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NÍVEL MESTRADO

EXSUDATOS DE PLANTAS: UM ESTUDO DE REVISÃO E EFEITO ANTI-HELMÍNTICO SOBRE O NEMATOIDE *Haemonchus contortus*

IRLLA CORREIA LIMA LICÁ

São Luís, MA

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Maranhão, como requisito para a obtenção do título de Mestre em Ciências da Saúde.

Orientadora: Profª. Dra. Alexandra Martins dos Santos Soares

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“Agradeço todas as dificuldades que enfrentei, não fosse por elas, eu não teria saído do lugar. As facilidades nos impedem de caminhar. Mesmo as críticas nos auxiliam muito.”

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LISTA DE ABREVIAÇÕES E SIGLAS

BSA - Albumina Sérica Bovina

CEUA - Comitê de Ética Animal

DPPH - 1,1-difenil-2-picrilidrazilo (do inglês, 1,1-Diphenyl-2-picrylhydrazyl)

EC_{50} - Concentração efetiva para 50% da população

EHA - Ensaio de eclosão de ovos (do inglês, egg hatching assay)

EPA - exsudato proteico *de Acacia mangium*

EPL - exsudato proteico de *Leucaena leucocephala*

EPM - exsudato proteico de *Mimosa caesalpiniaefolia*

EPS - exsudato proteico de *Stylosanthes capitata*

ESI–LC–MS/MS - espectrometria de massa

GAE – equivalente de ácido gálico (do inglês, gallicacid equivalent)

GE - equivalente de glicose (do inglês, glucose equivalent)

HPLC - cromatografia líquida de alta performance (do inglês, high-performance liquid chromatography)

L3 - larvas de terceiro estádio

LEIA - desembainhamento larvar (do inglês, larval exsheathment inhibition assay)

LP - (do inglês, Laticifer proteins)

MICs - concentrações mínimas inibitórias (do inglês, minimum inhibitory concentrations)

NSAIDS - antiinflamatórios não esteróides (do inglês, nonsteroidal anti-inflammatory drugs)

PR proteins - proteínas relacionadas à patogênese (do inglês, pathogenesis-related proteins)

QE - equivalente quercetina (do inglês, quercetin equivalent)

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RESUMO

A infecção por nematoides gastrintestinais é considerada o principal problema sanitário da criação de pequenos ruminantes, sendo *Haemonchus contortus* o endoparasito de maior prevalência e patogenicidade. O uso indiscriminado e continuado de produtos anti-helmínticos tem selecionado populações resistentes dos parasitos a esses produtos. Os produtos de origem natural em especial de plantas têm sido considerados como uma alternativa potencial no controle de parasitos gastrintestinais. Entretanto, estudos sobre identificação de proteínas obtidas de exsudatos de sementes de plantas são raros. Assim, o presente trabalho foi dividido em dois capítulos com intuito de ampliar estudos prospectivos sobre a busca de novos compostos biotivos derivados de exsudatos vegetais. No capítulo 1, fornecemos através de uma revisão, uma visão geral sobre os exsudatos liberados pelas propriedades biológicas plantas, suas e a presença de vários compostos, obtidos e analisados por diferentes procedimentos. No capitulo 2, objetivou-se identificar proteínas dos exsudatos proteicos de sementes de *Mimosa caesalpiniaefolia, Leucaena leucocephala*, *Acacia mangium* e *Stylosanthes capitata*, bem como avaliar sua ação anti-helmíntica sobre o nematoide gastrointestinal *H. contortus.* Após sanitização, as sementes foram embebidas em tampão acetato de sódio (0,1M, pH 5,0) por 24h a 10 ºC. Proteínas presentes nos exsudatos foram obtidas por fracionamento com sulfato de amônio (0-90%). Após precipitação, os exsudados foram incubados durante 4h a 10 °C e as proteínas recuperadas por centrifugação (12.000 x *g*, 4 °C 30 min). O sobrenadante foi descartado e o sedimento foi ressuspendido em tampão acetato de sódio 0,1 M, pH 5,0 e dialisados contra água (cut off: 2 kDa), liofilizados e armazenados (-20 ºC). O teor de proteínas nos exsudatos foi avaliado, assim como o efeito inibitório na eclosão de ovos (EHA) e inibição do desembainhamento larvar (LEIA) de *H. contortus.* Obteve-se 0,49; 0,06; 0,10; e 0,11 micrograma de proteínas exsudadas por grama de sementes, respectivamente, em *M. caesalpiniaefolia*; *L. leucocephala*; *A. mangium* e *S. capitata.* Embora os exsudatos não tenham inibido a eclosão de ovos, inibiram o desembainhamento de larvas de *H. contortus* com concentrações efetivas, EC_{50}, variando entre 0,26 e 0,61 mg/mL. Os exsudatos de *L. leucocephala* e *S. capitata* foram mais efetivos sobre a inibição do desembainhamento de larvas (EC_{50} 0,26 e 0,40 mg/mL, respectivamente). De acordo com os dados obtidos por espectrometria de massas dos exsudatos, detectou-se a presença diversas proteínas, dentre elas: protease, inibidor de protease, quitinase e lectina. Assim, através deste estudo sobre ação exsudatos de sementes sobre *H. contortus,* concluímos que estes apresentam diversas proteínas relacionadas com a defesa do vegetal e que podem estar relacionadas com a ação inibitória do desembainhamento larvar do nematoide, caracterizando-se como uma potencial alternativa para o controle deste parasito.

Palavras-chaves: Exsudação. Nematoide. Propriedades biológicas. Sementes.

ABSTRACT

Gastrointestinal nematode infection is considered the main sanitary problem of small ruminants *Haemonchus contortus* is the most prevalent endoparasite and pathogenicity. The indiscriminate and continued use of anthelmintic products has selected resistant populations of the parasites to these products. Products of natural origin in particular of plants have been considered as a potential alternative in the control of gastrointestinal parasites. However, studies on the identification of proteins obtained from exudates of plant seeds are rare. Thus, the present work was divided into two chapters in order to expand prospective studies on the search for new bioactive compounds derived from plant exudates. In chapter 1, we provide through a review, an overview on the exudates released by the biological properties of plants, their and the presence of several compounds, obtained and analyzed by different procedures. In Chapter 2, the objective was to identify proteins from the protein exudates of *Mimosa caesalpiniaefolia*, *Leucaena leucocephala*, *Acacia mangium* and *Stylosanthes capitata* seeds, as well as evaluate their anthelmintic action on the gastrointestinal nematode *H. contortus*. After sanitization, the seeds were soaked in sodium acetate buffer (0.1M, pH 5.0) for 24h at 10 °C. Proteins present in the exudates were obtained by fractionation with ammonium sulphate (0-90%). After precipitation, the exudates were incubated for 4 h at 10 °C and the proteins recovered by centrifugation (12,000 x *g*, 4°C 30 min). The supernatant was discarded and the pellet was resuspended in 0.1 M sodium acetate buffer, pH 5.0 and dialyzed against water (cut off: 2 kDa), lyophilized and stored (-20 ºC). The protein content in the exudates was evaluated, as well as the inhibitory effect of egg hatching (EHA) and larval exsheathment inhibition assay (LEIA) of *H. contortus*. There was obtained 0.49; 0.06; 0.10; and 0.11 micrograms of proteins exuded per gram of seeds, respectively, in *M. caesalpiniaefolia*; *L. leucocephala*; *A. mangium* and *S. capitata*. Although the exudates did not inhibit egg hatching, they inhibited the drawing of *H. contortus* larvae at effective concentrations, EC_{50}, ranging from 0.26 to 0.61 mg/mL. Exudates of *L. leucocephala* and *S. capitata* were more effective on inhibition of larvae larvae (EC_{50} 0.26 and 0.40 mg/mL, respectively). According to data obtained by mass spectrometry of the exudates, several proteins were detected, among them protease, protease inhibitor, chitinase and lectin. Thus, through this study on seed exudates on *H. contortus*, we conclude that they present several proteins related to plant defense and that may be related to larval exsheathment inhibition assay nematode, characterizing itself as a potential alternative to the control of this parasite.

Keywords: Exudation. Nematoid. Biological properties. Seeds.

1. INTRODUÇÃO

A caprinocultura é uma atividade econômica explorada em todos os continentes e tem sido estimulada no Brasil, na tentativa de garantir à população rural uma fonte de renda, além de fornecer carne e leite, importantes fontes proteicas na dieta do ser humano (HERMUCHE et al., 2012; MARANHÃO, 2013). Esta atividade faz parte do agronegócio nacional. Estimando-se que os rebanhos caprinos contam com um efetivo de, aproximadamente, 9 milhões de animais. Grande parte dos rebanhos está concentrada na região Nordeste, que responde por 57% do rebanho ovino e 91% do rebanho caprino (IBGE, 2014).

A infecção por nematoides gastrintestinais é considerada o principal problema sanitário da criação de pequenos ruminantes (CEZAR et al., 2008; IGARASHI et al., 2013), limitando a exploração economicamente viável destes animais (MINHO, 2014). Dentre os nematoides, *Haemonchus contortus* destaca-se como o endoparasito de maior prevalência e patogenicidade, provocando graves danos à saúde do animal, como anorexia, depressão, hemorragias, anemia crônica e morte. Entretanto, apesar de tais fatores ocasionarem prejuízos econômicos mundiais na ordem de 300 milhões de dólares (COSTA-JUNIOR; AMARANTE, 2015) na produção de caprinos, as parasitoses animais ainda são negligenciadas em termos de desenvolvimento de novos fármacos, vacinas e diagnósticos (CAMPBELL et al., 2011; SADDIQI et al., 2011; HEIM et al., 2015).

A principal forma de controle do *H. contortus* é realizada com anti-helmínticos sintéticos (OLIVEIRA et al., 2011). Entretanto, o uso inadequado de anti-helmínticos tem resultado na seleção de parasitos resistentes aos princípios ativos disponíveis no mercado. Além disso, podem induzir toxicidade para o hospedeiro vertebrado, apresentando ainda altos custos e causando poluição ambiental (TORRES; HOSTE, 2008). A constante preocupação com o uso dessas drogas tem estimulado alternativas de controle mais efetivas e de aquisição viável, destacando-se o uso de produtos de origem natural, seja animal ou vegetal, obtidos por meio de processos biotecnológicos (PILLUZA et al., 2014).

A fitoterapia pode ser uma importante aliada no controle estratégico desses nematoides gastrointestinais (MACEDO, 2013). O uso de plantas medicinais e seus

derivados constituem uma prática milenar e em constante expansão, posto que propriedades de ativos biológicos de muitas plantas podem funcionar como agentes protótipos para a obtenção e/ou síntese de novos fármacos com ação anti-helmíntica (GAÍNZA et al., 2015). Produtos fitoterápicos com atividade anti-helmíntica surgem como uma possibilidade de tratamento simples. Além disso, reduz custos, são biodegradáveis, não causam poluição ambiental e apresentam como vantagem, o desenvolvimento mais lento da resistência (NOGUEIRA et al., 2006; CHAGAS et al., 2011).

Embora o estudo de plantas para controle de *H. contortus* se baseie majoritariamente, em compostos não proteicos de baixa massa molecular (BARRAU et al., 2005, YONDO et al., 2013), proteínas surgem como uma opção potencial (LEVECKE et al., 2014), inclusive pelo fato de poderem ser produzidas em larga escala por técnicas de biologia molecular.

Proteínas são polímeros de aminoácidos encontrados em todos os organismos vivos (NELSON; COX, 2006). Desempenham funções diversificadas e apresentam inúmeros potenciais biológicos. Diferentes estudos comprovaram a ação antimicrobiana (KLAFKE et al., 2013), antitumoral (SILVA et al., 2014), carrapaticida (LIMA et al., 2005), nematicida (BRAGA et al., 2014), dentre outras atividades, das proteínas. Lectinas, proteases e quitinases são exemplos de proteínas de plantas com potenciais propriedades anti-helmínticas devido sua capacidade de se ligar e/ou quebrar importantes macromoléculas que constituem a cutícula do parasito *H. contortus* (SALLES et al., 2014).

Diante deste contexto, há fontes vegetais de proteínas pouco estudadas e que podem ser anti-helmínticos potenciais, como por exemplo, exsudatos de sementes de plantas (LICÁ et al., 2018). O processo de exsudação ocorre durante a germinação das sementes, na fase de embebição, com exteriorização de uma mistura complexa de moléculas grandes e pequenas. Dentre os mais comuns, encontram-se os açúcares, aminoácidos, ácidos orgânicos, flavonoides, esteróis e proteínas (NELSON, 1990; NELSON, 2004; SCHWEMBER; BRADFORD, 2010; FRY, 2012).

Em sementes, o exsudato representa uma fonte útil de moléculas de

defesa, que são principalmente péptidos/proteínas com a capacidade para inibir patógenos de solo, atuando na prevenção contra infecções (SCARAFONI et al. 2013; ROCHA et al., 2015).

Apesar de alguns estudos demonstrarem o potencial de exsudatos de sementes contra microrganismos patogênicos, raras são as identificações de proteínas nessas amostras, poucos são os estudos de suas atividades biológicas e ação sobre nematoides. Assim, o presente estudo foi desenvolvido com o intuito de descrever sobre os exsudatos liberados das plantas, suas propriedades biológicas e potencial farmacológicos, assim como a presença de vários compostos, obtidos e analisados por diferentes procedimentos; identificar proteínas dos exsudatos proteicos de sementes de *Mimosa caesalpiniaefolia, Leucaena leucocephala, Acacia mangium e Stylosanthes capitata,* bem como avaliar sua ação anti-helmíntica sobre o nematoide gastrointestinal *H. contortus.*

2. REFERENCIAL TEÓRICO

2.1 Helmintoses gastrintestinais em caprinos

As helmintoses gastrintestinais constituem o principal fator limitante para a produção de caprinos em todo o mundo (VIEIRA, 2003). As perdas causadas pelas verminoses são determinadas não somente pelos efeitos agudos da doença, que, em muitos casos, resultam em morte do animal infectado, mas, principalmente, pelos danos indiretos causados por infecções crônicas. Estes acarretam desenvolvimento corporal lento, perda de peso, redução na produção de carne e lã e aumento das despesas associadas ao controle de doenças (KRYCHAK-FURTADO, 2006).

Os fatores predisponentes às parasitoses animais são: idade, sendo os jovens e senis mais sensíveis; estado nutricional inadequado, épocas de maior estresse, como parto, lactação, desmame e nascimento; fatores do animal, como raça, tipo de parasita, manejo, superpopulação e a introdução de animais portadores nos rebanhos (SOUZA, et al., 2000).

Dentre os helmintos que parasitam o trato gastrointestinal de caprinos, encontram-se: *Trichostrongylus*, *Cooperia*, *Oesophagostomum* e gênero *Haemonchus* (ANDRIOLA et al*.,* 2011). Os nematoides pertencentes ao gênero *Trichostrongylus*, são pequenos e não ultrapassam sete milímetros de comprimento quando adultos. Nos caprinos, a espécie mais relevante é *Trichostrongylus axei*, qual se aloja no abomaso, tanto de ruminantes domésticos quanto silvestres, e no estômago de equinos. O gênero *Cooperia* apresenta distribuição mundial, aloja-se no intestino delgado dos ruminantes. As espécies prevalentes em caprinos são *Cooperia oncophora*, *Cooperia pectinata* e *Cooperia punctata*. O gênero *Oesophagostomum* engloba parasitos do intestino grosso de ruminantes e suínos. As principais espécies são *Oesophagostomum columbianum* e *Oesophagostomum radiatum* (DURO, 2010). No entanto, um dos vermes mais frequentes em criatórios de caprinos é o nematoide *H. contortus*, que geralmente ocasiona os maiores prejuízos (AMARANTE, 2004).

O nematoide *H. contortus* é um parasito pertencente ao filo Nematelminto;

Classe Nematoda; Superfamília Trichostrongyloidea; Gênero *Haemonchus*, que acomete pequenos ruminantes, principalmente os caprinos (GEORGE, 1998). *H. contortus* causa hemoncose (BRITO et al., 2009), enfermidade que provoca edema submandibular, progressiva perda de peso, fraqueza, desidratação e anemia severa, caracterizada pela queda do volume globular, palidez das mucosas, dificuldade respiratória e redução da eficiência reprodutiva (CARVALHO, 2011; YUKI, 2012; ENDO et al., 2014). Em casos mais graves de intensa infestação pode levar à morte súbita dos animais (CLIMENI et al., 2008), devido ao hábito hematófago do verme (FETTERER; RHOADS,1998; CARVALHO, 2011).

O ciclo biológico desse parasito (Figura 1) apresenta uma fase de vida livre e outra parasitária (JACQUIET et al., 1998). Seu ciclo de vida é iniciado pela eclosão dos ovos na massa fecal ocorrendo o desenvolvimento das larvas. Basicamente, as larvas de primeiro estádio (L_1) rompem os ovos, se alimentam das bactérias presentes nas fezes e sofrem duas mudas até chegarem a larvas de terceiro estádio (L_3), a forma infectiva. Na fase L_3 essas larvas apresentam motilidade, e assim saem da massa fecal se abrigando nas folhagens dos pastos, onde ficam suscetíveis para serem ingeridas pelos hospedeiros, os caprinos. No interior do corpo do animal a L_3 sofre outras duas mudas (L_4 e L_5) até alcançar o estádio adulto (JACQUIET et al., 1998).

