramine, D-mannitol, and dihydrate D-trehalose. This lectin was significantly effective against Gram-positive bacteria such as

Bacillus cereus, with an inhibition zone of 20 ± 0.25 mm at a lower concentration than that obtained with the control drug ampicillin [27].

ADL, a lectin extracted from the skin of salamander *Andrias davidianus,* showed antibacterial activity against *E. coli, Enterobacter aerogenes, S. aureus, B. subtilis,* and *Shewanella sp*. The mechanism of action by which this protein acts seems to be related to the inhibition of cell respiration. In fact, ADL inhibited glucose degradation pathways in different bacteria, including TCAC in *E. coli* and S. *aureus,* and HMP in *E. aerogenes, Shewanella sp. and B. subtilis*, which interrupts the vital processes of these organisms resulting in cell death [28].

3.1.2. Potentiation of antibiotic action

The species *Dioclea violacea* synthesizes a lectin with some specificity to mannose and glucose. This lectin showed no relevant antibacterial activity against multidrug-resistant bacteria such as *S. aureus, E. coli, and P. aeruginosa.* However, it was capable of potentiating the antibacterial effect of gentamicin, reducing the minimum inhibitory concentration (MIC) of the antibiotic against *S. aureus* and *E. coli* by 80.1% and 60.3%, respectively. The authors proposed that the lectin acts by delivering the drug to target cells through the recognition of carbohydrates in the membrane, which leads to the release of gentamicin, facilitating the entry of the antibiotic into the bacterial cytoplasm [29]. Similar findings were observed in a study conducted with a lectin obtained from *Canavalia ensiformis* (ConA), which had no significant antibacterial effect, but potentiated the activity of gentamicin against *S. aureus* and *E. coli* [30].

The antibiotic-potentiating activity of lectins has been consistently demonstrated. In this context, *Vatairea macrocarpa* lectin (VML) was able to reduce the MIC of norfloxacin, penicillin, and gentamicin against *S. aureus* [31]; Artocarpin, isolated from the heartwood of *Artocarpus heterophyllus,* showed moderate antibacterial activity against methicillin-resistant *S. aureus* (MRSA) *and E. coli* and weak antibacterial activity against *P. aeruginosa.* However, the lectin increased the antibacterial activities of antibiotics, producing synergistic effects when associated with norfloxacin against MRSA, *P. aeruginosa* and *E. coli,* as well as with tetracycline against MRSA and *P. aeruginosa* and with ampicillin against MRSA. These

findings indicate that lectins may be useful in the management of antibacterial resistance, a major current public health problem [32].

According to Silva et al. [33], bacteria become resistant to antibiotics through multiple mechanisms, including structural changes in the cell wall, efflux pump expression, ribosome mutations, and antibiotic inactivation by enzymatic activity. A study by these authors demonstrated that *Parkia platycephala* lectin (Figure 2) (PPL) modulated the antibacterial activity of gentamicin, reducing the MIC of the multiple drug resistance (MDR) bacteria *S. aureus* and *E. coli* by 61% and 36.9%, respectively. However, PPL was not able to modulate the activity of gentamicin against *P. aeruginosa*, which may be due to differences in the polysaccharides that form the extracellular wall of *P. aeruginosa.*

Community and hospital infections caused by β-lactamase-producing bacteria have increased worldwide. Hydrolysis of the β-lactam ring represents the most common mechanism of resistance of Gram-negative bacteria against third-generation cephalosporins. *Punica granatum* sarcotesta lectin (PgTeL) showed antibacterial activity against clinical and MDR isolates of *E. coli* expressing β-lactamases, exerting harmful effects on growth, cell structure and biofilm formation. In addition, the lectin showed synergistic effects when associated with the antibiotics such as ampicillin, carbenicillin, cefotaxime, cephalexin, and cefuroxime [34].

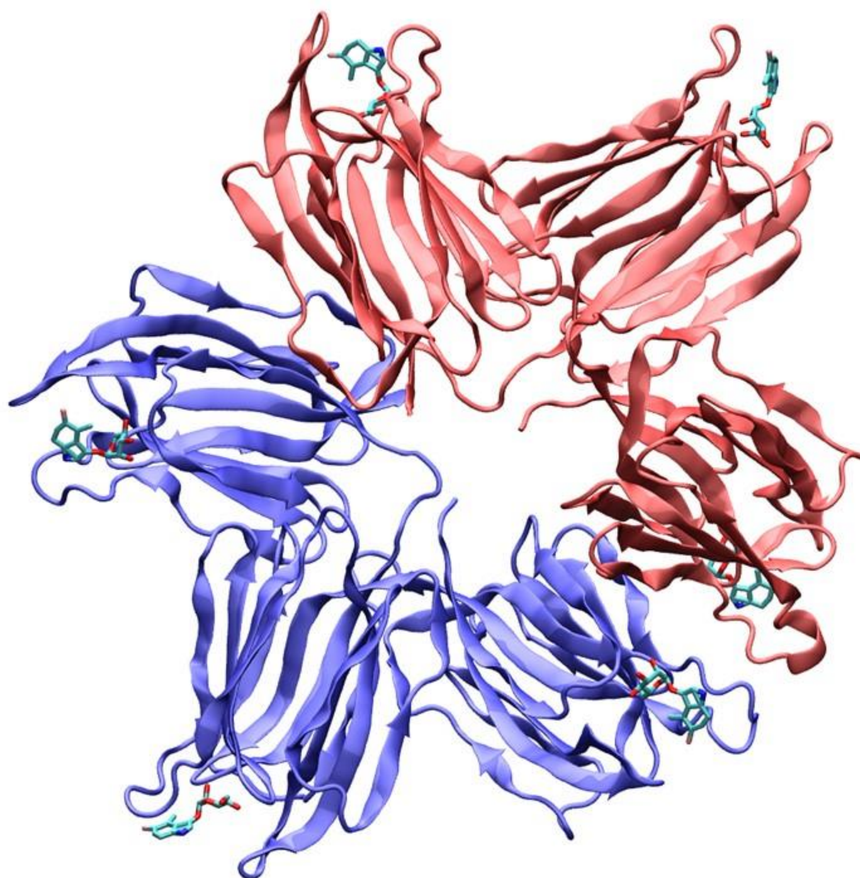

Figure 2: Structural representation of the *Parkia platycephala* seed lectin showing the quaternary arrangement of the domain with chains A (blue) and B (red). The lectin is complexed with 5-Bromo-4-chloro-3-indolyl-a-D-mannose (in cyan stick) (PDB ID: 1ZGS) [35].

3.1.3. Action of lectins by binding to the bacterial surface

Lectins can also prevent microorganisms from sticking to a surface, which can affect biofilm formation, since adhesion is the first step in this process [36]. The lectin extracted from *Bauhinia variegata* (BVL-I) seeds, as well as its recombinant form (rBVL-1), was found to inhibit the adhesion of oral bacteria such as *Streptococcus mutans* and *Streptococcus sanguinis* in about 86%. While the mechanisms underlying the mechanism of action by rBVL-I have not yet been elucidated, evidence suggests that BVL-I binds to carbohydrates present on the bacterial surface, occupying the binding site for the adhesion of microorganisms to the oral cavity [37].

Biofilm formation is an important mechanism of bacterial resistance, representing a significant cause of persistent and recurrent bacterial infections. Like aggregation induction, biofilm formation has been associated with increased mutation frequency and lower susceptibility to antibiotics. In a study conducted by da Silva et al. [38], the lectin PgTeL, showed strong antibacterial activity against non-resistant and resistant isolates (MRSA) of *S. aureus*, causing structural damage in both strains. Curiously, while biofilm formation by the non-resistant isolate was inhibited by more than 50% at a concentration of 200 μg/mL, this phenomenon was stimulated at lower concentrations. The author suggested that this stimulus may be due to a defense mechanism of the bacterium after coming into contact with the lectin at concentrations unable to affect its growth. However, the resistant strain inhibited biofilm formation at all tested concentrations.

The binding affinity of lectins to carbohydrates, make them attractive as inhibitors of the adhesion of bacteria to specific surfaces by competitiveness, as reported for LecA, a lectin produced by *P. aeruginosa* as a virulence factor associated with bacterial adhesion and biofilm formation. In research conducted by Palmioli et al. [39] it was possible to inhibit the formation of *P. aeruginosa* biofilm by using a galactose-based dendrimer (Gal18) at a concentration of 250 μM. The legume *Canavalia ensiformis* produces a specific lectin of mannose (Figure 3) (ConA) with remarkable antibiofilm activity against enterohemorrhagic *E. coli* (EHEC) and *Listeria monocytogenes*. It is remarkable that ConA was also able to reduce EHEC's mobility by 37% [40].

Preetham et al. [41] demonstrated that a lectin extracted from the shrimp *Penaeus semisulcatus* inhibited biofilm formation by *A. hydrophila, V. parahaemolyticus, S. aureus,* and *E. faecalis*. A lectin extracted from the marine sponge *Aplysina fulva* (AFL) was effective in inhibiting the emission of the biofilm by *S. aureus*, *Staphylococcus epidermidis*, and *E. coli*, but without affecting its plankton forms [42]. Research conducted by Marques et al. [43] showed that lectin isolated from the marine sponge *Chondrilla caribensis* reduced the total biomass of biofilms by the same strains without affecting the viability of these bacteria attached to the biofilm. In addition, this activity was completely inhibited in the presence of alpha-lactose, suggesting that the carbohydrate recognition domain (CRD) is involved in the antibiofilm activity.

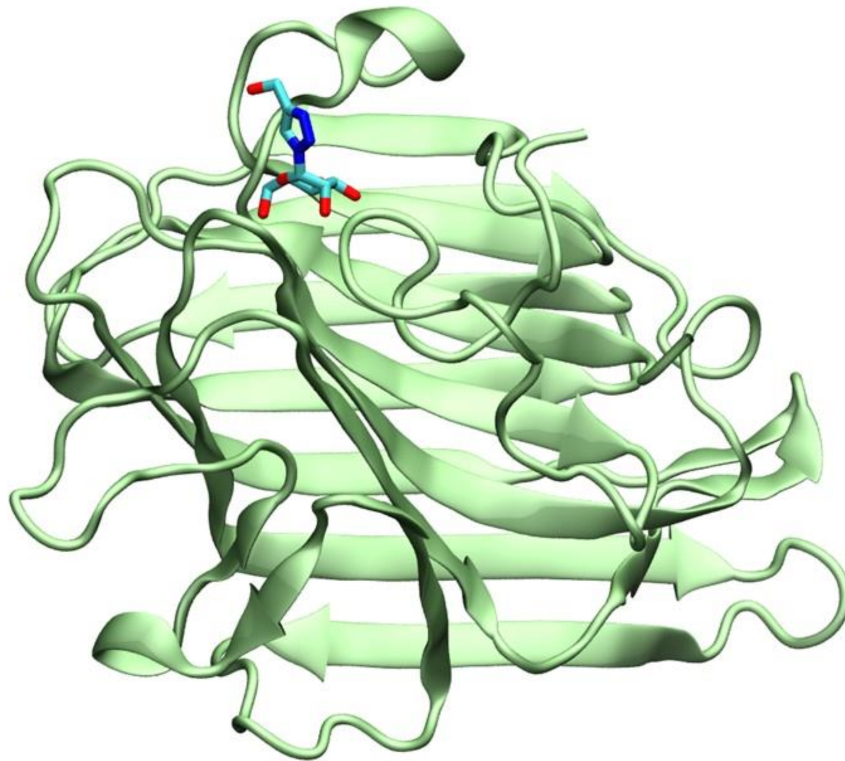

Figure 3: Structural representation of the (cartoon) complex Concanavalin-A (ConA) from *Canavalia ensiformis* with the 4-(hydroxymethyl)-1-(alpha-D-mannopyranosyl)-1H-1,2,3-triazole (a synthetic derivative of high-mannose – represented in stick). PDB ID: 4PF5 [44].

The search for substances capable of affecting biofilm formation has attracted the interest of researchers worldwide, as evidence indicates that antibiofilm therapy could minimize the emergence of microbial resistance. In this context, CasuL, a lectin extracted from the leaves of *Calliandra surinamensis* showed variable inhibitory effects on the growth of non-resistant *S. aureus,* MRSA, *E. coli,* and *Staphylococcus saprophyticus*. While *Escherichia coli* growth was not affected by lectin treatment, significant inhibition ($p < 0.05$) was observed for *S. saprophyticcus* and MRSA isolate, although the magnitude of inhibition was below 30%. However, the lectin significantly inhibited biofilm production by all tested bacteria, with an inhibition profile comparable to those obtained with the control drug tetracycline [45].

Still in this context, a lectin extracted A*plysia dactylomela* eggs (ADEL), binding specifically to galacturonic acid, was able to agglutinate in the presence of *S. aureus* and

reduce biofilm biomass by almost 40% without affecting cell viability. The formation of bacterial aggregates can cause a decrease in the number of adherent cells, which may explain why ADEL does not inhibit bacterial growth, but is, instead, effective in inhibiting biofilm production [10]. Studies with *Moringa oleifera* lectin (WSMoL) suggested that the effects of lectins on biofilm formation and bacterial growth can be influenced by the concentration. Thus, while the lectin extracted from the seeds of this plant inhibited biofilm production by *Serratia marcescens* at low concentrations (less than 1.3 $\mu g/ml^{-1)}$ without interfering with bacterial growth, it was found to significantly inhibit bacterial growth at a concentration of 2.6 $\mu g/ml^{-1}$, which is significantly better than the inhibitory concentrations demonstrated by the control drug gentamicin (10.4 and 20.8 $\mu g/ml^{-1}$) [26].

3.1.4. Plant lectins with antibacterial activity

Studies have shown that plants produce lectins as part of their defense mechanism. According to Hasan et al. [46], plants are more prone to microbial infections compared to animals, due to the lack of a well-developed immune system, as well as due to mobility incapacity. So, to ensure the perpetuation of the species, reproductive organs, such as fruit bodies and tubers, are developed to store a series of self-protective molecules against invading microbes. In their research, Hasan and colleagues showed that a lectin extracted from *Solanum tuberosum* (StL-20) had remarkable antibacterial activity against *E. coli, L. monocytogenes, S. enteritidis* and *S. boydii*. In addition, StL-20 was able to inhibit the formation of biofilm by *Pseudomonas aeruginosa* from 5 to 20%, the only bacterium used in this test, as lectin concentration increased from 2.5 to 15 µg/mL, becoming almost constant at 20 µg/mL.

A lectin extracted from the fruiting body *of Sparassis latifolia* showed higher activity against Gram-negative bacteria than against Gram-positive bacteria. The MICs for *resistant E.* coli, *S. aureus* resistant and *P. aeruginosa* were 100, 200 and 50 µg/mL, respectively, which were higher than those of the corresponding normal strains. In addition, it was observed inhibition of the transition from a random spiral to α-helix conformation after interaction with bacterial LPS., indicating that the lectin inhibited the activity in these microorganisms [47].

ApulSL is a lectin extracted from *Apuleia leiocarpa* seeds that specifically binds to N-acetylglycosamine, a chitin monomer. This lectin showed bacteriostatic activity against Gram-positive bacteria such as *B. subtilis, B. cereus, E. faecalis, M. luteus, S. pyogenes,* and *S. aureus,* as well as against Gram-negative bacteria such as *Xanthomonas campestris pv, campestris, Xanthomonas campestris pv. viticola*, *Xanthomonas campestris pv. malvacearum*, *K. pneumoniae*, *E. coli, P. aeruginosa*, and *S. enteritidis*. Since N-acetylglucosamine is present in both Gram-positive and Gram-negative bacteria, its interaction with the lectin may explain its antibacterial activity observed [48].

Lectins extracted from seeds of legumes *Vicia faba, Lens culinaris* (Figure 4) and *Pisum sativum*, which specifically interacts with mannose and glucose, showed antibacterial activity against *S. aureus, S. mutans, P. aeruginosa,* and *K. pneumoniae*, whose mechanisms possibly involves agglutination [49]. Agglutinating capacity was also reported for KRL, a lectin extracted from the rhizome *of Kaempferia rotunda*, which was effective against *B. subtilis, B. cereus, B. megaterium, Sarcina lutea*, *Klebsiella*, *E. coli, Shigella sonnei*, and *Salmonela typhi*. The agglutinating activity of KRL was inhibited in the presence of mannose. Additionally, this lectin also inhibited the growth of most of these bacteria, suggesting that it can recognize molecules on the surface of both Gram-positive and Gram-negative bacteria [50]. These findings were corroborated by the study of Rashel Kabir et al. [50], demonstrating that this lectin was able to agglutinate in the presence of *S. aureus* and *E. coli.* However, no agglutinating activity was reported for *Salmonella enteretidis*.

