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PURIFICAÇÃO, CARACTERIZAÇÃO E AVALIAÇÃO DA ATIVIDADE FÚNGICA DA LECTINA DE CLADÔNIAS DE *Opuntia ficus indica*

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ORIENTADORA: Profª. Drª. PATRÍCIA MARIA GUEDES PAIVA
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GISELLY MARIA DE SÁ SANTANA

"Purificação, caracterização e avaliação da atividade fúngica da lectina de cladônia de *Opuntia ficus indica*"

Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco

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“Precisamos ter paciência para poder fazer a vontade de Deus e receber o que ele promete.” Hb 10.36

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LISTA DE ABREVIATURAS

AH – Atividade Hemaglutinante

CE20% - crude extract 20%

Con A: Concanavalina A

EB20% - Extrato Bruto 20%

EDTA - Ácido etilenodiaminotetraacético

HA - Hemagglutinating activity

OfiL – lectina de *Opuntia ficus indica*

SDS –PAGE: Eletroforese em gel de poliacrilamida contendo sulfato sódico de dodecila

SHA - Specific Hemagglutinating Activity

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RESUMO

Lectinas são proteínas hemaglutinantes que se ligam a carboidratos, estas proteínas possuem propriedades biológicas, incluindo atividade antimicrobiana. O objetivo deste trabalho foi o isolamento da lectina de cladônias de *Opuntia ficus indica* (OfiL) e a avaliação de sua atividade antifúngica. A atividade hemaglutinante (AH) do extrato bruto de cladônias (EB20%) foi avaliada utilizando eritrócitos de coelho, galinha e humanos. A AH foi avaliada na presença de carboidratos e íons e em diferentes temperaturas e valores de pH. OfiL foi isolada por cromatrografia em coluna de quitina e Sephadex G-25. OfiL foi submetida a eletroforese em gel de poliacrilamida em condições desnaturantes (SDS-PAGE). EB20% e OfiL foi avaliada quanto a atividade antifúngica usando espécies de fungos de *Colletotrichum gloeosporioides*, *Candida albicans*, *Fusarium descencelulare*, *F. lateritium*, *F. moniliforme*, *F. oxysporum* and *F. solani*. A AH de EB20% foi detectada com eritrócitos de coelho, galinha e humanos tipo A e O. A AH foi elevada em pH 5,0, termostável, inibida por glicoproteínas e estimulada com Ca^{2+} ou Mg^{2+}. OfiL foi purificada em cromatografia em coluna de quitina e Sephadex G-25, resolvendo em um única banda protéica de 8.4 kDa em SDS-PAGE. EB20% e OfiL apresentaram atividade antifúngica frente a todos os fungos testados. A atividade da lectina foi mais ativa para *C. albicans*. A inibição da AH por glicoproteínas revelou a presença de lectina em cladônias de *O. ficus indica*. Atividade antifúngica de extrato de cladônias e OfiL indica o seu potencial biotecnológico.

Palavras chaves: lectina, *Opuntia ficus indica*, cladônias, atividade antifúngica.

ABSTRACT

Lectins are hemagglutinating proteins with carbohydrate binding sites; these proteins have biological properties including antimicrobial activity. The aims of this work were the isolation of *Opuntia ficus indica* lectin (OfíL) and evaluation of its antifungal activity. Hemagglutinating activity (HA) of cladode crude extract (CE20%) was evaluated using rabbit, chicken or human erythrocytes. The HA assay was also made in presence of carbohydrates or ions and at different temperatures and pH values. OfiL was isolated by chromatography of CE20% on chitin or Sephadex G-25 columns. OfiL was submitted to polyacrylamide gel electrophoresis for denatured proteins (SDS-PAGE). CE20% and OfiL were evaluated for antifungal activity using *Colletotrichum gloeosporioides*, *Candida albicans*, *Fusarium descencelulare*, *F. lateritium*, *F. moniliforme*, *F. oxysporum* and *F. solani*. CE20% HA was detected with rabbit, chicken and human erythrocytes, A and O types. The HA was high at pH 5.0, thermostable, inhibited by glycoproteins and stimulated with Ca^{2+} or Mg^{2+}. OfiL purified by chitin (13 folds) as well as by Sephadex G-25 (10 folds) columns was resolved as single 8.4 kDa polypeptide by SDS-PAGE. CE20% and OfiL showed antifungal activity against all tested fungi. The lectin was mainly active on *C. albicans*. The inhibition of HA with glycoproteins revealed that activity of *O. ficus indica* cladodes is due to lectin presence. Antifungal activity from extract and OfiL indicates cladode biotechnological potential.

Keywords: lectin, *Opuntia ficus indica*, cladodes, antifungal activity.

1. INTRODUÇÃO

1.1 LECTINAS

1.1.1 Histórico

O primeiro relato sobre lectinas foi descrito por Stillmark em 1888. A partir de uma preparação protéica parcialmente pura, obtida de *Ricinus communis* (mamona), a qual denominou ricina, ele testou seu efeito em sangue e observou que ao adicionar esta lectina à amostra sangüínea as células vermelhas se agrupavam (BELTRÃO, 2001). Eritrócitos de diferentes animais foram testados e apresentaram diferentes reações frente a esta lectina. Outra toxina, a crotina, isolada também por Stillmark a partir de sementes de *Croton tiglium*, apresentou uma atividade aglutinante diferente para células de fígado, epiteliais e leucócitos quando comparada à da ricina. Foi observado erm um trabalho realizado por Hellin (1891) que extratos tóxicos de sementes de *Abrus precatorius* continham lectina (abrina) (SHARON e LIS, 1987; SHARON, 1989).

Em 1913, um estudo comparativo da ação da ricina, crotina e abrina, desenvolvido por Kobert, comprovou as observações de Stillmark. Apenas na terceira década do último século (1935), foi descoberta uma lectina com reconhecimento específico para eritrócitos humanos (SHARON, 1989).

Em 1947, Boyd, a partir de seus estudos com fitohemaglutininas, considerou uma aplicação prática das lectinas grupo-sangüíneo específicas na medicina e começou a investigá-las como moléculas de reconhecimento. Um dos grandes estudos se deu em 1960, Nowell verificou que a lectina de *Phaseolus vulgaris* (PHA-fitohemaglutinina) estimulava linfócitos, que quando

expostos à essa lectina tinha um crescimento ativo e proliferação *in vitro* , sendo este estudo de grande impacto para a imunologia (SHARON e LIS, 1987).

Essas pesquisas não só demonstraram as propriedades distintas destas moléculas na sorologia, como também tornaram possíveis os primeiros passos quanto à base molecular de suas atividades. As descobertas decorrentes destas novas abordagens experimentais aumentaram em muito o entendimento e a aplicação das lectinas nas mais diversas áreas. As lectinas encontraram aplicações práticas em biologia e medicina (SHARON, 1998).