A cutícula do ovo do *H. contortus* é formada por três camadas: uma vitelínica; uma quitinosa e uma formada por lipídios e algumas proteínas (MANSFIELD et al., 1992). O principal constituinte estrutural do ovo é o colesterol, responsável pela fluidez e a permeabilidade da membrana. As proteínas presentes podem desempenhar diversas funções como: respiração celular, comunicação entre células, crescimento, transporte e reconhecimento celular (CARVALHO, 2011). A eclosão dos ovos é iniciada por estímulos ambientais e pelas enzimas da eclosão, liberadas pelo próprio embrião (ROGERS; BROOKS, 1977). Estas enzimas presentes no fluido de eclosão de *H. contortus* estão incluídas nos grupos das proteases, lipases, quitinases, α e β- glicosidases e leucinas aminopeptidases (ROGERS & BROOKS, 1977). A inibição destas enzimas causam redução ou inibição total do processo de eclosão (ROGERS & SOMMERVILLE, 1962).

As larvas possuem uma cutícula que forma o exoesqueleto (PAGE & JOHNSTONE, 2007), que é um revestimento externo essencial para o crescimento, locomoção e sobrevivência dos nematoides. Essa estrutura pode diferir entre espécies ou mesmo entre as fases de vida de uma mesma espécie, no entanto, apresenta uma forma estrutural básica constituída de proteínas com pequena quantidade de lipídios e carboidratos (BIRD & BIRD, 1991). O processo de perda da bainha ou desembainhamento ocorre após a ingestão das larvas pelo animal, onde a mudança de pH, temperatura e exposição a dióxido de carbono dentro do animal, estimula a secreção de fluido rico em enzimas promovendo a digestão da bainha e a liberação das larvas no abomaso (ROGERS, 1962; 1977).

O desenvolvimento das larvas de *H. contortus* ocorre em condições quentes e úmidas (WHITTIER et al., 2009) com a temperatura ideal variando entre 18 e 26°C e a umidade entre 80 e 100% (ONYIAH e ARSLAN, 2005). Essas características ambientais influenciam diretamente no desenvolvimento larvar e na eclosão dos ovos, podendo retardar o processo de eclosão de dias a vários meses, ou ainda provocar o ressecamento das larvas de estágios iniciais ou inviabilizar a migração das larvas L_3, ou seja, como o desenvolvimento do parasito é dependente de condições meteorológicas favoráveis, a sobrevivência das fases larvais pré-infectiva ou parasitárias é de poucos dias. Entretanto, um fenômeno denominado hipobiose, estagna o metabolismo das larvas, interrompendo o desenvolvimento larval até o reestabelecimento de um meio ideal para sua sobrevivência. Permitindo que as larvas, após infecção, permaneçam protegidas por até vários meses no interior dos seus hospedeiros (WHITTIER et al., 2009).

Os adultos deste nematóide possuem tamanhos que variam entre 1 a 3 cm de comprimento e podem ser facilmente identificados devido a sua localização específica no abomaso (AMARANTE, 2005). Possuem uma cavidade bucal armada com uma lanceta que permite perfurar e fixar-se no intestino do animal, além disso, os machos apresentam um raio dorsal assimétrico em sua bolsa copuladora e espículos curtos e cuneiformes. As fêmeas possuem útero longo e branco, repleto de ovos que se espirala em torno do intestino cheio de sangue (ALMEIDA, 1935; LICHTENFELS et al., 1994). A vulva localiza-se próximo à extremidade da cauda, as

estruturas morfológicas das abas vulvares variam entre as espécies e subespécies (GEORGE, 1998; BOWMAN, 2006).

Assim, o estudo das características estruturais do ovo e da cutícula dos nematoides é uma importante ferramenta para o entendimento da penetração de substâncias antiparasitárias, bem como seu modo de ação e mecanismos de desenvolvimento da resistência.

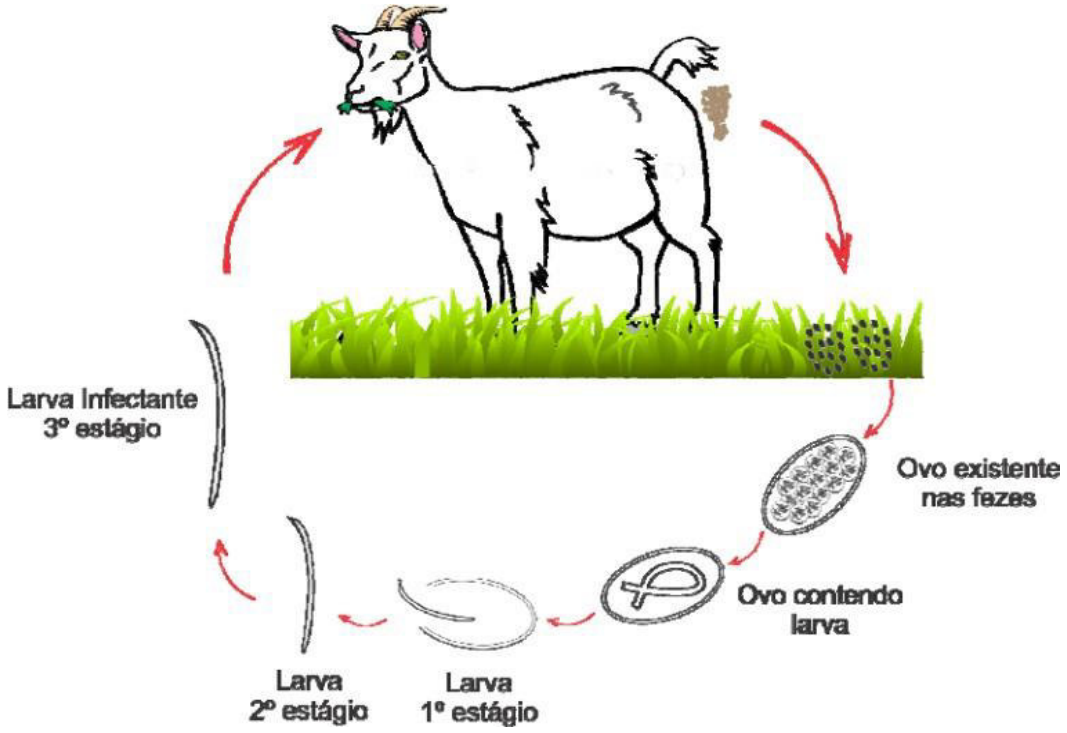

Figura 1: Representação esquemática do ciclo de vida do nematoide gastrintestinal *Haemonchus contortus*. Fonte: Vasconcelos, 2014.

2.2 Controle de nematoides gastrintestinais e resistência aos anti-helmínticos comerciais

O controle dos parasitos gastrintestinais é feito basicamente com produtos químicos comercialmente disponíveis. Os anti-helmínticos utilizados com mais frequência em pequenos ruminantes classificam-se nos seguintes grupos: benzimidazóis e pró-benzimidazóis; imidotiazóis; tetrahidropirimidinas; salicilanilidas;

avermectinas e milbemicinas, e organofosforados (LANUSSE, 1996). No entanto a aplicação destes produtos vem sendo realizado de maneira inadequada, provocando a resistência dos nematoides a cada aplicação, dificultando seu controle (OLIVEIRA et al., 2011).

O desenvolvimento de populações nematoides resistentes aos anti-helmínticos é uma consequência inevitável de seu uso (WALLER, 1995) e relaciona-se diretamente à contribuição genética dos nematoides que sobrevivem aos sucessivos tratamentos anti-helmínticos que serão capazes de transmitir essas características as gerações futuras (VAN WYK et al., 2002). O aumento da resistência a anti-helmínticos tem ocasionado grandes impactos econômicos para a pecuária brasileira e mundial (COSTA-JUNIOR & AMARANTE, 2015), chegando a 300 milhões de dólares (SINDAN, 2013).

Entende-se por resistência anti-helmíntica o aumento do número de indivíduos em uma população que é capaz de suportar doses de um determinado composto químico que mostra-se por meio de estudos letal à maioria de uma população normalmente sensível da mesma espécie (MELO et al., 2003). Muitos podem ser os fatores desta resistência, dentre eles cita-se principalmente a falha no controle parasitário (SANGSTER, 2001; MELO et al., 2003), uso intenso das drogas, subdoses medicamentosas e o uso continuo do mesmo principio ativo (WALLER, 1995).

O primeiro relato de resistência a anti-helmínticos em caprinos no Brasil foi no Rio Grande do Sul (DOS SANTOS; GONÇALVES, 1967 apud FARIA et al., 1997). Estudos posteriores no Nordeste brasileiro indicaram o mesmo perfil em Pernambuco e Bahia. No Ceará, outros relatos de resistência anti-helmíntica em caprinos e em ovinos demonstraram que esse problema está se disseminando (COSTA-JUNIOR; AMARANTE, 2015).

A importância de novas pesquisas para o controle alternativo é também respaldada pelo grande investimento econômico com os tratamentos anti-helmínticos convencionais, além da elevada prevalência de populações de helmintos resistentes. Fator também primordial e pouco considerado é o risco de resíduos de anti-helmínticos na carne, no leite e no meio ambiente (KRYCHAK-FURTADO,

2006).

Na tentativa de minimizar os problemas causados pela resistência, diversos métodos de controle parasitário vêm sendo propostos, como o controle integrado nas pastagens através da rotação de piquetes ou do uso de diferentes espécies de animais no mesmo piquete, o tratamento de animais somente quando ocorrem sinais clínicos evidentes ou morte por parasitismo no rebanho (PINHEIRO, 2000) e o controle seletivo feito através do método FAMACHA baseado nos sinais clínicos de anemia em ovinos (VAN WYK et al., 1997) e caprinos (VATTA et al., 2001).

Neste contexto surge também o controle integrado com o uso de fitoterápicos, apresentando como vantagens: suprimento sustentável, baixo custo, biodegrabilidade, grande possibilidade de utilização da biodiversidade da flora nacional, além da fácil aceitação da população (CHAGAS et al., 2011; MACEDO, 2015).

2.3 Atividade anti-helmíntica de plantas

Pesquisas na área de medicina têm sido cada vez mais alvos de estudos em todo o mundo. Assim, viabiliza-se a descoberta de novos fitoterápicos, assim como seus possíveis mecanismos de ação antiviral, antimicrobial ou parasiticida, por exemplo (LAM; NG, 2009; FAROOQUI et al., 2015; MACEDO et al., 2013).

Especificamente na área de medicina veterinária, em geral, as pesquisas com plantas medicinais objetivam a redução de problemas sanitários no controle de várias doenças que comprometem a produtividade dos animais (COSTA-JUNIOR; AMARANTE, 2015).

Dentre os fatores que levam a procura por produtos alternativos, destaca-se o uso incorreto e/ou abusivo das drogas sintéticas, que podem desencadear reações adversas. Neste contexto, o uso de produtos naturais vem sendo utilizado como alternativa no controle das parasitoses em caprinos e ovinos (MOLENTO et al., 2013).

A fitoterapia em medicina veterinária tem sido indicada, principalmente, para reduzir os custos dos tratamentos químicos e prolongar a vida útil dos produtos anti-

helmínticos disponíveis no mercado, pois diminui a pressão de seleção sobre os isolados de nematódeos (YOSHIHARA et al., 2013). Para tanto, tabela 1 traz um resumo de plantas com atividade anti-helmíntica sobre parasitos gastrintestinais utilizadas nos últimos anos.

Tabela 1. Espécies vegetais com ação sobre parasitos gastrointestinais.

Nome científico	Espécie de nematóide	Referência
Leucaena leucocephala	*Haemonchus contortus*	Alonzo-Diaz et al., 2011; Soares et al., 2015
Lantana Camara	*Haemonchus contortus*	Macedo et al., 2012
Alpinia zerumbet	*Haemonchus contortus*	Macedo et al., 2012
Tagetes minuta	*Haemonchus contortus*	Macedo et al., 2012
Mentha villosa	*Haemonchus contortus*	Macedo et al., 2012; Hassum et al., 2013
Carapa guianensis	*Haemonchus sp, Oesophagostomum sp e Trichostrongylus sp.*	Farias et al., 2010
Mentha piperita	*Ascaridia galli*, *Raillietina* sp	Golynski, 2003
Carica papaya	*Capillaria spp; Ascaridia galli*, *Ascaridia caninum*	Buttle et al., 2011; Shaziya et al., 2012
Artemisia vestita	*Haemonchus contortus*	Irum et al., 2015
Euphorbia helioscopia	*Haemonchus contortus*	Lone et al., 2012
Juniperus pinchotii	*Haemonchus contortus*	Whitney et al., 2013
Arachis pintoi *Cratylia argentea*	*Haemonchus contortus*	Von Son-de Fernex et al., 2012

2.3.1 Exsudatos de sementes e atividade anti-helmíntica

As plantas desenvolveram sofisticados mecanismos de defesa, a maioria das quais, concentradas nas sementes, uma vez que estes são os veículos de propagação e sobrevivência das espécies (CARLINI; GROSSI-DE-SÁ, 2002). Nos tecidos de sementes podem se acumular, constitutivamente ou após a indução, uma ampla gama de compostos defensivos que conferem resistência contra predadores fitófagos e infecção por vírus, bactérias, fungos, nemátodos (HEATH, 2000). Partes destes compostos são liberados pelas sementes como forma de defesa química, em um processo denominado exsudação.

Metabólitos secundários, peptídeos e proteínas, são compostos liberados pelas sementes durante a exsudação (SCARAFONI et al. 2013) e podem ser potenciais no desenvolvimento de novos produtos antiparasitários (ROCHA et al., 2015).

Diferentes estudos apontam os exsudatos como fontes de compostos antimicrobianos (DABOS et al., 2010; SUK et al., 2011; CAMPOS et al., 2012), anti-inflamatórios (CHOVATIYA; MEDZHITOV, 2014; ALLIJN et al., 2016) e anti-oxidantes (CHOUGALE et al., 2011; PINTUS et al., 2013). Adicionalmente, exsudatos de sementes apresentam ação contra fitonematóides (DOSSEY, 2010, ROCHA et al, 2015). Apesar de pouco estudado, exsudatos vegetais têm demonstrado excelentes resultados no tratamento dos parasitos gastroinstetinais (STEPEK et al., 2007a; BUTTLE et al., 2011; PEACHEY et al., 2016).

Algumas pesquisas científicas conduzidas com diferentes espécies vegetais demonstram propriedades antiparasitárias em ruminantes (NIEZEN et al., 1995; ATHANASIADOU et al., 2001; HOSTE et al., 2006; MINHO et al., 2008a; MANOLARAKI et al., 2010; ALONSO-DÍAZ et al., 2011; OLIVEIRA et al., 2011, VON SON-DE FERNEX et al., 2012; SOARES; et al., 2015).

Dentre as plantas estudadas como repositórios de potenciais compostos anti-helmínticos, estão as leguminosas (KAHIYA et al., 2003; ATHANASIADOU et al., 2005; MARTÍNEZ-ORTÍZ-DEMONTELLANO et al., 2010), como por exemplo *Mimosa caesalpiniaefolia, Leucaena leucocephala, Acacia mangium e Stylosanthes capitata*. As sementes das leguminosas são ótimas fontes de proteínas comparadas

a outras espécies de plantas (JOHN, 1992 *apud* HABBEN & LARKINS, 1995). Entre os tipos de proteínas de sementes das leguminosas, várias têm função protetora, estrutural ou metabólica. Dentre os protetores, os inibidores de proteases são, provavelmente, os mais abundantes e amplamente distribuídos, sendo particularmente comuns em sementes de leguminosas, proporcionando resistência contra patogénos (SASAKI, 2008). Existem também as β-1,3-glucanases e endoquitinases, que parecem ter propriedades antifúngicas; lectinas, que se ligam à glicoproteínas do intestino e interferem na absorção dos nutrientes; proteínas inativadoras de ribossomo; proteínas inibidoras de poligalacturonase, entre outras (SASAKI, 2008). Entretanto não há registros sobre estudos de propriedades anti-helmínticas de exsudatos de sementes sobre nematoides gastrointestinais de ruminantes.

2.3.2 Vegetais em estudo

2.3.2.1 *Mimosa caesalpiniaefolia* Benth.

M. caesalpiniaefolia, é nativa do Nordeste brasileiro, ocorrendo naturalmente nos estados do Maranhão, Piauí, Rio Grande do Norte e Ceará, é conhecida como unha-de-gato, sansão do campo, cebiá e sabiá (RIBASKI et al., 2003), de acordo com o estado onde ocorre. O sabiá possui muitos acúleos recurvados e de pontas agudas, as sementes possuem casca marrom-avermelhada (Figura 2) com látex e quando adultas a casca é cinza acastanhada sem látex, as folhas são muito nutritivas e palatáveis apresentando até 17% de proteína bruta.

Esta espécie possui uma importância significativa na Região Nordeste por sua resistência à seca e crescimento rápido, deste modo sua folhagem é utilizada na alimentação de grandes e pequenos ruminantes nos períodos secos (CARVALHO, 2007) por possuir um alto valor nutritivo (STAMFORD et al., 1997) as folhas apresentam ainda um alto potencial alelopático (PINÃ-RODRIGUES; LOPES, 2001).

Sua madeira é utilizada na produção de estacas (LEAL JÚNIOR et al., 1999) e

por apresentar uma alta massa específica e elevado poder calorífico é utilizada na produção de carvão (GONÇALVES, et al.,1999), a parte interna da casca é utilizada na medicina popular na forma de chá para doenças estomacais e também é utilizada como cicatrizante. Estudos com extratos de folhas da *M. caesalpiniaefolia* demonstraram eficácia contra o nematóide *H. contortus.* Canova (2016) atribuiu à atividade anti-helmintica desses extratos a presença de taninos condensados e de lectinas.

Figura 2: Exemplares de sementes da espécie *Mimosa caesalpiniaefolia* Benth.
Fonte: Próprio autor.

2.3.2.2 *Leucaena leucocephala* (Lam.) de Wit

L. leucocephala é nativa da América Central e da Península de Yucatán do México. Sendo encontrada naturalizada na maioria das áreas tropicais e subtropicais do mundo (LIM, 2012).