The *Moringa oleífera* seeds lectin (WSMoL), which has a specific affinity for D (+) – fructose, inhibited the growth of pathogenic bacteria, exhibiting significant activity against *Bacillus sp., Bacillus pumillus, Pseudomonas Stutzeri*, and *Serratia marcescens.* However, bactericidal effects were observed only against *Bacillus sp., B. pumillus, Bacillus megaterium, P. fluorescens*, and *S. marcescens*. In addition, WSMoL altered the membrane permeability of all bacteria tested, including those in which no bactericidal effect was observed [26].

In a study by Ferreira et al. [51], WSMoL showed high activity against *S. aureus*, probably due to the high level of peptide glycan in the cell wall of this bacteria, while *E. coli* was not sensitive to the treatment. On the other hand, a lectin extracted from seeds of *Sterculia foetida* L. showed bacteriostatic activity against Gram-positive and Gram-negative bacteria, including *B. subtilis* and *P. aeruginosa*, which were more sensitive to treatment with lectin [52]. A study by Oliveira et al. [53] demonstrated that the lectin of Eugenia *uniflora* L.

(EuniSL) seeds has more significant antibacterial activity than the crude extract of the same plant indicating that this lectin may be the main active component of the extract.

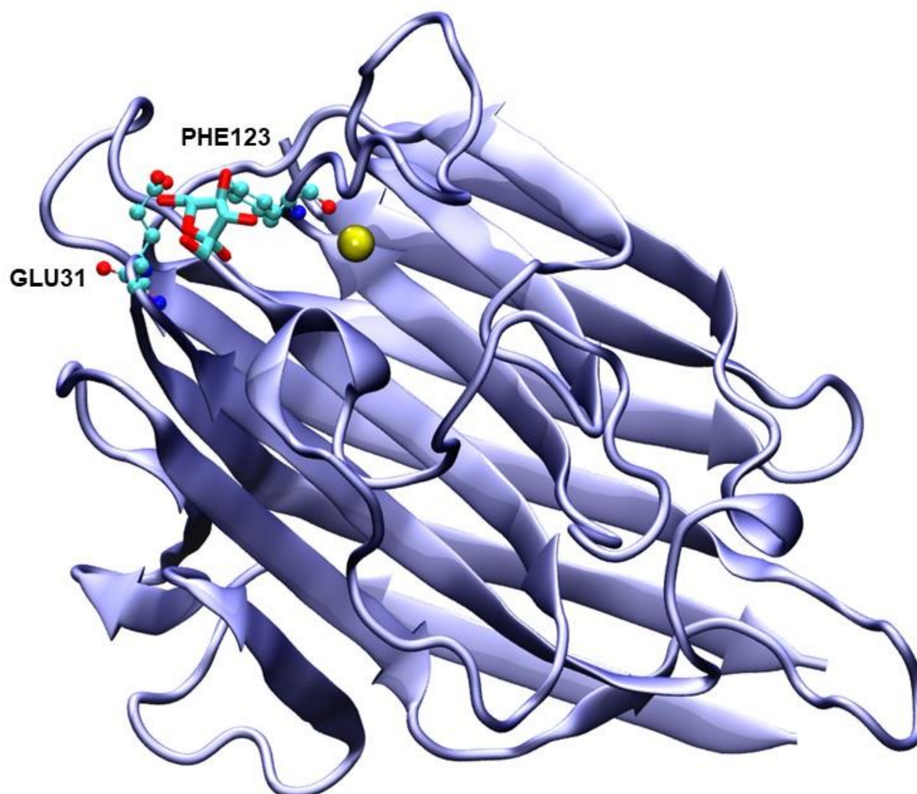

Figure 4: Representation of the lentil lectin (*Lens culinaris*) complexed with sucrose (in stick). The amino acids within 3 Å are represented in ball-and-sticks. The Ca^{2+} (yellow sphere) is a cofactor required for the activity of most vegetable lectins. Oxygens are colored in red, carbons in cyan, and nitrogen atoms in blue. PDB ID: 1LES [54].

Although lectins are mainly found in sexual organs and fruit bodies of plants, they can be expressed by various tissues. Thus, a lectin extracted from the *leaves of Lantana camara*, with specific affinity to chitin, demonstrated antibacterial activity against *P. aeruginosa, E. coli* and *K. pneumoniae,* achieving better results when compared to the reference antibiotic Ampicillin [55].

Some activities reported in the literature for crude extracts plants are attributed to the presence of lectins. SteLL, a lectin isolated from the leaves *of Schinus terebinthifolia* showed antibacterial activity against *P. aeruginosa, E. coli* and *K. pneumoniae,* as well as against *S.*

aureus, P. mirabilis and *S. enteritidis*. However, bactericidal activity was detected only against S. *aureus, E. coli* and *Proteus mirabilis.* In addition, the antibacterial activity of SteLL was stronger than that of *Schinus terebinthifolia* leaf extract, indicating that the lectin is a major active component with antibacterial activity [7].

In a study by Moura et al. [56], lectins were extracted from three parts of the *Myracrodruon urundeuva*, corresponding to the leaf (MuLL), bark (MuBL) and heartwood (MuHL), which showed antibacterial activity against resistant *S. aureus* strains (LAC USA300) and non resistant (8325-4). MIC results ranged from 12.5 to 25 µg/mL^{-1} for all lectins against the non-resistant strain, a result inferior to the antibiotics cefotaxime, cefoxitin, cefuroxime and ciprofloxacin (1 to 2 µg/mL^{-1}). However, lectins were better against the resistant strain compared to drugs. Furthermore, at the concentration of 100 µg/mL^{-1}, the lectins were bactericidal, while only ciprofloxacin presented this result.

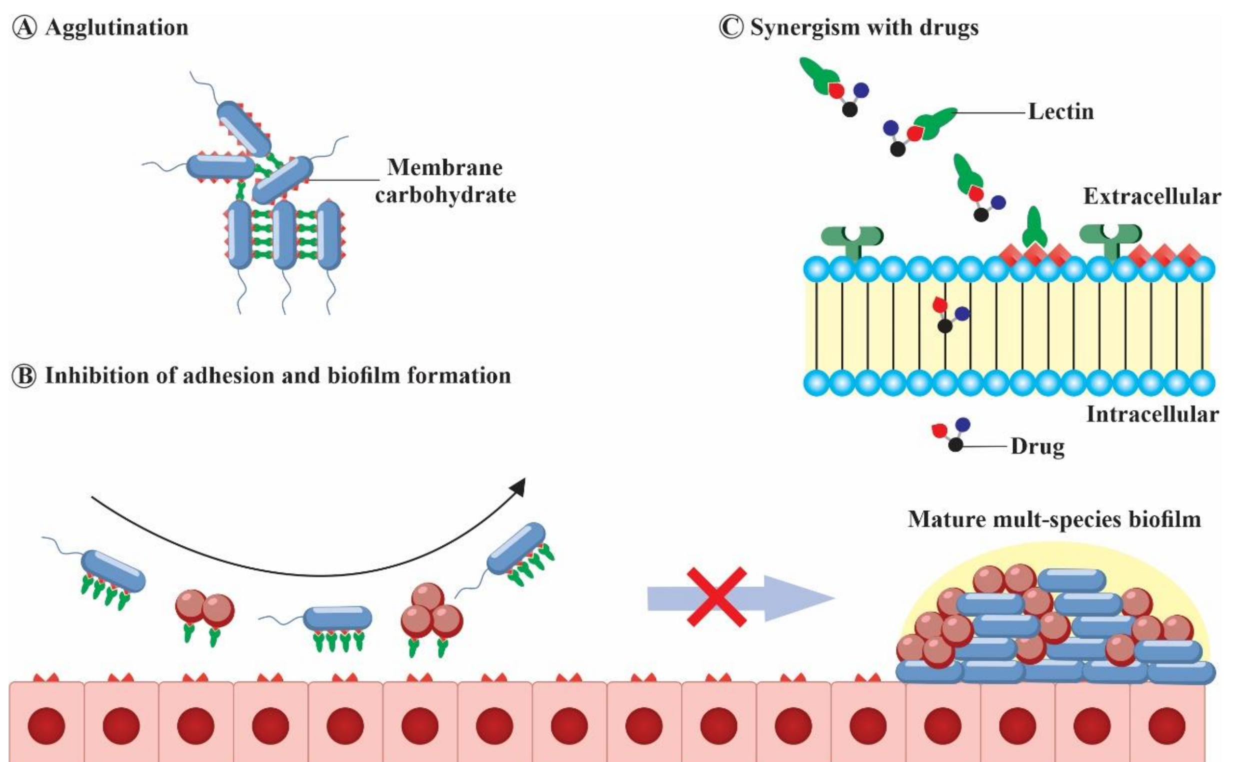

Figura 5. Suggested mechanism of lectin bioactivity against bacteria. **A)** Agglutination - Upon finding membrane carbohydrates on the surface of microorganisms, lectins bind to them, agglutinating the cells together, reducing motility and facilitating the activity of antimicrobial drugs. **B)** Inhibition of adhesion and biofilm formation – Lectins prevent the sites responsible for attaching microorganism to a surface from playing their role, inhibiting adhesion. Adhesion is the first step in the formation of microbial biofilms, and once this adhesion is inhibited, biofilm formation is also affected. **C)** Synergism with drugs – It is suggested that lectins act by delivering the drug close to the surface of the microorganism,

thus facilitating its entry into the cell. The binding performed by the lectins is reversible, allowing them to release the drug upon encountering the microorganism's membrane carbohydrates.

3.2. Lectins with antifungal activity

3.2.1. Fungi with medical importance

Regarding antifungal activity, plant-derived lectins as the most reported in the literature, corroborating the evidence that lectins participate in plant defense against insects, bacteria, and fungi [57]. Plant defense mechanisms, such as hypersensitivity response and acquired systemic resistance, are induced after the recognition of specific molecules derived from pathogens (avirulence proteins), restricting the proliferation of aggressor agents [58,59].

The antifungal activity of *Silene latifolia* lectin was demonstrated against yeasts of *Candida albicans, Candida catenulate, Candida glabrata*, and *Candida rugosa*, as well as against hyphae-generating strains of *Fusarium oxysporum* and *F. solani,* with significant inhibition of mycelium growth in the *Fusarium* strains. Like the antibacterial mechanism reported for some lectins, *S. latifólia* lectin also presented conformational changes in α-helix after binding with manana or laminarin, indicating that these components play an important role in the antifungal activity of lectin [47].

Research conducted by Klafke et al. [60] evaluated the effects of lectins obtained from the leaves of *Abelmoschus esculentus* and the seeds *of Canavalia brasiliensis* (Conbr II), *Mucuna pruriens*, *Clitoria fairchildiana*, *Dioclea virgate*, and *Bauhinia variegata* (BVL) against isolates of *C. albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Cryptococcus gattii*, *Cryptococcus neoformans*, *Malassezia pachydermatis*, *Rhodotorula sp*.and *Trichosporon sp*. The six lectins showed fungicide activity only against *C. parapsilosis*, as the MIC for the other species was higher than 500 μg/mL^{-1}.

Suspensions containing *Candida* blastoconidia were incubated with 500 μg/mL^{-1} of *A. esculentus* lectin (AEL-FITC), which strongly bonded to blastoconidia *of C. parapsilosis.* However, the lectin showed weak interaction with *C. tropicalis* and did not bind to *C. albicans.* The differences observed can be explained by the specificity of lectin to certain

surface glycans, as well as to the variable composition of cell walls vary among fungal species, which can influence both physicochemical and biological properties [60].

Pinheiro et al. [11] demonstrated that *Talisia esculenta* lectin (TEL) inhibited the growth of *Microsporum canis* at concentrations above 0.125 mg/mL^{-1}, reaching 100% inhibition at the concentration of 2 mg/mL^{-1}. Affinity tests revealed that D-mannose and N-acetyl-D-glycosamine inhibited the antifungal activity of TEL. Experiments were also performed using hair infected with *M. canis* to determine whether the lectin could inhibit the growth of fungi obtained from arthroconidia present in the animal hair. The results obtained showed growth inhibition of 73% in 15 samples of infected hair treated with the lectin at a concentration of 500 mg/mL^{-1}, which is comparable with the effect observed in the control group, where 80% inhibition was obtained. Additionally, the lectin showed affinity to macroconidia and arthroconidia, which may result from its binding to chitin molecules in the conidia wall, preventing its complete development.

The Lectins jackin and frutackin, extracted from the seeds of *Artocarpus integrifolia* and *Artocarpus incisa,* respectively, were reported to inhibit the growth of *F. moniliforme* and *Saccharomyces cerevisiae*, demonstrating agglutinating activities that were inhibited only in the presence of chitin and N-acetyl-D-glycamine. Jackin also inhibited the germination of *F. moniliform* at a concentration of 2.25 mg/mL. At the same concentration, this lectin impaired the normal development of hyphae by preventing mycelium from producing spores, which in turn resulted in sterile fungi. Qualitative experiments performed with frutackin found a similar activity to that reported for Jackin. These lectins have high similarity and according to data obtained by fluorescence spectrum, both showed exposed tryptophan residues, indicating that they could freely interact with the chitin column through a hydrophobic interaction between the tryptophan lateral chain and glycosamine rings [61]. Importantly, the affinity of jackin and frutackin to chitin may be responsible for their antifungal activities.

In fact, chitin-binding lectins are more likely to have antifungal activity. Accordingly, the specific chitin-binding lectin extracted from the seed of *M. oleifera* WSMoL, showed significant fungicide effects against *C. albicans*, *C. glabrata*, *C. krusei,* and *C. parapsilosis*. It was observed a decrease in the viability of fungal cells, with more pronounced effects against *C. glabrata* and weaker activity against *C. albicans* and *C. parapsilosis* in comparison with the isopropyl alcohol control. The lectin was found to induce necrosis and apoptosis in all isolates, as evidenced by the presence of double-colored cells (indicative of strong cell

damage) in colorimetric tests. The induction of apoptosis by the lectin may be linked to mitochondrial dysfunction, involving depolarization of transmembrane potential, the release of apoptogenic factors, and impaired oxidative phosphorylation. In fact, the results showed membrane potential depolarization in all isolates, with *C. krusei* being the most sensitive species with regard to the antifungal effects of WSMoL [62].

CasuL, a lectin extracted *from Calliandra surinamensis,* showed antifungal activity against *C. krusei,* with MIC and Minimum Fungicide Concentrations (CFM) of 125 and 250 µg/mL respectively, and drastic morphological changes, with retraction of cytoplasmic content and cell rupture. Since Calcofluor is a fluorochrome capable of binding to chitin, it can be used to reveal changes in the cell wall integrity of yeasts and hyphae. Additionally, *C. krusei* cultures treated with CasuL showed a decrease in the fluorescence signal, associated with discontinuous staining in the wall and cytoplasm, evidencing loss of cell wall integrity [45].

Research by Gomes et al. [63] tested the antifungal activity of four chitin-binding lectins extracted from *Dioclea violacea* (Dviol), *Dioclea rostrata* (DRL), *Canavalia brasiliensis* (ConBr), and *Lonchocarpus sericeus* (LSL), against isolates obtained from the vaginal secretion of pregnant and non-pregnant women with or without symptoms of vulvovaginal infection, totaling 30 samples belonging to the genera *Candida*, *Rhodotorula*, *Thicrosporon*, and *Kloeckera*. As shown in Table 2, the lectins presented antifungal concentrations ranging from 2 to 256 µg/mL.