1.1.2 Conceito

Lectinas são proteínas ou glicoproteínas, diferente de anticorpos e enzimas, que especificamente ligam-se reversivelmente a carboidratos sem alterar a estrutura do ligante, resultando em aglutinação de células ou precipitação de polissacarídeos e glicoconjugados (GOLDSTEIN et al., 1980; PEUMANS e VAN DAMME, 1998). O termo lectina é derivado do latim, *legere* (que significa escolhido, selecionado), e reflete etmologicamente sua propriedade de aglutinar grupos sangüíneos (MATSUI *et al*., 2001). Lectinas apresentam um ou mais sítios de ligação para carboidratos (DIXON, 1981). Sendo assim, lectinas seriam uma classe de proteínas de origem não imunológica, de distribuição ubíqua na natureza, que reconhecem carboidratos livres ou ligados a superfícies celulares através de sítios de ligação nos quais a hidrofobicidade é a principal força de atração (KENNEDY *et al.* 1995). Lectinas que possuem estruturas semelhantes e apresentam formas moleculares com mobilidades eletroforéticas diferentes, pertencentes a uma mesma espécie, formam grupos de proteínas denominadas isolectinas (LIS & SHARON, 1981; LIS & SHARON, 1986). O termo isoforma foi proposto para

lectinas pertencentes à mesma espécie, cuja a heterogeinidade da origem genética não foi bem definida (PAIVA & COELHO, 1992).

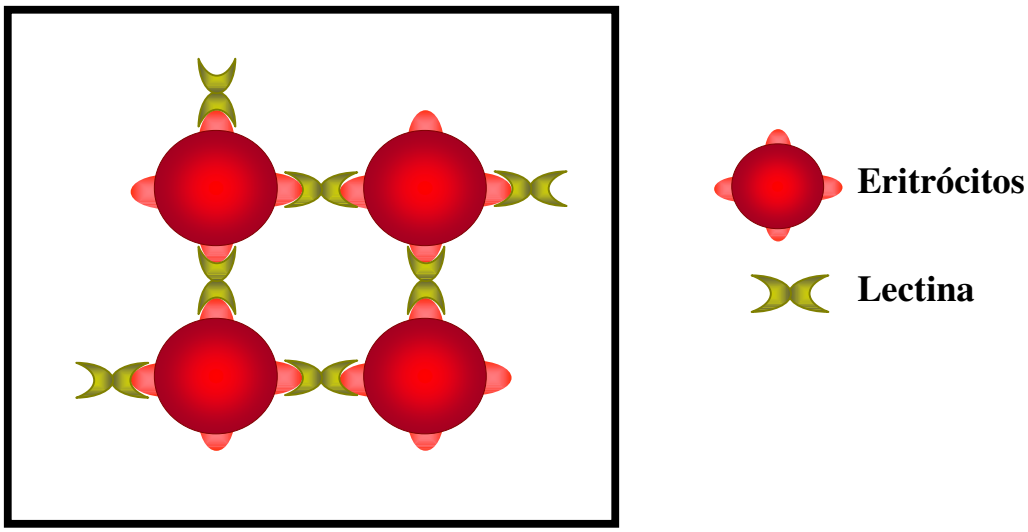

Figura 1– Representação esquemática da aglutinação de eritrócitos por lectinas.

1.1.3 Classificação

Segundo Peumans & Van Damme (1998), a especificidade de lectinas é determinada conforme o monossacarídeo que apresentar maior ação inibitória, estando assim classificadas em: grupo manose, grupo fucose, grupo galactose/N-acetilgalactosamina, grupo N-acetilglicosamina, grupo ácido siálico e complexo glicano (Tabela 1).

Há uma outra classificação para as lectinas vegetais, em relação a sua estrutura. As merolectinas, proteínas que apresentam um único sítio ligante para carboidratos, como a haveína, incapazes de aglutinar células ou de precipitar glicoconjugados por ser monovalente. As hololectinas, proteínas que apresentam apenas domínio de ligação a carboidratos, porém, possuem dois ou mais sítios de ligação. Fazem parte desse grupo, as lectinas que aglutinam células e/ou precipitam glicoconjugados, que compreende a maioria das lectinas conhecidas,

como a Con A. As quimerolectinas são proteínas que possuem além do domínio ligante a carboidratos, outro domínio com atividade catalítica ou outra atividade biológica que independe do domínio ligante do carboidrato, como a quitinase 1 (PEUMANS e VAN DAMME, 1998b). As superlectinas são um grupo especial de quimerolectinas; são proteínas de fusão com dois domínios de ligação a carboidratos, os quais são estruturalmente diferentes e reconhecem carboidratos distintos (PEUMANS, *et al.*, 2001).

Tabela 1: Famílias de lectinas de plantas: ocorrência e especificidade.

GRUPO	ESPECIFICIDADE	LECTINA
Fucose	Fucose	*Ulex europaeus* aglutinina I
N-acetilglicosamina	GlcNAc	*Triticum aestivum* (germen de trigo)
Galactose/N-	Galactose>>GalNAc	*Artocarpus integrifolia* (jacalina)
acetilgalactosamina	Gal = GalNAc	Clerodendron trichotomum
	Gal<<GalNAc	*Glycine max* (feijão de soja-Soybean)
Manose	Manose/Glicose	*Cratylia mollis* (Cramoll) *Canavalia ensiformis* (Con A)
	Manose/Maltose	*Calystegia sepium*
	Manose	*Galanthus nivalis*
Ácido Siálico	Neu5Acα(2,6)Gal/GalNAc	*Sambucus nigra*
	Neu5Acα(2,3)Gal/GalNAc	*Maackia amurensis*
	Ácido Siálico	*Triticum aestivum*
Complexo Glicano	Glicoproteína	*Phaseolus Vulgaris* (PHA)

GAL, galactose; GalNAc, N-acetilgalactosamina; GlcNAc, N-acetilglicosamina.

1.1.4 Ocorrência

As lectinas são amplamente distribuídas na natureza, sendo encontrada em uma grande variedade de organismos como plantas, vírus, bactérias, fungos, invertebrados (VIJAYAN & CHANDRA, 1999) e vertebrados (HARRISON, 1991; MAKKER *et al.*, 2002). Porém, o maior número de lectinas já estudadas foi em plantas (WHITTAKE, 1969). Na planta as lectinas são purificadas a partir da semente, a qual é uma das principais fontes de lectina (FREIRE *et al.*, 2002, REGO *et al.*, 2002, KONOZY *et al.*, 2003). Podem ser encontradas também em folhas (SAITO *et al.*, 1993; MORIYAMA *et al.*, 2003), cascas (KAKU *et al.*, 1990), entrecascas (HUANG *et al.*, 2002; ROJO *et al.*, 2003), raízes (NAEEM *et al.*, 2001), frutos, rizomas (TATENO *et al.*, 2003), bulbo, vagem, talo e flores (LIU *et al.*, 2002).

1.1.5 Função

Lectinas exercem uma variedade de efeitos biológicos nas células, porém os mais extensivamente estudados foram: aglutinação e estimulação mitogênica (LICASTRO *et al.*, 1991). Contudo, considerando as diferenças estruturais e especificidade a carboidratos entre as famílias de lectinas, é muito improvável que todas as lectinas tenham os mesmos ou similares papéis fisiológicos (PEUMANS e VAN DAMME, 1998). Em animais, há indícios que as lectinas participem do processo de endocitose, transporte intracelular de glicoproteínas e defesa contra microrganismos (RUDIGER *et al.*, 2000). Já nas plantas as lectinas desempenham importantes funções como proteína de reserva, mediadores da simbiose planta-microrganismo e mecanismo

de defesa contra fitopatógenos, animais e insetos predadores (WANG e NG, 2003; LIMPENS e BISSELING, 2003).

