UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

DEJANI MAÍRA PANAZZOLO

ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DE *SOLANUM AMERICANUM* E *SOLANUM CORYMBIFLORUM* COM POTENCIAL USO EM ENFERMIDADES DE REBANHOS LEITEIROS

Porto Alegre
2020

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DE *SOLANUM AMERICANUM* E *SOLANUM CORYMBIFLORUM* COM POTENCIAL USO EM ENFERMIDADES DE REBANHOS LEITEIROS

Dejani Maíra Panazzolo
Zootecnista - UFSM
Mestre em Zootecnia – UFRGS

Tese apresentada ao Programa de Pós-Graduação em Zootecnia como um dos requisitos para a obtenção do título de Doutora em Zootecnia
Área de Concentração: Produção Animal

Orientadora: Vivian Fischer

Porto Alegre (RS), Brasil
Junho de 2020.

CIP - Catalogação na Publicação

Panazzolo, Dejani Maíra
ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DE SOLANUM AMERICANUM E SOLANUM CORYMBIFLORUM COM POTENCIAL USO EM ENFERMIDADES DE REBANHOS LEITEIROS / Dejani Maíra Panazzolo. -- 2020.
122 f.
Orientadora: Vivian Fischer.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2020.

1. vacas leiteiras. 2. biofilmes bacterianos. 3. alcaloides. 4. Solanum americanum . 5. Solanum corymbiflorum . I. Fischer, Vivian, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Dejani Maíra Panazzolo
Mestre em Produção Animal

TESE

Submetida como parte dos requisitos
para obtenção do Grau de
DOUTORA EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 30.06.2020
Pela Banca Examinadora

VIVIAN FISCHER
PPG Zootecnia/UFRGS
Orientadora

Andrea Machado Leal Ribeiro
UFRGS

Itabajara da Silva Vaz Junior
UFRGS

Wladimir Padilha da Silva
UFPEL

Homologado em: 13/11/2020
Por

DANILO PEDRO STREIT JR.
Coordenador do Programa de
Pós-Graduação em Zootecnia

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

Agradecimentos

À Deus, pela saúde, força e coragem para buscar meus objetivos.

À minha família, pelo apoio e suporte em todos os momentos.

Ao Dr. Harold Ospina Patiño (*In memoriam)* e à Dra. Liliana Essi pelas palavras de estímulo, e por serem grandes incentivadores do meu propósito.

À minha orientadora e amiga, Dra. Vivian Fischer, pelo apoio e ensinamentos durante estes sete anos de convívio.

Aos docentes Dr. José Ângelo Zuanazzi, Dr. Alexandre José Macedo, Dr. Sidnei Moura e Dra. Mirian Apel pela oportunidade de trabalharmos juntos.

Aos grupos de pesquisa GSR e Nuplac, e ao Laboratório de Farmacognosia, dos quais pude fazer parte, aprender, ensinar, trocar experiências e fazer amigos.

À Dra. Marina Scopel, Elen Oliveira e Dra. Luciana Tallini, pela ajuda, experiências e amizade.

Aos docentes Dr. Itabajara Vaz Jr. e Dra. Andrea Machado Leal Ribeiro pelas sugestões, me impulsionando a ver e fazer meu melhor.

À UFRGS, à CAPES e ao PPG Zootecnia pela oportunidade e suporte para projetar e desenvolver minhas ideias.

A todos que indiretamente participaram da minha jornada e crescimento profissional.

E para aqueles que me desestimularam e disseram que eu não conseguiria, muito obrigada, eu não teria insistido se não fosse por vocês!

Our doubts are traitors and make us lose
good we oft might win, by fearing to
attempt.”

Willian Shakespeare

ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DE *SOLANUM AMERICANUM* E *SOLANUM CORYMBIFLORUM* COM POTENCIAL USO EM ENFERMIDADES DE REBANHOS LEITEIROS[1]