Caracterizam-se por medir entre 5 a 10 metros de altura, apresentar folhas alternas bipinadas, as flores possuem corola e estames brancos, vagens agrupadas

e achatadas de coloração marrom-escura, com um bico no ápice com sementes de coloração marrom (Figura 3) (PANDEY; KUMAR, 2013). No Brasil, é utilizada como forragem para pequenos animais devido sua riqueza em proteínas e nutrientes (SAFWAT et al., 2014).

Figura 3: Exemplares de sementes da espécie *Leucaena leucocephala* (Lam.) de Wit**.** Fonte: Próprio autor.

Diferentes partes das plantas *L. leucocephala* são usadas na medicina popular tradicional como antifúngica (FENNER et al., 2006). As folhas são uma rica fonte de proteínas e contêm 3% de tanino, caroteno, leucenol e 3-4% de glicéidos de flavonol (PANDEY; KUMAR, 2013). Os estudos mostraram que extratos de sementes de *L. leucocephala* tem atividade antidiabética (SYAMSUDIN et al., 2010) e contra o parasito *H. contortus* (OLIVEIRA et al., 2011; VON SON-DE-FERNEX et al., 2014).

2.3.2.3 *Acacia mangium* Willd.

A. mangium é uma espécie de árvore florida na família das ervilhas Fabaceae, que é nativa do Nordeste do Queensland na Austrália, na Província

ocidental de Papua Nova Guiné, Papua e nas Ilhas Maluku orientais (FERREIRA, 1990). Caracteriza-se por ter crescimento rápido e por ser utilizada para o reflorestamento em regiões tropicais e atua também na regeneração de áreas degradadas devido à sua capacidade de reciclar nutrientes e alta produção de biomassa (COLONNA et al., 1991). É imprópria para plantios em regiões de alto déficit hídrico e com ocorrências de geadas fortes, desenvolve-se bem em solos pobres, erodidos, com pouca drenagem e pH abaixo de 3,5. Entretanto, segundo Ferreira (1990) tem grande potencial de cultivo no Brasil, já havendo recomendação de procedências adequadas para as regiões Amazônicas e Vale do Rio Doce em Minas Gerais.

Figura 4: Exemplares de sementes da espécie *Acacia mangium* Willd. Fonte: Próprio autor.

Árvores de acácia têm em média 41% de proteínas (COLONNA et al., 1991), sendo distribuídas em todas as partes da planta, concentrados principalmente na casca do caule e sementes apesar de serem pequenas (Figura 4). Espécies deste gênero vêm sendo alvo de constante pesquisa sobre ação antiparasitária contra *H. contortus* (KAHIYA et al., 2003; MAX, 2010).

2.3.2.4 *Stylosanthes capitata* Vog.

S. capitata amplamente distribuída pelo continente americano, apresentando grande variação de formas e tipos, resultantes da evolução de ecótipos submetidos às diferentes condições de clima, solos e pressões bióticas (KARIA et al., 2002).

Esta espécie são excelentes forrageiras e por isso tem se destacado por sua adaptação a solos ácidos e de baixa fertilidade, alta capacidade de fixação biológica do nitrogênio (mutualismo com algumas espéices de bactérias), tolerância a seca e elevada produtividade (ANDRADE; VALENTIM, 2007). *S. capitata* tem hábito de crescimento cespitoso, podendo atingir até um metro de altura. A cor das flores varia do bege ao amarelo.

Figura 5: Exemplares de sementes da espécie *Stylosanthes capitata* Vog. Fonte: Próprio autor.

3. OBJETIVOS

3.1 Geral

Levantar o potencial biológico e farmacológico de exsudatos de plantas através de uma revisão bibliográfica, bem como, identificar proteínas dos exsudatos proteicos de sementes de *Mimosa caesalpiniaefolia, Leucaena leucocephala, Acacia mangium e Stylosanthes capitata e* avaliar sua ação anti-helmíntica sobre o nematoide gastrointestinal *H. contortus.*

3.2 Específicos

- ✓ Descrever sobre os exsudatos liberados das plantas, suas propriedades biológicas e potencial farmacológicos, assim como a presença de vários compostos;
- ✓ Avaliar a atividade inibitória de eclosão de ovos e desembainhamento larvar do nematóide *H. contortus* por exsudatos proteicos das sementes de *M. caesalpiniaefolia, L. leucocephala, A. mangium e S. capitata*;
- ✓ Identificar as proteínas presentes nos exsudatos proteicos por espectrometria de massas.

4. RESULTADOS

Os resultados do presente trabalho estão apresentados sob forma de capítulos, consistindo em artigos científicos nas seguintes situações:

✓ Capítulo 1: Biological properties and pharmacological potential of plant exudates.
Situação: Publicado na revista Food Research International (Qualis A2 em Medicina I; Fator de Impacto: 3.086). Volume 105, March 2018, Pages 1039-1053. Doi: https://doi.org/10.1016/j.foodres.2017.11.051.

✓ Capítulo 2: Seeds exudate different classes of proteins and possess anthelmintic activity toward the nematode *Haemonchus contortus.*
Situação: Submetido na revista PLOS ONE (Qualis A2 em Medicina I; Fator de Impacto: 3.057).

CAPÍTULO I

Biological properties and pharmacological potential of plant exudates

Neste capitulo consta o artigo publicado na revista Food Research International (Qualis A2 em Medicina I; Fator de Impacto: 3.086). Volume 105, March 2018, Pages 1039-1053. Doi: https://doi.org/10.1016/j.foodres.2017.11.051.

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Review

Biological properties and pharmacological potential of plant exudates

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ABSTRACT

Exudates released from plants, consist of complex mixtures of organic and inorganic molecules that have been used in traditional medicine from several years. They may vary among genera, species or within a genus and mainly include latex, sap, gums, resins, seed or root exudates. Plant exudates are known to possess several biological activities including, antimicrobial, anti-inflammatory, antioxidant, wound healing and anti-nociceptive. Exudates oozed out from plants have also been used as ingredients in medicines, food, perfumes and cosmetics. The present review provides brief overview about the exudates released from plants, their biological properties and beneficial effects for human beings. Due to the presence of various compounds, different methodologies and procedures have been employed for their collection and analyses. Literature studies suggest that plant exudates have extensive therapeutic potential for curing diseases with minimal toxic effects. This aspect could be taken into account in prospective studies regarding the search of new products derived from plant exudates with pharmaceutical value.

1. Introduction

Traditional medicines derived from various plants species have been frequently used in daily health care system in developing countries since a long time (Breitbach, Niehues, Lopes, Faria, & Brandao, 2013; Ferreira et al., 2014; Júnior & Pinto, 2005). Knowledge obtained from ethnopharmacological studies have been employed to cure several diseases in traditional system of medicine and also known as the efficient means for drug discovery. The resurgence of public interest for plant based natural compounds due to their lesser side effects and better compatibility with the human body has resulted in finding out alternative ways to obtain bioactive compounds, which could be effective and safe as compared to synthetic drugs (Malik et al., 2011; Malik, Mirjalili, Fett-Neto, Mazzafera, & Bonfill, 2013; Malik, Bhushan, Sharma, & Ahuja, 2014; Malik, Biba, Gruz, Arroo, & Strnad, 2014; Gallego et al., 2017). The widespread use of synthetic drugs has resulted in development of resistance against the pathogens. It has become one of the world's most serious public health problems (Gumz, Schoneweg, & Arcanjo, 2015; Varaldo, 2002; Wise, 2003). It is difficult to eradicate some pathogenic bacteria and in several cases, use of more than one antibiotic is required, which may leads to side effects and other problems (Golkar, Bagasra, & Pace, 2014; Gould & Ball, 2013; Malfertheiner et al., 2007).

Natural products, mainly from plants represent potential alternative to treat various diseases caused by micro-organisms. The use of plants for therapeutic purposes is based on popular and scientific knowledge. These are also acts as suppliers of isolated active substances, such as total or purified extracts (Atanasov et al., 2015; Carmona & Pereira, 2013). Phytotherapeutic remedies are cost-effective with minimal toxicity and reduced health risks. These are also readily available in the market as compared to synthetic drugs (Khandaker, Sajan Das, Akhter, & Shahriar, 2016).

Among various plant- derived natural bioactive compounds, exudates employed routinely for primary health care in developing countries (Joy, Thomas, Mathew, & Skaria, 1998). These are the complex mixtures of large and small molecules, including carbohydrates, proteins, amino-acids, volatile compounds or inorganic ions released from healthy plants during the process of exudation (Mirhosseini & Amid, 2012; Neumann & Römheld, 2007; Uren, 2007). Exudates are known to possess several medicinal properties and have also been used as ingredients in medicines, food, perfumes and cosmetics (Iqbal & Fry, 2012). They may vary greatly among genera, between species or within a genus, and their function in plants is not fully understood (Boer & Ella, 2000). Certain legumes exude specific flavonoids and isoflavonoids, which activate genes responsible for nodulation and promote chemotaxis (Bais, Weir, Perry, Gilroy, & Vivanco, 2006; Hassan & Mathesius, 2012). Other members of family *Poaceae* exude carbohydrates and amino acids that are energy sources and nutrients for

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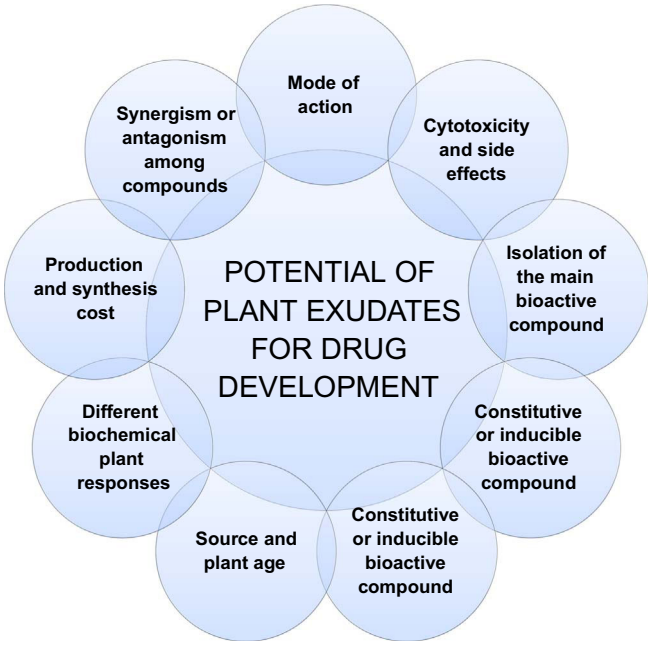

Fig. 1. Various factors to be taken into account in assessing the potential of plant exudates for drug development.

microorganisms thus increasing the population of plant growth promoting bacteria in rhizosphere (Gray & Smith, 2005; Souza, Ambrosini, & Passaglia, 2015). Weert et al. (2002) found that exudation of some organic acids, such as malic, pyroglutamic, succinic and fumaric acid and amino acids including; L-aspartic acid, L-glutamic acid, L-isoleucine, L-leucine and L-lysine by tomato plants influence flagellar motility of *Pseudomonas fluorescens*. The substances released during exudation may exert a chemo-attractant effect on microorganisms present in soil or even prevent the seed from being colonized by phyto-pathogens (Lugtenberg, Rozen, & Kamilova, 2017; Ma, Oliveira, Freitas, & Zhang, 2016; Nóbrega, Santos, Cunha, Carvalho, & Gomes, 2005; Okubara & Paulitz, 2005). We are not describing here this subject in detail since there are number of excellent reviews published during the last decade on interaction between plant exudates and microbes (Baetz & Martinoia, 2014; Huang, Chaparro, Reardon, & Zhang, 2014; Walker, Bais, Halligan, Stermitz, & Vivanco, 2003).

Ethnopharmacological studies have indicated the use of plant exudates in traditional medicines but there are only a few scientific records or merits of these products. Fig. 1 depicts the various factors need to take into account in order to use plant exudates for drug development. Root exudates are the most studied compounds but in relation to understand the interactions between plants and microbial communities in the rhizosphere or for molecular mechanisms (Badri & Vivanco, 2009; Deshpande, Pontaroli, Chaluvadi, Lu, & Bennetzen, 2011; Doornbos, Van Loon, & Bakker, 2012; Huang et al., 2014; Semchenko, Saar, & Lepik, 2014; Walker et al., 2003). Although, there are scientific reports discussing about the therapeutic potential of exudates released from plants but there is no any detailed review focusing on the medicinal uses of plant exudates. Therefore, the aim of present paper is to assess the various biological activities and pharmacological potential of plant exudates. Different types of exudates released from plants, their method of collection and analyses are described. All the available reports on various activities of plant exudates including; antimicrobial, anti-inflammatory, antioxidant, wound healing, anti-nociceptive will be discussed in detail. Future prospects of these plant exudates for drug discovery and various factors concerning them are highlighted.

1.1. Different types of exudates released from plants

Depending on the physical and chemical characteristics, exudates are generally classified as resins, sap, latex or gums (Coppen, 1995; Langenheim, 2004). Additionally, there are other plant exudates such as seed exudates and root exudates. Latex, also known as milk sap is a cytoplasmic exudate of specialized cells, called laticiferals (Konno, 2011; Rudall, 1987). The chemical composition of latex acts as a defense to fight against fungi and viruses through its constituents (Pereira, Valdirene, Fernandes, Maurício, & Xavier-Filho, 1999). More than 20,000 plant species from over 40 families exude latex, which accounts for about 8.9% of all angiosperm plants (Lewinsohn, 1991). The best-known example is latex obtained from rubber, which has solids content over 50% of the weight of latex. Latex contains a variety of chemicals, such as terpenoids, alkaloids, rubber, cardenolides as well as various proteins and enzymes; such as proteases, chitinases, and glucosidases (Konno, 2011). Some chemical compounds in latex, such as morphine (an alkaloid from poppy latex) and cardenolides (from milkweed latex) show apparent toxicity against animals, including insects (Konno et al., 2004, 2006; Ramos et al., 2007, 2010; Wasano et al., 2009). Such toxic chemicals are suggested to have defense roles (Farrell, Dussourd, & Mitter, 1991). Some of the species that produce latex include: *Asclepias syriaca* L. (milkweed), *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (rubber tree) and *Lactuca sativa* L. (Dussourd, 1995; Dussourd & Eisner, 1987).

The resins are solid or semisolid amorphous materials, generally comprising a complex blend of organic compounds called terpenes. They are insoluble in water but soluble in certain organic solvents (Lambert, Heckenbach, Wu, & Santiago-Blay, 2010; Langenheim, 2004; Paparozzi, 2005). They can occur in almost any organ or tissue of the plant species (Rikkinen et al., 2016). Important families that produce resins are: *Burseraceae*, *Dipterocarpaceae*, *Leguminosae* (mainly, *Caesalpinioideae*), *Styracaceae* and two families of conifers, each with an important resin producing genus, *viz*. *Araucariaceae* (*Agathis*) and *Pinaceae* (Lambert et al., 2010).

Vegetable gums are solids consisting of mixtures of polysaccharides, which are water soluble or absorb water and swell to form a gel or gelatin, when placed in water (Bhosale & Osmani, 2014; Yang & Zhang, 2009). They are translucent and amorphous substances often produced by plants as a protection after an aggression (Buckeridge, Tiné, Santos, & Lima, 2000). Many plants growing in semi-arid conditions produce gummy exudates in large quantities, when cortex is assaulted. This is to seal cutting and prevent dehydration (Buckeridge et al., 2000). Gums can be obtained from the shells, or even from seeds, such as guar gum of *Cyamopsis tetragonoloba* (L.) Taub. and carob gum (or carob tree) of *Ceratonia siliqua* L., both obtained from the seed endosperm. Other natural gums are mucilages produced by algae (*e.g.* alginates, agar, carrageenan) and bacteria (dextran, xanthan) (Bhosale & Osmani, 2014).

Sap is a fluid transported in xylem cells (vessel elements) or phloem sieve tube elements of the plant (Robert & Shmuel, 2009). These cells transport water and nutrients throughout the plant. Sap should not be confused with latex, resin or cell sap; it is a different substance, produced separately and possesses different components and functions (Douglas, 2006). Xylem sap consists primarily of a watery solution of hormones, mineral elements and other nutrients. Transport of sap in xylem is characterized by its movement from roots towards leaves (Douglas, 2006). Phloem sap consists primarily of sugars, hormones, and mineral elements dissolved in water (Douglas, 2006).

2. Methodology

Scientific publications from various recognized databases including Scopus, Google Scholar, ACS, PubMed, Wiley, Scielo and Web of Science were surveyed and analyzed. Anthelmintic, nematicide, antifungal, fungicide, antimicrobial, antioxidant, anti-ulcerogenic, wound

healing, exudation, plant exudate, resin, latex, gum, seed, root, pharmacology, biological activities are among the keywords used for the literature search. Inclusion criteria limited to studies involving *in vitro* or *in vivo* experiments related to the action of plant exudates against bacteria, fungi, nematode or studies regarding pharmacological potential and applications of plant exudates Additional data from books, theses and dissertations are also included in the following sections of this review.