1.1.6 Purificação

A preparação de extratos é a primeira etapa realizada para purificar lectinas, sendo utilizada solução salina (KONOZY *et al.*, 2003) ou solução tampão (KABIR *et al.*, 1993; OLIVEIRA *et al.*, 2002). Os extratos que apresentem atividade hemaglutinante podem ter suas lectinas parcialmente purificadas através de procedimentos, tais como, fracionamento salino com sulfato de amônio e diálise exaustiva (KABIR, 1998; COELHO & SILVA, 2000).

Outra técnica utilizada na purificação é a cromatrografia; a cromatografia de troca iônica que separa as moléculas de acordo com sua carga elétrica (WANG *et al.*, 2003), a de gel filtração, que separa através do tamanho molecular da proteína (ROJO *et al.*, 2003) e a cromatografia de afinidade, técnica mais utilizada atualmente, explora a especificidade de ligação a carboidratos (TATENO *et al.*, 2003).

1.1.7 Caracterização

Lectinas são caracterizadas através de processos eletroforéticos, ensaio de inibição da atividade hemaglutinante (AH) fazendo uso de monossacarídeos simples ou carboidratos complexos. Além da avaliação da estabilidade térmica e efeito de íons e pH na AH. O pH tem efeito variado sobre as lectinas; em alguns casos não afeta a atividade (WITTSUWANNAKUL *et al.*, 1998) e em outros a lectina perde sua atividade em determinada faixa de pH, como é o caso

da lectina de *Erythrina speciosa* (KONOZY *et al*., 2003). Quanto ao efeito da temperatura, algumas lectinas permanecem estáveis até 55-65 ºC e a partir de então, com a elevação da temperatura, a atividade hemaglutinante decai até ser abolida, como no caso das lectinas de *Luetzelburgia auriculata* e *E. speciosa* (OLIVEIRA *et al.*, 2002; KONOZY *et al*., 2003). Lectina ativa após aquecimento a 95 ºC também foi isolada (SUSEELAN *et al.*, 2002).

Muitas lectinas contêm metais e, em alguns casos, existe evidência da necessidade de íons para sua atividade (SHARON e LIS, 1990). A lectina de *E. speciosa*, por exemplo, é uma metaloproteína que contém Ca^{+2} e Mn^{+2}, quando tratada com EDTA sua AH é totalmente abolida sendo a mesma restaurada após a adição de Ca^{+2} e Mn^{+2} (KONOZY *et al*., 2003). Por outro lado, a lectina *Helianthus tuberosus* L. (SUSEELAN *et al.*, 2002) não teve sua atividade abolida quando tratada com EDTA e não necessitou de íons metálicos tais como Ca^{+2}, Mn^{+2} e Mg^{+2} para a mesma.

Há uma grande versatilidade entre as lectinas quanto a seqüência e composição de aminoácidos na cadeia polipeptédica; ao número de subunidades na estrutura protéica e especificidade do sítio de ligação para mono e oligossacarídeos (SHARON & LIS, 2004).

Através da eletroforese pode-se determinar propriedades tais como: ponto isoelétrico e massa molecular aproximada (NEMOTO & SATO, 1998), assim como caracterizá-la como uma glicoproteína através da coloração com reativo de Schiff (COELHO & SILVA, 2000). A eletroforese em gel de poliacrilamida para proteínas nativas (sob condições não desnaturantes) caracteriza a proteína em relação à carga líquida. Lectinas básicas (SULTAN *et al*., 2004) e ácidas (SANTOS *et al*., 2005) foram reveladas com esta técnica.

Eletroforese em gel de poliacrilamida sob condições desnaturantes é utilizada para análise da pureza e da massa molecular de subunidades protéicas (NEMOTO & SATO, 1998;

REYNOSO-CAMACHO *et al.*, 2003). Este método faz uso do detergente sulfato sódico de dodecila (MACHUKA *et al*.; 1999; PAJIC *et al*., 2002).

Para a detecção das bandas protéicas no gel são utilizados corantes como negro de amido ou azul de Coomassie (GUZMAN-PARTIDA *et al*., 2004).

1.1.8 Aplicações

As lectinas possuem várias e importantes aplicações; é o grupo mais versátil de proteínas de plantas usadas em pesquisas biomédica e biológica básica e aplicada. Lectinas têm sido empregadas na determinação de tipos sangüíneos (MO *et al*., 2000), como agente mitogênico (WANTYGHEM *et al*., 1986) e na purificação de eosinófilos de lavados pulmonar (SHINAGAWA & ANDERSON, 2000).

Por causa do efeito danoso dos agentes quimioterápicos na terapia do câncer tem sido dada uma atenção especial aos inibidores de crescimento de origem natural; o interesse pelo efeito antitumoral de lectinas tem aumentado consideravelmente (ABDUKLLAEV & DeMEJIA, 1997).

As lectinas também são usadas como imunossupressoras no transplante de medula óssea (REMANI *et al*., 1994), como sondas estruturais revelando a organização das superfícies celulares e mudanças durante envelhecimento e patologias (ASTOUL *et al*., 2000; NISHIMURA *et al*., 2000), inibidoras do crescimento fúngico (FREIRE *et al*., 2002), na atividade antiproliferativa NGAI e NG, 2004), no diagnóstico de patologias em tecidos cerebrais, como mudanças degenerativas convencionais (NISHI *et al*., 2003), e tumor meningotelial (BELTRÃO *et al*., 2003). Devido a presença de carboidratos na superfície celular há a aplicação das lectinas

para separação de populações celulares biologicamente distintas; a propriedade de ligação a carboidratos das lectinas pode ser também utilizada em técnicas preparativas e analíticas para caracterização, seqüeciamento e purificação de carboidratos e glicoconjugados (PEUMANS e VAN DAMME, 1998; PAIVA *et al.*, 2003). São também empregadas como moléculas bioadesivas no endereçamento de drogas (BIES *et al*., 2004) e na análise de imunoglobulinas humanas (DAZIEL *et al*., 1999) .

1.2 *Opuntia fícus indica* Mill

Opuntia fícus indica (Figura 2) é uma planta da família Cactaceae, conhecida como palma. É um arbusto perene, ereto, ramoso, composto de artículos ou segmentos carnosos, superpostos uns aos outros, espatulados, comprimidos, achatados, ovalado-oblongos, de cor verde-claro e atravessados por um eixo lenhoso muito distinto, armados de espinhos de 2 cm de comprimento (Corrêa, 1984).