Autora: Dejani Maíra Panazzolo
Orientadora: Dr. Vivian Fischer

Resumo: A mastite é uma inflamação da glândula mamária que tem como causa principal infecções bacterianas. É a doença que mais utiliza antimicrobianos no sistema de produção, comprometendo a sustentabilidade e gerando problemas de saúde pública. Seguindo o conhecimento tradicional de povos nativos, que utilizam solanáceas para tratamento de mastite, o objetivo desse estudo foi realizar uma pesquisa bio-guiada sobre a atividade antimicrobiana e antioxidante a partir da elaboração de extratos: aquoso (AQU), metanólico (MET) e alcaloídico (ALK), das espécies de *Solanum americanum* (SA) e *Solanum corymbiflorum* (SC). Os extratos foram utilizados em ensaio de microdiluição em caldo e coloração com cristal violeta, para avaliar a multiplicação bacteriana e a formação do biofilme de *Staphylococcus aureus* e *Escherichia coli,* e à reação com o radical DPPH, para avaliar a capacidade antioxidante. Os dados de absorbância foram obtidos por densidade ótica e submetidos à análise de variância (ANOVA). As médias foram comparadas pelos testes de Dunnett e Tukey, adotando um nível de significância de $P<0.05$. Seguindo a pesquisa bio-guiada, os extratos que apresentaram atividade biológica foram submetidos à caracterização química através de cromatografia em camada delgada (CCD), cromatografia líquida de alta eficiência (CLAE) e espectrometria de massas (EM) de alta resolução. O extrato AQU de SA estimulou a multiplicação de *S. aureus* e *E. coli*. O extrato MET de SC estimulou a formação do biofilme para *S. aureus,* mas reduziu essa capacidade em relação à *E. coli*. O extrato ALK de ambas as espécies inibiu a formação de biofilme de *S. aureus*. Com base na comparação de dados quanto à massa exata, via de fragmentação e ao espectro UV, foi possível indicar a presença do alcaloide *N-trans*-feruloiltiramina (*m/z* 314.1382) no extrato ALK de SA. Todos os extratos de SA e o extrato MET de SC à 250 *μ*g/mL apresentaram mais de 50% de atividade antioxidante. Os resultados obtidos nestes ensaios *in vitro* podem estar relacionados ao conhecimento tradicional que utiliza folhas de SC no tratamento e redução dos sintomas da mastite. O presente estudo evidenciou a inibição da formação do biofilme de *S. aureus* e a ação antioxidante como potenciais modos de atuação dos compostos ativos.

Palavras-chave: alcaloides; biofilmes bacterianos; produção animal; solanáceas

[1] Tese de Doutorado em Zootecnia - Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (122 p.). Junho, 2020.

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *SOLANUM AMERICANUM* AND *SOLANUM CORYMBIFLORUM* WITH POTENTIAL USE ON DAIRY COWS DISEASES[2]

Author: Dejani Maíra Panazzolo
Supervisor: Dr. Vivian Fischer

Abstract: Mastitis is a mammary gland inflammation caused mainly by bacterial infections. It is the disease that most uses antimicrobials in the production system, compromising sustainability and generating public health problems, such as the development of resistant strains. Following the traditional knowledge of native tribes, who use solanaceous to treat mastitis, the aim of this study was to conduct a bio-guided research on antimicrobial and antioxidant activity based on elaboration of extracts: aqueous (AQU), methanolic (MET), and alkaloid (ALK), from the species *Solanum americanum* (SA) and *Solanum corymbiflorum* (SC). The extracts were used in a broth microdilution assay and stained with violet crystal, to evaluate the bacterial growth and biofilm formation of *Staphylococcus aureus* and *Escherichia coli*, and to the reaction with the radical DPPH, to evaluate the antioxidant capacity. The absorbance data were obtained by optical density and subjected to statistical analysis (ANOVA). The means compared by the Dunnett and Tukey tests, adopting a significance level of $P<0.05$. Following bio-guided research, extracts that showed biological activity were subjected to chemical characterization through thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and high-resolution mass spectrometry (HRMS). AQU extract of SA stimulated the growth of *S. aureus* and *E. coli*. MET extract of SC stimulated biofilm formation for *S. aureus* but reduced this ability for *E. coli*. The ALK extract from both species inhibited biofilm formation *S. aureus*. Based on the data comparison of exact mass, fragmentation pathway and UV spectrum, it was possible to indicate the presence of the alkaloid *N-trans*-feruloyltyramine (m/z 314.1382) in the ALK extract of SA. All the extracts of SA and the MET extract of SC at 250 μg/mL showed more than 50% of antioxidant activity. The results obtained in these *in vitro* tests may be related to the traditional knowledge that uses SC leaves in the treatment and reduction of mastitis symptoms. The present study showed the inhibition of the biofilm formation of *S. aureus* and the antioxidant action as potential modes of action of the active compounds.

Keywords: alkaloids; animal production; bacterial biofilm; solanaceous

[2] Doctoral Thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (122 p.). June, 2020

Sumário

Lista de Quadros

Lista de Tabelas

Capítulo II

Supplementary material

Capítulo III

Lista de Figuras

Capítulo I

Capítulo II

Supplementary material

Capítulo III

Lista de abreviaturas

AQU: Extrato aquoso
MET: Extrato metanólico
ALK: Extrato alcaloídico
SC: *Solanum corymbiflorum*
SA: *Solanum americanum*
ANOVA: Análise de variância
CCD: Cromatografia em camada delgada
CLAE: Cromatografia líquida de alta eficiência
EM: Espectrometria de massas
UV: Ultravioleta
MRSA: *S. aureus* resistente à meticilina
IIM: Infecções Intramamárias
CTA: Conhecimento tradicional associado
CCS: Contagem de células somáticas
DEL: Dias em leite
LPS: Lipopolissacarídeo
MHC: Complexo de histocompatibilidade
IL: Interleucina
IFN: Interferon
TNF: Fator de necrose tumoral
ERO: Espécies reativas do oxigênio

CAPÍTULO I

1. Introdução

A produção animal é um importante setor consumidor de antimicrobianos, basicamente utilizados de forma terapêutica, profilática e como aditivos zootécnicos (PEREIRA; SCUSSEL, 2017). Doenças infecciosas são as principais causas do uso terapêutico, e, dentre elas, a mastite é a principal causa deste uso em rebanhos leiteiros. Entende-se por mastite a inflamação da glândula mamária desencadeada por uma infecção ou trauma, caracterizada por alterações físicas, químicas e bacteriológicas do leite, além de alterações patológicas no tecido glandular (ZHAO; LACASSE, 2008). É a doença que mais causa prejuízos para a cadeia produtiva do leite, independentemente do nível produtivo ou tecnológico da propriedade. Causando perdas tanto para o produtor quanto para a indústria leiteira, principal razão pela qual é uma das doenças mais estudadas mundialmente (GREEN; BRADLEY, 2013).