3. Pharmacological activities of plant exudates

3.1. Methods of collection and analyses of plant exudates

Different methodological procedures are used for their collection and analyses by employing various analytical techniques including high-performance liquid chromatography (HPLC), electrospray ionization liquid chromatography-tandem mass spectrometry (ESI–LC–MS/MS and GC/MS) (Dundek et al., 2011; Rocha et al., 2015). Some of the common methods employed for exudates collection and analyses would be briefly discussed here. Gums are collected directly from the trees and soaked in distilled water at 60 °C or treated with a solution of sodium salt (NaCl) to homogenize the contents and obtain suspension (Campos et al., 2012; Yao, Cao, & Wu, 2013). The crude resin, a viscous secretion is collected by tapping the trees (Termentzi, Fokialakis, & Skaltsounis, 2011). Seed exudates can be obtained from whole seeds after surface-sterilized with ethanol or sodium hypochlorite and soaked after thoroughly washed with distilled water or buffers. For instance, soybean seeds were soaked in 6 mL of 0.1 M sodium acetate buffer at pH 5.0, temperature 28 °C, and 70% relative humidity. The exudates collected after removing the seeds and the volumes were recorded (Rocha et al., 2015). The root exudates can be prepared placing sterile seedlings on stainless steel gauze on the bottom of a cylinder after stipulated days of growth (Lugtenberg, Kravchenko, & Simons, 1999). Root exudates generally require continuous filtration and centrifugation to remove dirt, solids and microbial cell debris (Dundek et al., 2011). The method of collection of exudates released from plants according to their purpose and use has been reviewed in detail by Vranová, Rejšek, Skene, Janouš, and Formánek (2013). The plant stems or roots can be cut off to obtain xylem sap from the cut surface. The plants are placed in horizontal position to collect the sap dripping from the cut surface in tubes (Rep et al., 2002; Satoh, Iizuka, Kikuchi, Nakamura, & Fujii, 1992). The sap is obtained from adult trees in vertical manner. The latex can be collected in a plastic container having distilled water and maintained at 5 °C. Latex is centrifuged to remove water insoluble polymers and the supernatant is recovered and freeze-dried to be used for biological or pharmacological tests (Buttle et al., 2011; Pereira et al., 1999).

3.2. Anthelmintic activity

Nematoids (Phylum Nematoda), which include parasites of animals and plants accounts for about billions dollars loss of several crops of economic interest worldwide every year (Nicol et al., 2011; Williamson & Hussey, 1996). The most widely used method in controlling nematodes during recent years is the application of synthetic nematicides (Andres, Gonzalez-Coloma, Sanz, Burrilo, & Sainz, 2013; Briar, Wichman, & Reddy, 2016). These synthetic compounds are harmful to human health as well as to the environment, in addition to be expensive (Nico, Jiménez-Díaz, & Castillo, 2004). Moreover, the problem of developing resistance to anthelmintics is a worldwide concern (Klauck et al., 2014; Shalaby, 2013; Vercruysse et al., 2011; Waller, 1994). Nematicides of plants are considered as an alternative in the treatment of parasitic infections caused by nematodes. For instance, the indigenous system of medicine reports a series of plants for anthelmintic efficacy (Akhtar, Zafar, Khanb, & Muhammad, 2000). Several plant species, including *Pisum sativum* and *Glycine* max produce compounds such as proteins, alkaloids and other secondary metabolites during the exudation processwhich are toxic to nematodes (Hammond, Fielding, & Bishop, 1997; Hiltpold, Jaffuel, & Turlings, 2015; Rocha et al., 2015). These exudates can be induced by external factors like biotic and abiotic stressors (Amin, 2015). The different compounds present in plant latex act as a defense against pathogens and may have nematicidal potential (Pereira et al., 1999). Latex from *Carica papaya* L. latex is effective in controlling helminth infections in animals (Buttle et al., 2011; Moraes, Levenhagen, Costa-Cruz, Costa, & Rodrigues, 2017 and Stepek, Lowe, Buttle, Duce, & Behnke, 2006). The anthelmintic effect of latex from fruits of *C. papaya* was evaluated against *Ascaridia galli* in an experimental infection in chickens (Schrank, 1788). A single dose of papaya latex (20%) at 1447.89 mg per bird was found to be 100% effective (Mursof & He, 1991). The papaya latex contains four cysteine proteases (Stepek et al., 2006), which collectively have anthelmintic effects, especially in chicken nematodes (Buttle et al., 2011; Stepek et al., 2007). Similarly, Satrija, Nansen, Bjørn, Murtini, and He (1994) investigated the antihelmintic activity of papaya latex against natural *Ascaris suum* (Goeze, 1782) infection in pig. Sixteen naturally infected pigs based on fecal egg count and body weight were divided into four groups. The results of this study showed that papaya latex at dose levels of 6–8 g per kg body weight was effective against *A. suum*.

Buttle et al. (2011) demonstrated that papaya latex has potent anthelmintic activity capable of killing the adult parasitic nematode *Haemonchus contortus* (Rudolph, 1803) from the sheep abomasums. The activity was detected after administration of 117 μmol of active papaya latex supernatant for 4 days. The *in vivo* anthelmintic efficacy of crude papaya latex against rodent nematodes resident in various parts of the gastrointestinal tract has been reported and the bioactivity was attributed to cysteine proteinases (Stepek et al., 2007). *In vitro* and *in vivo* experiments have shown the efficacy of the papain latex supernatant against the adult stages of parasitic nematodes in equines (Peachey et al., 2016) and *Heligmosomoides polygyrus* (Dujardin, 1845) in mice (Satrija, Nansen, Murtini, & He, 1995). At 15 mg/mL, the aqueous latex of *Jatropha curcas* L. showed anthelmintic activity that was attributed to the predominant effect of the plant-containing piperazine citrate (Parmar, Johari, & Rathore, 2014).

Plants are constantly threatened by nematodes. A series of studies have been carried out to develop nematicides from plant seeds (Gifoni et al., 2012; Rocha et al., 2015). *Moringa oleifera* Lam. (family *Moringaceae*) is known to be resistant to the attack of pathogens (Gifoni et al., 2012). The seed exudates of *M. oleifera* contain several molecules involved in plant defense (Gifoni et al., 2012). Seed exudate in *Glycine* max (L.) Merr. (soybean) has been reported to inhibit the motility of *Meloidogyne incognita* (Karssen, 1996) at 60–500 mg of protein/L, which is mainly attributed to the presence of various proteins (Rocha et al., 2015).

The exudation of organic compounds by plant roots has been a known fact for many decades (Bais et al., 2006; Neumann & Römheld, 2007). The major compounds of root exudate are carbohydrates, organic acids and amino acids (Carvalhais, Fedoseyenko, Haijirezai, Borriss, & Vonwiren, 2011; Grayston, Vaughan, & Jones, 1997). Extensive studies have shown that these exudates can influence and affect the activity of soil organisms in rhizosphere including nematodes (Amin, 2015). Chemicals in root exudates such as tannic acids, flavonoids, glycosides, fatty acids and volatile organic molecules can attract nematodes to roots or result in repellence, motility inhibition or even death (Yang et al., 2016).

A good example of this mechanism is the parasitic relationship between *M. incognita* and the roots of tomato plants (Yang et al., 2016). These exudates contain nematicidal substances, leading to death or reducing the mobility of nematodes (Rocha et al., 2015). Hubbard et al. (2005) reported that exudate of green pea root tip (*Pisum sativum* L.) caused a loss of mobility and induced transient and reversible stillness in several nematodes. The same response occurred when second juvenile phase (J2) was incubated in the presence of exudate.

Brinke, Heininger, and Traunspurger (2011) verified an alternative

test for nematodes using gum as a gelling agent instead of agar. The characterization of nematode toxicity tests is performed by liquid media, which allows the rapid screening of potential toxicities and provides information on the susceptibility of all nematodes (Boyd, Cole, Anderson, & Williams, 2003; Hoss, Baumgarte, Tebbe, Nguyen, & Jehle, 2008; Sochova, Hofman, & Holoubek, 2007; Traunspurger et al., 1997).

Ferula assa-foetida L., a medicinal plant belonging to family *Apiaceae* and native to central Asia is used among indigenous people because of its wide range of biological properties, including anthelmintic activity (Mahendra & Bisht, 2012; Upadhyay, Parveen, Dhaker, & Kumar, 2010). For instance, a gummy mass is obtained from *F. assa-foetida* resin after extraction with ethanol. This gummy mass when dissolved in water (at concentrations of 0.25, 0.5, 1.0 and 2.0%) caused significant mortality of J2 of *Meloidogyne javanica* Treub. and *M. incognita* under laboratory conditions (Zia-Ul-Haq, Mansoor, & Akhter, 2010).

El-Sherbiny and Zen-El-Dein (2012) verified the nematicidal activity of the crude aqueous extract of *F. assa-foetida* resin against *M. incognita*. The extract presented lethal concentration 50% and 90% (LC50 and LC 95) of 110.8 and 519.5 ppm, respectively under *in vitro* experiments. The authors also verified *in vivo* nematicidal properties of the extract in open air for controlling the eggplant nematodes.

3.3. Antimicrobial action

The use of natural substances to control diseases is a centuries old activity. One group of natural compounds with potential antimicrobial activity is the plant exudates and their components. There are only a few reports on antimicrobial activity of natural gums. Dabos, Sfika, Vlatta, and Giannikopoulos (2010) assessed the antibacterial activity of a natural resin excreted from the trunk and branches of the mastic bush (*Pistacia lentiscus* L.), named mastic gum against *Helicobacter pylori* (Goodwin et al., 1989) *in vivo*. They reported that the monotherapy did not achieve acceptable eradication rates but the mastic gum could be used as an alternative in patients unwilling to undergo the conventional eradication therapy regime. In general, the mechanism of action is not proposed but some studies have been performed in cashew tree gum, an exudate from *Anacardiaceae* plant species. Cashew tree gum displays antimicrobial activity against *Escherichia coli* (Escherich, 1885), methicillin-sensitive and resistant *Staphylococcus aureus* (Rosenbach, 1884), *Listeria innocua*, *Pseudomonas aeruginosa* (Schroeter, 1872), *Enterococcus faecium* (Orla-Jensen, 1919) (minimum inhibitory concentrations (MICs) varying from 20 to 60 mg/mL). Cells of *E. coli* and methicillin-resistant *S. aureus* collapsed and became rougher after treatment with the pure cashew gum at 10 mg/mL. The authors suggested that the disintegration of bacterial cell structure could probably be due to interaction with cell wall (Campos et al., 2012).

Some derivate products of gums possess activity against bacteria and fungi. Peach (*Prunus persica* L.) gum derived oligosaccharides, prepared from peach gum polysaccharides by hydrolysis using hydrogen peroxide had high antimicrobial activities against *Bacillus subtilis* (Cohn, 1872), *S. aureus* and *E. coli* at concentration of 100 μg/mL. However, the antibacterial activity of these oligosaccharides was not elucidated due to the complex constituents and structures of peach gum polysaccharides (Yao et al., 2013). Derivates of gum karaya (*Sterculia urens*) have potential to be used as stabilizers and antibacterial agents in food and other industries (Padil, Senan, & Cernik, 2015).

Natural resins can display antibacterial, antifungal and antiprotozoa activity. For instance, *Commiphora myrrha* L. (oleo-gum-resin), *Operculina turpethum* L. (glycosidic resin) and *Pinus roxburghii* Sarg. (oleo-resin) display antibacterial activities against gram-positive and gram-negative bacteria with different sensitivities and this fact could be the reason for using these plants by indigenous people against infections (Shuaib, Ali, Ali, Panda, & Ahmad, 2013). Furthermore, investigations on the cytotoxicity of resins as well other plant exudates are of utmost importance together with antibacterial activity experiments (Soderberg, Johansson, & Gref, 1996).

Resin acids are abundant and low-cost natural resins from pine and conifer trees. The antimicrobial activity of the resin acids and derivates has been previously documented (Savluchinske-Feio, Curto, Gigante, & Roseiro, 2006; Smith, Williamson, Zloh, & Gibbons, 2005). These natural resins exhibit strong antimicrobial activities against a broad spectrum of bacteria with MICs varying between 0.7 and 40 mM due to their hydrophobicity and unique structure. Resin acids have strong antimicrobial activities (ranged between 0.7 and 40.0 μM) and display selective lysis of microbial membranes over mammalian membranes (Wang et al., 2012).

The antimicrobial properties of plant saps were previously described in experiments carried out with latex sap from *C. papaya* against *Candida albicans* (Giordani, Cardenas, Moulin-Traffort, & Regli, 1996). It has been reported that fungistatic action of *C. papaya* latex against *C. albicans* was due to cell wall degradation. Urushiol, a major organic component in the sap of lacquer tree (*Rhus verniciflua* F. Barkley), showed *in vitro* antibacterial activity against *H. pylori* by disrupting the bacterial cell membrane (Suk et al., 2011). The MIC of urushiol monomer against *H. pylori* strains was 0.064 to 0.256 mg/mL under *in vitro* conditions. For *in vivo* experiments, 2–4 polymer urushiol presented a lower MIC (0.128 mg/mL) that of urushiol monomer. Although seven days treatment with a concentration below 0.128 mg/mL per day might be safe in mice but the use of urushiol as a therapeutic agent against *H. pylori* need to be assessed to avoid the induction of allergic reactions (Suk et al., 2011). The search of new antimicrobials can be done using physiological plant process as model. For instance, plant response to pathogen infection can be a source of bioactive compounds from plants, which are useful for human beings and could be used in studies related to development of new antimicrobial compounds. For example, pathogenesis-related proteins (PR proteins) appeared in xylem sap of fungus-infected tomato (*Solanum lycopersicum* L.) (Rep et al., 2002).

Antifungal and antibacterial compounds (secondary metabolites, proteins) are also present in the nectar (Gershtein et al., 2015; Sasu, Wall, & Stephenson, 2010). In general, secondary metabolites are assumed to be nectar-protective compounds but PR proteins (such as chitinases, glucanases and thaumatin-like proteins) are the majority of nectar proteins, named nectarins (Heil, 2011). Chitinases and glucanases degrade chitin and β-1,3 glucans, respectively, the most prominent cell wall components of microbes. Thaumatincan inhibit hyphal growth and sporulation of various fungi (Escalante-Pérez et al., 2012). The defense proteins necessary to prevent infections in nectar can be interesting compounds with potential antimicrobial activities towards broad range of bacteria and fungi. However, it is important to mention that some potential antimicrobial proteins are present in nectar but most of the researches did not purify the bioactive protein to determine their potential activity. For instance, a lipase has been identified in *Jacaranda mimosifolia* (Don, 1822) nectar, which could be related to the fatty acids cleavage for pollinator attraction or to play roles in plant defense. However, the enzyme could not to be purified from nectar for further antimicrobial experiments (Kram, Bainbridge, Perera, & Carter, 2008).

Plant latex has antibacterial and antifungal properties (Ishnava, Chauhan, Garg, & Thakkar, 2012; Manoorkar & Gachande, 2014; Sumathi, 2011; Van Deenen, Prüfer, & Schulze, 2011). The essential oil from *F. assa-foetida* latex displays antifungal activity with MICs varying from 85 to 90 μg/mL and antibacterial activity with MICs between 80 and > 200 μg/mL. It has been suggested that *F. assa-foetida* oil can be used as a natural additive in foods to prevent pathogens growth (Kavoosi & Rowshan, 2013). In some plants, the antibacterial activity of latex is due to the presence of various components such as proteases, chitinases, osmotin, alkaloids, glycosides, diterpenes and saponins (Upadhyay, 2015). However, most of the studies did not isolate potential compounds for confirming antibacterial tests. Several studies have scientifically supported the usage of latex as a remedy for various fungal and bacterial infections in traditional medicine (Kareem, Akpan,

& Ojo, 2011; Vimal & Das, 2015). Additionally, the antimicrobial activity of plant latex has been reported against resistant human pathogen such as methicillin-resistant strain *S. aureus* (Aref et al., 2010; Suhaili, Yeo, Yasin, Badaludin, & Zakaria, 2011).

The solvent used to recover the plant exudates may affect the successful extraction of antimicrobial compounds. For instance, *Himatanthus articulatus* (Vahl) Woodson latex obtained with water displayed antifungal properties (Sequeira, Vital, Pohlit, Pararols, & Cauper, 2009). However, the solvent for extraction of bioactive compounds from plant latex varies from species to species. For example, extracts from *Calotropis gigantia* (L.) Dryand. latex were obtained with different polarity solvents and the chloroform extracted fraction showed inhibitory effect against two carcinogenic bacteria: *Streptococcus mutans* (Clarke, 1924) and *Lactobacillus acidophilus* (Moro, 1900) with MICs: 0.032 and 0.52 mg/mL, respectively. The methyl nonanoate, a saturated fatty acid, was revealed as the bioactive compound (Ishnava et al., 2012). The methanol extract from *J. curcas* latex was found to be the most effective with MIC ranging from 0.39 and 6.25 mg/mL for *S. aureus* and *Serratia marcescens* (Bizio 1823), respectively. The potential antimicrobial activity could be attributed to saponins and tannins (Suhaili et al., 2011). In addition to extraction method, the quantity of chemical substances varies with age and sex of the plant species for example in *Rheum emodi* (Malik et al., 2010).

Interestingly, the active components in plants latex showed changes their bioactivities to some extent, when combined together (Khusro, Aarti, Raj, & Panicker, 2014; Khusro, Preetam Raj, & Panicker, 2014). For instance, when two or three plant extracts were combined, their activity reduced against some tested pathogenic organisms. Additionally, the aqueous crude extracts of ginger and garlic tested in combination did not show antimicrobial effects but their ethanolic extracts combined together led to *S. aureus* and *Bacillus* species inhibition (Onyeagba, Ugbogu, Okeke, & Iroakasi, 2004).

Plant latex can also be used in replacement of chemical methods for nanoparticles synthesis. Substances obtained by chemical synthesis may present adverse effects in medical applications. Thus, the green synthesis of metallic nanoparticle solutions induced by *Euphorbiaceae* latex offers a rational approach towards antimicrobial application and integration to biomedical devices (Valodkar et al., 2011). The synthesis of silver nanoparticles based natural rubber has also been reported and can be used for making antimicrobial materials (Rathnayake, Ismail, Azahari, De Silva, & Darsanasiri, 2014). Marcus, Green, Goulter, and Manners (1999) have purified peptides from *Macadamia integrifolia* Maiden & Betche seed exudates with antimicrobial activity but to the best of our knowledge, the purification of antimicrobial compounds from seeds or roots exudates have not been performed in other studies. There are several researches that strongly suggests the induction of antimicrobial substances in these exudates after a simulated attack, pathogenic bacteria or fungal challenge or other stress condition (Basu et al., 2006; Charmont, Jamet, Pont-Lezica, & Canut, 2005; Walker et al., 2003; Walker et al., 2004). For instance, an induced root exudation of phenolic compounds with antifungal activity in response to barley (*Hordeum vulgare* L.) root infection with *Fusarium graminearum* (Schwein.) Petch at 2 days postinoculation has been reported by Lanoue et al. (2010).