Esta planta é indiscutivelmente americana, porém não se tem certeza do seu *habitat* primitivo. Já desde longo tempo deveria ser cultivada no México, quando chegaram ali os conquistadores, pois estes encontraram diversas variedades, certamente resultantes de uma cultura inteligente e prolongada; os espanhóis, reconhecendo o valor da planta, a levaram para outros pontos do continente, assim como para as Antilhas, a Itália e a própria Espanha. Os portugueses introduziram no Brasil, em Angola, na Índia e de certo em outras regiões, sendo que em toda parte se aclimatou perfeitamente. Esta planta vegeta bem sob temperaturas mais elevadas bem como suporta 6° a 8°C abaixo de zero.

O. fícus indica resiste às secas mais prolongadas, graças a considerável quantidade de água armazenada nas cladônias (Corrêa, 1984). Este tecido é muito procurado e bem aceito por

todos os animais, como forragem aquosa e refrigerante, de alto valor em todas as regiões que, como o Nordeste brasileiro, estão sujeitos às secas periódicas que dessecam os cursos de água e calcinam as demais plantas forrageiras. A rapidez e a vitalidade desta espécie, vegetando exuberantemente em terras de qualidade inferior e em zonas áridas, tornaram praticamente rendosa a instalação da indústria pastoril e pecuária onde ela não parecia possível, sendo aproveitada por todos os animais inclusive as aves domésticas (Corrêa, 1984).

Várias propriedades de cladônias de *O. fícus indica* vem sendo descritas como diurético(Galati et al., 2002), anti-úlcera(Galati et al., 2001) cicatrizante (Trombetta et al., 2006), como também vem sendo investigada a sua utilização na dieta animal (Tegegne et al., 2007), a composição química da mucilagem (Sepúlveda et al., 2007) e seus metabólitos secundários (Saleem et al., 2006).


Figura 2- Aspectos de *Opuntia ficus indica*

2. OBJETIVOS

2.1. **OBJETIVO GERAL**

Purificar, caracterizar e avaliar a atividade fúngica da lectina de cladônias de *Opuntia ficus indica*.

2.2. **OBEJETIVOS ESPECÍFICOS**

- Avaliar a presença de lectina em cladônias de *O. ficus indica* utilizando eritrócitos de humanos, coelho e galinha.
- Extrair a lectina de cladônias de *O. ficus indica.*
- Definir a especificidade mono e/ou oligossacarídica da lectina.
- Determinar efeito de pH, íons e temperatura na atividade hemaglutinante.
- Identificar o padrão da proteína em eletroforese para proteínas em condições nativas e em condições desnaturantes e redutoras.
- Avaliar a atividade fúngica de cladônias de *O. ficus indica.*

3. REFERÊNCIAS

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4. ARTIGO

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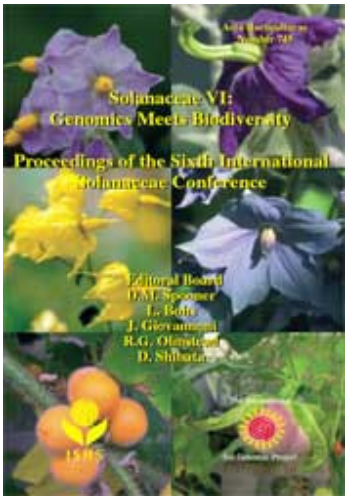

Isolation of lectin from *Opuntia ficus indica* cladodes

Isolation of lectin from *Opuntia ficus indica* cladodes

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ABSTRACT

Lectins are hemagglutinating proteins with carbohydrate binding sites; these proteins have biological properties including antimicrobial activity. The aims of this work were the isolation of *Opuntia ficus indica* lectin (OfiL) and evaluation of its antifungal activity. Hemagglutinating activity (HA) of cladode crude extract (CE20%) was evaluated using rabbit, chicken or human erythrocytes. The HA assay was also made in presence of carbohydrates or ions and at different temperatures and pH values. OfiL was isolated by chromatography of CE20% on chitin or Sephadex G-25 columns. OfiL was submitted to polyacrylamide gel electrophoresis for denatured proteins (SDS-PAGE). CE20% and OfiL were evaluated for antifungal activity using *Colletotrichum gloeosporioides*, *Candida albicans*, *Fusarium descencelulare*, *F. lateritium*, *F. moniliforme*, *F. oxysporum* and *F. solani*. CE20% HA was detected with rabbit, chicken and human erythrocytes, A and O types. The HA was high at pH 5.0, thermostable, inhibited by glycoproteins and stimulated with Ca^{2+} or Mg^{2+}. OfiL purified by chitin (13 folds) as well as by Sephadex G-25 (10 folds) columns was resolved as single 8.4 kDa polypeptide by SDS-PAGE. CE20% and OfiL showed antifungal activity against all tested fungi. The lectin was mainly active on *C. albicans*. The inhibition of HA with glycoproteins revealed that activity of *O. ficus indica* cladodes is due to lectin presence. Antifungal activity from extract and OfiL indicates cladode biotechnological potential.

INTRODUCTION

Cladodes of *Opuntia ficus indica* Mill., Cactaceae family are used in folk medicine for their antiulcer and wound-healing activities. Diuretic (Galati et al., 2002), antiulcer (Galati et al., 2001) as well as wound-healing (Trombetta et al., 2006) activities from cladodes were already described. *O. ficus indica* cladodes were also investigated as food components to animals (Tegegne et al., 2007) and by determination of chemical composition of mucilage (Sepúlveda et al., 2007) and secondary metabolites constituents (Saleem et al., 2006). Plant bioactive molecules include lectins, which are proteins or glycoproteins with carbohydrate binding sites and erythrocyte agglutination properties (Kennedy et al., 1995). The lectin carbohydrate binding property stimulates the biotechnological use of these molecules (Paiva et al., 2003). The lectin effect on growth of microorganisms has been evaluated. Lectins with antifungal activity were isolated from bacteria (Van Dellen et al., 2002), animals (Suzuki et al., 2002) and plants (Trindade et al., 2006). The aims of this work were the isolation of lectin from *O. ficus indica* cladodes and evaluation of its antifungal activity.

MATERIALS AND METHODS

Cladodes from Limoeiro City (State of Pernambuco, Northeast of Brazil) were dried and powdered. Extraction of proteins was performed when cladode powder (10 g) was homogenized (16 h, 4° C) in 0.15 M NaCl (50 ml). After filtration through a gauze followed by centrifugation at 4,000 g for 15 min, the supernatant was collected and termed crude extract (CE20%). The

protein concentration was estimated according to Lowry et al. (1951) using bovine serum albumin (31 a 500 μg/ml) as standard. Absorbance at 280 nm was also measured.

Hemagglutinating activity (HA) was evaluated according to Paiva & Coelho (1992) with rabbit, chicken or human erythrocytes. Titer was defined as the lowest sample concentration, which showed hemagglutination. Specific HA (SHA) was calculated from the ratio of titer to protein concentration (mg/ml). HA was also evaluated at different pH values (2.5, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10 and 11). The effect of temperature on HA evaluated CE20% heated at 30, 40, 50, 60, 70, 80, 90 and 100 ºC. HA inhibition assays were performed with 0.2 M carbohydrate (galactose, glucose, fructose, lactose, mannose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, N-acetylglucosamine, raffinose, sucrose and trehalose) or 1 mg/ml glycoprotein (casein, fetuin, ovalbumin, bovine serum albumin and fetal bovine serum) solutions. To evaluate the effect of different divalent ions on HA, the assays were performed with 0.01, 0.02 and 0.04 M Ca^{2+}or Mg^{2+} solutions. Additionally, CE20% was dialyzed against 0.005 M EDTA for 16 h at 4 ºC followed by 0.15 M NaCl for 6 h at 4 ºC to eliminate EDTA. Aliquots (50 μl) of dialyzed CE20% were serially diluted with 0.15 M NaCl containing 0.01, 0.02 and 0.04 M Ca^{2+}or Mg^{2+} before erythrocyte addition.