Consistent evidence has indicated that targeting the inflammatory process has a significant impact when considering the management of fungal infections. In this context, two major immunological properties should be considered: resistance (the ability to limit fungal load) and tolerance (the ability to limit host damage caused by the immune response) [64]. Rodriguez-De la Noval et al. [65] conducted *in vitro* experiments to investigate the protective effect of Lectin-Fc fusion proteins (IgG) with affinity to β-1,3-glucan or chitin polysaccharides, including Dectin-1-Fc (IgG2a), Dectin-1-Fc (IgG2b), and WGA-Fc (IgG2a). The results demonstrated that lectins linked to *Aspergillus fumigatus* conidia in germination presenting intense WGA-Fc (IgG2a) activity but had weak effect on hyphae development. Additionally, biofilm production was inhibited by up to 14% with Dectin-1-Fc (IgG2b) and 20% with WGA-Fc after 48 h of treatment. Importantly, the opsonization of conidia in germination with both lectin-Fc fusion proteins increased the deposition of C3 proteins

(complement system component with important roles in innate immunity) on the conidia, resulting in increased phagocytosis of conidia by macrophages after opsonization. These findings were further confirmed by *in vivo* tests using infected C57BL/6 mice were treated with 10 µg of lectin-Fc (IgG), which protected the mortality by about 20%. In general, the three lectin-Fc (IgG) proteins exhibited significant antifungal activity against *A. fumigatus,* demonstrating their potential to be used as biopharmaceutical molecules in antifungal therapies [65].

Previous work by Chikalovets et al. [66] found that a mussel lectin, identified as Crenomytilus grayanus (CGL), had its expression elevated in the mantle of animals infected with *Pichia pastoris,* reaching peak concentration after12 hours, and returning to the original pattern within 24 hours. The higher level of expression of CGL in the mantle implies that this lectin could have a significant contribution to the prevention of microbial reproduction since the mantle is constantly being washed with seawater containing pathogens and pollutants. Additionally, *in vitro* tests with fungi of the genera *Aspergillus* (3), *Penicillium* (4), *Trichoderma* (2) and *Mycelia* (1) cultured in the presence of CGL demonstrated that the agglutinating activity of this lectin was inhibited in the presence of galactose, evidencing its specificity. Furthermore, antifungal assays indicated inhibition of germination in three of the ten evaluated strains.

While many lectins have their antifungal activities attributed to their ability to interact with, studies have demonstrated that these molecules can interact with other components on the cell surface of fungi, inducing morphological alterations (such as the transition from yeast to filamentous forms), increasing the production of reactive oxygen species (ROS) and inhibiting biofilm production. In this context, a lectin extracted from *Helianthus annuus* (Helja) seeds (0.1 µg/µL) inhibited the growth of *C. albicans*, reducing cell viability by 82% after 48h. In addition, this lectin inhibited morphological transition and biofilm production in *C. albicans*, indicating that the treatment with this lectin can inhibit the pathogenicity of this microorganism [67].

The activity of different lectins against *Candida* strains has been consistently demonstrated. Lectins extracted from *Vicia faba* (Figure 6)*, Lens culinaris*, and *Pisum sativum* with different degrees of purity showed promising antifungal activity against *C. albicans* [49]. MaL, a lectin extracted from the seeds of *Machaerium acutifolium* showed antifungal activity against *C. albicans*, *C. parapsilosis,* and *C. tropicalis*. In addition, *C.*

parapsilosis cultures treated with this lectin (at 18 μM) failed to form visible colonies in Sabouraud agar medium. The lectin significantly altered the membrane permeability and induced the production of ROS. Additionally, scanning electron microscopy (SEM) analyses revealed that the same cultures treated with the lectin at a concentration of 9 μM were characterized by the presence of significantly elongated cells presenting pores in the cell wall and fragmented DNA, indicating that the mechanisms underlying the toxic effects of MaL against *C. parapsilosis* involve cell death by apoptosis [68].

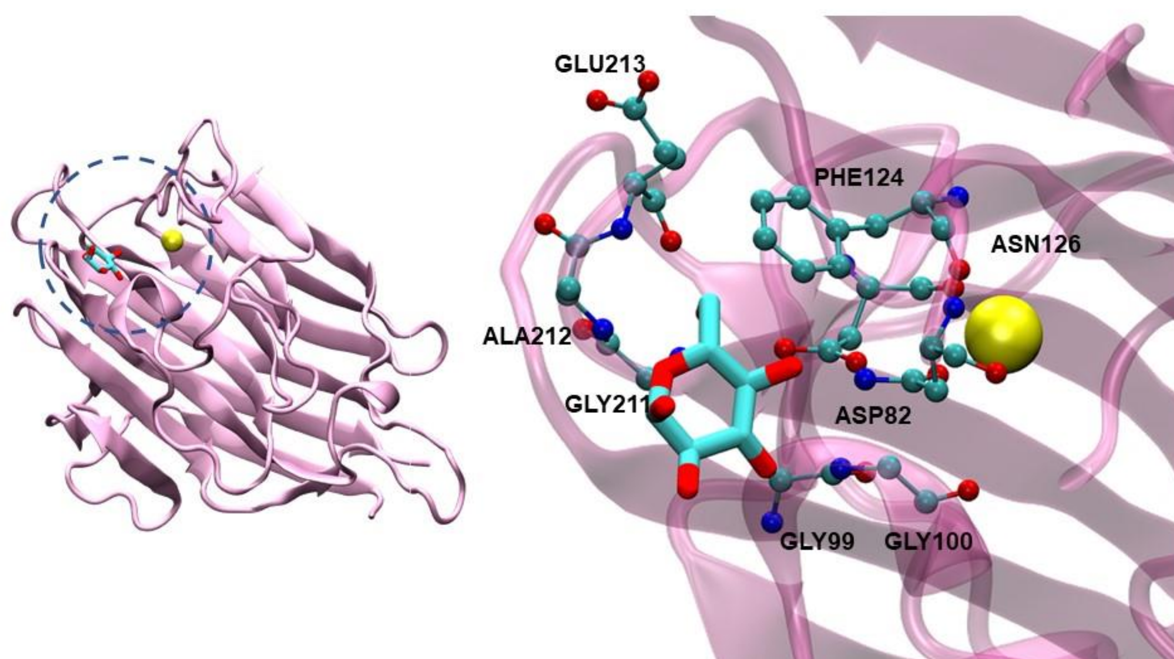

Figure 6: The structural representation of the chain A of *Vicia faba* lectin protein complexed with a D-glucose is on the left panel. The right panel is a zoomed picture of the active site showing the residues near the glucose (3.5 Å of distance). Ca^{2+} (yellow sphere) is also presented in the near region. PDB ID: 2B7Y [69].

A jackalin-related lectin extracted from the seeds of *Helianthus annuus* L. (Helja), which is known as an efficient mannose ligand, showed important effects against pathogenic yeasts such as *C. tropicalis*, C. *parapsilosis*, C. *albicans,* and *Pichia membranifaciens* at a concentration of 200 µg/mL. inhibiting the growth *Candida* strains in up to 50%, while the growth of *P. membranifaciens* was inhibited in 98%. According to the study, the lectin presents a common antifungal mechanism against all the fungal strains, by inducing alterations in the membrane permeability [70].

Many lectins have been classified as metalloproteins due to the presence of metal cations. In fact, metal ions such as Ca^{2+}, Mg^{2+} and Mn^{2+} are cofactors required for activity (especially hemagglutination) of most vegetable lectins [71]. The hemagglutinating activity of *Sophora alopecuroides* lectin (SAL) was inhibited in the presence of D-galactose. Additionally, the agglutination performed by this lectin was potentiated by the addition of Mn^{2+}, indicating that the ion act as a cofactor for SAL activity. Importantly, the lectin inhibited mycelial growth in *Penicillium digitatum* and *Alternaria alternata* cultures after a 24-hour treatment period [72].

3.2.2. Fungi with agriculture importance

In general, lectins can inhibit fungal growth by affecting spore germination and mycelium growth. Lectins showing this type of activity include those obtained from the seeds of *Dracaena guianensis* (Dgui), *Canavalia ensiformis* (ConA), and *Canavalia maritima* (ConM). These lectins present similar properties, acting as mannose/glucose ligands. ConM and Dgui have 90% and 86% similarities in their protein sequence with ConA, respectively. However, only Dgui was found to effectively delay conidia germination and appressorium formation in *Colletotrichum gloeosporioides*, which has a significant impact on the activation of defense mechanisms against pathogen infection. According to the authors, the ability of Dgui, but not ConA or ConM, to specifically inhibit the germination of *C. gloeosporioides* may be due to the differential specificities of these lectins related to complex carbohydrates and glycoproteins. In addition, there may exist subtle differences present in the region of carbohydrate recognition of molecules in relation to the atomic distances involved in hydrogen bonding and van der Waals interactions between lectins and carbohydrates [58].

Capsicum frutescens lectin exhibited strong inhibitory activity in the growth and germination of spores and hyphae of *Fusarium moniliformee*, and *Aspergillus flavus,* while no effect against *Fusarium graminearum*, *Fusarium solani*, *Physalospora piricola*, and *Botrytis cinerea* was observed (Table 2). The sugar-binding specificity of this lectin is similar to that of ConA, both being glucose and mannose ligands in addition to presenting similar molecular mass. However, the lectin of the pepper seed also binds to galactose and fucose, although with less avidity [73].

Ang et al. [74] analyzed the antifungal effects of a lectin extracted from *Phaseolus vulgaris* seed (CPBL) against six species of fungi, including *Phyllosticta citriasiana*, *Magnaporthe grisea*, *Bipolaris maydis*, *Valsa mali*, *Mycosphaerella arachidicola* and *Setosphaeria turcica.* The authors demonstrated that the lectin, at a concentration of 30 µM, inhibited mycelial growth in *V. mali* (by 30.6%) but failed to inhibit this phenomenon in the other strains. Additionally, it was reported that the agglutinating activity of this lectin was inhibited in the presence of glucosamine, besides being an Mg^{2+}-dependent effect.

The binding affinity of MTL to fungal components was examined by an enzyme-linked lectin (ELLA) assay. Fungi of the genera *Fusarium* (2), *Trichoderma* (2), *Haematonectria* (2), *Aspergillus* (1), and *Alternaria* (1), numbered from M1 to M8, were cultured in the presence of MTL demonstrated strong affinity, except for strain number 7. MTL was also able to decrease conidia germination, as well as to strongly inhibit the growth of strains M3, M5, M6, and M8 [17].

A lectin extracted from the red bean *Phaseolus vulgaris* showed antifungal activity against *Coprinus comatus*, *F. oxysporum,* and *Rhizoctonia solani*, which was evidenced by the formation of inhibition zones at the concentrations of 60 and 300 µg/mL. While the agglutinating activity of this lectin activity could not be inhibited by simple sugars, it was inhibited by glycoproteins such as lactoferrin, ovalbumin, and thyroglobulin. According to the author, the fact that this lectin shows a certain degree of structural similarity with chitinases could be, at least partially, responsible for its antifungal activity, since chitinases are known to adversely affect hyphae growth, leading to cell wall rupture through the release of chitin oligosaccharides from the cell wall and cytoplasm leakage [61].

Chitin is a biopolymer composed of repeated GlcNAc abundantly found in nature, especially in the composition of insect exoskeletons, fungal cell walls, nematode eggs, marine diatomaceous and shells of crustaceans and zooplankton. A chitin-binding lectin extracted

from *Solanum integrifolium* inhibited the development of fungi such as *Rhizoctonia solani* and *Colletotrichum gloeosporioides*, with inhibition zones of 8 and 12 mm, respectively [63].

Evidence has indicated that, like the interaction with bacterial components, the interaction of lectins with fungal carbohydrates may involve different degrees of affinity [75]. Thus, it was observed that a lectin extracted from the root of *Portulaca elatior* (PeRoL), which has a great affinity to trehalose, but also bind to galactose, glucose, mannose, and N-acetylglucosamine, showed fungicide activity against fungi of the genus *Candida* [24]. Lunatin, a lectin extracted from the seeds of *Phaseolus lunatus,* inhibited the growth of *Pythium aphanidermatum*, *Fusarium solani*, *F. oxysporum*, and *Botrytis cinerea.* Curiously, this lectin had its hemagglutinating activity inhibited by D-galactose, D-fructose, D-glucose and mannitol [75].

Chen et al. [76] demonstrated that the *Phaseolus coccineus* lectin (PCL) inhibited the growth of *Gibberalla sanbinetti*, *Sclerotinia sclerotiorum*, *Helminthosporium maydis*, and *Rhizoctonia solani*. However, the hemagglutinating activity of this lectin was inhibited by sialic acid, which resulted in an abrupt increase in the lectin MIC, indicating that binding to sialic acid may be significantly involved in the antifungal activity of PCL. *Amaranthus viridis* Linn lectin (AVL), inhibited the growth of *Botrytis cinerea* and *Fusarium oxysporum* at 100 and 200 µg/disc, respectively but had no significant activity against *Rhizoctonia solani*, *Trichoderma reesei*, *Alternaria solani*, and *F. graminearum*. The lectin had its activity inhibited in the presence of T-antigen, N-acetyl-D-galactosamine, and N-acetyl-D-lactosamine, in addition glycoproteins asialofetuin and fetuin. Since a mixture of GlcNAc oligomers of chitin hydrolysate did not inhibit agglutination, it was suggested that the antifungal action of this lectin may not involve its binding to chitin molecules [77].

A galactose-specific lectin extracted from the roots of *Astragalus mongholicus* (AMML) was reported to inhibit the mycelium growth in *Botrytis cincerea* cultures with an IC_{50} of 1.2 µM, in addition to presenting significant antifungal activity against *Colletorichum sp*. and *Drechslera turcica* at the concentration of 100 µg/well. Furthermore, AMML showed weak ribonuclease activity against yeast tRNA at 1.25 U/mg. Although this lectin was initially described as a galactose-specific molecule, it was also capable of binding to L-ramnose and celobiosis, indicating that AMML shows plasticity with regard to the binding to carbohydrates [57].

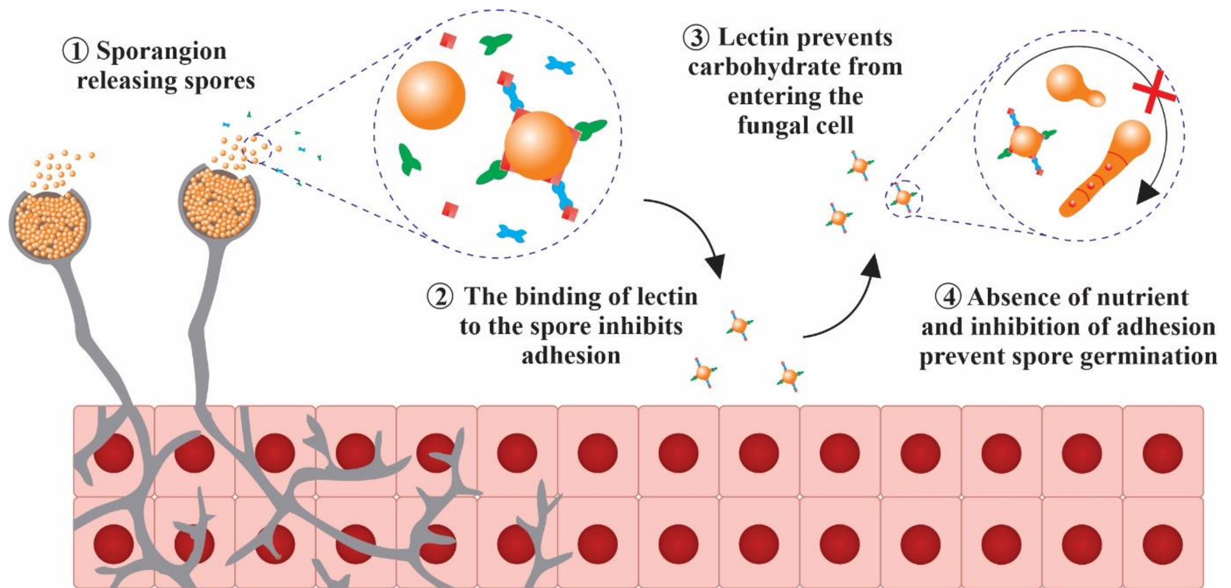

Figure 7. Suggested mechanism for bioactivity of lectins against fungi. **1** – The release of spores occurs by the sporangios. **2** – Lectins quickly bind to carbohydrates on the surface of spores, inhibiting adhesion to a surface. **3** – Lectins bind to extracellular carbohydrates, reducing the availability of nutrients to the spore. **4** – The absence of nutrients, added to the inhibition of adhesion to a surface, prevent the germination of spores from occurring.