Root exudate from *Vigna unguiculata* (L.) Walp seedlings contain diverse defense proteins and inhibited the growth of *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen (Nóbrega et al., 2005). The secretion of PR proteins in exudates is correlated closely with plant developmental stage. In an experiment conducted by De-la-Pena et al. (2010), PR proteins such as chitinases, glucanases, myrosinases and others, represented 27% of the total proteins from root exudates of *Arabidopsis thaliana* (L.) Heynh. These PR proteins showed enhanced secretion during flowering and almost undetectable levels in the flowering-defective mutants. This point out the necessity to understand the physiological state of plant that produces bioactive proteins effective against different bacteria and fungi pathogenic to plants and animals.

Differences in resistant and susceptible plants to infection by microorganisms may lead to different biochemical defense responses. Root exudates of the susceptible and less susceptible *Phaseolus vulgaris* L. cultivars were able to increase and inhibit, respectively, the growth of fungi (El-Gali, 2015). Thus, for the development of prospective studies regarding the discovery of antimicrobial compounds from plant exudates, this fact must be considered.

3.4. Anti-inflammatory and antinociceptive effects

Inflammation is a protective response of the organism to cell and tissue damages, which initiates a process of homeostasis restore. However, uncontrolled inflammation triggers many chronic diseases such as cardiovascular diseases, diabetes, respiratory diseases, mental disorders, autoimmune diseases and cancers (Chovatiya & Medzhitov, 2014; Medzhitov, 2008). Since inflammation is the foundation of most chronic diseases and thus it may act as a potential therapeutic target as reported by Allijn et al. (2016). The current anti-inflammatory drugs possess several side-effects. Due to these reasons, the search for novel anti-inflammatory medicines is necessary. Natural products are an important alternative source of products with bioactive compounds (Gautam & Jachak, 2009). Along with these products, there are the exudates which are traditionally used as anti-inflammatory agents.

Calotropis procera (Aiton) Dryand. (Asclepiadaceae) is a succulent shrub producing large quantities of latex rich in biologically active compounds *e.g.* steroids, tannins, flavonoids, alkaloids, saponins, cardenolides and lignans (Aliyu et al., 2015; Nadia et al., 2015). *C. procera* latex has the potential to ameliorate inflammation associated with arthritic conditions (Kumar & Roy, 2007; Kumar, Chaudhary, Ramos, Mohan, & Matos, 2011). Laticifer proteins (LP) obtained from *C. procera* has been reported as protective agent in an inflammatory process induced by a lethal bacterial infection. The protective effect of LP at 60 mg/kg i.p. was achieved by a single administration at 24 h before experimental infection with *Salmonella enteric* (ex Kauffmann & Edwards, 1952) serovar *Typhimurium* (Lima-Filho et al., 2010). Freitas et al. (2012) showed that the latex-methanol extract of *C. procera* at 5 mg/kg, i.p. protect against 5-fluorouracil-induced oral mucositis, and inhibit the expression of proinflammatory mediators. Acylated lignans isolated from the latex of *C. procera* showed significant anti-inflammatory action with the highest activity in (+)-pinoresinol 4-*O*-[6″-*O*-protocatechuoyl]-*β*-D-glucopyranoside and showed half-maximal (50%) inhibitory concentration (IC50) values of 7.6 μM and 2.7 μM against lipoxygenases; 5-LOX and 15-LOX, respectively (Abdel-Mageed et al., 2016). Anti-inflammatory and anti-nociceptive action was exhibited by the soluble protein fraction of callus and root tissues extracts of *C. procera* (Teixeira et al., 2011). *C. procera* latex dichloromethane and ethyl acetate fractions exhibited anti-inflammatory properties. Reduced neutrophil migration (67% with dichloromethane and 56% with ethyl acetate fractions) in rats was observed in carrageenan-induced peritonitis and it was suggested that cyclopeptides from ethyl acetate fraction may be responsible for the detected activity (Juca et al., 2013).

Arya and Kumar (2005) demonstrated that aqueous and methanol extracts of dried latex in *C. procera* inhibited oedema & cellular infiltration and exerted anti-inflammatory effects mainly by inhibiting histamine & bradykinin and partly by inhibiting prostaglandin E_2. The potent anti-inflammatory activity possessed by *C. procera* latex was found to be elicited by accidental exposure or after local administration as observed in experimental models (Alencar et al., 2004; Arya & Kumar, 2004; Kumar & Basu, 1994; Kumar & Sehgal, 2007; Sangraula, Dewan, & Kumar, 2001; Shivkar & Kumar, 2004; Singh, Kumar, Dewan, & Kumar, 2000).

The occurrence of pro- and anti-inflammatory actions was evidenced in *C. procera* latex by de Alencar et al. (2016). They demonstrated that pro-inflammatory activity of the latex can be separated from the anti-inflammatory using a simple protocol based on

centrifugation and dialyses. The fraction named dialysis latex was responsible for the induction of inflammatory response, whereas the non-dialyzable latex fraction suppressed this effect. Both dialysis and non-dialyzable fractions of the latex exhibited antinociceptive properties (Dewan, Kumar, & Kumar, 2000; Dewan, Sangraula, & Kumar, 2000; Soares et al., 2005). Non-dialyzable latex inhibited inflammatory response accompanied by reduction of pro-inflammatory mediators and cellular influx (Kumar et al., 2015).

The topical application of triterpene tirucallol at 0.25, 0.5 or 1.0 mg/ear, isolated from *Euphorbia lactea* Haw. latex exerted anti-inflammatory effect, inhibited edema formation and neutrophil migration (Fernandez-Arche, Saenz, Arroyo, de la Puerta, & Garcia, 2010). Similar mechanism of action was reported in a study with the latex of *Euphorbia helioscopia* L. at 200 mg/kg, which showed anti-nociceptive and anti-inflammatory effects (59.38%) (Saleem, Ahmad, Ahmad, Hussain, & Bukhari, 2015). The latex from *Hancornia speciose* Gomes showed anti-inflammatory activity through the inhibition of inflammatory mediators in the formalin inflammation and paw oedema models (Marinho, Alviano, Matheus, Alviano, & Fernandes, 2011).

The oleo-gum-resin obtained from the roots of *F. assa-foetida* (commonly known as assa-foetida) is known to possess anti-inflammatory activity. A wide range of chemical compounds including sugars, sesquiterpene coumarins and polysulfides have been isolated from this plant species. Most of the bioactive compounds reported from asafoetida belong to the sesquiterpene coumarins class (Iranshahy & Iranshahi, 2011). Recent studies revealed the anti-inflammatory action of oleo-gum-resin from *F. assa-foetida* (Bagheri, Dashti, & Morshedi, 2014; Bagheri, Hedesh, Mirjalili, & Dashti, 2016). Cashew gum (CG), a complex heteropolysaccharide extracted from *Anacardium occidentale* L. has been shown to be protective effect against gastrointestinal damage, characterized by inhibition of inflammation due to increase of adherent mucus in mucosa and decrease of oxidative stress. The highest protection was observed against gastric lesions induced by naproxene in male wistar rats pretreated with CG at10 mg/kg, p.o. (Carvalho et al., 2015).

Resinous exudates, myrrh and frankincense produce in *Commiphora* and *Boswellia* (family *Burseraceae*), respectively has long been used as a remedy in the traditional medicines in different countries. These are also prescribed simultaneously in Chinese traditional medicines due to their similar curative effects and to treat blood stagnation and inflammation diseases, relief of swelling as well as pain (Shen & Lou, 2008). The gummy exudate from *Boswellia serrata* Roxb. ex Colebr. has been used in Ayurvedic medicine and traditional Chinese medicine (TCM) for inflammatory diseases including bronchial asthma and rheumatoid arthritis (Ammon, 2006; Shen & Lou, 2008). Pharmacological activities of *B. serrata* gum resin and its main active compound boswellic acid, is also well documented (Ernst, 2008). The extract of *B. serrata* is effective against ulcerative colitis (Gupta et al., 1997), chronic colitis (Gupta et al., 2001), rheumatoid arthritis (Sander, Herborn, & Rau, 1998). Crohn's disease (Gerhardt, Seifert, Buvari, Vogelsang, & Repges, 2001), osteoarthritis (Kimmatkar, Thawani, Hingorani, & Khiyani, 2003; Sengupta et al., 2008; Sontakke et al., 2007) and collagenous colitis (Madisch et al., 2007).

B. serrata extract attenuates inflammation *via* immunomodulation (Ammon, 2010; Gayathri, Manjula, Vinaykumar, Lakshmi, & Balakrishnan, 2007; Umar et al., 2014). Shenvi, Kiran, Kumar, Diwakar, and Reddy (2015) synthesized hybrid molecules containing non-steroidal anti-inflammatory drugs (NSAIDS) and boswellic acids from *B. serrata* resin and reported their anti-inflammatory and anti-arthritic activities. A positive correlation of antioxidant activity with the mechanism involved in protecting intestinal epithelial barrier from inflammatory damage was found in a study with *B. serrata* oleo-gum-resin extract (at concentration of 0.1–1.0 μg/mL) and its active derivative acetyl-11-keto-β-boswellic acid (at 0.027 μg/mL) in an experimental model using Caco-2 cell monolayer exposed to H_2O_2 or to INF-γ + TNF-α (Catanzaro et al., 2015). Terpenoids purified from ethanolic extract of oleo-gum-resin of *Boswellia ovalifoliolata* have been reported to possess potent anti-inflammatory potential (Chib et al., 2014). The hexanic extract from *Commiphora erythraea* (Ehrenb.) Engl. resin and one of its fractions (H-3) showed anti-inflammatory activity. Pure furanosesquiterpenoids isolated from H-3 reduced the oedematous response by 26–32% at the dose of 0.3 μmol/cm^2 (Fraternale et al., 2011). Kimura et al. (2001) isolated triterpene myrrhanol A from *Commiphora mukul* (Hook. ex Stocks) Engl. gum resin and demonstrated its potent anti-inflammatory effect in adjuvant-induced air-pouch granuloma of mice. The 50% inhibitory dose of myrrhanol A was 2.80 mmol/kg for carmine content, 5.83 mmol/kg for granuloma weight, and 1.75 mmol/kg for pouch fluid weight. Its effects were more marked than those of hydrocortisone and 50% aqueous methanolic extract of the crude drug. The *C. mukul* gum resin extract was also efficient in the treatment of osteoarthritis (Manjhi, Gupta, Sinha, Rawat, & Rai, 2016). An outcome study reported significant improvement of osteoarthritis in the patients who received *C. mukul* resin extract capsules (500 mg, 3.5% guggulsterones) thrices a day along with meals for two months (Singh et al., 2003). According to literature, the pharmacological activities of laticiferous plants are attributed to their active constituents such as proteins, terpenes, flavonoids and phenolic components.

3.5. Anti-ulcerogenic

Turkish sweetgum, styrax liquidus, a resinous exudate (balsam) obtained from the wounded barks of *Liquidambar orientalis* L. tree showed protection against ethanol-induced gastric ulcer model at 150 and 300 mg/kg doses with ulcer inhibition ratio 83.3% ($p < 0.001$) and 66.7% ($p < 0.01$), respectively. The main component of balsam was styrene (81.9%) (Gurbuz et al., 2013). In Turkish folk medicine, sweetgum is used for treatment of peptic ulcers after mixing with honey (Honda et al., 1996). Styrax has medicinal uses dating back to the Aztec Empire. The ancient Aztecs used it as a treatment for skin infections and other ailments (Lingbeck, O'Bryan, Martin, Adams, & Crandall, 2015).

3.6. Antioxidant properties

Antioxidants are the molecules that protect cells from oxidative damage either reacting directly with free radicals or indirectly by inhibiting the activity of free radical generating enzymes. Natural products derived from plants are considered reliable antioxidant resources to cure number of diseases with lesser side effects that are often associated with the use of synthetic drugs. Exudates released from several plants species namely, *Ficus carica* L., *Euphorbia* species possess potent antioxidant activities (Chougale, Bhosale, Jadhav, & Padul, 2011). It has been reported that *Euphorbia characias* L. latex possesses proteins that act as antioxidant enzymes to protect the plants against pathogens (Pintus, Medda, Rinaldi, Spanò, & Floris, 2010). Pintus et al. (2013) found antioxidant activities of *E. characias* latex by determining total content of free-radical scavenging, polyphenol and flavonoid molecules and acetylcholinesterase inhibitory activities. Chougale et al. (2011) screened latexes of 16 plant species for antioxidant activity and found that ethanolic extracts of all the plant latexes showed antioxidant activity that varied depending on the plant species. *Euphorbia genicalata* L. and *Ficus religiosa* L. 1753 not Forssk. 1775 latexes had highest activities (93.74% and 88.23% inhibition, respectively) followed by *Ervatamia* species (82.21%), *Thevetia* species (72.88%), *Michelia champaca* (L.) Baill. ex Pierre (72.23%) and *Ficus bengalensis* L. (70.39%). Their study suggested that plant latexes could be used for medicinal purposes. Antioxidant activity of the resinous exudate and flavonoids isolated from *Heliotropium taltalense* (Phil.) I.M. Johnst. has been carried out by Modak, Rojas, and Torres (2009) using homogeneous system; *i.e.* by evaluating the absorbance changes of an ethanolic solution of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and by heterogeneous system *i.e.* using a micellar solution of sodium dodecyl sulfate. Chemical analysis of exudates resinous isolated from *H. taltalense* showed the presence of filifolinol and its ester derivative filifolinyl senecionate and the

Table 1
Biological activities of exudates oozed by various plant species.

Family	Plant species	Plant part	Exudate	Active compounds	Effective dose	Type of study	Activity	Reference(s)
Anacardiaceae	*Anacardium occidentale* L.	Tree trunk	Cashew gum	–	10 mg/kg	*In vivo* (male wistar rats)	Gastroprotective and anti-inflammatory	Carvalho et al., 2015
	Pistacia atlantica subsp. *kurdica*	–	Pistacia gum	–	100, 200 and 400 mg/kg p.o.	*In vivo* (rats)	Anti-inflammatory	Minaiyan, Karimi, & Ghannadi, 2015
	Pistacia lentiscus L.	Seed	Pure mastic gum	–	1,05 g	Human trial	Antimicrobial	Dabos et al., 2010
		–	Mastic gum	–	–	*In vitro* cell culture	Antioxidant and anti-inflammatory	Triantafyllou et al., 2011
Apiaceae	*Ferula assa-foetida* L.	–	Aqueous extract of gum	–	3 mg/mL	*In vitro* (guinea-pig ileum)	Antispasmodic	Fatehi, Farifteh, & Fatehi-Hassanabad, 2004
		–	Aqueous extract of gum	–	0.3–2.2 mg/100 g body weight	*In vivo* (rats)	Hypotensive	
		Crown	Resin, essential oil from oleo-gum resin	–	0.028–0.058 mg/mL	*In vitro*	Antimicrobial	Kavoosi & Rowshan, 2013
		–	Resin, crude extract of the oleo-gum resin	–	100.8 and 364.2 ppm	*In vitro*	Anthelmintic	El-Sherbiny & Zen-El-Dein, 2012
		–	Oleo-gum-resin extract	–	50 mg/kg	*In vivo* (mice)	Analgesic	Bagheri et al., 2014
			Oleo-gum resin extract	–	10 mg/kg	*In vivo* (mice)	Antinociceptive	Bagheri et al., 2016
				–	2.5 mg/kg	*In vivo* (mice)	Anti-inflammatory	
Apocynaceae	*Calotropis gigantea* (L.) Dryand.	Apical part	Latex, chloroform extract	–	0.028–0.058 mg/mL	*In vitro*	Antimicrobial	Ishnava et al., 2012
		Apical part	Latex, crude aqueous extract	–	62–125 μg/mL	*In vitro*	Antimicrobial	Kumar, Loganathan, & Rao, 2010
		Whole plant	Latex	(+)-pinoresinol 4-*O*-[6″-*O*-vanilloyl]-*β*-D-glucopyranoside	13.4–39.8 μM	*In vitro*	Antiviral	Parhira et al., 2014
	Calotropis procera (Aiton) Dryand.	Branch; leaf	Latex, in isotonic saline	–	0.02 mL/Kg body weight	*In vivo*	Antihelmintic	Al-Qarawi, Mahmoud, Haroun, & Adam, 2001; Mahmoud, Haroun, Sobaih, Omer, & Adam, 2001
		Branch; leaf	Latex, chopped leaves	–	100 g/Kg	*In vitro*	Antihelmintic	Parihar, Babalola, & Rehman, 2014
		Aerial part	Dry latex	–	500 mg/kg	*In vivo* (rats)	Anti-diarrhoeal	Kumar, Dewan, Sangraula, & Kumar, 2001
		Aerial part	Latex	Laticifer proteins	5 mg/kg	*In vivo* (hamsters)	Anti-inflammatory	Freitas et al., 2012
		Leaf and stem	Latex	Acylated lignans	7.6 and 2.7 μM against 5-LOX and 15-LOX, respectively	*in vitro*	Anti-inflammatory	Abdel-Mageed et al., 2016
		Callus and root cultures	Latex	Callus proteins, root proteins	1 mg/kg	*In vivo*	Anti-inflammatory	Teixeira et al., 2011
		Callus and root cultures	Latex	Callus proteins, root proteins	1 mg/kg (callus proteins), 5 and 25 mg/kg (root proteins)	*In vivo*	anti-nociceptive	
		Aerial part	Latex (dichloromethane, ethyl acetate, and aqueous fractions)	Cyclopeptides (in ethyl acetate fraction)	10.0 mg/kg	*In vivo*	Anti-inflammatory	Juca et al., 2013
		Aerial part	Latex	Laticifer proteins	5 or 50 mg/kg	*In vivo* (mice)	Anti-inflammatory	de Alencar et al., 2016
		Aerial part	Latex	Laticifer proteins	25 mg/kg	*In vivo* (rats)	Anti-inflammatory	Kumar et al., 2011
		Aerial part	Latex	Laticifer proteins	25 mg/kg	*In vivo* (rats)	Anti-inflammatory	Kumar et al., 2015
	Hancornia speciosa Gomes	Tree trunk	Latex	–	0.1–1.3 mg/kg	*In vivo*	Anti-inflammatory	Marinho et al., 2011
		Tree trunk	Latex	Chlorogenicacid and naringenin-7-*O*-glucoside	5%	*In vivo*	Osteogenic	Dos Santos Neves et al., 2016
	Himatanthus articulatus (Vahl) Woodson	Bark	Crude latex	–	1.0 mg of extract/disc	*In vitro*	Antimicrobial	Sequeira et al., 2009
	Wrightia tinctoria R.Br.	–	Latex	Latex proteases	10 mg/kg	*In vivo*	Wound healing	Yariswamy et al., 2013