For lectin isolation CE20% sample (22 mg of protein) was applied to a chitin (6.5 x 1.5 cm) or Sephadex G-25 (21.0 x 1.0 cm) column equilibrated (flow rate of 20 ml/h) with 0.15 M NaCl. The columns were washed with the equilibrium solution until absorbance (measured at 280 nm) was less than 0.05. Afterwards, *O. ficus indica* lectin (OfiL) was eluted from column with 1 M acetic acid (chitin) or 0.5 M NaCl (Sephadex G-25). Collected fractions (2.0 ml) were evaluated by spectrophotometry at 280 nm. Fractions with HA (OfiL) were pooled and dialysed

against 0.15 M NaCl (6 h at 4°C). Purification was measured as the ratio between SHA in the stage and SHA of CE20%. Yield was measured by the ratio of HA values.

Polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) was performed on 10% (w/v) gel according to Laemmli (1970). Polypeptide of OfiL (50 µg of protein) and molecular mass standards (bovine serum albumin, 66,000 Da; carbonic anhydrase, 29,000 Da; cytocrome c, 12,500 Da and aprotinin, 6,500 Da were stained with Coomassie Brilliant Blue.

The antifungal activity was evaluated with *Candida albicans* (DAUFPE) *Colletotrichum gloeosporioides* (DAUFPE), *Fusarium descencelulare* (URM-3006), *F. lateritium* (URM-2491), *F. moniliforme* (URM-3226), *F. oxysporum* (URM-2489) and *F. solani* (URM-2480) which were isolated from Culture Collections of Departamento de Micologia and Departamento de Antibióticos from the Universidade Federal de Pernambuco, Brazil. Antifungal activity was performed according to Wang and Ng (2005) modified assay. Purified lectin was filtered using a 0.45 µm sterile syringe filter. Following, CE20% (50 µL; 550 µg) or OfiL (50 µL; 10 µg) was spread on solidified YNB medium in Petri plates (100 x 15 mm). A fungal mycelium disk (0.625 cm in diameter) was disposed in the center of Petri plate. All assays were carried out in triplicate. A 0.15 M NaCl solution and 10 ppm Cercobin were used as negative and positive controls, respectively. The plates were incubated at 28 °C for 72 h. A transparent ring around the paper disc revealed antifungal activity. Zones of growth inhibition around discs were measured (in mm).

RESULTS AND DISCUSSION

O. ficus indica is cultived in the Northeast of Brazil as an important food source for animals. The economic importance and claimed biological properties of the plant stimulated our studies.

The CE20% agglutinated chicken, rabbit and human types A and O erythrocytes at different degrees. The best HA was detected with chicken erythrocytes (32^{-1}). Vegetal tissues are good source of proteins and lectin was already isolated from *Cactus machaerocereus eruca* (Zenteno et al., 1991).

CE20% HA was completely inhibited with ovoalbumin and fetal bovine serum. The monosaccharides galactose, glucose, mannose and methyl-α-D-manopyranoside also inhibited HA (titer changed from 32^{-1} to 8^{-1}). Similarly, the lectina from *Serpula vermiculares* was inhibited by carbohydrate and glycoproteins (Molchanova et al., 2006). The inhibition of HA with carbohydrates indicates that erythrocyte agglutination involves lectin (Kennedy et al., 1995). Linkage of monossacharide or glycoprotein to lectin carbohydrate binding sites avoids binding of lectin to cell surface glycoconjugates and HA is not detected.

Cladode HA was performed under different experimental conditions aiming to define the most stable environment to evaluate different biotechnological applications of lectin.

CE20% HA was not altered to large pH range and different temperatures, revealing high protein stability. Evaluation of these conditions are important since interfere on hydrogen bridges and ionic interactions between amino acids that maintain protein structure. Disruption of forces result in protein denaturation with lost of HA. Similar to cladode HA lectins isolated from *Helianthus tuberosus* (Suseelan et al., 2002) and *Ganoderma lucidum* (Thakur et al., 2007) were stable after heating and pH variation, respectively.

The effect of ion on HA was also investigated. CE20% HA was increased after addition of Ca^{2+} (titer of 128^{-1} with 0.02 to 0.0 4 M) or Mg^{2+} (titer of 128^{-1} with 0.04 and 0.08 M) and abolished after treatment of CE20% with chelant agent EDTA. When Ca^{+2} was added to inactive EC20%, the HA was restored to 16^{-1} (0.005 to 0.02 M) and 32^{-1} (0.04 and 0.08 M). The results indicated that cladode lectin is a metalloprotein and that the HA is ion dependent. The lectin from *Canavalia ensiformis*, concanavalin A, contains sites for Ca^{2+} and Mn^{2+} involved in the interaction lectin-carbohydrate (Vetri et al., 2007).

Chromatography of CE20% (11 mg of protein with SHA of 3.0) on chitin column produced a single active peak (Figure 1A) containing 0.3 mg of purified OfiL. By this stage the lectin SHA (40) had increased 13 fold in relation to CE20%. Chromatographic profile of Sephadex G-25 column showed two active peaks, one unadsorbed and another eluted with 0.5 M NaCl (Figure 1B) containing 0.2 mg of OfiL with SHA of 29 (purification factor of 10). HA yield of 15 and 5% were obtained with chitin and Sephadex G-25 chromatographies, respectively. SDS-PAGE resolved OfiL as single polypeptide of molecular mass 8.4 kDa (Figure 2) revealing that chromatographic matrices were efficient to purify the lectin.

OfiL affected the growth of all assayed fungi, although it was mainly active on *C. albicans* (Table 1). Binding of OfiL to chitin column stimulated the evaluation of its antifungal activity since chitin is a component of cell wall of these microorganisms (Graham and Sticklen, 1994). Chitin-binding lectins isolated from the genus *Artocarpus* also showed antifungal activity (Trindade et al., 2006).

CONCLUSIONS

Cladodes from *O. ficus indica* contains a stable and ion dependent lectin (OfiL) with antifungal activity.

ACKNOWLEDGEMENTS

The authors express their gratitude to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for research grants. Also, the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE) for financial support. The authors are deeply grateful for the technical assistance of Maria Barbosa Reis da Silva.

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Table

Table 1. Antifungal activity (%) from *O. ficus indica* cladode preparations

Fungus	CE20%	OfiL
Colletrotrichum gloesporioides	54	34
Candida albicans	53	59
Fusarium moniliforme	40	ND
Fusarium oxysporum	44	19
Fusarium solani	24	12

Inhibition percentual calculated in relation to fungi growth at 0.15 M NaCl (negative control). ND, activity not detected.