3.3. Lectins with antiprotozoal activity

The role of lectins in the control of protozoa is not yet completely defined and, in this context, targeted research is likely moving more slowly than that addressing the effects of lectin against other microorganisms such as bacteria and fungi. Nevertheless, consistent evidence has demonstrated that lectins present immunomodulatory properties, stimulating the production of cytokines that play important roles in the host defense against certain parasites.

Accordingly, Thomazelli et al. [13] obtained promising results against *Leishmania amazonensis* using the lectin ConA extracted from *Canavalia ensiformis.* This lectin was found to increase the phagocytosis of amastigotes by peripheral blood mononuclear cell (PBMC)-derived macrophages, in addition to potentiating the elimination of promastigotes in a post-infection period of 24, 48 and 72 h. The treatment with ConA increased the production of cytokines such as IFN-γ IL-6, TNF-α, IL-4, IL-2 and IL-10, as well as restored the levels of these mediators following *Leishmania* infection, which significantly contributes to overcoming *L. amazonensis* infection.

Other lectins with significant immunomodulatory effects are ScLL and ArtinM, extracted from *Synadenium carinatum* and *Artocarpus heterophyllus,* respectively. These proteins increased the production of cytokines such as IL-12 (ScLL and ArtinM) and IL-10 (ArtinM) by bone marrow-derived macrophages (BMDM), which was comparable to the LPS. They also stimulated the production of nitric oxide (NO), a mediator with key roles in pathogen killing [12].

The marine sponge lectin from the *Chondrilla caribensis* (CCL) was effective against *Leishmania infantum* promastigote, causing direct damage to the parasite structure (IC50 =1.2 µM). This lectin interacts with galactose residues, this carbohydrate is a component of the lipophosphoglycan (LPG) and glycoinositol phospholipid (GIP), glycans present on the surface of *Leishmania* promastigotes. *Leishmania's* membrane components change throughout its cell cycle. In the amastigote form, LPG expression is reduced, while glycoinositol phospholipid (GIP) is present in both forms. In molecular docking tests, CCL was able to interact with GIP, revealing that lectin bioactivity is maintained throughout the *L. infantum* cell cycle. Furthermore, CCL was able to induce the production of ROS, which leads to cell death in the parasite [78]. The *Parkia pendula* lectin (PpeL) inhibited the development of *L. infantum* promastigote (IC50 =10.5 µM). Its activity could be inhibited in the presence of α-methyl-mannoside, indicating that its leishmanicidal activity activity may occur through interaction with mannose residues present in LPG and GIP in *L. infantum* membrane [79].

The snake *Bothrops leucurus*, produce a lectin (BLL) that also showed affinity to galactose, reaveling activity against *Leishmania amazonensis* and *Leishmania brasiliensis*. In the tests by Aranda et al. [80], BBL showed immunomodulatory activity, by reducing the survival of amastigotes, as well as reducing the amount of infected cells (1.6 µM), a similar result obtained in the treatment with pentamidine. Furthermore, infected and uninfected macrophages treated with BLL had their production of cytokines (IL-10, INF-Ɣ, TNF-α, IL-6 and IL-1β) and NO increased. When the lectin was used together with galactose, its action was reduced, indicating that its bioactivity depends on its carbohydrate recognition domain (CRD). In cytotoxicity tests, BLL was more selective to parasites than host cells.

Lectins have also been demonstrating experimentally promising molecules in the control of toxoplasmosis, a disease with high prevalence and mortality rates in Brazil. Studies demonstrated that the *in vivo* treatment of C57BL/6 mice infected with *Toxoplasma gondii* with these lectins stimulated the production of Th1, Th2 and Th17 cytokines. Of note,

regulation of the Th1 response is required to control inflammatory tissue damage. Additionally, the control of *T. gondii* infection requires a balance between the production of pro-inflammatory and anti-inflammatory cytokines, including IL-12, IFN-γ, and IL-10 [12]. Immunomodulatory effects were also demonstrated by *Bothrops pauloensis* lectin (BpLec), which increased IL-6 secretion by HeLa cells after infection with *T. gondii* taquizoites. BpLec also reduced the secretion of the migration inhibitory factor (MIF), probably by interacting with proteins on the parasite surface [81].

Earlier reports have indicated that lectins have the potential to be used in the treatment of sexually transmitted infection caused by *Trichomonas vaginalis,* since both surface and secreted proteins of *T. vaginalis* present N-glycan residues that can function as binding sites to lectins [82]. It is postulated that due to its antiviral and antiparasitic properties, lectins could treat simultaneously infections by HIV, HSV, and *T. vaginalis*. This evidence is supported by reports demonstrating that cyanovirin-N and griffithsin, lectins with antiretroviral properties, were capable of binding to N-glycans of *T. vaginalis,* leading to the agglutination of the parasite. In addition, these lectins, as well as galactin-1, reduced the recovery of *Tritrichomonas foetus* in a mice model of vaginal infection, demonstrating a significant potential for the treatment of infection.

In addition to the immunomodulatory effects, lectins were found to cause direct damage to the structure of parasites. Thus, lectin extracted from the seeds of *Phaseolus vulgaris* demonstrated significant toxicity against *T. vaginalis* trophozoites, leading to complete loss of the membrane structures, as well as the destruction of the nucleus and cytoplasm. *T. vaginalis,* the etiological agent of trichomoniasis, is a parasite that colonizes the urogenital tract of both female and male individuals. It was demonstrated *P. vulgaris* lectin presented more significant effects than metronidazole (the standard reference drug for the treatment of this condition in humans), leading to pronounced alterations in the structure of trophozoites, including swelling, and plasma membrane disruption, and redistribution of pinocytotic and phagocytic vacuoles, with large empty areas in the cytoplasm [83].

As demonstrated for bacteria and fungi, lectins can also induce protozoa agglutination. The ability of lectins to select and complex with microbial glycoconjugates made them useful as cell probes capable of detecting a variety of constituents. In this context, an experiment carried out by Moura et al. [84] showed that a lectin isolated from the marine sponge *Cliona varians* (CvL) agglutinated *Leishmania chagasi* promastigotes, revealing that galactose

receptors are present at this stage of the parasite life cycle. Further research demonstrated that *Phaseolus vulgaris* lectin showed leishmanicidal activity against *Leishmania donovani,* eliminating 100% of promastigotes after 24 hours of treatment, similar to the effect obtained with amphotericin B [85].

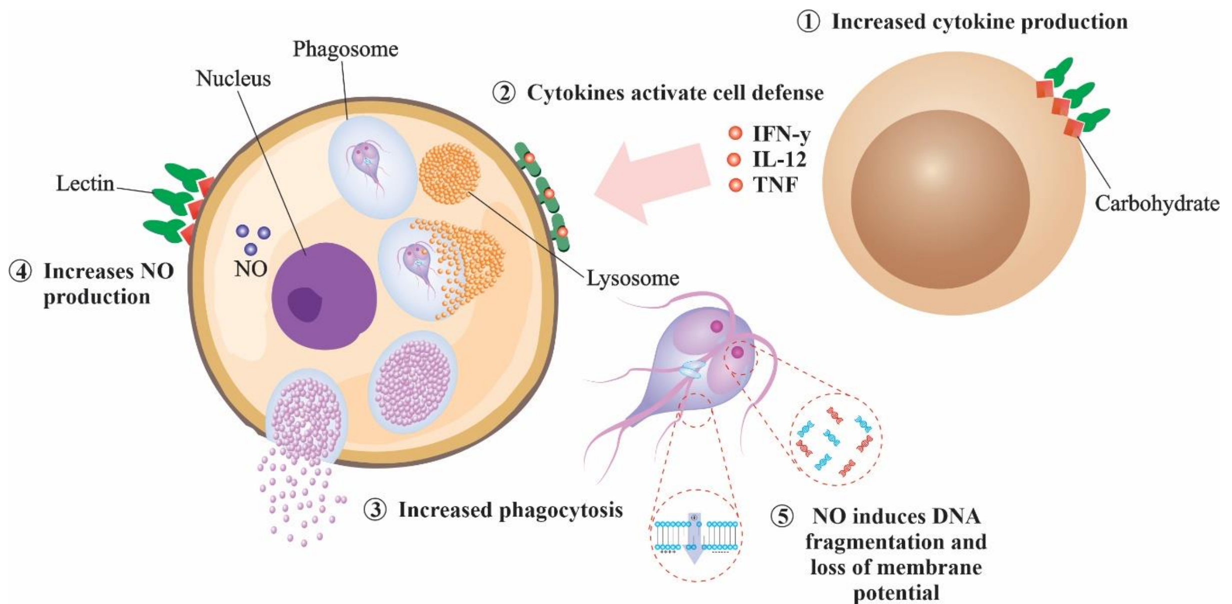

Figure 8. Suggested mechanism of lectin bioactivity against protozoa. **1** – Lectins stimulate the production of cytokines. **2** – Cytokines activate defense cells, like macrophages. **3** – Macrophages increase their phagocytic activity. **4** – Lectins stimulate macrophages to produce nitric oxide (NO). **5** – NO induces DNA fragmentation and loss of parasite membrane potential, leading to apoptosis.

4. Perceptions, conclusions, and perspectives

Lectins represent biomolecules with multiple pharmacological potentialities, being particularly effective against microorganisms, such as biofilm-producing bacteria and fungi. In addition, their immunomodulatory properties may represent, with the advancement of research, an alternative to current antibiotic therapy. It is noteworthy to emphasize that there are still many gaps with regard to the properties of lectins and therefore, this literature review gathered data in order to provide a better understanding of how these proteins act in different organisms.

Most studies included in this work indicated that lectins present affinity to specific carbohydrates, which only partially explain the bioactivities reported for lectins. Although most studies suggest that the antiprotozoal activity of lectins occurs due to

immunomodulatory mechanisms, this evidence is limited by the low number of articles investigating this subject. Lectins have the ability to induce agglutination in a variety of cells. However, lectin-induced agglutination in erythrocytes may represent a significant toxic effect to humans, which remains to be better investigated.

In general, plant lectins are more effective in inhibiting the growth of microorganisms, as well as in interfering with the production of biofilms and modulating the antimicrobial activity of commercial drugs. On the other hand, lectins extracted from animals (mainly fish and mussel) seems to have important roles in the defense against invading agents as their expression is increased during infection. Nevertheless, *in vivo* research is urgently required for the elucidation of both the mechanisms of action and toxic effects of lectins, especially when considering the interaction of lectins with carbohydrates in several cell types.

In conclusion, the multiple biological properties of lectins make them interesting molecules in the context of scientific research, especially in antimicrobial drug development. It is expected that the advances in lectin engineering will result in the production of recombinant lectins with the potential to be used as therapeutic agents against current health problems such as antimicrobial microbial resistance.

Declarations

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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ANEXOS

Table 1: Antibacterial activity of lectins reported in the literature. The concentrations mentioned are the lowest with relevant activity against the bacteria tested.

Species	Lectin	Bioactivity	Bioactivity Concentration	Affected microorganisms	References
Vatairea macrocarpa (Benth.) Ducke	VML	Modulation + penicillin; Modulation + gentamicin; Modulation + norfloxacin	14.94 µM; 1.18 µM; 4.71 µM.	*Staphylococcus aureus; Staphylococcus aureus; Staphylococcus aureus.*	[31]
Dioclea violacea Benth.	DVL	Modulation + gentamicin	0.39 µM; 0.49 µM.	*Staphylococcus aureus; Echerichia coli.*	[29]
Pinctada fucata martensii (Dunker, 1880)	PmCTL-1	Growth inhibition	11.18 µM; 11.18 µM; 11.18 µM.	*Micrococcus luteus; Staphylococcus aureus; Bacillus subtilis.*	[19]
Misgurnus anguillicaudatus (Cantor, 1842)	rMaCTL	Agglutinating	0.04 µM; 0.08 µM; 0.04 µM; 0.08 µM.	*Aeromonas hydrophila; Escherichia coli; Vibrio anguillarum; Staphylococcus aureus.*	[9]

Canavalia ensiformis (L.) DC.	ConA	Modulation + gentamicin	0.50 µM; 0.78 µM.	*Staphylococcus aureus; Escherichia coli.*	[30]
Lantana camara L.	LCL	Growth inhibition	10 µg (7.1 mm); 10 µg (7.3 mm); 10 µg (6,9 mm).	*Klebsiella pnumoniae; Pseudomonas aeruginosa; Escherichia coli.*	[55]
Vicia faba L.	ND*	Growth inhibition	0.87 µM; 0.87 µM; 3.47 µM.	*Staphylococcus aureus; Klebsiella pneumonia; Pseudomonas aeruginosa.*	[49]
Lens culinaris Medik.	ND*	Growth inhibition	0.14 µM; 0.28 µM; 1.09 µM.	*Staphylococcus aureus; Klebsiella pneumonia; Pseudomonas aeruginosa.*	[49]
Pisum sativum L.	ND*	Growth inhibition	7.35 µM; 3.68 µM; 7.35 µM.	*Staphylococcus aureus; Klebsiella pneumonia; Pseudomonas aeruginosa.*	[49]
Penaeus semisulcatus De Haan, 1844 [in De Haan, 1833-1850]	Semisulcatus lectin	Antibiofilm	1.52 µM; 1.52 µM; 1.52 µM; 1.52 µM.	*Aeromonas hydrophila; Vibrio parahaemolytic*	[41]

				us; Staphylococcus aureus; Enterococcus fecalis.	
Portulaca elatior Mart. ex Rohrb	PeRoL	Growth inhibition	0.25 µM; 0.12 µM; 0.98 µM.	*Enterococcus faecalis; Pseudomonas aeruginosa; Staphylococcus aureus.*	[24]
Punica granatum L.	PgTeL	Growth inhibition	0.24 µM; 0.48 µM;	*Staphylococcus aureus 8325-4; Staphylococcus aureus LAC USA300;*	[38]
Aplysina fulva (Pallas, 1766)	AFL	Antibiofilm	0.52 µM; 8.33 µM; 16.67 µM.	*Staphylococcus aureus; Staphylococcus epidermidis; Escherichia coli.*	[42]
Oplegnathus punctatus (Temminck & Schlegel, 1844)	rOppCTL	Agglutinating	5.19 µM; 5.19 µM; 5.19 µM; 5.19 µM; 5.19 µM; 5.19 µM.	*Bacillus subtilis; Staphylococcus aureus; Escherichia coli; Vibrio anguillarum; Edwardsiella tarda;*	[2]

				Aeromonas hydrophila.	
Parkia platycephala Benth.	PPL	Modulation + gentamicin	0.51 µM; 0.40 µM.	*Staphylococcus aureus; Escherichia coli.*	[33]
Canavalia ensiformis (L.) DC.	ConA	Antibiofilm	3.92 µM; 3.92 µM.	*Escherichia coli; Listeria monocytogenes.*	[40]
Procambarus clarkii (Girard, 1852)	PcLec6	Agglutinating	2.58 µM; 2.58 µM.	*Staphylococcus aureus; Vibrio alginolyticus.*	[1]
Stenopsyche kodaikanalensis Swegman & Coffman, 1980	*Stenopysche kodaikanalensis* lectin	Agglutinating; Bacteriolytic	1.67 µM; 1.67 µM.	*Bacillus subtilis; Bacillus flexus.*	[25]
Chondrilla caribensis f. hermatypica Rützler, Duran & Piantoni, 2007	CCL	Antibiofilm	7.35 µM; 0.92 µM; 29.41 µM.	*Staphylococcus aureus; Staphylococcus epidermidis; Escherichia coli.*	[43]
Aplysia dactylomela Rang, 1828	ADEL	Antibiofilm	0.26 µM.	*Staphylococus aureus.*	[10]
Calliandra surinamensis Benth.	Casul	Antibiofilm	0.13 µM; 1.04 µM; 0.26 µM; 0.13 µM.	*Staphylococcus aureus; MRSA; Escherichia coli; Staphylococcus*	[45]

				saprophyticcus.	
Mytilus trossulus Gould, 1850	MTL	Agglutinating	2.8 µM.	*Vibrio proteolyticus.*	[17]
Moringa oleifera Lam.	WSMoL	Growth inhibition	0.54 µM; 17.27 µM.	*Staphylococcus aureus; Escherichia coli.*	[26]
Bauhinia variegata L.	nBVL	Anti-adhesive	6.25 µM; 6.25 µM.	*Streptococcus mutans; Streptococcus sanguinis.*	[37]
Artocarpus heterophyllus Lam.	Artocarpine	Growth inhibition	0.97 µM; 0.97 µM; 3.89 µM.	*MRSA; Escherichia coli; Pseudomonas aeruginosa.*	[32]
Sparassis latifolia Y.C. Dai & Zheng Wang 2006	*Sparassis latifolia* lectin	Growth inhibition	8.33 µM; 4.17 µM; 2.08 µM.	*Staphylococcus aureus; Escherichia coli; Pseudomonas aeruginosa.*	[47]
Andrias davidianus (Blanchard, 1871)	ADL	Respiration inhibition	735.29 µM; 735.29 µM; 735.29 µM; 735.29 µM; 735.29 µM.	*Escherichia coli; Enterobacter aerogenes; Staphylococcus aureus; Bacillus subtilis; Shewanella sp.*	[28]