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Table 1 (*continued*)

Family	Plant species	Plant part	Exudate	Active compounds	Effective dose	Type of study	Activity	Reference(s)
Burseraceae	*Boswellia carteri* Birdw.	–	Oleo-gum-resin, Bowsellic acid fraction	Palmitic acid, lupeol, β-boswellic acid, 11-keto-β-boswellic acid, acetyl β-boswellic acid, acetyl 11-keto-β-boswellic acid, acetyl-α-boswellic acid, 3-oxo-tirucallic acid and 3-hydroxy-tirucallic acid	1 g/kg body weight/day	*In vivo* (rats)	Anti-fibrotic	Ali & Mansour, 2011
	Boswellia ovalifoliolata N.P.Balakr. & A.N. Henry	–	Oleo-gum-resin	6R,7R-epoxy-1-oleanen-3-ol and 3α-hydroxy-tirucall-8,24-dien-21-oic acid	1 μM	*In vitro*	Anti-inflammatory	Chib et al., 2014
	Boswellia serrata Roxb. ex Colebr.	–	Gum resin, alcoholic extract	–	150 mg/kg	*In vivo*	Antidiabetic	Shehata, Quintanilla-Fend, Bettio, Singh, & Ammon, 2011
		–	Gum resin	Boswellic acids	100 and 200 mg/kg	*In vivo*	Anti-arthritic	Umar et al., 2014
		–	Gum resin	Hybrid molecules of boswellic acids with non-steroidal anti-inflammatory drugs	10 mg/kg	*In vivo/in vitro*	Anti-inflammatory and anti-arthritic	Shenvi et al., 2015
		–	Gum resin, methanolic extract + dextrin + glycerin	–	–	*In vivo*	Wound healing	Khalil, Abd, & Hussein, 2016
		–	Oleo-gum resin	11-keto-β-boswellic acid (KBA) and 3-acetyl-11-keto-β-boswellic acid (AKBA)	0.1–10 μg/mL (extract) and 0.027 μg/mL (AKBA)	*In vitro*	Anti-inflammatory	Catanzaro et al., 2015
		Stem bark	Gum resin	AKβBA and other boswellic acids, phenolic compounds	3 mg dwt/mL	*In vitro*	Antioxidant and antithrombotic	Kokkiripati et al., 2011
	Bursera copallifera (Sessé & Moc. ex DC.) Bullock	–	Resin	3-Epilupeol formiate, α-amyrin acetate, 3-epilupeol acetate, lupenone, 3-epilupeol and α-amyrin	–	*In vitro/in vivo*	Anti-inflammatory	Romero-Estrada et al., 2016
	Commiphora erythraea (Ehrenb.) Engl.	–	Resin	Myrrhone, 3-methoxy-furanogermacradien-6-one, 2-methoxy-furanogermacren-6-one	0.3 μmol/cm^2	*In vitro/in vivo*	Anti-inflammatory and antioxidant	Fraternale et al., 2011
	Commiphora myrrha (Nees) Engl.	–	Resin rich methanolic extract	–	100–1000 μg/mL	*In vitro*	Antimicrobial	Shuaib et al., 2013
Caricaceae	*Carica papaya* L.	Fruit	Latex in distilled water	–	1447.89 mg/bird	*In vitro*	Anthelmintic	Mursof & He, 1991
	Carica papaya L.	Fruit	Latex in deionized water	–	45 mg of protein	*In vitro*	Antifungal	Giordani et al., 1996
Convolvulaceae	*Operculina turpethum* (L.) Silva Manso	–	Crude resin	–	100 to 1000 μg/mL	*In vitro*	Antimicrobial	Shuaib et al., 2013
Dipterocarpaceae	*Shorea robusta* Gaertn.	–	Resin, hidroethanolic extract	–	300 mg/kg	*In vivo*	Anti-inflammatory and antipyretic	Wani et al., 2012
		–	Resin, ethanolic extract in petroleum jelly (10 and 30% w/w)	–	10 and 30% w/w	*In vivo*	Wound healing	Wani et al., 2012

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Table 1 (*continued*)

Family	Plant species	Plant part	Exudate	Active compounds	Effective dose	Type of study	Activity	Reference(s)
Euphorbiaceae	*Euphorbia antiquorum* L.	Stem	Latex, methanolic extract	–	–	*In vitro*	Antibacterial and Antifungal	Sumathi, 2011
	Euphorbia helioscopia L.	Leaf and stem	Latex	flavonoids	200 mg/kg (anti-inflammatory) and 300 mg/kg (anti-pyretic)	*In vivo*	Anti-inflammatory and anti-pyretic	Saleem et al., 2015
	Euphorbia umbellata (Pax) Bruyns	–	Latex, hexane, chloroform, ethyl acetate and methanol fractions	–	1.87–30.5 mg/mL	*In vitro*	Cytotoxic	Luz et al., 2016
	Hevea brasiliensis (Willd. ex A.Juss.) Müll.Arg.	Tree	Latex	Proteins in C-serum phase	20 mg/mL	*In vitro*	Antioxidant	Kerche et al., 2016
	Jatropha curcas L.	Leaf; stem/branch	Latex in water	–	15 mg/mL	*In vitro*	Anthelmintic	Parmar et al., 2014
		Leaf; stem/branch	Latex, methanol extract	–	0.39 and 6.25 mg/mL	*In vitro*	Antimicrobial	Suhaili et al., 2011
	Jatropha neopauciflora	–	Latex	–	500 and 750 mg/kg	*In vitro*	Anti-inflammatory	Hernandez-Hernandez et al., 2017
	Pax	–	Latex	–	5.4 μg/mL	*In vitro*	antioxidant	
		–	Latex	–	50%	*In vitro*	wound-healing	
	–	Crude latex	–	2 mg/mL	*In vitro*	antimicrobial		
Leguminosae	*Glycine max* (L.) Merr.	Seed	Seed exudate	Proteins	500 mg/mL	*In vitro*	Anthelmintic	Rocha et al., 2015
	Vigna unguiculata (L.) Walp.	Root	Root exudate	Proteins	100 μg/mL	*In vitro*	Antifungal	Nóbrega et al., 2005
Moraceae	*Artocarpus heterophyllus*	Leaf	Latex	–	100 g/Kg soil	*In vivo*	Antihelmintic	Parihar et al., 2014
	Lam.	Fruit stem	Latex	Heat-shock/chaperone protein	18.5 to 370 μg/mL	*In vitro*	Human blood coagulation inhibitor	Siritapetawee & Thammasirirak, 2011
	Ficus benghalensis L.	Leaf	Latex	–	100 g/Kg soil	*In vivo*	Antihelmintic	Parihar et al., 2014
	Ficus carica L.	Leaf	Latex	–	10 mg/mL	*In vitro*	Antiproliferative	Hashemi et al., 2011
		Fruit	Latex, hydroethanolic, ethyl acetate, dichloromethane extracts		17 μg/mL	*In vitro*	Cytotoxic	Khodarahmi, Ghasemi, Hassanzadeh, & Safaie, 2011
		–	Latex	6-*O*-acyl-*β*-D-glucosyl-*β*-sitosterols (6-AGS)	50 μg/mL	*In vitro*	Cytotoxic	Rubnov, Kashman, Rabinowitz, Schlesinger, & Mechoulam, 2001
		Fruit	Latex	–	–	Human trial	Antiwart	Bohlooli, Mohebipoor, Mohammadi, Kouhnavard, & Pashapoor, 2007
		–	Latex, extract	–	100–400 μg/ml	*In vitro*	Anti-angiogenic	Mostafaie et al., 2010
		Fruit	Latex, methanolic extract	–	500 and 750 μg/mL	*In vitro*	Antimicrobial	Aref et al., 2010
	Ficus elastica Roxb. ex Hornem.	Leaf	Latex	–	100 g/Kg soil	*In vivo*	Antihelmintic	Parihar et al., 2014
Pinaceae	*Pinus merkusii* Jungh. & de Vriese	–	Oleo-resin	–	30 μg/ml	*In vitro*	Antibacterial and cytotoxic	Soderberg et al., 1996
	Pinus roxburghii Sarg.	–	Oleo-resin, methanol extract	–	100–1000 μg/mL	*In vitro*	Antimicrobial	Shuaib et al., 2013
Poaceae	*Hordeum vulgare* L.	Root	Root exudate	Phenolic compounds	0.1–100 μM	*In vitro*	Antimicrobial	Lanoue et al., 2010
Proteaceae	*Macadamia integrifolia* Maiden & Betche	Seed exudate	Seed exudate	Peptide	5–10 mg/mL	*In vitro*	Antimicrobial	Marcus et al., 1999
Rosaceae	*Prunus domestica* L.	–	Gum, gum-loaded, stabilized gold and silver nanoparticles	–	–	*In vitro* and *in vivo*	Anticancer, antibacterial, urease-inhibition, anti-inflammatory, analgesic	Islam et al., 2017
	Prunus persica (L.) Batsch	Trunk tree	Gum derived oligosaccharides	–	100 mg/mL	*In vitro*	Antioxidant	Yao et al., 2013
	Prunus persica (L.) Batsch	Trunk tree	Gum derived oligosaccharides	–	100 μg/mL	*In vitro*	Antibacterial	
Xanthorrhoeaceae	*Aloe harlana* Reynolds	Leaf	Latex	7-*O*-methylaloeresin A and aloin	–	*In vitro*	Antioxidant	Asamenew, Bisrat, Mazumder, & Asres, 2011
	Aloe harlana Reynolds	Leaf	Latex	Anthrone and chromone	0.06–3.6 mM	*In vitro*	Antimicrobial	

flavonoids naringenin, 3-*O*-methylgalangin and 7-*O*-methyleriodictiol. Their results showed that the antioxidant molecules react more quickly when inserted in the micelle. These findings probably are due to hydrophobic effects and electrostatic interaction related to the solubilization of organic solutes (Modak et al., 2009). In another study by Kumar, Srivastava, Singh, Mathad, and Thind (2014), sap extracted from *Musa acuminata* Colla. (banana) pseudostem contains significant amount of antioxidants along with carbohydrate, protein and phenolic compounds and could be used for preparation of various antioxidant formulations in pharmaceutical industries. Resin of *Bombax ceiba* L. has been reported to possess antioxidant properties, which could be attributed to the presence of tannins and flavonoids in plants (Rao et al., 2015).

3.7. Wound healing properties

Exudates from plants have been used in traditional medicines to heal wounds. The wound healing properties of exudates have been attributed to the presence of tannins. It has been reported that exudates from *Dacryodes edulis* H.J. Lam caused a significant increase in cell proliferation when applied to open wounds and ringworms (Hutchinson, Dalziel, & Hepper, 1963). Ligha and Fawehimi (2008) showed the ability of latex exudates of *J. curcas* Linn in acceleration of wound healing. They studied the wound healing properties based on histopathological parameters including neutrophils, macrophages, lymphocytes and fibroblasts and observed the significant increase in the numbers of inflammatory cells and fibroblasts. Recently, Hernandez-Hernandez et al. (2017) evaluated the biological properties of *Jatropha neopauciflora* Pax. latex involved in the wound-healing process. The work has revealed that *J. neopauciflora* latex promoted the wound-healing process, probably by avoiding microorganism infections, inhibiting inflammation and acting as antioxidant. The latex contains phenols (6.9 mg gallic acid equivalent (GAE)/mL), flavonoids (12.53 μg quercetin equivalent (QE)/mL), proteins (7.62 μg/mL) and carbohydrates 18.52 μg glucose equivalent (GE) in its chemical constitution. The healing and antimicrobial effects of aqueous extract of the stem bark from *Bowdichia virgilioides* Kunth in the therapy of skin wounds has been explored by Agra et al. (2013). Topical application of *Wrightia tinctoria* R. Br. latex proteases also enhances the wound healing process (Yariswamy et al., 2013). Table 1 summarizes the pharmacological activities of different exudates oozed by various plant species.

4. Applications in pharmaceutical industry

Natural gums are the important tree-based polysaccharides in markets, especially gum arabic, gum karaya, gum tragacanth, guar gum and kondagogu gum that have been used as food additives and pharmaceutical ingredients for centuries (Padil et al., 2015).

Gum arabic, obtained from the exudates of acacia tree is the oldest and best known of all natural gums and was once extensively used in the pharmaceutical industry. However, it was replaced by celluloses and modified starches in many applications (Verbeken, Dierckx, & Dewettinck, 2003). Although, the gum arabic does not possess antimicrobial properties but it can be used in drug conjugates. For example, amphotericin B - gum arabic conjugates were stable, non-hemolytic, non-toxic and showed good anti-fungal activity *in vitro* (Nishi et al., 2007). When studies were carried out regarding the antimicrobial delivery systems based on electrostatic complexes of cationic ε-polylysine and anionic gum arabic, it was verified that high levels of gum arabic led to improved physical stability, but reduced antimicrobial activity. Thus the complexes must be carefully formulated to balance these two effects so as to optimize their antimicrobial activity, without adversely affecting product appearance or stability (Chang & Mcclements, 2014). Additionally, gum arabic can be used in the development of eugenol oil antimicrobial nanoemulsions, as food grade natural emulsifiers (Hu, Gerhard, Upadhyaya, Venkitanarayanan, & Luo, 2016).

Gun karaya is most consumed in the pharmaceutical industry due to its function as an adhesive in leakproof sealing rings for post-surgical drainage pouches or ostomy bags (Verbeken et al., 2003). The gum tragacanth is a dried exudation obtained from stems and branches of *Astragalus gummifer* Labill. and other Asiatic species of *Astragalus*. It has also been widely used in pharmaceutical and food industry as texturant additive, emulsifier, thickener and stabilizer. It is an effective suspending agent and prevents insoluble materials, which are usually the active ingredients from settling-out in aqueous mixtures. The gum facilitates the absorption of water-insoluble components, thus improving the action of pharmaceutical (Verbeken et al., 2003). Tragacanth gum also has potential to be used as a polymeric wall for producing antimicrobial nanocapsules loaded with plant extracts, including common extracts used in traditional medicine (for example *Chamomile* extract) (Ghasempour, Alizadeh, & Bari, 2012).

Guar gum polysaccharide is a biodegradable, non-toxic, low cost and renewable raw material. Although, pure guar gum is inactive against bacteria but it can be used as a biomaterial to produce hydrogels for antibacterial and dye removal applications (Sharma et al., 2015). Films prepared using chitosan and guar gum also have antimicrobial properties (Rao, Kanatt, Chawla, & Sharma, 2010).

5. Perspectives and conclusions

Plant exudates have potential for drug development, which are less harmful to human health as well as environmentally safe as compared to synthetic compounds. However, this can be achieved only after many *in vitro* and *in vivo* experiments for the concerned plant exudate from that specific plant species. It is also crucial to identify molecular and receptor mechanisms involved in it. The chemical composition of the plant exudates is complex and varies significantly on source and plant's age. Moreover, susceptible and disease-resistant plant cultivars have different biochemical responses to microorganisms, thus producing different compounds. These aspects could be taken into account in prospective studies regarding the search of new drugs from plant exudates. It is important to isolate bioactive compounds from plant exudates in order to assess their biological activities and synergism. Similarly, it would be beneficial to find out the other biological activities in plant exudates for better understanding of their mode of action. For instance, the substances released during exudation may exert a chemo attractant effect on microorganisms besides the antimicrobial effect, or possess pro- and anti-inflammatory actions. Furthermore, studies should be focused on the cytotoxicity of plant exudates with pharmacological activities as well the protection of bioactive compound using encapsulation process. It can be concluded that plant exudates possess various biological activities and may have therapeutic potential to cure many diseases.

Conflict of interest

The authors declare no conflicts of interest.

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CAPÍTULO II

Seeds exudate different classes of proteins and possess anthelmintic activity toward the nematode *Haemonchus contortus*

Neste capitulo consta o artigo submetido na revista PLOS ONE (Qualis A2 em Medicina I; Fator de Impacto: 3.057).