Figures

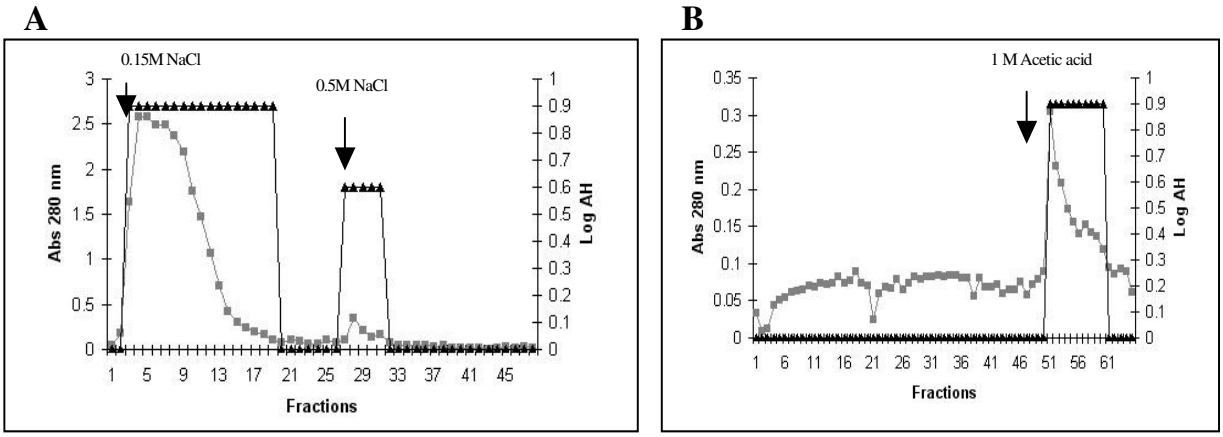

Fig. 1. Isolation of OfiL by Sephadex G-25 (A) and chitin (B) columns. A sample of CE20% (11 mg in 0.15 M NaCl) was applied to each column equilibrated with the same solution. Arrows indicate addition of eluents. Fractions of 2.0 ml were collected. Absorbance at 280 nm (■), HA (▲).

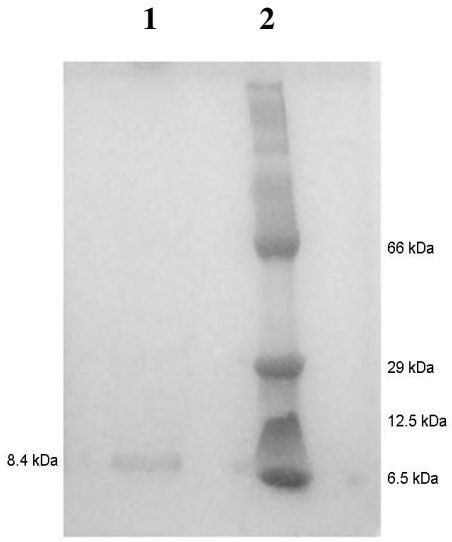

Fig. 2. SDS-PAGE pattern of OfiL.OfiL (1) and molecular mass markers (2) were loaded onto a SDS-PAGE (10%) and the gel was stained with Coomassie Brilliant Blue.

5. CONCLUSÕES

Cladônias de *O. ficus indica* contêm uma lectina estável a diferentes valores de pH e temperatura, característica estrutural que estimula a investigação da aplicação biotecnológica da lectina. A atividade antifúngica sobre fungos fitopatogênicos indica o seu potencial uso para controle de pragas agrícolas.

6. ANEXOS

6.1 Normas para redação de artigos para a revista *Acta Horticulturae*

ISHS Authors Guide: Publishing in Acta Horticulturae

GENERAL INFORMATION

ISHS Publication Policy

All oral presenters, including invited and key-note speakers, **must** submit a manuscript for the Acta. If this is not agreed to by the author(s), the work can be presented as a poster. Authors of posters are welcomed to submit their manuscript for ***Acta Horticulturae*** but posters cannot be published as such. The manuscript should be submitted a few weeks before the symposium starts or at the symposium in order to avoid unnecessary delay in the reviewing process and editing of the ***Acta Horticulturae*** volume.
The ISHS claims traditional copyright protection of all material published in ***Acta Horticulturae***, except where copyright is held by a third party such as a governmental agency and, upon request, permits fair scientific use as long as the author and ISHS are credited. The authors of an Acta article are required to obtain permission if copyrighted material is used.

Length of the Printed Paper

All symposium verbal and poster presentations are eligible for publication in the proceedings if a suitable manuscript is prepared according to the **"ISHS Authors Guide"** and submitted on time to the Convener of the meeting. The convener will send the manuscript to the Editorial Board for reviewing. Manuscripts should be as concise as possible in order to reduce to a minimum the number of pages of ***Acta Horticulturae***. As a general rule the maximum recommended length of an invited paper is 16 pages and of a submitted oral paper or poster is 8 pages, including figures and tables. An average page of text will contain about 800 words.
Manuscript reviewers will cut unnecessary information and will advise on the number of pages each manuscript should have taking into account its content and characteristics. For any length over the recommended number of pages the convener will have the right to charge 75,- euro per additional page.

Language

English is the official language of ***Acta Horticulturae***. However, if the original contribution is presented at a bilingual symposium, a manuscript in Spanish or French is also acceptable, provided it includes a one page extended abstract in English. An abstract in French or Spanish can be added to manuscripts in English.

Spelling

ISHS has no preference whether English or American spelling is used although uniformity within each paper is required. Latin words or phrases are in italics, with the exception of very common expressions such as "i.e.," "e.g.," "et al.," "in vitro," "ex vitro" and "etc. The expression "etc." for "and so forth" should be used only with series, such as 1, 2, 3, etc.

Units

Use the metric system exclusively. Use abbreviation L for liter, mg/L for milligram(me) per liter, ml for milliliter, and t for tonne (metric ton). SI units can be used where appropriate.

Font and Type Size

Use **Times New Roman** font exclusively. Titles are printed in 14 point but the rest of the manuscript, including tables should be 12 point. When italic typeface is required use italic type, not underline.

Plant Names

Scientific names are to be included for all plant species and are to be in italic font except for the abbreviations "var.", "subsp.", "f.", etc. which indicate rank at infraspecific level (e.g., *Cedrus libani* subsp. *atlantica, Phytophthora parasitica* var. *nicotianae*). Author citation should only be used when helpful for historical or taxonomic reasons, and then it should only be used when the name is first mentioned in the body of the text (do not use author citation in the abstract or title). Author names are to abbreviated in accordance with the international standard provided by Brummitt, R.K., & Powell, C.E., "Authors of Plant Names", Royal Botanic Gardens, Kew 1992. An on-line version of this work may be consulted via http://www.rbgkew.org.uk/data/authors.html.