Apuleia leiocarpa (Vogel) J.F.Macbr.	ApulSL	Growth inhibition	0.81 µM; 0.81 µM; 1.62 µM; 3.23 µM; 0.81 µM; 1.62 µM; 3.23 µM; 3.23 µM; 3.23 µM; 3.23 µM; 0.20 µM; 0.40 µM; 0.40 µM.	*Bacillus subtilis; Bacillus cereus; Enterococcus faecalis; Escherichia coli; Klebsiella pneumoniae; Micrococcus luteus; Pseudomonas aeruginosa; Salmonella enteritidis; Staphylococcus aureus; Streptococcus pyogenes; Xanthomonas campestris pv. peasants; Xanthomonas campestris pv. malvacearum; Xanthomonas campestris pv. viticola.*	[48]
Aspergillus gorakhpurensis Kamal & Bhargava 1969	*A. Gorakhpurensis* lectin	Growth inhibition	50.14 µM; 50.14 µM; 50.14 µM.	*Bacillus cereus; Staphylococcus aureus; Escherichia*	[27]

				coli.	
Kaempferia rotunda L.	KRL	Growth inhibition	1.72 µM; 1.72 µM.	*Staphylococcus aureus; Escherichia coli.*	[86]
Solanum tuberosum L.	StL-20	Growth inhibition	1.25 µM; 0.50 µM; 0.50 µM; 1.25 µM.	*Escherichia coli; Listeria monocytogenes; Salmonella enteritidis; Shigella boydii.*	[46]
Litopenaeus vannamei (Boone, 1931)	LvCTl3	Agglutination	5.56 µM; 5.56 µM; 5.56 µM.	*Vibrio alginolyticus; Vibrio parahaemolyticus; Bacillus subtilis*	[14]
Sterculia foetida L.	SFL	Growth inhibition	7.53 µM; 15.06 µM; 7.53 µM; 15.06 µM; 7.53 µM; 15.06 µM.	*Bacillus subtilis CCT 0516; Escherichia coli ATCC 2536; Pseudomonas aeruginosa ATCC 23243; Staphylococcus aureus ATCC 25619; Pseudomonas aeruginosa*	[52]

				ATCC 8027; Staphylococcus aureus ATCC 25925.	
Schinus terebinthifolius Raddi - synonymy of *Schinus terebinthifolia* Raddi	Stell	Growth inhibition	0.13 µM; 2.05 µM; 0.26 µM; 0.13 µM; 0.26 µM; 0.03 µM.	*Pseudomonas aeruginosa; Escherichia coli; Klebsiella pneumoniae; Staphylococcus aureus; Proteus mirabilis; Salmonella enteritidis.*	[7]
Moringa oleifera L.	WSMoL	Growth inhibition	69.06 µM; 69.06 µM.	*Staphylococcus aureus; Escherichia coli.*	[51]
Bothrops leucurus (Wagler, 1824)	BIL	Growth inhibition	1.05 µM; 2.08 µM; 4.17 µM.	*Staphylococcus aureus; Enterococcus faecalis; Bacillus subtilis.*	[21]

Kaempferia rotunda L.	KRL	Growth inhibition	2.59 µM; 2.59 µM; 2.59 µM; 2.59 µM; 20.69 µM; 20.69 µM.	*Shigella Sonnei; Bacillus cereus; Bacillus subtilis; Bacillus megaterium; Klebsiella sp.; Sarcina lutea.*	[50]
Eugenia uniflora L.	EuniSL	Growth inhibition	0.02 µM; 0.25 µM; 0.25 µM; 0.02 µM; 0.02 µM; 0.25 µM; 0.25 µM.	*Staphylococcus aureus; Streptococcus sp.; Bacillus subtilis; Klebsiella sp.; Pseudomonas aeruginosa; Corinebacterium bovis; Escherichia coli.*	[53]
Holothuria (Metriatyla) scabra Jaeger, 1833	HSL	Growth inhibition	0.01 µM; 0.03 µM; 0.04 µM; 0.05 µM; 0.11 µM; 0.11 µM.	*Staphylococcus sp.; Streptococcus sp.; Shigella sp.; Klebsiella sp.; Serratia sp; Escherichia coli.*	[16]

Fenneropenaeus chinensis (Osbeck, 1765)	rFc-hsL	Growth inhibition	5 µM; 5 µM; 2.5 µM; 2.5 µM; 5 µM; 10 µM; 20 µM; 20 µM.	*Bacillus subtilis;* *Bacillus cereus;* *Bacillus megaterium;* *Bacillus thuringiensis;* *Micrococcus luteus;* *Staphylococcus aureus;* *Escherichia coli;* *Klebsiella pneumoniae.*	[15]
Fenneropenaeus chinensis (Osbeck, 1765)	rmFc-hsL	Growth inhibition	0.6 µM; 2.4 µM; 0.6 µM; 0.6 µM; 4.8 µM; 0.6 µM; 4.8 µM; 1.2 µM.	*Bacillus subtilis;* *Bacillus cereus;* *Bacillus megaterium;* *Bacillus thuringiensis;* *Micrococcus luteus;* *Staphylococcus aureus;* *Escherichia coli;* *Klebsiella pneumoniae.*	[15]

Bufo arenarum Hensel, 1867 - synonymy of *Rhinella arenarum* (Hensel, 1867)	LBP1	Growth inhibition	25 µg/11±0.8; 25 µg/16±0.8; 25 µg/20±0.7; 25 µg/12±0.7.	*Escherichia coli K12 strain 4100; Escherichia coli; Proteus morganii; Enterococcus faecalis*	[22]
Bufo arenarum Hensel, 1867 - synonymy of *Rhinella arenarum* (Hensel, 1867)	LBP2	Growth inhibition	25 µg/12±0.5; 25 µg/17.5±0.8; 25 µg/19±0.7; 25 µg/12.5±0.7.	*Escherichia coli K12 strain 4100; Escherichia coli; Proteus morganii; Enterococcus faecalis*	[22]
Myracrodruon urundeuva Allemão	MuLL	Growth inhibition	3.57 µM; 1.79 µM.	*Staphylococcus aureus* LAC USA300; *Staphylococcus aureus* 8325-4.	[56]
Myracrodruon urundeuva Allemão	MuBL	Growth inhibition	1.79 µM; 0.89 µM.	*Staphylococcus aureus* LAC USA300; *Staphylococcus aureus* 8325-4.	[56]
Myracrodruon urundeuva Allemão	MuHL	Growth inhibition	1.79 µM; 1.79 µM.	*Staphylococcus aureus* LAC USA300; *Staphylococcus*	[56]

				aureus 8325-4.	

**ND: Not determined*

Table 2: Antifungal activity of lectins reported in the literature. The concentrations mentioned are the lowest with relevant activity against the tested fungi.

Species	Lectin	Bioactivity	Bioactivity Concentration	Microorganisms	References
ND*	Dectin-1-Fc (IgG2a)	Germination inhibition	0.20 µM.	*Aspergillus fumigatus*	[65]
ND*	Dectin-1-Fc (IgG2b)	Germination inhibition	0.20 µM.	*Aspergillus fumigatus*	[65]
Triticum aestivum L.	WGA-Fc (IgG2a)	Germination inhibition	0.20 µM.	*Aspergillus fumigatus*	[65]
Machaerium acutifolium Vogel	MaL	Growth inhibition	18 µM; 9 µM.	*Candida albicans;* *Candida parapsilosis;*	[68]
Vicia faba L.	ND*	Growth inhibition	1.74 µM.	*Candida albicans.*	[49]
Lens culinaris Medik.	ND*	Growth inhibition	0.28 µM.	*Candida albicans.*	[49]
Pisum sativum L.	ND*	Growth inhibition	14.71 µM.	*Candida albicans.*	[49]
Moringa oleifera Lam.	WSMoL	Growth inhibition	1.38 µM; 1.38 µM; 1.38 µM; 1.38 µM.	*Candida albicans;* *Candida glabrata;* *Candida krusei;* *Candida parapsilosis.*	[62]
Portulaca elatior Mart. ex Rohrb	PeRoL	Fungicide	0,48 µM; 0,48 µM; 0,48 µM; 0,48 µM.	*Candida albicans;* *Candida parapsilosis;* *Candida krusei;* *Candida tropicalis.*	[24]
Helianthus annuus L.	Helja	Growth inhibition	0.6 μM^{-7}.	*Candida Albicans*	[67]

Solanum integrifolium Lam.	CBL	Growth inhibition	1.52 µM.	*Rhizoctonia solani;*	[63]
Calliandra surinamensis Benth.	Casul	Growth inhibition	2.60 µM.	*Candida Krusei*	[45]
Phaseolus lunatus L.	Lunatin	Growth inhibition	4.12 µM; 4.12 µM; 4.12 µM; 4.12 µM;	*Pythium aphanidermatum;* *Fusarium solani;* *Fusarium oxysporum;* *Botrytis cinerea.*	[75]
Mytilus trossulus Gould, 1850	MTL	Germination inhibition	28 µM; 28 µM; 28 µM; 28 µM.	*Trichoderma ~~M3~~;* *Haematonectria ~~M5~~;* *Haematonectria ~~M6~~;* *Alternaria ~~M8~~.*	[17]
Sparassis latifolia Y.C. Dai & Zheng Wang	*Sparassis latifolia* lectin	Growth inhibition	4.17 µM; 1.04 µM; 4.17 µM; 2.08 µM; 8.33 µM; 4.17 µM.	*Candida albicans;* *Candida catenulate;* *Candida glabrata;* *Candida rugosa;* *Candida albicans 14001;* *Candida albicans 14007.*	[47]
Crenomytilus grayanus Dunker	CGL	Germination inhibition	55.56 µM; 55.56 µM; 55.56 µM.	*Aspergillus ~~M3~~* *Penicillium ~~M7~~;* *Trichoderma ~~M9~~.*	[66]
Phaseolus vulgaris L.	CPBL	Growth inhibition	30 µM.	*Valsa mali*	[74]
Helianthus annuus L.	Helja	Growth inhibition	12.50 µM; 12.50 µM; 12.50 µM; 12.50 µM.	*Candida albicans;* *Candida tropicalis;* *Candida parapsilosis;* *Pichia membranifaciens.*	[70]

Canavalia brasiliensis Benth.	ConBr	Growth inhibition	0.08 µM.	*Candida parapsilosis.*	[60]
Mucuna pruriens (L.) DC.	*Mucuna pruriens* lectin	Growth inhibition	0.03 µM.	*Candida parapsilosis.*	[60]
Clitoria fairchildiana R.A. Howard	*Clitoria fairhcildiana* lectin	Growth inhibition	0.02 µM.	*Candida parapsilosis.*	[60]
Dioclea virgata (Rich.) Amshoff	*Dioclea virgate* lectin	Growth inhibition	0.15 µM.	*Candida parapsilosis.*	[60]
Bauhinia variegata L.	BVL	Growth inhibition	3.91 µM.	*Candida parapsilosis.*	[60]
Abelmoschus esculentus (L.) Moench	*Abelmoschus esculentus* lectin	Growth inhibition	0.05 µM.	*Candida parapsilosis.*	[60]
Dioclea violacea Benth.	DVL	Growth inhibition	0.61 µM; 1.23 µM; 1.23 µM; 1.23 µM; 1.23 µM; 4.90 µM; 1.23 µM; 1.23 µM; 0.31 µM; 1.23 µM; 0.31 µM; 0.61 µM; 9.81 µM.	*Candida albicans URM4987; Candida albicans URM4986; Candida URM4979 azyma; Candida guilliermondii URM4975; Marine candida URM4976; Candida membranaefaciens URM4983; Candida obtusa URM4982; Candida robusta URM4972; Candida*	[87]

				shehatae URM4978; Candida tropicalis URM6090; Candida tropicalis URM4989; Kloeckera apiculata URM5002; Rhodotorula glutinis URM5092.	
Dioclea rostrata Benth.	DRL	Growth inhibition	4.98 µM; 2.49 µM; 0.16 µM; 2.49 µM;	*Candida guilliermondii URM4975; Candida membranaefaciens URM4983; Candida shehatae URM4978; Kloeckera apiculata URM5002.*	[87]
Canavalia brasiliensis Benth.	ConBr	Growth inhibition	0.31 µM; 0.31 µM; 0.31 µM; 0.08 µM; 0.31 µM; 0.08 µM; 0.31 µM; 9.87 µM; 0.08 µM.	*Candida albicans URM4987; Candida URM4979 azyma; Candida guilliermondii URM4975; Candida membranaefaciens URM4983; Candida obtusa URM4982; Candida shehatae*	[87]

				URM4978; Candida tropicalis URM4989; Kloeckera apiculata URM5002; Trichosporon cutaneum URM4973.	
Sophora alopecuroides L.	SAL	Growth inhibition	3.125 µM; 3.338 µM.	*Penicillium digitatum; Alternaria alternata*	[72]
Dioclea guianensis Benth.	Dgui	Germination inhibition	3.85 µM.	*Collectotrichum gloesporioides.*	[58]
Curcuma amarissima Roscoe	*Curcuma amarissimo* lectin	Growth inhibition	0.54 µM; 1.08 µM; 1.08 µM.	*Collectrotrichum cassiicola; Exserohilum turicicum; Fusarium oxysporum.*	[71]
Talisia esculenta (A. St.-Hil.) Radlk.	TEL	Growth inhibition	2.08 µM.	*Microsporum canis.*	[11]
Phaseolus coccineus L.	PCL	Growth inhibition	8.33 µM; 8.33 µM; 8.33 µM; 8.33 µM.	*Gibberella sanguinetti; Sclerotinia sclerotiorum; Helminthosporium maydis; Rhizoctonia solani.*	[76]
Capsicum frutescens L.	*Capsicum frutescens* lectin	Inhibition of hyphae growth	33.90 µM; 33.90 µM.	*Fusarium moniliforme; Aspergillus flavus.*	[73]
Amaranthus viridis L.	AVL	Growth inhibition	100 µg/disc; 200 µg/disc.	*Botrytis cincerea; Fusarium oxysporum.*	[77]
Artocarpus integrifolius L.f.	Jackin	Inhibition of germination.	160.71 µM.	*Fusarium moniliforme.*	[88]

Artocarpus incisa	Frutackin	Inhibition of germination.	160.71 µM.	*Fusarium moniliforme.*	[88]
Astragalus mongholicus Bunge	AMML	Growth inhibition	20 µg/well; 100 µg/well; 100 µg/well; 100 µg/well.	*Botrytis cincerea;* *Fusarium oxysporum;* *Collectorichum sp.;* *Drechslera turcia.*	[57]
Phaseolus vulgaris L.	*Phaseolus vulgaris* lectin	Growth inhibition	60 µg/disc; 60 µg/disc; 60 µg/disc.	*Coprinus comatus;* *Fusarium oxysporum;* *Rhizoctonia solani.*	[61]

**ND: Not determined*

Table 3: Antiprotozoal activity of lectins reported in the literature. The concentrations mentioned are the lowest with relevant activity against the protozoa tested.