Seeds exudate different classes of proteins and possess anthelmintic activity toward the nematode *Haemonchus contortus*

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Abstract

Nematodes have developed resistance to the most common drugs commercially available. This study aimed to identify and evaluate the action of the protein exudates from *Mimosa caesalpiniaefolia*, *Leucaena leucocephala*, *Acacia mangium* and *Stylosanthes capitata* seeds on the gastrointestinal nematode *Haemonchus contortus*. The exuded proteins were precipitated with ammonium sulfate (0-90%), dialyzed exhaustively against distilled water, lyophilized and assessed for anthelmintic activity. Although no egg hatching inhibition was observed, all exudates showed efficacy to inhibit the larval exsheathment of *H. contortus* larvae with an EC_{50} varying from 0.61 to 0.26 mgP mL^{-1} in the studied seed exudates. Proteomic analysis revealed the presence of proteases, protease inhibitors, chitinases, lectin among other proteins in the exudates. Most of the exuded proteins belong to the oxidative stress / plant defense and to energy / carbohydrate metabolism. We conclude that the bioactive proteins exuded from the seeds of *M. caesalpiniaefolia*, *L. leucocephala*, *A. mangium* and *S. capitata* display a stage specific anthelmintic action against *H. contortus*.

Key words: Exudation. Defense proteins. Nematode. Proteomic. MS/MS analysis

Introduction

Gastrointestinal nematode (GIN) infection represents one of the major threats to small ruminant production worldwide [1,2]. The blood-sucking nematode *Haemonchus contortus* is the most important parasite of sheep and goat [3,4,5]. *H. contortus* cause anaemia, reduced productivity and can lead to death in heavily infected animals [6,7]. It was estimated that due to haemonchosis, world losses could reach up to 400 million dollars (USD) annually [8,9].

The most commonly used procedure in nematode control is the application of synthetic nematicides. However, the control of *H. contortus* in sheep and goats has become less effective due to the high levels of resistance to anthelmintic drugs [10,11]. In the search for alternatives to conventional anthelmintics, plant natural products have been widely investigated [12,13,14,15,16].

Plants have a variety of chemical defense mechanisms and protection against predators [17,18]. Among the bioactive plant macromolecules, the anthelmintic proteins have been highlighted [19], as they are active toward *H. contortus* [20]. The segment of therapeutic proteins is one of the fastest growing in the pharmaceutical market. This study goes through several generations of develop commercially viable products [21,22]. The simplicity of production at relatively low costs, reduced risks of side effects, and high bioavailability are some commercial advantages of the use of proteins [23,24].

Mimosa caesalpiniaefolia, Leucaena leucocephala, *Acacia mangium* and *Stylosanthes capitata* are legume species, belonging to the Fabaceae family, with wide distribution [25,26]. Different parts of these plants are used by traditional medicine to control many diseases including helminth infections [27,28,29,30].

There are still useful sources of poorly studied plant proteins, which may present high anthelmintic potential, as seed exudates [31]. Exudates, including sap, latex, resins, gums, seed or root exudates are known to possess several medicinal properties [32]. Seed exudates are constituted of mainly peptides / proteins and secondary metabolites [33]. They act as chemotactic factors and also possess the ability to inhibit soil pathogens preventing infections [34,35].

Chitinases, protease inhibitors and proteases are example of proteins exuded from plants [36,37]. For instance, the soybean seed exudates contain active proteins

against the Root-Knot Nematode *Meloidogyne incognita* [38]. Recently, we have shown that proteins exuded from *Myracrodruon urundeuva* seeds display anthelmintic activity toward *H. contortus* [19].

The objective of this study was to assess the anthelmintic activity of the exudates from seeds of *M. caesalpiniaefolia*, *L. leucocephala*, *A. mangium* and *S. capitata*, and analysis their proteome profiles as an attempt to relate exuded proteins with activity.

Materials and methods

Plant material

The seeds of *M. caesalpiniaefolia, L. leucocephala, A. mangium* and *S. capitata* obtained from certified species, were purchased from Arbocenter (Birigui, SP, Brazil). They were selected according to size, weight and the absence of morphological damage.

Exudates preparation

The seeds were weight (80 g) and placed in twenty 50 mL plastic tubes (4 g / tube). The seeds were surface sterilized with 70% (v/v) ethanol for 5 minutes and rinsed three times with distilled water. Next, the seeds were soaked in 0.1 M sodium acetate, pH 5.0 (1:3 seed g / volume buffer) for 24 h at 10 °C. Exudates were collected, the volumes were recorded and the seeds were discarded [38]. The exudates were submitted to precipitation by ammonium sulfate at 90% saturation [39]. The precipitation was carried out in an ice bath under continuous stirring and the salt was added gradually. After addition of the salt, the exudates were incubated for 4 h at 10 °C and proteins recovered by centrifugation (12,000 *x g*, 4 °C 30 min). The supernatant was discarded and the pellet was ressuspended in 0.1 M sodium acetate, pH 5.0 and dialyzed (cut off 2 kDa)

for 24 h against distilled water (with six water changes) at 10 °C in a proportion of 1:50 (v / v). The protein content was determined using bovine serum albumin (BSA) as the standard protein [40]. After protein quantification the samples were freeze-dried and named protein exudates (PE).

Anthelmintic Activity

Nematodes

Eggs and third instar larvae (L3) of *H. contortus* were obtained from sheeps infected experimentally [41, 42]. The experimental procedures were performed according to the guidelines of the Animal Ethics Committee (CEUA) of the Federal University of Maranhão and were approved under the protocol number 23115.014307/201671.

Egg hatching assay

Egg hatching assay (EHA) was carried out in 96-well sterile plates [43], with modifications. The PE were diluted in PBS (10 mM sodium potassium phosphate buffer, pH 7.2, containing 125 mM NaCl). Aliquots (100 µL) of a suspension containing approximately 100 fresh eggs in distilled water were incubated with (100 µL) PE at final concentrations varying from 1.0 to 0.06 mg mL^{-1}, in quadruplicate. Distilled water and PBS were used as control. The plates containing different exudates and controls were incubated for 48 h at 27 ºC. Eggs and first-stage larvae (L1) were counted under a dissecting microscope at 40× magnification.

Larval exsheathment inhibition assay (LEIA)

This assay (LEIA) was performed according to Bahaud et al. [44]. First, 6 mg of PE were soaked in 1.0 mL PBS. The PE were incubated with 100 larvae L3 of *H. contortus*, at 1.2, 0.6, 0.3, 0.15, 0.075, mg mL^{-1}, at 27 °C, and 80% relative humidity for 3 hours. Next, the larvae were washed in distilled water for 3 minutes at 2,540 *x g*. Then, 160 µL of 2% (v/v) sodium hypochlorite solution (containing 2.5% active chlorine) were added. Every 20 minutes, exsheathment was stopped by addition of 30 µL lugol. The assay was carried out in quadruplicate and monitored for 60 minutes. The PBS buffer was used as control. The kinetic of larval exsheathment in the different experimental treatments was then monitored by microscopic observation (40x) (Primo Vert). The percentage of sheathed larvae was identified at 10-minute intervals for 60 minutes.

Label-free proteomic analysis (LC-ESI-MS/MS) from the seed exudates

To assess the protein profile of the seed exudates from *M. caesalpiniaefolia*, *L. leucocephala*, *A. mangium* and *S. capitata* a proteomic approach was used according to Araújo et al. [20]. Before the LC-ESI-MS/MS analysis, 50 µg from each exudate were dissolved in 20 µL 0,4 M ammonium bicarbonate containing 8 M urea. Dithiothreitol [5 µL of a 50 mM solution] and iodoacetamide [5 µL of a 150 mM solution] were used to carry out reduction and alkylation, respectively. Samples were digested overnight with trypsin at a proportion of 1:50 (trypsin/exudate). The peptides generated by trypsin were analyzed by reverse-phase chromatography column (RPC) coupled to an LTQ-Orbitrap XL mass spectrometer (MS) on an nLC-Easy II system. Before loading the samples, the RPC was pre-equilibrated with a mobile phase A [0.1% (v/v) formic acid in water]. After adsorption, peptides were eluted with a linear gradient of 2% to 40% acetonitrile with mobile phase B [0.1% (v/v) formic acid in acetonitrile]. The MS/MS analysis was

done by MS/MS mode using Linear Trap Quadrupole (LTQ) after fragmentation of peptides induced by collision-induced dissociation. The MS/MS profiles were evaluated using the Peaks Studio, and the LC-ESI-MS/MS raw data were processed and proteins identified using the UniProt Viridiplantae database (entry: 3275215). The searches were done using the following parameters: maximum of two missed cleavage by trypsin, carbamidomethylation (C) and methionine oxidation (M) as fixed and variable specific modification; fragment mass error tolerance of 0.6 Da; and the false discovery rate (FDR) with protein levels at ≤1%, and at least two peptides identified for each protein in both technical replicates performed to each exudate. To reduce the natural redundancy in identified proteins list proteins identified with the same values for -10lgp; coverage, #peptides; #uniques, PTM and avg mass were used to remove redundant proteins. Gene Ontology (GO) using the UniProtKB database as a reference provides protein classification according to their biological process.

Statistical analysis

Protein quantification was performed in triplicate and data were expressed as mean ± standard deviation. For the anthelmintic assays, the data were initially transformed into Log (X), normalized and then non-linear regression was calculated to obtain an effective concentration (EC) to inhibit 50% larval exsheathment using GraphPad Prism 7.0 software (GraphPad Inc., San Diego, CA , USA) and expressed as mean and confidence interval (95%). The level of significance was set at 0.05% [45].

Results

Protein content

176 The content of soluble total exuded proteins from *M. caesalpiniaefolia, L.*
177 *leucocephala, A. mangium* and *S. capitata* was 0.49, 0.06, 0.10, and 0.11 mg of
178 proteins/ g seed, respectively.
179
180 **Inhibitory activity of exudates on egg hatching and larval exsheathment of *H.***
181 ***contortus***
182 The PE did not inhibit egg hatch (data not shown), but showed efficacy to inhibit
183 larval exsheathment of *H. contortus* (Table 1) with EC_{50} varying from 0.61 to 0.26 mg
184 mL^{-1}.
185
186 **Table 1.** Anthelminthic activity (EC_{50}) obtained from *Mimosa caesalpiniaefolia,*
187 *Leucaena leucocephala, Acacia mangium and Stylosanthes capitata* exudates on
188 *Haemonchus contortus* larval exsheathment.
189

Seed	EC_{50} mg mL^{-1} (CI 95%)
M. caesalpiniaefolia	0.61 (0.5202 – 0.7133)
L. leucocephala	0.26 (0.1677 – 0.3889)
A. mangium	0.52 (0.4635 – 0.5853)
S. capitata	0.40 (0.3680 – 0.4227)

190
191
192

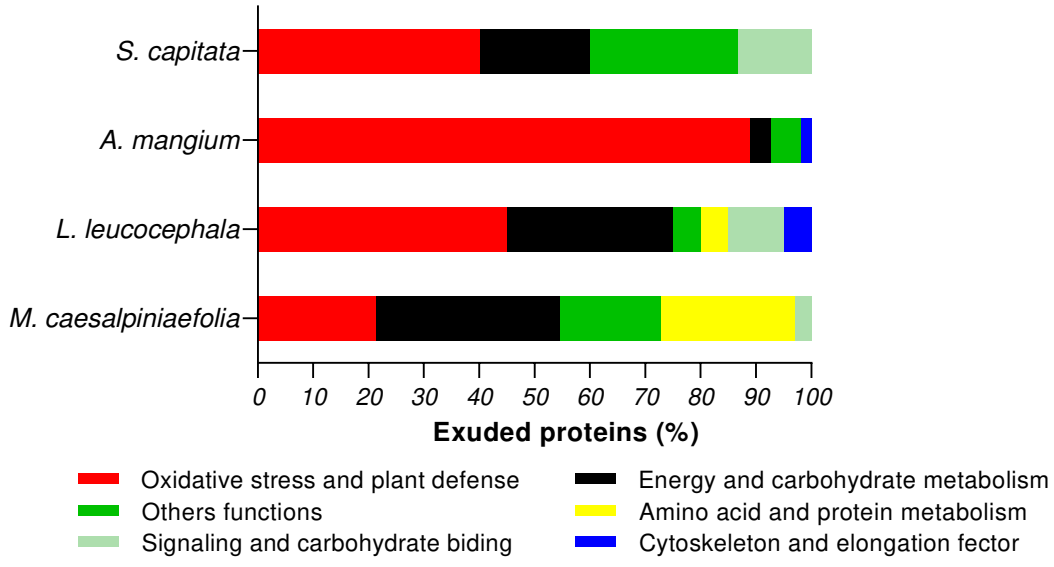
193 EC_{50} = Effective concentration for 50% of the population in milligrams per milliliters;
194 95% CI = 95% confidence interval
195
196 **Proteome profile**
197 Overall, 142 proteins of all PE were identified. Specificallly, 33 (23%), 40
198 (28%), 54 (38%) and 15 (11%) proteins were respectively identified in *M.*
199 *caesalpiniaefolia, L. leucocephala, A. mangium* and *S. capitata* (S1 Table). Isomerases,
200 elongation factors, lectins, pathogenesis-related proteins, superoxido dismutases, seed

storage proteins, chitinases, proteases, protease inhibitors, calmodulins, beta-amylases are among the identified proteins exuded by the seeds (S1 Table).

The biological classification of the exuded proteins reveals that they are classified into five main classes: amino acid and protein metabolism; cytoskeleton and elongation factors; oxidative stress and plant defense, oxidative stress and plant defense, signaling and carbohydrate binding and other functions (Fig. 1).

A total of 54%, 73.2%, 92.5% and 60% of the exuded proteins, respectively from *M. caesalpiniaefolia, L. leucocephala, A. mangium* and *S. capitata* belong to two classes: oxidative stress and plant defense and energy and carbohydrate metabolism (Fig. 1).

Fig 1. Biological classification by GO analysis of proteins identified from (A) *M. caesalpiniaefolia,* (B) *L. leucocephala,* (C) *A. mangium* and (D) *S. capitata* seed exudates analyzed by LC-ESI-MS/MS.



Discussion

Interactions of seeds with soil-born organisms may be antagonistic (e.g pathogens) or synergistic (e.g symbiosis with rhizobia) [46,47,48]. Pathogens like fungi and nematodes, make soil a hostile environment to seeds. To survive, seeds developed strategies to defend from pathogen infection [49,50]. Proteins are in the front line of defense in seeds toward pathogens [51,52]. Besides to the importance in defense, these proteins can display interesting activities with great potential to biotechnological applications. Among these activities, it was already reported that seed exude proteins that inhibit the nematodes development [53, 20]. Thus, to better understand the importance of exuded seed proteins for biotechnological purpose, in this work the proteins exudated from *M. caesalpiniaefolia*, *L. leucocephala, A. mangium* and *S. capitata* seeds were identified and their anthelmintic efficacy was assessed toward the *H. contortus* egg hatching and larval exsheathment.

In general low amounts of proteins are exuded from seeds [33, 19]. According to Nelson [54] not all proteins are present in the exudate at same time. Molecules are released early or later during imbibition. The quantity and quality of the material released during exudation may be influenced by a number of environmental and morphological factors, such as pH, temperature, and seed size [37].

Although the *H. contortus* egg is an important stage for the control of the life cycle [55], the exudates obtained in this work were not effective in inhibiting the hatching up to 1 mg mL^{-1} (data not shown). The cuticle of the *H. contortus* eggs is formed by three basic layers: a yolk; a chitin layer and a layer formed by lipids and proteins [56,57]. Protease, lipase, chitinase, α and β-glycosidases, and leucine aminopeptidase are present in *H. contortus* hatching fluid [58]. Inhibition of these enzymes can promote reduction or abolish the hatching process [59]. Some of these

243 enzymes are present in the seed exudates and perhaps, for this reason, the exudates were
244 not effective in this life-stage.

245 The anthelmintic activity against *H. contortus* was also assessed by the ability of
246 exudates to inhibit other important process for the nematode development, the larval
247 exsheathment. All exudades inhibited *Haemonchus contortus* larval exsheathment
248 (Table 1). The larval exsheathment is the second step of the nematodes molting process.
249 It is responsible for a vital step to nematodes: the infection in the definitive host [60].

250 After proteomic analysis, excluding non-identified proteins and those proteins
251 that do not attempt to the established criteria, an average of 35.25 proteins were
252 identified in each PE (S1 Table). Similar results were obtained by Rocha et al. [38] with
253 the identification of 41 proteins by applying ESI-LC-MS/MS to evaluate the protein
254 profile of soybean (*Glycine max*) seed exudates.

255 There is no specific pattern of proteins present in the studied seeds, since the
256 different species showed a particular protein composition in exudates (S1 Table) with
257 anthelmintic activity as protease [61,62] protease inhibitor [63], chitinase [64], lectin
258 [65] are present in the exudates of the studied species. Indeed, chitinases are present in
259 the *A. mangium* and *L. leucocephala*; proteases in *M. caesalpiniaefolia* and *A.*
260 *mangium*; protease inhibitors in *L. leucocephala*, *A. mangium* and *M. caesalpiniaefolia*;
261 lectin in *S. capitata* (S1 Table).

262 The cuticle is the outmost layer of the body wall that protects the organism and
263 selectively regulates the flow of fluids through the body wall during nematode
264 development [66]. Proteases and chitinases can be related with ovicidal activity by
265 degradation of egg shall [67, 61, 38]. However, the studied exudates did not inhibited
266 egg hatching.

Due to the importance of proteases to larval exsheathment [68,69,60], the nematode developmental process is completely susceptible to the protease inhibitors present in PE. Protease inhibitors were also identified in two other proteomic studies on seed exudates [38,19]. The presence of protease inhibitor in the exudates of soybean (*G. max*) and *M. urundeuva*, and anthelmintic activity against *Meloidogyne incognita* and *H. contortus*, respectively, were observed [20,39]. Protease inhibitors are known to confer natural protection against nematode attack. Thereby, they have been purified from plants aiming biotechnological application [70,71].