Common names may be used for well-known plants once the scientific name has been provided (e.g., apple, pear, potato, rose, tomato). Cultivated varieties which are the product of selection and/or breeding are to be referred to as "cultivars" and not "varieties". Cultivar names are to be written in accordance with the International Code of Nomenclature for Cultivated Plants. The current (2004) edition is obtainable from ISHS via http://www.actahort.org/books/647/. In particular, the part of a name which denotes the cultivar is it be placed within single quotation marks. The abbreviation "cv." is not to be used within a name (e.g., Malus ×domestica 'Golden Delicious', not Malus ×domestica cv. Golden Delicious).

If indicating hybrid status, the multiplication symbol should be used before the name of the genus or the species epithet as appropriate (e.g., ×Cupressocyparis leylandii, Mentha ×piperita), or within the formula denoting the hybrid (e.g., Mentha aquatica × M. spicata). If the multiplication symbol is not available in your font set, use the letter "x" in lower case, but leave a space between it and the word to which it should be applied (e.g., x Cupressocyparis leylandii, Mentha x piperita). Neither the multiplication symbol nor the letter "x" are to be in italics.

Use the letter "x" to indicate a cross such as "red x yellow" and for the term "by" in measurements (2 cm x 4 cm). Use italic n and x when indicating sporophytic or basic chromosome number (e.g., 2n=4x = 48).

Headings Ranks and Format

Papers contain one to four headings, all aligned at the left hand margin, as follows:

RANK ONE Use boldface and all capital letters. Use a space before this rank but subsequent paragraph(s) continue without a space. Subsequent paragraphs within this section are indented without spaces between paragraphs. Headings such as **INTRODUCTION**, **MATERIALS AND METHODS, RESULTS, DISCUSSION** are Rank one headings. Do not use a period after this heading.

Rank Two This heading subdivides **RANK ONE** headings, thus there must be at least two or more Rank Two subheads. Titles are **boldface** with the first letter of important words in capital letters and the others in lower case. Rank Two headings are separated by a space above the heading as in **RANK ONE** headings. No period **after Rank Two** headings. The paragraph starts on the first line after the Rank Two heading and is indented.

1. Rank Three. This heading may be used to divide Rank Two headings. Initiate this heading with Arabic numerals (1,2,3 etc.). with numbers and title in **boldface** with the beginning of each word in capital letters. The subheading ends with a period. The paragraph continues on the same line. Do not separate this heading with blank lines.

Rank Four. This heading subdividing **Rank Three headings** will be used rarely. Align left, end with a period, and continue on same line. The font is *italic*, non-boldface, with the beginning of each word in capital letters. Do not separate with blank lines.

Paper

Use first quality white paper for your printout. The printable area on your sheet of paper is **strictly fixed** (15.3 x 23.5 cm = 6.02 x 9.25 inches) irrespective of paper size. For A4 size paper this printable area is obtained by entering following margin settings in the "page set-up" of your word-processor: **top: 2.7 cm (1.06"); bottom: 3.5 cm (1.38"); left: 2.8 cm (1.10"); right: 2.9 cm (1.14")**.

Papers should be printed preferably on laser-writers but Ink-jet printers also give satisfactory results. Papers produced on a type-writer will not be accepted.

Spacing and Indentations

The final text should be single spaced but a double spaced manuscript may be submitted for editing by the convener. Titles of subheadings should **not** be underlined. Text should be **"justified"** in order to fill the entire printable area.

Provide a hanging indent (0.6 cm) on the second line of the Keywords and Literature Cited references. First lines of all paragraphs should have a 1.25 cm indentation except those that immediately follow rank three and rank four subheadings. Do not include blank lines between paragraphs within a section.

ORGANIZATION OF A RESEARCH PAPER

Title

Titles are printed in boldface in 14 point type. If the paper is in Spanish or French with extended English abstract, (see **Language** above) the title is in **boldface** in English and not in

boldface for other languages. Use capital and lower case for the first word in the titles except for articles (“a” and “the”), prepositions (“of,” “in” “on.” “during,” “between”), and conjunctions (“and” and “but”), except when they are the first word. Gene symbols, which normally begin with lower case letters are not capitalized in titles nor is the first word of specific epithets in binomials. Do not include authorities for binomials in titles. Keep titles as concise as possible. Binomials will be in boldface Italics.

Bylines

The byline under the title includes the name of author(s) (without titles) and affiliations. The given name of authors may be either written out in full or listed by initials. Initials are followed by a period. If two initials are listed, do not include a space between them but provide a space before the family name. The family name is always presented after the given name, even for those countries that use a different sequence (Spanish names are alphabetized by the paternal family name. Accents should be kept in names so as not to violate their spelling rules). The affiliation or address of author is included below the name. The address of the author may be in the language of the country, but spell out the country name in English.

For multi-authored papers keep the affiliation of each author separately; when space permits, these can be listed side by side; if not, underneath each other. If there are two authors, separate the author name by “and,” e.g. A.B. Smith and C.D. Jones; three authors would be A.B. Smith, C.D Jones and E.F. Brown. Do not use footnotes in the bylines.

Footnotes should be avoided in bylines. They might be appropriate when there are two departments in one institution.

Journal Paper Numbers

Journal paper numbers, or reference numbers, if needed, are placed in the Acknowledgement section (see below).

Keywords

This is a rank 2 heading followed by colon (**Keywords:** apple, pear). List five to seven key words **not used** in the title. Remember that electronic search engines focus on Title and Keywords. The second line of keywords is a hanging indent (0.6 cm).

Abstract

Use a rank 2 heading for **Abstract**. An abstract in English, limited to 200-300 words in a single paragraph, **all boldface**, **is required in all cases**. Indent the first line of the abstract. The abstract should contain a concise but comprehensive statement of the problem and results. The entire abstract should be in **boldface**. *The title and abstract will be freely available on the ISHS website and should be considered an advertisement for the paper as it may be all that most viewers will read. Thus, it should be carefully and accurately written.*

Introduction

This should include a statement of the problem, a brief survey of previous work, and the scope and purpose of the investigation. References to previous work should be included.

Materials and Methods (Experimental Procedures)

This section should be included in papers describing experiments but may not be required in review papers. Describe concisely the plant materials, the growing technique, methods used, and lay-out of experiments. Include the name of all chemicals and compounds. An indication of the statistical methods used to analyze data should be included

Results and Discussion

This is the heart of the paper. The section(s) may either be presented as a single section or divided into separate **Results and Discussion** sections. If separate, describe experimental results in the **Results** section and reserve interpretations, speculations, and conclusions for the **Discussion** section. At the end of the paper attempt to answer questions formulated in the introduction and conclude with a summary of results and an assessment of future research or prospects.

Acknowledgements

This is reserved for journal paper numbers, source of funding, and name of project, if required. Acknowledgement of help from colleagues or professional associates is appropriate but avoid acknowledgement of routine secretarial help or family members.

Citations and Literature Cited

1. Format. Citations to references in the text are listed chronologically surrounded by parentheses with the following format: (Peters, 1950; Jones and Smith, 1990; Brown et al., 1999a). If there are two authors with the same name that have published in the same year, initials may be used to avoid confusion. Note: "et al." is used for three or more authors.