Species	**Lectin**	**Bioactivity**	**Bioactivity Concentration**	**Microorganisms**	**References**
Canavalia ensiformis (L.) DC.	ConA	Immunomodulation	0.39 µM.	*Leishmania amazonensis*	[13]
Synadenium carinatum Boiss.	ScLL	Immunomodulation	0.01 µM.	*Toxoplasma Gondi*	[12]
Artocarpus heterophyllus Lam.	ArtinM	Immunomodulation	0.01 µM.	*Toxoplasma Gondi*	[12]
Phaseolus vulgaris L.	*Phaseolus vulgaris* lectin	Direct damage to the structure of the microorganism	3.97 µM.	*Trichomonas vaginalis*	[83]
Bothrops pauloensis AMARAL	BpLec	Immunomodulation	0.13 µM.	*Toxoplasma Gondi*	[81]
Phaseolus vulgaris L.	PHA	Direct damage to the structure of the microorganism	1.59 µM.	*Leishmania Donovani*	[85]
Cliona varians (Duchassaing & Michelotti, 1864*)*	CvL	Agglutination	8.77 µM.	*Leishmania chagasi*	[84]
Bothrops leucurus (Wagler, 1824)	BLL	Immunomodulation	1.55 µM. 1.3 µM.	*Leishmania amazonensis; Leishmania brasiliensis.*	[80]
Chondrilla caribensis (Rützler, Duran & Piantoni)	CCL	Direct damage to the structure and induction of ROS	1.2 µM.	*Leishmania infantum*	[78]

Parkia pendula (Willd.) Benth. Ex Walp	PpeL	Inhibition of promastigote development.	10.5 µM.	*Leishmania infantum*	[79]

**ND: Not determined*

CAPÍTULO III – Lectins ConA and ConM extracted from *Cavanalia ensiformis* (L) DC and *Canavalia rosea* (Sw.) DC inhibit planktonic *Candida albicans* and *Candida tropicalis*
Link: https://www.elsevier.com/journals/biochimie/0300-9084/guide-for-authors

Victor Juno Alencar Fonseca[1]; Ana Lays Braga[1]; Ray Silva de Almeida[1]; Taís Gusmão da Silva[1]; Josefa Carolaine Pereira da Silva; Luciene Ferreira de Lima[1]; Maria Helena da Cruz Santos[2], Romério Rodrigues Silva[2], Claudener Souza Teixeira[2]; Maria Flaviana Bezerra Morais-Braga[1]

[1]*Universidade Regional do Cariri, URCA, Cel Antônio Luis, 1161, 63105-000, Pimenta, Crato, CE, Brazil.* victorjunobiomedico@gmail.com; ana.lays@hotmail.com; rayalmeidasilva2306@gmail.com; gusmao@urca.br; carol.bio1881@outlook.com; luciene.ferreira@urca.br; flavianamoraisb@yahoo.com.br.
[2]*Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal do Cariri, Crato, CE, Brazil.* Santos.mariahelenacruz@gmail.cruz; romeriorodrigues95@hotmail.com; claudener@gmail.com.

Correspondence author: victorjunobiomedico@gmail.com

Abstract

Natural compounds extracted from plants represent valuable sources for the maintenance of human health. Among the various substances of plant origin, we highlight lectins, which participate in the defense against microorganisms and in signaling the damage caused by pathogens to the cell surface and/or intracellular in plants. This study aims to analyze the potential of lectins ConA and ConM, extracted from seeds of *Canavalia ensiformis* (L.) DC and *Canavalia rosea* (Sw.) DC, respectively, isolated and combined in subinhibitory concentration to the antifungal fluconazole against standard strains of *Candida albicans* and *Candida tropicalis*, as well as their effect on the morphological transition of the species. The antimicrobial tests were performed by microdilution against *Candida* spp. ConA and ConM solutions were microdiluted at concentrations ranging from 0.5 to 512 µg/mL. The test to verify the combined lectin/fluconazole effect was performed by microdilution, however, using subinhibitory concentrations of lectins and with antifungal ranging from 0.5 to 512 µg/mL. The ability to inhibit the morphological transition of *Candida* spp. was evaluated by microcultivation in a moist chamber. The results of the minimum inhibitory concentration revealed no antifungal activity against the tested strains (≥ 512 µg/mL). However, lectins modulated the action of fluconazole, reducing the IC_{50} of the drug against *C. albicans* by

more than 50%. Lectins were also able to discretely modulate the morphological transition of the tested strains, showing that these proteins may play an important role in processes linked to pleomorphism in *Candida* species.

Keywords: Plant Proteins; Opportunistic Pathogens; Antifungal.

1. Introduction

Over the years, the different factors linked to the diffusion and spread of invasive candidiasis have gradually changed worldwide, the main reason being the emergence of various *Candida* species [1], where there are already more than 200 yeasts of this genus [2], in which the pathogens *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and the most recent, *Candida Auris* [3] are included.

The incidence of these microorganisms varies between 2 and 14 per 100,000 people worldwide, and according to population surveys, at least 10-20% of those infected die. This problem has been observed both in developing and developed countries, reaching a high mortality rate globally [4]. In Brazil, this number has been increasing every year. In 2017, 62 deaths from candidiasis were recorded, reaching 92 in 2019 adding all regions of the country [5].

It is remarkable that, although science and medicine have developed over the years, these microorganisms pose an increasing threat. Species such as *C. Albicans* and *C. tropicalis* have characteristics that make them difficult pathogens to eradicate, such as the production of biofilms and their plasticity regarding morphology. Under favorable conditions, they can undergo a transition from their yeast form to hyphae, a phenomenon known as cellular morphogenesis, which is often associated with virulence, aiding in colonization, tissue invasion, and immune system evasion [6-8].

Moreover, some of their characteristics, such as the presence of efflux pumps and ability to form biofilm [6] lead to increased fungal resistance to drugs, with the class of antifungal agents having a limited number of drugs with different fungal components as targets [9]. The plasticity of fungal cells of the *Candida* genus has often been associated with their increased virulence, and for this reason, there is great interest in researching compounds capable of inhibiting these factors, being more favorable to reduce the virulence of the fungus,

allowing the host immune system to eliminate the pathogen, an effective strategy in response to antifungal resistance [6,7].

Many of these responses can be found in nature, where living things must respond promptly to environmental stress, whether physical, chemical, or biological in nature, a way to confer protection against predators and/or pathogens. As part of these defense mechanisms are lectins, which can recognize and interact with carbohydrates present in the membranes of cells and microorganisms, promoting cell signaling that can confer greater resistance to pathogens in animals and plants [10,11]. Legumes produce seeds rich in bioactive lectins, proteins that assist them in their defense, the main representative of these lectins being ConA, extracted from *Canavalia ensiformis* [12].

Lectins make reversible binding to specific free carbohydrates or cell wall polysaccharides and cell membrane glycoconjugates [13,14], and due to this binding to carbohydrates, also present in the cell wall or cell membranes of several microorganisms, they perform a variety of biological activities [15].

Considering the promising antimicrobial effect of lectins, including already reported in the literature against viruses, [16], bacteria [17], protozoa [18], and fungi [19] is that this study seeks to analyze the antifungal potential, intrinsic and combined to drug, of the lectins ConA and ConM, extracted from seeds of *Canavalia ensiformis* and *Canavalia Rosea* (cited in the literature and lectin database by the synonymy *Canavalia Maritima* Thouars) respectively, against standard strains of *C. Albicans* and *C. tropicalis* strains, respectively, as well as their effect on inhibiting a virulence factor of the genus, the morphological transition.

2. Material and Methods.

2.1 Used Strains and preparation of the materials.

Seeds were peeled and macerated until obtaining a fine powder appropriate for the protein extraction process. The purification process was performed by affinity chromatography in a Sephadex G-50 matrix, using a spectrophotometer to measure the absorbance at a wavelength of 280 nm, to monitor the concentration of the proteins obtained,

as described by Ramos et al. [20]. The flour obtained from the seeds was treated with specific solvents and dialyzed with distilled water, a process described by Teixeira et al. [21].

The fungal strains used were *C. Albicans* (INCQS 40006 - ATCC 10231) and *C. tropicalis* (INCQS 40042 - ATCC 13803), obtained from the Culture Collection of the National Institute Quality Control in Health (INCQS), Oswaldo Cruz Foundation. Aliquots were collected from them using a platinum loop and then solubilized in saline solution until a turbidity standard equivalent to 0.5 on the McFarland scale was obtained, except for the lectin effect test on the morphological transition of *Candida* spp, where the strains were inoculated in Yeast Extract Peptone Dextrose (YPD) medium - interlab, enriched with 5% serum and subsequently plated on Sabouraud Dextrose Agar (SDA) - KASVI.

The antifungal of choice was Fluconazole - isofarma® at a concentration of 0.2%, equivalent to 2 mg/mL. For the tests, fluconazole was diluted in sterile distilled water to the matrix concentration of 1024 µg/mL. The lectins went through the same dilution process, taking care to homogenize the solution gently, without using a vortex. All culture media were weighed on precision scales and prepared according to the manufacturer, diluted in distilled water, except the Sabouraud Dextrose Broth - ISOFAR medium, which was prepared doubly concentrated.

2.2 Verification of intrinsic antifungal effect of lectin and fluconazole - viability curve and determination of the inhibitory concentration.

Flat bottom 96-well plates were used to perform the broth microdilution method as described by Javadpour et al. [22]. A serial dilution process was performed, and the concentrations of lectins and fluconazole ranged from 512 to 0.5 µg/mL. The last wells of the plates were reserved for growth control of the microorganisms. Dilution controls of the products (using saline solution instead of inoculum) and sterility of the medium were also conducted. The plates were incubated at 37 °C for 24 hours. After this period, the growth of microorganisms was observed, as evidenced by the presence of turbid medium on the plates. For quantitative tests, the plates were taken for reading in an ELISA spectrophotometer apparatus (Termoplate®), with a wavelength of 630 nm [23]. The results provided the minimum inhibitory concentration (MIC) of the tested products, as well as the IC_{50}. The tests were performed in quadruplicate.

2.3 Determination of minimum fungicidal concentration.

To determine whether the products alone and in combination were able to affect the viability of *Candida* cells, the minimum fungicidal concentration (MFC) was determined. Using a pipette, 5 µL from each well of the MIC test plate (except for the sterility control and product dilution) were transferred to Petri dishes containing SDA medium, distributing according to the guide card at the bottom of the plate. After 24 h of incubation, the plates were inspected for any *Candida* colony formation [24]. The concentration at which there was no growth of fungal colonies will be considered the MFC of the product.

2.4 Verification of the antifungal effect of the lectin/fluconazole combination - viability curve and determination of the inhibitory concentration.

To execute the test, the lectins were used in subinhibitory concentration (MC/8, where MC is the Matrix Concentration of the evaluated products) according to the methodology used by Coutinho et al. [25], with minor modifications. The commercial drug (fluconazole) was used in the drug combination test in serial dilution at concentrations ranging from 512 to 0.5 µg/mL. The last wells of the plates were reserved for growth control of the microorganisms. Dilution controls of the product in combination with fluconazole were also performed, in addition to the sterility control of the medium. The whole test was performed in quadruplicate and the plates were incubated at 37 °C for 24 hours. The reading was performed in an ELISA spectrophotometry apparatus (Termoplate®) with a wavelength of 630 nm and the results will be used to obtain a cell viability curve [23].

2.5 Effect of lectins on the morphological transition of *Candida* spp.

Sterile micromorphological chamber slides were prepared for yeast observation. Three milliliters of Potato Dextrose Agar (PDA) medium - Becton Dickinson & Co. USA, depleted by dilution were added to the chambers, containing the natural product concentrations (lectins) MC and MC/4, one concentration, which according to Houghton et al. [26], it is clinically relevant (256 µg/mL) and another which would be the matrix concentration (1024

μg/mL). Aliquots of the inocula were taken from the Petri dishes to make two parallel streaks on the solid medium, which were then covered with a sterile coverslip. The chambers were incubated for 24 h (37 °C) and inspected under a light microscope with a 10x objective. A camera was attached to the L-2000I-TRINO/6633 - Bioval microscope to capture images. A yeast growth control (hyphae stimulated by depletion) was performed, as well as a control with the antifungal drug fluconazole for comparison purposes. The assays were produced according to Sidrim & Rocha [27] and Mendes [28], with some modifications.

2.6 Statistical analysis.

Statistical analysis was performed using GraphPad Prism 9 software (Free trial GraphPad Software, Inc., La Jolla, CA, USA). Data were expressed as geometric means. Statistical significance was assessed with a two-way ANOVA test followed by Bonferroni post hoc test (where $p < 0.05$ and $p < 0.0001$ are considered significant and $p > 0.05$ not significant).

To evaluate virulence, the total area of the streaks was measured using ToupView software (ToupTek Photonics® - China), checking the areas where there was hyphae growth. Next, measurements were taken of all the hyphae filaments found in five random areas in each streak, for each concentration. The average length of the hyphae filaments was taken and analyzed by ANOVA followed by Bonferroni correction for multiple comparisons, comparing the values according to the concentration of the product [29].

3. Results

3.1 Antifungal tests.

After the 24-hour period in the oven, the 96-well plates were removed and analyzed for turbidity of the medium. The results revealed fungal growth in all wells. After analysis in ELISA on the 630 nm filter to determine the Minimum Inhibitory Concentration (MIC), inhibition of fungal growth could not be observed in any of the concentrations tested (Table 1), both for lectins and fluconazole, although the latter achieved slightly better values, but

without significance, indicating that, at these concentrations, lectins do not present antifungal activity when used by themselves. Due to the absence of significant inhibition of lectins throughout the concentrations evaluated, the MFC was performed only with the antifungal fluconazole, revealing a fungistatic action at the concentration of 512 µg/mL and no fungicidal effect (data not shown).

Table 1. Inhibitory Concentration of 50% of microorganisms (µg/mL) by Lectins and Fluconazole alone and combined.

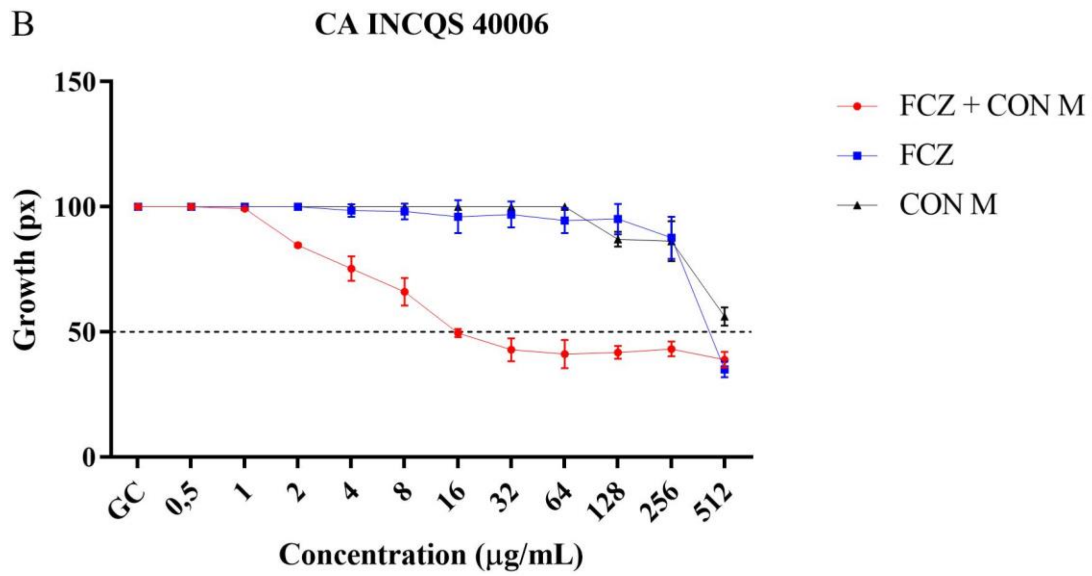
Microorganismos	**IC_{50} of microorganisms for evaluated compounds**				
	CON A	CON M	FCZ	CON A + FCZ	CON M + FCZ
CA INCQS 40006	539.1	607.2	432.2	19.6	17.6
CT INCQS 40042	502.5	405.5	275.1	567.9	575.9

FCZ: Fluconazole; CA: *Candida albicans*; CT: *Candida tropicalis*; CON A: lectin obtained from *Canavalia ensiformis*; CON M: lectin obtained from *Canavalia rosea*; INCQS: National Institute Quality Control in Health.