Table 1 shows that the *S. capitata* PE displayed high inhibition of the larval exsheathment of *H. contortus* larvae. However, different from the other studied exudates, protease inhibitors were not detected in *S. capitata* PE (S1 Table). There are at least two hypothesis for this result. First, not all proteins are detected by MS/Ms analysis. It is possible that protease inhibitors are present in low concentration in *S. capitata* PE and could not be detected. Second, the anthelmintic activity might be displayed by other protein class. A candidate protein for this anthelmintic activity is a lectin identified in *S. capitata* PE (S1 Table). The anthelmintic activity of lectins was previously reported in several studies [65,72]. Rocha et al. [38] attributed the anthelmintic activity of *G. max* seed exudates to the presence of lectin identified by MS/MS analysis.

The presence of lectin in the PE of *S. capitata*, *M. caesalpiniaefolia* an *L. leucocephala*, could be related to the anthelmintic action. Glycoproteins exist in the old cuticle and can be target for exuded lectins by interaction with their glycidic portion. Once establish such interactions, it can change the structure of old cuticle. In addition carbohydrates are important for *H. contortus* development. For instance, Schallig and

van Leeuwen [73] suggested that many glycoconjugates in L3 *H. contortus* larvae may act as protectors during the larval exsheathment of *H.contortus*.

The data of the present study lead to the following question: Which exuded proteins would be involved in anthelmintic activity against *H. contortus*? The biological classification of the PE (Figure 1) gives some clues. The majority of proteins identified by proteomic analysis of soybean exudates (*G. max*) was classified in plant defense group [39]. In this work *A. mangium* seed exudates have 88.7% of their proteins classified in the group of oxidative stress and plant defense. However, analyzing all the identified proteins in PE from all the seed exudates, the majority was classified in two main groups: 56% related to oxidative stress and plant defense and 20% to energy and carbohydrate metabolism. Probably, the presence of bioactive proteins belonging to different classes is a possibility to be studied, with the objective of advancing the development of anthelmintic products from seed exudates.

Conclusion and Perspective

In conclusion, as the proteins from *M. caesalpiniaefolia*, *L. leucocephala*, *A. mangium*, and *L. leucocephala* seed exudates inhibit *H. contortus* exsheathment but not egg hatching, the effect is life-stage specific. The inhibition observed may be related to proteins involved to energy / carbohydrate and oxidative stress/ plant defense. This work reinforces that seed exudates possess promising bioactive proteins for the development of new biotechnological strategies to treat ruminants infection by *H. contortus*. These data encourage further investigations to identify the bioactive proteins in the exudates responsible for the anthelmintic effect. In addition, further mechanistic and toxicity studies are also a high priority.

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566 **Supporting information**

567 **S1 Table.** Proteins identified from *M. caesalpiniaefolia, L. leucocephala, A. mangium and S.capitata* seed exudates by LC-ESI-MS/MS.

Plant Specie	Protein Description	Accession Number	Score (-10lgP)	Coverage (%)	Peptides Identified	Molecular Weight (Da)
Amino acid and Protein metabolism						
M. caesalpiniaefolia	Carboxypeptidase	A0A1D1YCE7_9ARAE	62.10	4	4	41995
	26S proteasome non-ATPase regulatory	A0A1S3VLX4_VIGRR	37.42	2	2	60482
	Cytosol aminopeptidase family protein	A0A061GI42_THECC	80.88	12	2	17250
	Peptidyl-prolyl cis-trans isomerase	A0A165Y2C6_DAUCA	44.58	4	2	41541
	Probable cysteine protease RDL3	RDL3_ARATH	44.92	2	2	55006
	probable serine protease EDA2	A0A1S3USY2_VIGRR	59.16	4	3	77638
	Subtilisin serine protease 2	A0A061GRM3_THECC	78.48	2	5	81483
	Subtilisin-like serine protease	A0A072TJK5_MEDTR	75.89	6	2	34985
L. leucocephala	Peptidyl-prolyl cis-trans isomerase	M8ACI8_TRIUA	110.06	19	3	18236
	Peptidyl-prolyl cis-trans isomerase	S5WBW2_9FABA	53.21	4	2	26718
Cytoskeleton and elongation factor						
L. leucocephala	desiccation protectant protein	A0A1S4DFE3_TOBAC	98.41	13	2	18322
	Elongation factor 1-alpha	A0A1D1YFT8_9ARAE	91.35	5	2	50218
A. mangium	Rubber elongation factor	W0JBG5_HEVBR	114.27	43	5	14712
Energy and Carbohydrate metabolism						
M. caesalpiniaefolia	fasciclin-like arabinogalactan	A0A1S3TD69_VIGRR	81.25	13	6	25839
	Fasciclin-like arabinogalactan	A7KQG9_EUCGR	48.32	5	2	26054
	Glucan endo-1,3-beta-glucosidase-like protein	A0A072VAP9_MEDTR	36.72	10	2	10041
	glucose and ribitol dehydrogenase	A0A1S3V523_VIGRR	51.87	4	2	31683
	Malate dehydrogenase	A0A0K1R7F5_ZOSMR	32.10	10	2	12328
	Malate dehydrogenase	A0A1U8AJC1_NELNU	58.77	8	4	35833
	Phosphoglycerate kinase	M5X0L9_PRUPE	35.69	4	2	47568

	Triosephosphate isomerase	Q38IW8_SOYBN	44.61	5	2	27204
	Alpha-galactosidase	A0A059BIP2_EUCGR	42.39	4	2	45845
	Alpha-galactosidase	A0A061E9L0_THECC	44.52	2	2	73050
	Beta-galactosidase	A0A0R0HQX5_SOYBN	85.42	4	4	84890
L. leucocephala	Alpha-galactosidase	M0TTK4_MUSAM	73.31	4	4	46659
	Beta-amylase	A0A1U8HJH0_CAPAN	39.35	2	2	72311
	Beta-galactosidase	A0A1S4DQ09_TOBAC	59.29	2	3	84225
	Enolase	A0A0B2QAW5_GLYSO	157.77	7	5	48996
	Glucan endo-1,3-beta-glucosidase	G7JQL1_MEDTR	67.39	4	2	36466
	Glutathione S-transferase	A0A0B2QDE1_GLYSO	110.42	13	4	25359
	Glyceraldehyde-3-phosphate dehydrogenase	F8UWQ6_THYVU	121.82	17	2	21092
	Glyceraldehyde-3-phosphate dehydrogenase	A0A0A0YQF5_9APIA	135.38	8	3	36622
	Glycoside hydrolase	A0A1R3GD69_9ROSI	72.90	3	3	77396
	Malate dehydrogenase	A0A059BV12_EUCGR	50.93	3	2	36055
	Ribulose bisphosphate carboxylase small chain 3	RBS3_FRIAG	40.44	4	2	19615
	Xyloglucan endotransglucosylase/hydrolase	A0A059BV12_EUCGR	61.11	3	2	36055
A. mangium	NAD(P)-binding rossmann-fold protein	A0A072UT49_MEDTR	74.00	11	2	27563
	Malate dehydrogenase	A0A068U3Q5_COFCA	43.04	5	2	33002
S. capitata	Acyl-CoA-binding protein	M8D834_AEGTA	77.21	10	2	13219
	Fructose-bisphosphate aldolase	T2B9M0_ARAHY	59.30	3	3	38383
	Malate dehydrogenase	A0A0A1G6F9_9FABA	114.66	9	2	36105
Oxidative stress and Plant Defense						
M. caesalpiniaefolia	Xyloglucan-specific fungal endoglucanase inhibitor protein	Q8GT67_SOLLC	82.25	10	5	30721
	Brassinosteroid insensitive 1-associated receptor kinase 1	A0A0A0QTG7_9ASTR	35.61	2	2	67851
	Cysteine proteinase inhibitor	G7JLQ2_MEDTR	48.31	10	2	13177
	Cysteine proteinase inhibitor	A0A1S4AB43_TOBAC	37.15	9	2	15301
	Cysteine proteinase inhibitor	B9SYV2_RICCO	66.06	13	4	12872

	Lactoylglutathione lyase	A0A059CFQ8_EUCGR	59.79	8	4	32361
	Lipoxygenase	G3GC08_9ASPA	34.71	1	2	94820
L. leucocephala	Chitinase KBchit5-3-1	Q8LK49_LEULE	158.26	14	4	34514
	Chitinase	Q8MD06_LEULE	176.20	18	4	34908
	Cysteine proteinase inhibitor	K4C291_SOLLC	61.87	12	2	14132
	Endochitinase	A6ZJJ1_PARPC	113.52	11	2	29064
	Glucan endo-1,3-beta-glucosidase-like protein	A0A0B2R8X2_GLYSO	60.62	5	2	21935
	Kunitz proteinase inhibitor	U5NDP6_TRIRP	52.33	5	2	23319
	Kunitz-type trypsin inhibitor	ID5A_PROJU	77.12	9	2	15468
	Lipoxygenase	Q4JME6_ARAHY	72.18	1	2	97476
	Lipoxygenase	G3GC08_9ASPA	84.66	1	2	94820
	Lipoxygenase	Q6X5R7_NICAT	62.09	2	2	97215
	pathogenesis-related protein PR-4-like	A0A1U8HSB9_GOSHI	66.99	5	2	22296
	Pathogenesis-related thaumatin family protein	A0A072UDD5_MEDTR	69.82	5	2	26228
	Proteinase inhibitor	D3YBD3_TRIRP	42.13	1	2	81772
	Thaumatin-like protein 1	TLP1_PRUPE	38.39	4	2	25765
	Trypsin inhibitor	ITRY_ENTCO	60.42	7	2	19465
	Trypsin inhibitor	ITRY_LEULE	248.02	64	35	20104
	Trypsin inhibitor	Q9AVS0_PEA	63.27	12	2	11296
	14-3-3 protein	A0A1R3JIS8_9ROSI	66.91	5	2	28410
A. mangium	Acidic chitinase	O65330_ELAUM	146.44	10	7	35737
	Acidic class II chitinase	Q8H985_CITJA	120.21	14	5	32143
	Basic chitinase 1-1	Q6IVX8_9CARY	211.23	21	28	37899
	Basic class I chitinase	Q6R8K3_MUSAC	105.97	8	6	50358
	Basic endochitinase A	A0A1D1XQL6_9ARAE	155.04	14	14	38493
	Basic endochitinase A	A0A199W684_ANACO	143.18	11	13	68622
	Bowman-Birk type wound-induced trypsin inhibitor	IBBWT_MEDSA	127.89	22	9	6337
	chitinase 10-like	A0A1U8L297_GOSHI	170.68	17	16	30443
	Chitinase 4	A0A1D1ZB20_9ARAE	43.57	4	3	32207

	Chitinase B	V5TEI0_DIOMU	92.50	16	4	33396
	Chitinase class I	B0ZC08_CASGL	265.25	29	66	34377
	Chitinase	W0TSA0_ACAMN	319.79	60	125	34702
	Chitinase	Q43752_CITSI	106.37	12	4	31910
	Chitinase	Q7X9R8_EUOEU	85.46	12	3	33640
	Chitinase	B2X051_FRAAN	145.33	15	9	30700
	Chitinase	Q8MD06_LEULE	145.64	19	13	34908
	Chitinase	P93327_MEDTR	285.40	42	100	34924
	Chitinase	E2J9M1_MORAL	108.21	12	6	38516
	Chitinase	Q6SZS3_ORYSA	167.99	9	13	34392
	Chitinase	Q43853_ULMAM	182.36	23	28	34951
	Class I chitinase	Q9M7H4_ARABL	143.26	13	13	33288
	Class I chitinase	Q9M7F9_9BRAS	65.23	6	2	31990
	Class I chitinase	E5LCJ0_CASEQ	259.61	23	58	34110
	Class I chitinase	Q9ZP10_CICAR	266.14	37	79	35473
	Class I chitinase	D7RTU7_GOSHI	129.87	9	11	34666
	Class I chitinase	B9VQ31_PYRPY	73.45	8	2	34023
	Class I chitinase	U3PW23_THECC	165.68	18	16	34852
	Class Ia chitinase	K9MY71_9FABA	294.11	54	114	34579
	Class Ib chitinase	Q1W6C5_9CARY	101.36	7	4	34130
	Class II chitinase	Q4Z8L7_WHEAT	195.74	27	29	28260
	Class IV chitinase	A9ZMK1_NEPAL	94.14	8	5	29027
	Endochitinase A2	CHI2_PEA	272.30	40	85	34678
	Endochitinase EP3	CHI5_ARATH	88.98	5	4	29436
	Endochitinase MCHT-2	Q9AVA8_CUCME	76.77	7	3	33967
	Endochitinase	P06215	224.96	27	45	35444
	endochitinase-like	A0A1U8MN37_GOSHI	57.81	8	2	34926
	endochitinase-like	A0A1U8N548_GOSHI	116.93	10	7	33531
	Multicopper oxidase	A0A1R3HZ71_9ROSI	27.60	2	2	60280

	osmotin-like protein OSML13	A0A1U8G8A9_CAPAN	29.03	4	2	26792
	Pathogenesis related protein	A0A1X7QH12_VICFA	98.98	13	5	34505
	Putative chitinase	Q8VXF0_MUSAC	205.22	27	31	33410
	Putative chitodextrinase	A0A023SGB5_EUCUL	200.84	19	25	34720
	Putative class I chitinase	A0A1C9TAC9_9SPER	90.67	18	4	16129
	Putative class I chitinase	Q949H3_HEVBR	193.20	25	36	31647
	Putative class I chitinase	Q76IR2_TAXDI	178.02	16	16	33779
	Trypsin inhibitor	ITRY_ACACO	83.09	11	3	19584
	Trypsin inhibitor	ITRY_ENTCO	84.95	11	3	19465
	Small rubber particle protein	A0A1U7Z4X5_NELNU	32.04	8	2	15677
S. capitata	Lactoylglutathione lyase	A0A166F496_DAUCA	89.35	8	2	32633
	Pathogenesis-related 10	Q93XI0_LUPAL	71.14	9	2	16949
	Pathogenesis-related protein 10b	K4LMW7_LENCU	57.47	9	2	17172
	Superoxide dismutase [Cu-Zn] 1	P24704_SODC1_ARATH	115.30	24	6	15098
	Superoxide dismutase [Cu-Zn]	A0A0H4IRB5_9FABA	87.61	6	2	21108
	Thaumatin-like protein-like protein	E2D2C7_9FABA	152.87	28	8	22947
	Signalling and Carbohydrate binding					
M. caesalpiniaefolia	Concanavalin A-like lectin/glucanases	A0A1R3GCV1_9ROSI	64.05	6	2	22682
L. leucocephala	Annexin	G7LF86_MEDTR	69.78	3	2	35646
	Calmodulin	Q6DMS1_SALMI	86.58	11	3	16731
	Concanavalin A-like lectin/glucanases superfamily	A0A1R3GCV1_9ROSI	67.19	6	2	22682
	Conglutin beta 2	CONB2_LUPAN	48.98	1	2	70701
S. capitata	Calmodulin-related protein	M8B5W8_AEGTA	55.93	4	2	46429
	Lectin 1	P23558_LEC1_LABAL	35.88	4	2	27151
		Other Functions				
M. caesalpiniaefolia	BnaA10g27260D protein	A0A078HR75_BRANA	34.08	5	2	27189
	glycine-rich cell wall structural	A0A1S3VGC8_VIGRR	44.48	2	2	43877
	Kiwellin	L7TY92_ACTER	37.63	8	2	22301
	Phosphatidylethanolamine-binding protein	B9IGD8_POPTR	35.32	7	2	18526

	Plastocyanin	M0U319_MUSAM	47.57	9	2	16996
	Translationally controlled tumor-like protein	D5K163_TACME	39.22	30	2	5389
L. leucocephala	Cytochrome c, class IA/ IB	A0A1R3JXJ8_9ROSI	78.86	13	2	12254
	Short-chain dehydrogenase/reductase	A0A072UJ88_MEDTR	40.49	4	2	28119
A. mangium	Desiccation-related protein	A0A1R3G8E3_9ROSI	102.55	8	4	32357
	Polygalacturonase-inhibiting protein	A0A1S3UBJ8_VIGRR	38.66	1	2	101987
	Small rubber particle protein	A0A1U7Z4X5_NELNU	32.04	8	2	15677
S. capitata	Allergen Pru protein	I3T3P8_MEDTR	112.49	8	4	25305
	Allergen V5/Tpx-1-related protein	A0A1R3JBH6_9ROSI	81.27	9	2	17477
	Major latex allergen Hev b 5	Q39967_ALL5_HEVBR	78.86	11	2	16090
	Seed storage protein Ara h1	N1NG13_ARAHY	62.45	2	2	71345

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5. CONSIDERAÇÕES FINAIS

Os exsudatos de plantas têm potencial para o desenvolvimento de fármacos, que são menos prejudiciais à saúde, bem como ambientalmente seguros em comparação com compostos sintéticos. No entanto, isto pode ser conseguido apenas após muitas experiências *in vitro* e *in vivo* para o exsudado de plantas em questão a partir de espécies de plantas específicas.

Assim, no panorama atual, com a diminuição da eficácia dos produtos antiparasitários disponíveis, muitas são as pesquisas que buscam novos compostos ou moléculas com atividade contra os parasitos. Dessa forma, as espécies vegetais podem ser uma rica fonte de material para prospecção de atividade sobre os parasitos.

Até agora, muitos estudos forneceram evidências de que proteínas apresentam potencial para utilização como parte de um programa de controle de nematóides gastrointestinais. As proteínas aqui relatadas apresentam mecanismo de ação diferenciado dos demais produtos do mercado, assim, essas moléculas poderiam ser utilizadas para o tratamento de cepas resistentes aos atuais medicamentos disponíveis comercialmente.

Em conjunto, nossos dados indicam que a secreção seletiva de proteínas por sementes de *M. caesalpiniaefolia, L. eucocephala, A. mangium e S. capitata* podem contribuir para o desenvolvimento de produtos anti-helmínticos contra *H.contortus.*

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