Citations to personal communications include the surname or initials of the person and are only to be included within the text, **not** in the Literature Cited section. The date is optional. Thus: (A.B. Peters, pers. commun.) or (A.B. Peters, pers. commun., 2001).

Consider **Literature Cited** as a Rank 2 heading,. Literature cited should only include references used in the paper. List the authors in alphabetical order, letter by letter, and in chronological order for publications of the same author(s). Do **not** use a comma before "and" after the penultimate author. Do not use an issue number if the journal uses consecutive numbers for each volume. In the format that follows, note that in all cases the given name or initials follow the family name.

Journal Paper:

Navazoi, J.P. and Simon, P.W. 2001. Diallel analysis of high carotenoid content in cucumber. J. Amer. Soc. Hort. Sci. 126:100-104.

Van Os, E. and Benoit, F. 1999. Stare of the art of Dutch and Belgian greenhouse horticulture and hydroponics. Acta Hort. 481:765-767

Book:

Darrow, G.M. 1966. The Strawberry: History, Breeding and Physiology. Holt, Rinehart and Winston, New York.

Chapter in Book:

Daubeny, H.A. 1996. Brambles. p.109-190. In: J. Janick and J.N. Moore (eds.), Fruit

Breeding, Vol. 3, Nuts. Wiley, New York.

Chapter in Conference Proceedings:
Aviram, M. and Fuhrman, B. 1998. Tomato lycopene and β-carotene inhibit LDL oxidation. Proc. Tomato and Health Seminar. Pamplona, Spain 25-28 May. p. 45-52.

Website:
Food and Agricultural Organization. 2002. www.fao.org

2. Abbreviations. Do not abbreviate single word journals. Do not abbreviate states or provinces of countries. When in doubt do not abbreviate. Commonly used abbreviations are as follows:

Abstract - Abstr.
Academia - Acad.
Advances - Adv.
Agriculture - Agri.
Agronomy - Agron
American - Amer.
Annals - Ann.
Annual - Annu.
Archives - Arch.
Biochemistry - Biochem.
Biol. - Biol.
Circular - Cir.
Communication - Commun.
Conference - Conf.
Congress - Congr.
Contribution - Contrib.
Culture - Cult.
Department - Dept.
Dissertation - Diss.
Gazette - Gaz.
Genetics - Genet.
Horticulture, -ae, -al - Hort.
Institute - Inst.
International - Intl.
Japanese - Japan.
Journal - J.
Laboratory, -ies - Lab.
Letters - Lett.
Magazine - Mag.
Management - Mgt.
Market - Mkt.
Marketing - Mktg.
Molecular - Mol.
National - Natl.
Physiology - Physiol.
Plantae, -arum - Plant.
Progress - Prog.
Publication(s) - Publ.
Report - Rep.
Reporter - Rptr.
Research - Res.
Review (s), Revue(s) - Rev.
Scientia - Scientia
Scientific - Scientific
Series - Ser.
Station - Sta.
Statistics, -ical - Stat.
Supplement(s) - Suppl.
Technical, -que - Tech.
Technology, -ical - Technol.
United Kingdom - UK
United States of America - USA
US Department of Agriculture -U.S. Dept. Agr.
University - Univ.
Yearbook - Yearb.
Zeitschrift- Z.

Tables and Figures

Tables and figures are normally included at the end of the article in that sequence. Prefix the table section with the word **Tables** and the figure section with the word **Figures**. Captions are provided directly above each table and below each figure with hanging indents. They are numbered consecutively with Arabic numbers, and aligned with the width of the Table or Figure, or to the full width of the page if the figure or table occupies more than half of the width of the page. Thus, Table 1, Table 2 etc. and Fig. 1, Fig. 2. etc. If the table or figure is not original, give the source at the end of the caption, e.g. Source: Jones et al. 2001.

1. Tables. Use tables sparingly. Titles of tables go above the table. Place all headings to the center of their column.
The size of the table should not exceed the standard page width and length, but tables may be placed portrait or landscape format.
Solid lines are used in the heading and in the bottom of the table but are to be avoided in the body, but, if necessary, use dotted lines.
The units of the data must be indicated in parentheses in the table headings. If table footnotes are needed, use superscript Arabic numbers 1, 2, 3, etc. The sources of tables should be in the caption (see model).

Proper format for tables in *Acta Horticulturae* should include 4 parts: (1) caption, (2) masthead, (3) body, and (4) footnotes. This can best be demonstrated with two examples, listed as Table 1 and Table 2 in the **sample article** file.

Caption. The caption should be understandable without recourse to the paper itself. The caption has only the first word capitalized (except for proper names) and ends in a period. The caption may be more than a single sentence. The source of the table, if necessary to include, is indicated in the caption (see Table 2).
Masthead. In general, tables are best read up and down. Each column of the table must be explained by a masthead heading. The masthead is enclosed top and bottom by two lines extending to the each edge of the table (see Table 1 and 2). Horizontal lines within the masthead can be used to separate groups under a common heading (see Table 2). The units of each column need to be clearly indicated, e.g., No. fruit; Fruit wt. (g); Harvest index (%). Masthead headings should be located on the bottom of the masthead cell.
Body. Avoid internal lines in the body of the table. Center values under the masthead heading. Use rounding to avoid unwarranted precision. Means may be separated by using lower case letters (5% significance) or upper case letters (1% significance). Indicate statistical tests and significance by footnotes, preferably superscript 1, 2, 3, etc. [If letters are used, start at the end of the alphabet (z, y, x, etc.).] The body of the table is enclosed in a line.
Footnotes. Footnotes go underneath the body of the table. Put each footnote on a separate line.

2. Figures. Titles of figures go underneath the figure. Figures may be submitted electronically but provide a hard copy since resolution may be imperfect. If a figure is outsized it may be reduced photographically. Be sure to include clear, sharp pictures. Figures, graphs and drawings normally should be all in black and white, not color. Color photographs can only be printed after a special agreement with the conveners and ISHS Secretariat and

there will be a charge to authors.

ARTICLE SUBMISSION

See our **sample article** provided for format. Submit a hard copy (printout preferably on A4 size paper) as well as an electronic version of the article in a commonly used word processor format (preferably MSWord or alternatively WordPerfect). For print-technical reasons and to enhance the output quality where possible please include a separate (electronic) copy of any picture-, image- or other graphic object files in high resolution and add the source files for charts, tables etc. that you used in your article.

6.2. Indicadores de produção 2007-2008

6.2.1 Resumos em congressos

Resumo apresentado na SBBq 2007

PARTIAL CHARACTERIZATION OF FROND AND RHIZOME LECTINS FROM *Microgramma vaccinifolia* [1]*Santana, G. M. S.*; [1]*Albuquerque, L.P.*; [1]*Coelho, L. C. B. B. and* [1]*Paiva, P. M. G.*

Resumo apresentado no V Congresso Brasileiro de Micologia

AVALIAÇÃO DA ATIVIDADE ANTI-FÚNGICA DE EXTRATO DE CLADÔNIAS (*Opuntia fícus indica)* CONTENDO LECTINA. **Giselly Santana ; Lidiane Albuquerque; Diogo Simões; Luana Coelho; Patrícia Paiva**