3.2 *Canavalia ensiformis* and *Canavalia rosea* lectins modulate the action of fluconazole

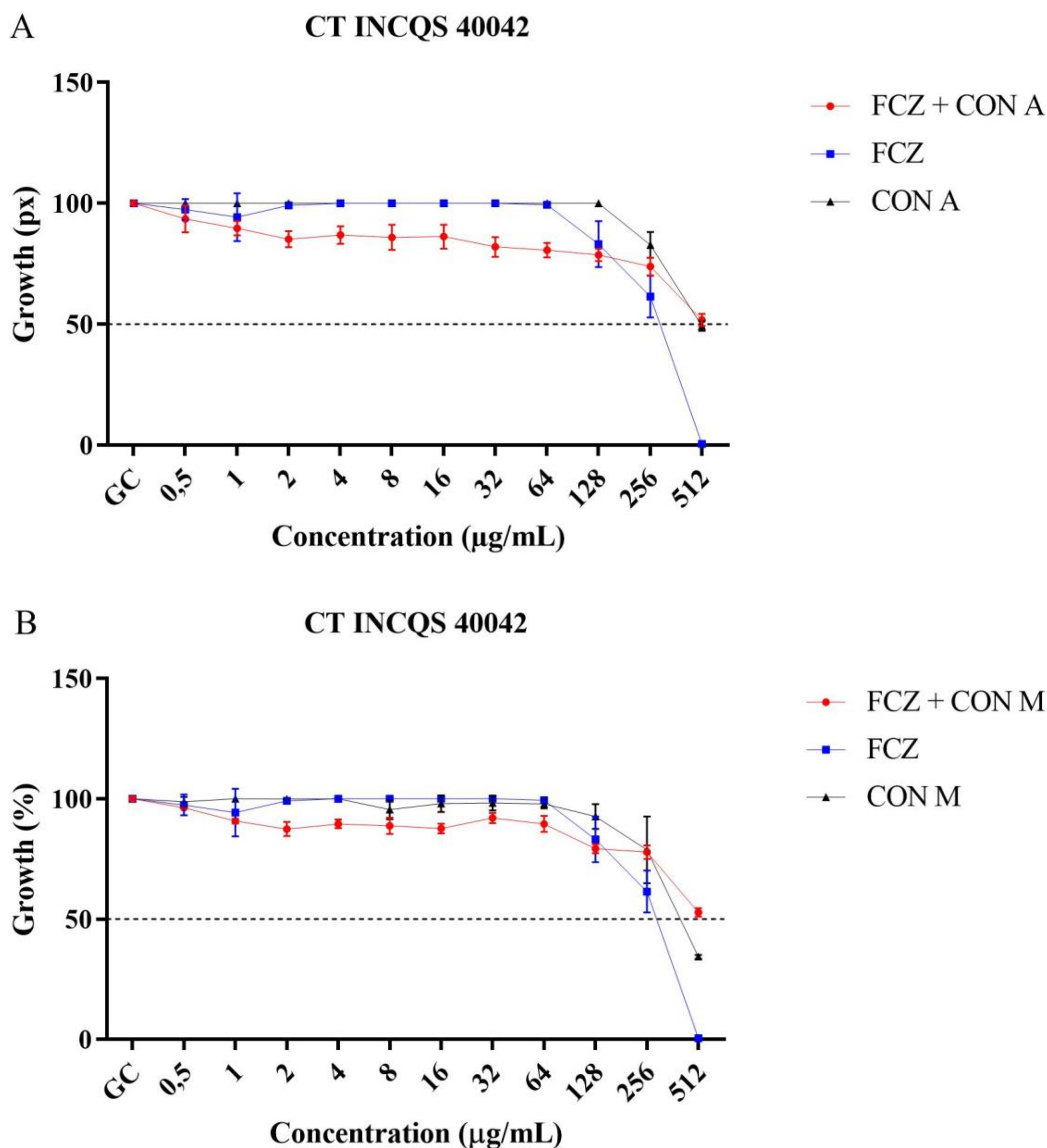
The association of lectins at subinhibitory concentrations with fluconazole increased the activity of the antifungal against *C. albicans* 40006, reducing by more than 50% the amount of antifungal required to inhibit 50% of the fungal growth of *C. albicans* at concentrations ranging from 16 to 512 µg/mL, while for *C. tropicalis* the inhibition was slight at first and then stagnated, indicating that the yeasts adapted to the combined treatment (Figure 1 and 2).

Figure 1. Modulation of the antifungal effect of fluconazole complexed with Con A (A) and Con M (B) against *C. albicans* 40006.



CA INCQS 40006: *Candida albicans* ATCC 10231; Con A: *Canavalia ensiformis* lectin; Con M; *Canavalia rosea* lectin; FCZ: Fluconazole; FCZ + Con A: Fluconazole + *Canavalia ensiformis* lectin; FCZ + Con M: Fluconazole + *Canavalia rosea* lectin; GC: Growth control.

Figure 2. Modulation of the antifungal effect of fluconazole complexed with Con A (A) and Con M (B) against *C. tropicalis* 40042.



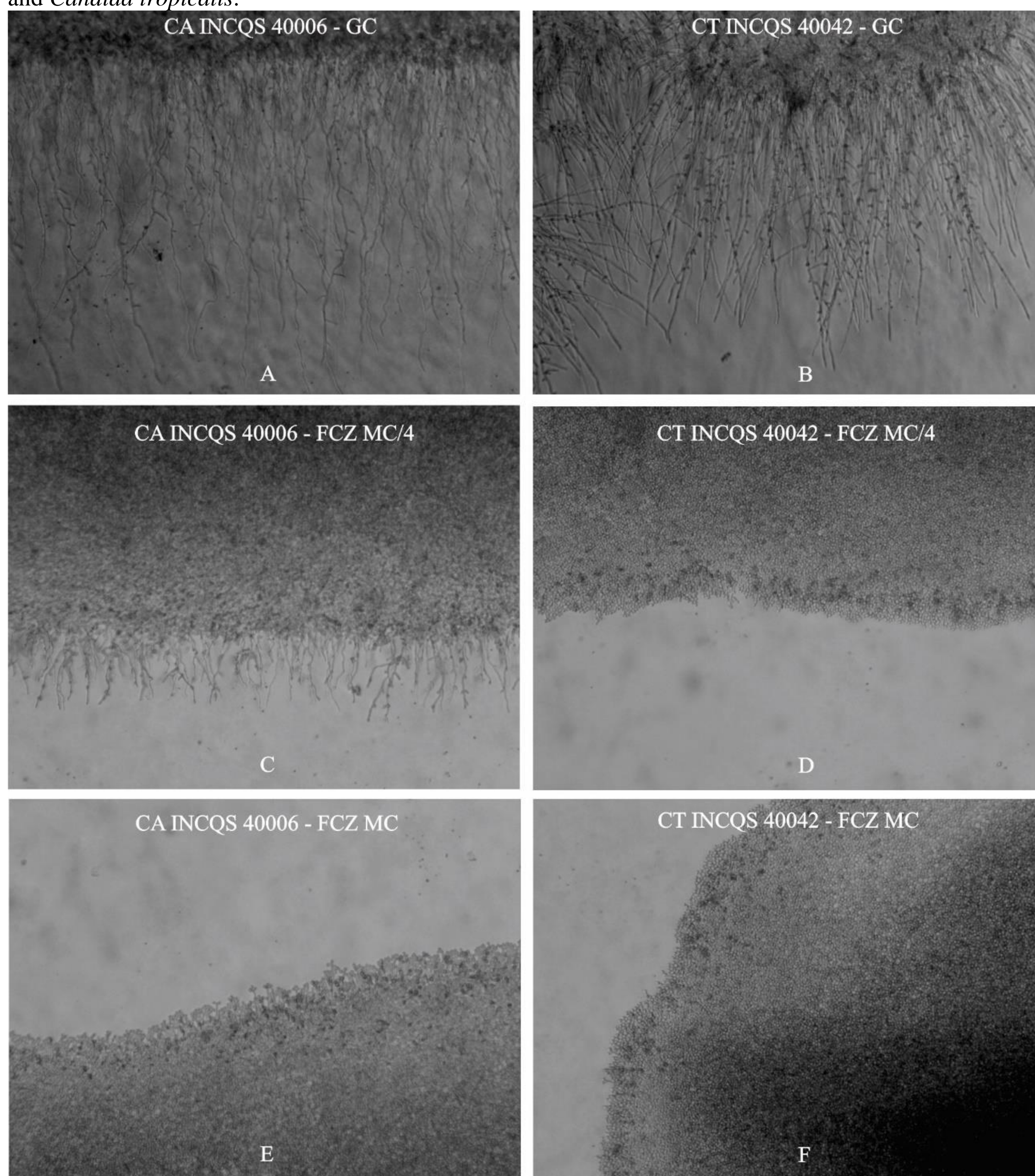
CT INCQS 40042: *Candida tropicalis* ATCC 13803; Con A: *Canavalia ensiformis* lectin; Con M; *Canavalia rosea* lectin; FCZ: Fluconazole; FCZ + Con A: Fluconazole + *Canavalia ensiformis* lectin; FCZ + Con M: Fluconazole + *Canavalia rosea* lectin. GC: growth control.

3.3 Action of *Canavalia ensiformis* and *Canavalia rosea* lectins on the morphological transition of *Candida* spp.

The morphological transition from yeast to filamentous in the growth controls with *C. albicans* and *C. tropicalis* could be observed at all edges of the streaks (100% growth). Lectin solutions were used at MC and MC/4 concentrations (1024 µg/mL and 256 µg/mL, respectively), and growth was observed at all edges of the streaks, except at the MC/4

concentration of ConM against *C. albicans* (55.6% growth). In the controls with fluconazole used in the same concentrations, a 100% inhibition in the morphological transition of the fungi was observed, with a discrete growth only in MC/4 of *C. albicans*, which showed 100% growth in all edges of the streak, but with a reduction in the length of the hyphae of more 78% compared to the growth control (Figure 3).

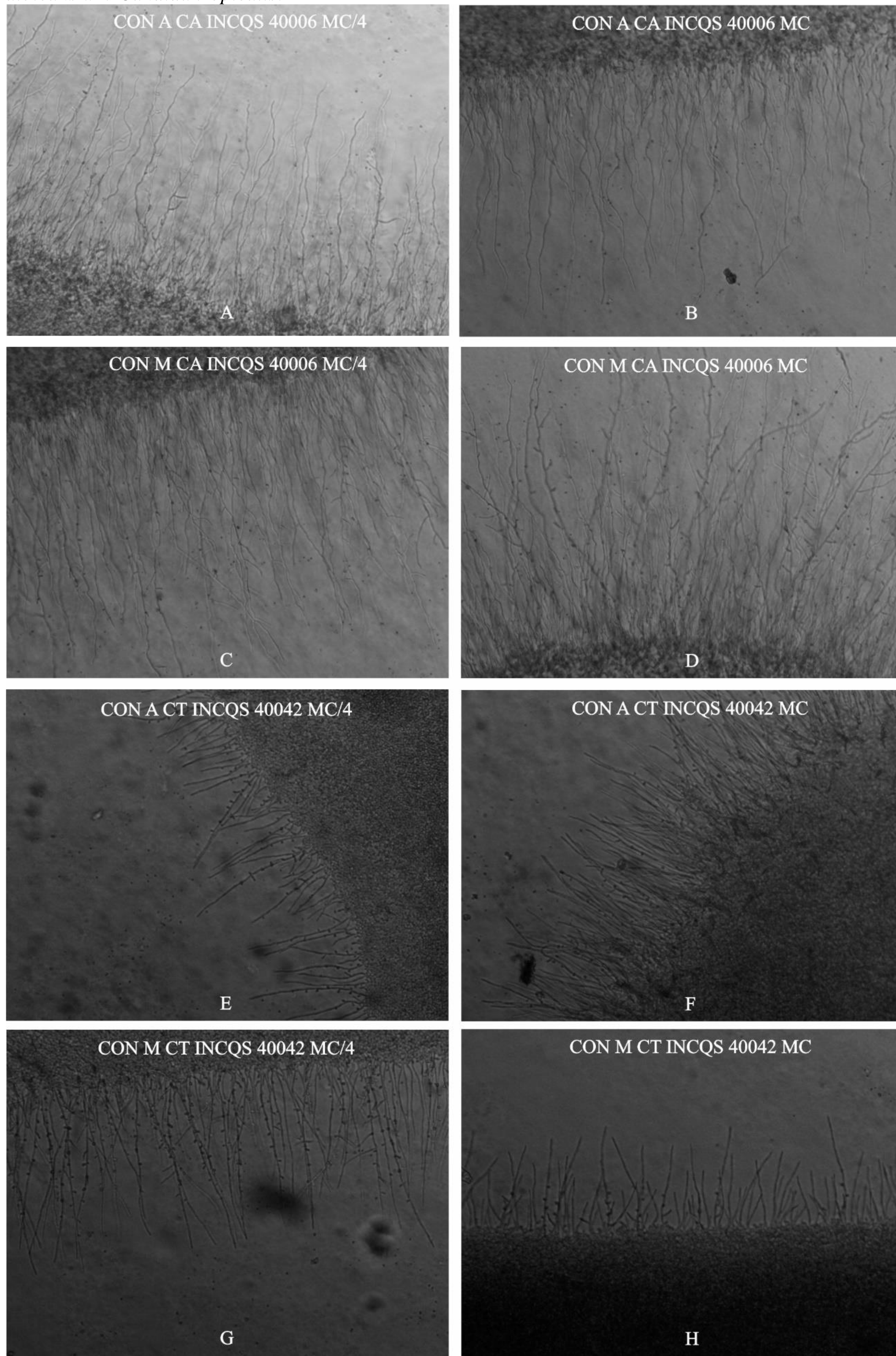
Figure 3: Images of growth control and fluconazole effect on the morphological transition of *Candida albicans* and *Candida tropicalis*.



Growth control and control with fluconazole on the morphology of *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803 at concentrations of MC/4 and MC (256 and 1024 µg/mL respectively). Images observed under an optical microscope on a 10x objective. GC: Growth control; FCZ: Fluconazole Control; INCQS: National Institute Quality Control in Health.

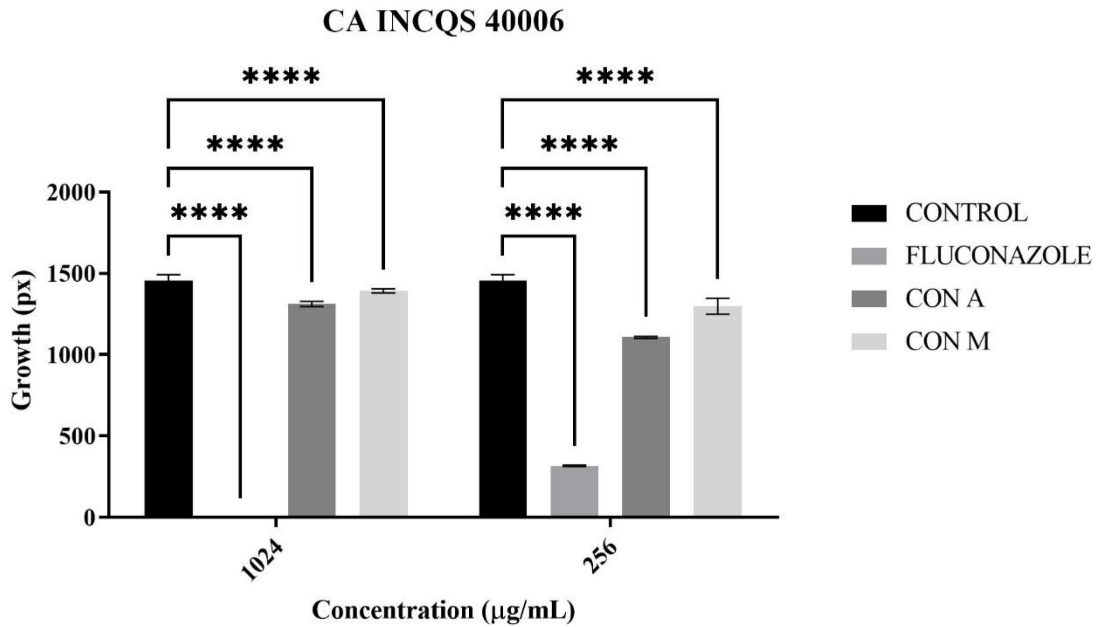
The results expressed in Figure 4 show that, compared to the growth control, the products were statistically relevant, without completely inhibiting the transition, but reducing the final length of the hyphae. However, it was observed that at lower concentrations of MC/4, the results were more promising than at higher concentrations of the product (MC), except for the MC of ConM against *C. tropicalis*, which showed a considerable reduction of 42.6% in hyphae length, a value almost three times greater than that presented in MC/4 (14.7%) (Figures 5 and 6).

Figure 4: Images of the action of the ConA and ConM lectins in the morphological transition of *Candida albicans* and *Candida tropicalis*.



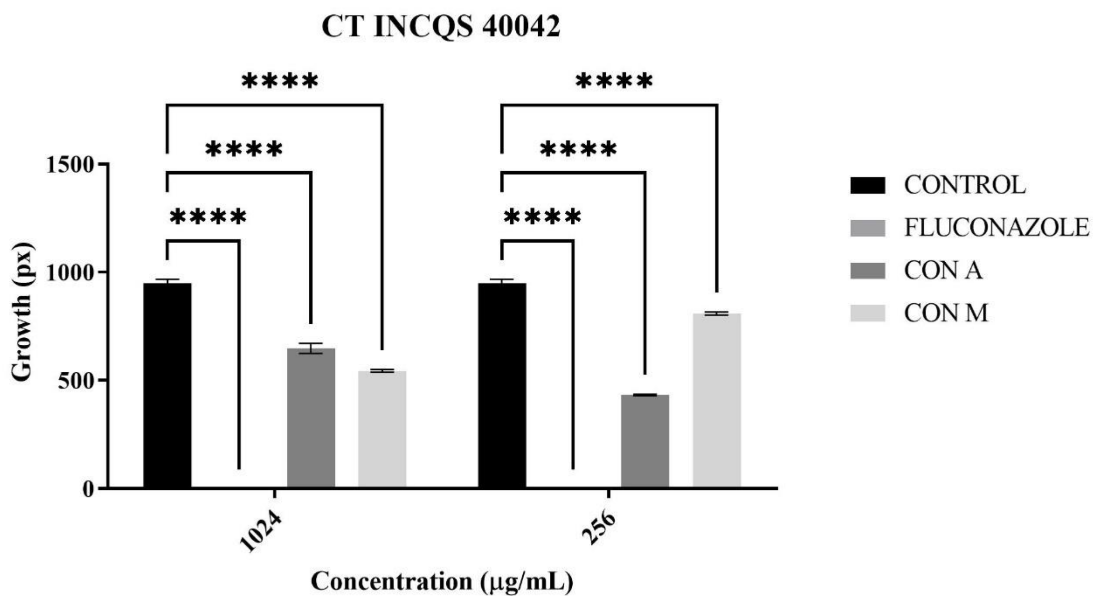
Effect of ConA and ConM lectins from *Canavalia ensiformis* and *Canavalia rosea* on the morphology of *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803 at concentrations of MC/4 and MC (256 and 1024 µg/mL respectively). Images observed under an optical microscope on a 10x objective.

Figure 5: Graphs of the effect on the morphological transition of ConA and ConM lectins against *Candida albicans* compared to growth control.



CA INCQS 40006: *Candida albicans* ATCC 10231; Con A: *Canavalia ensiformis* lectin; Con M; *Canavalia rosea* lectin.

Figure 6: Graphs of the effect on the morphological transition of ConA and ConM lectins against *Candida tropicalis* compared to growth control.



CT 40042: *Candida tropicalis* ATCC 13803; Con A: *Canavalia ensiformis* lectin; Con M; *Canavalia rosea* lectin.

4. Discussion

In research conducted by Klafke et al. [30], lectins extracted from seeds of *Canavalia brasiliensis* (Conbr), *Mucuna pruriens*, *Clitoria fairchildiana*, *Dioclea virgate*, and *Bauhinia variegata* (BVL) were tested against isolates of *C. albicans*, *C. tropicalis*, *Candida parapsilosis*, *Cryptococcus gattii*, *Cryptococcus neoformans*, *Malassezia pachydermatis*, *Rhodotorula* sp. and *Trichosporon* sp, with significant activity only against *C. parapsilosis* at concentrations ranging from 0.97 to 125 µg/mL. In our research, the lectins extracted from *Canavalia ensiformis* (ConA) and *Canavalia rosea* (ConM) seeds were tested against standard strains of *C. albicans* and *C. tropicalis*, is not showing antifungal activity at the concentrations tested.

It is important to note that both ConA and ConM lectins have an affinity for mannose and glucose [15], components of the cell wall, but lack affinity for N-acetylglucosamine, which constitutes the chitin, a homopolysaccharide found in insect exoskeletons, fungal cell walls, nematode eggs, marine diatoms, and the shells of crustaceans and zooplankton [31]. Lectins that bind to chitin seem to have more promising fungicidal activity. This is the case of *Phaseolus vulgaris* lectin (PHA), which inhibited the growth of *Coprinus comatus*, *Fusarium oxysporum*, and *Rhizoctonia solani* [32]. According to Ye et al. [32], the red bean lectin showed some degree of structural similarity to chitinases. This may account in part for its antifungal activity because chitinases are known to adversely affect hyphae growth, leading to cell wall disruption, the release of chitin oligosaccharides from the cell wall, and cytoplasm leakage.

Another chitin-specific lectin is extracted from *Molinga oleifera* seed (WSMoL), which showed fungicidal activity at concentrations of 80, 20, 40 and 80 µg/mL for *C. albicans*, *C. glabrata*, *Candida krusei* and *C. parapsilosis*, respectively [33]. The absence of antifungal activity of ConA and ConM, compared to the activities reported in the literature for other lectins, may be due to the absence of the interaction of ConA and ConM with chitin, and the variation in cell wall carbohydrate composition among fungal species [30].

Synergistic activity was observed when subinhibitory concentrations of ConA and ConM lectins associated with fluconazole were used, reducing the IC_{50} of the antifungal by more than 50% against *C. albicans*. Combination drug therapy is commonly recommended in the treatment of infectious agents in intensive care units, considering that not all pathogens are

sensitive to monotherapy [34]. In agreement with the data were obtained, the research conducted by Ferreira et al. [35] also showed antifungal activity in the combination of ApuL lectin, extracted from *Alpinia purpurata*, with fluconazole, reducing the IC_{50} by 8-fold. Santos et al. [36] proposes that lectins act by delivering the drug to target cells through carbohydrate recognition in the membrane, which leads to drug release, facilitating the entry of the antifungal into the microbial cytoplasm. The lack of activity in combination therapy against *C. tropicalis* may be due to the high resistance to azolic compounds that this species has, specifically to fluconazole [37].

It is interesting to note that although fluconazole by itself did not show positive results at the concentrations tested in MIC, it was able to completely inhibit the dimorphism presented by the species tested at the higher concentrations of the product. This occurs because ergosterol biosynthesis is the target of the class of azole antifungals, which suggests a relationship between components of the ergosterol biosynthesis pathway with fungal filamentation [7].

Studies have reported that many small molecules are able to modulate morphogenesis in *C. albicans* and that these acts directly or indirectly on the pathways of morphological transition from the yeast to filamentous phase [6, 38]. The most reported example in the literature is farnesol, a quorum sensing molecule that is produced by fungi and can block filamentous growth by acting on the cAMP-PKA pathway and other regulatory factors responsible for hyphae formation [6,38]. It was shown that *C. albicans* cells treated with azoles produced high levels of farnesol, a direct consequence of the sterol biosynthetic intermediate accumulation that indirectly stimulates the overproduction of farnesol. [39].

Although the mechanisms by which lectins act on fungal cell walls is not completely elucidated, there are reports of these proteins having activity in morphological transition and antibiofilm, a factor directly linked to dimorphism in species such as *C. albicans*. This is the case of lectin extracted from *Helianthus annuus* (Helja) seeds, which inhibited the morphological change from yeast to filamentous in *C. albicans*, an important attribute for the pathogenicity of this microorganism. Helja also inhibited the development of biofilm formed by *C. albicans* by 40% when used in the early phases (adhesion to surface) of biofilm formation and by 30% in the intermediate phase (attached and secreting extracellular matrix components) [40].

In the present study, it is possible to see the confirmation of what has been observed in the literature that lectins can act on the morphological transition of *C. albicans* and *C. tropicalis*, having presented statistically significant results for MC and MC/4 concentrations of ConA and ConM. However, the results were better when observed at MC/4 and this may be related to the production of reactive oxygen species (ROS). Although these compounds can be toxic to many microorganisms, recent studies have shown that at subtoxic levels, H_2O_2 promoted hyphae development and that C. albicans may itself generate these ROS, contributing to its morphogenesis, a condition in response to a stress stimulus [41].

It is also possible that this result is related to the affinity of these lectins for polysaccharides that make up the cell wall of *Candida* spp. such as mannose, glucose, and chitin [42]. This type of affinity may act competitively to other ligands of these polysaccharides. Tunicamycin, for example, has a blocking effect on the formation of N-acetylglucosamine, significantly altering the glycosylation process of proteins [43]. According to Kadosh [7], these findings suggest that polysaccharide remodeling on the cell surface of *C. albicans* may play a crucial role in its morphogenesis.

5. Conclusion

Although ConA and ConM did not show antifungal activity by themselves, they showed an important synergistic action with the antifungal drug fluconazole, mainly against *C. albicans*. They were also able to reduce the development of hyphae during the morphological transition of *C. albicans* and *C. tropicalis*. It is necessary to elucidate the mechanisms by which lectins can act and to complement the results obtained with antibiofilm tests since this virulence factor is directly linked to the fungal dimorphism of these species. It is interesting to note that the best results for the morphological transition test were obtained from the lowest concentrations. This may represent an adaptation of the fungus by the level of stress exerted by the high concentration of the substances, which may have led to the expression of genes that act directly or indirectly to promote the filamentous form. More specific tests to elucidate these mechanisms should be carried out and, given the growing interest of medicine in obtaining new substances with different pharmacological targets, these lectins may represent great importance for the development of new drugs.

Declarations

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CAPÍTULO IV – CONSIDERAÇÕES FINAIS

4.1. Discussão Geral

Fungos do gênero *Candida* são responsáveis pela candidíase invasiva, que acomete pessoas em todo mundo, levando a óbito até 20% dos acometidos. Como relatado no capítulo II, existem mais de 200 espécies desse gênero, onde estão incluídos os patógenos *Candida albicans*, *Candida tropicalis*, *Candida krusei* e *Candida parapsilosis*, e o mais recente, *Candida Auris*.

Os fatores que tornam esses micro-organismos tão bem-sucedidos em sua sobrevivência a ambientes hostis, como o corpo humano, estão ligados aos seus fatores de virulência. No capítulo I e II foi discutido a importância da plasticidade que esses fungos apresentam, sendo capazes de alterar sua forma leveduriforme para pseudohifas e hifas verdadeiras. Esse processo permite a evasão do sistema imune e invasão dos tecidos adjacentes, quando em um hospedeiro. a formação de hifas também está ligada a ligação do micro-organismo a uma superfície, o que dá início ao desenvolvimento de biofilmes, uma espécie de comunidade microbiana que se comunicam através de moléculas de *Quorum Sensing* e garantem a resistência a estresse físico e químico.

Esses fatores de virulência, ligados ao aparecimento recente de cepas multirresistentes a antifúngicos, tem levado cientistas que buscarem alternativas aos tratamentos vigentes. As lectinas, biomoléculas capazes de interagir com carboidratos específicos, tem estado em evidência, e representam grande potencial para o desenvolvimento de futuros medicamentos. De fato, sua característica as torna interessantes por serem capazes de interagir com componentes presentes nas membranas plasmáticas de células e micro-organismos.

De acordo com as pesquisas realizadas e apresentadas no capítulo II, essas lectinas podem ser extraídas de diversas fontes, como plantas, animais e até micro-organismos. Esses metabolitos primários apresentam diversas bioatividades. os mecanismos de aglutinação parecem ocorrer frequentemente e podem levar a uma redução na motilidade de micro-organismos ou mesmo sua adesão a uma superfície. Quando organismos são infectados por um patógeno, elas podem ser produzidas de modo a promover sinalização e ativação de macrófagos, modulando a resposta imune.

A depender das características de alguns medicamentos, as lectinas podem atuar de forma sinérgica, tornando os microrganismos mais susceptíveis as drogas comumente utilizadas para tratar algumas doenças, como a candidíase. Vários relatos na literatura podem ser encontrados no capítulo II com essa atividade. Além disso, no capítulo III foram realizados testes *in vitro* que mostram as capacidades moduladoras de lectinas ConA e ConM, extraídas de sementes de *Canavalia*, reduzindo a IC_{50} do antifúngico fluconazol frente as espécies de *Candida albicans*.

Outro resultado apresentado pelas lectinas de Canavalia foi a redução discreta da transição morfológica das espécies de *Candida*, estando de acordo com os diversos relatos de literatura apresentados no capítulo II, que mostram lectinas capazes de inibir a germinação de esporos de fungos, bem como reduzir a infectividade desses patógenos ao inibir fatores iligados ao seu dimorfismo. Embora as lectinas ConA e ConM não tenham sido ativas quando usadas sozinhas contra esses micro-organismos, existem vários relatos na literatura que provam que essas lectinas podem inibir o desenvolvimento não só de fungos, mas também de bactérias e protozoários.

Diversos fatores podem estar ligados a falta de atividade para os fungos testados no capítulo III. É possível que a composição química das paredes fúngicas apresentem características distintas das cepas relatadas na literatura. Além disso, os resultados obtidos nos testes da transição morfológica sugerem uma adaptação do fungo, mediante ao estresse gerado pelo contato com a lectina, que leva a resistência desses patógenos às lectinas.

4.2. Conclusões Gerais

As lectinas representam biomoléculas com múltiplas potencialidades, sendo capazes de inibir o desenvolvimento de micro-organismos por diferentes mecanismos. A maior parte dos estudos apresentam testes de afinidade com carboidratos específicos, que podem explicar apenas parcialmente as bioatividades relatadas para as lectinas. Essa interação pode levar a inibição do crescimento dos micro-organismos, bem como a inibição da formação de biofilmes, ou mesmo estimulando os mecanismos de defesa em testes *in vivo*. As lectinas podem ser usadas para modular a ação de drogas antimicrobianas, e dessa forma representar uma alternativa a terapia combinatória de medicamentos.

Embora os resultados sejam promissores, é necessária a validação de alguns mecanismos de ação, que não estão bem elucidados, como a modulação de lectinas

complexadas a fármacos. Além disso, testes de afinidade mais específicos, como ligação direta a quitina, devem ser realizados a fim de justificar algumas as atividades relatadas que depende da interação com componentes da membrana, como aglutinação e inibição de adesão.

4.3. Perspectivas e investigações futuras

Diante dos resultados apresentados no capítulo III, é interessante que ocorra a repetição de alguns testes utilizando meios de cultura enriquecidos com outros nutrientes diferentes da glicose, pois as lectinas ConA e ConM tem afinidade com esse monossacarídeo, o que poderia levar a competição nos sítios de ligação a carboidrato, impedindo a interação da proteína com o micro-organismo, uma vez que a literatura tenha relatado muito bem a atividade antifúngica de lectinas ligantes de glicose e manose.

Com o promissor resultado da terapia combinada com o antifúngico fluconazol, se faz necessário a utilização de outros antifúngicos juntamente com as lectinas, como o cetoconazol e itraconazol, de modo a conduzir pesquisas futuras com cepas multirresistentes. É importante notar que, devido a afinidade a carboidrato que essas lectinas possuem, é possível que essas proteínas apresentem algum nível de citotoxicidade. Testes de viabilidade celular, através de ensaios colorimétricos, como o MTT, são ideais para triar moléculas que possuem efeitos na proliferação celular ou efeitos tóxicos que eventualmente podem conduzi-las a morte.

Considerando que *Candida albicans* pode se adaptar a uma condição de estresse e produzir espécies reativas de oxigênio que promovem a sua transição entre a fase leveduriforme e hifal, é importante avaliar as capacidades antioxidantes das lectinas ConA e ConM. O método do DPPH é interessante por ser considerado de fácil realização, preciso e rápido, sendo adequado determinação da capacidade antioxidante de substâncias puras, como as lectinas.

Ao considerar a importância dos resultados na redução do comprimento das hifas relatados no capítulo III, testes antibiofilme se fazem necessários. Os processos ligados ao desenvolvimento de hifas estão diretamente ligados a fase inicial de desenvolvimento de biofilmes fúngicos das espécies de *Candida*, como a adesão a uma superfície, e por isso, é possível que as lectinas ConA e ConM apresentem atividade antibiofilme, como muitas lectinas relatadas na literatura.