Como a produção de leite é uma atividade que demanda alto custo e o produtor trabalha com uma baixa margem de lucro, a capacidade produtiva é o ponto crucial entre manter-se ou abandonar a atividade. Ressalta-se que a mastite por *S. aureus* apresenta baixa taxa de cura no rebanho (<25%) e vacas com recidivas geralmente são abatidas precocemente (RAINARD, 2005). Além dos custos, há uma progressiva preocupação com saúde pública, uma vez que a resistência aos antimicrobianos tem sido fortemente associada à transmissão horizontal entre humanos e animais (RICHARDSON et al., 2018).

A baixa taxa de cura está associada aos fatores de virulência desenvolvidos por microrganismos patogênicos. Dentre os vários mecanismos, o que mais afeta a produção leiteira é a capacidade de algumas cepas em formar biofilmes, através da expressão de genes que regulam a produção de adesinas (BUDRI et al., 2015; MELO et al., 2013). Esse fato, associado a períodos de imunossupressão do sistema imunológico favorecem a instalação e desenvolvimento da patogenia. SILVA et al., (2016) sugerem que compostos do metabolismo secundário de plantas podem regular a expressão de genes inibindo fatores de virulência, como a formação de biofilme. Estes produtos naturais de plantas tem sido utilizados na alimentação animal como aditivos fitogênicos sendo potenciais substituintes aos antimicrobianos utilizados como melhoradores de desempenho (LILLEHOJ et al., 2018). Antioxidantes são recomendados para suplementação na dieta de vacas leiteiras

para auxiliar no fortalecimento do sistema imunológico no período de transição (SPEARS; WEISS, 2008).

Produtores de leite da região sul do Brasil utilizam-se do Conhecimento Tradicional Associado (CTA) a uma espécie de solanácea endêmica utilizada por tribos indígenas para tratamento de inflamações decorrentes de infecções, incluindo a mastite (KELLER; PRANCE, 2012; KELLER, 2003). Estudos recentes com folhas e frutos de *Solanum corymbiflorum* em modelos murinos apresentaram forte atividade anti-inflamatória, corroborando com o uso tradicional para tratar doenças de pele em humanos (PIANA et al., 2016a, 2016b). A etnoveterinária é uma prática bem aceita para descoberta de novos princípios ativos através dos compostos fornecidos na dieta (MUBARACK et al., 2011). Portanto, o presente estudo objetivou avaliar duas espécies de solanáceas consumidas pelos ruminantes, *Solanum americanum* e *Solanum corymbiflorum*, quanto à atividade antimicrobiana, formação de biofilme e atividade antioxidante *in vitro,* além de realizar a caracterização fitoquímica do extrato promissor através do ensaio bio-guiado.

No primeiro capítulo é apresentada uma revisão bibliográfica sobre o uso de antimicrobianos na produção animal, resistência antimicrobiana, pré-disposição a infecções no período periparto, caraterização da mastite bovina englobando temas como fatores de virulência e imunologia da glândula mamária. Em seguida uma breve definição dos compostos secundários e seu uso na nutrição animal. Por fim, a importância das solanáceas e a caracterização das espécies utilizadas no estudo. O segundo capítulo, em formato de artigo, aborda o efeito dos extratos sobre a multiplicação bacteriana e a formação do biofilme sobre as principais bactérias causadoras de mastite, assim como a caracterização química do extrato de alcaloides, que apresentou capacidade de inibir a formação do biofilme de *S. aureus.* O terceiro capítulo, apresenta a avaliação da capacidade antioxidante e o comportamento cinético dos extratos, juntamente com as considerações finais, referências bibliográficas e material suplementar da tese.

2. Revisão bibliográfica [3]

2.1 Antimicrobianos na produção animal

A produção animal é um importante setor consumidor de antimicrobianos, utilizados com objetivos mais amplos do que na medicina humana. No rebanho leiteiro a forma terapêutica, assim como nos humanos, é usada no controle do agente patogênico para tratamento de doenças infecciosas do animal ou do rebanho, como mastite, retenção de placenta e metrite. A forma profilática consiste na prevenção contra possíveis infecções, e a forma metafilática conhecida como tratamento de animais de contato, visa utilização de antimicrobiano em todo rebanho para evitar a disseminação do agente, tendo como exemplo clássico para estes dois usos a terapia vaca seca em vacas leiteiras. Os antimicrobianos também são usados como aditivo zootécnico; neste caso, os animais não estão acometidos com doenças, a finalidade é melhorar o desempenho através de promotores de crescimento fornecidos na dieta (PEREIRA; SCUSSEL, 2017).

A principal causa de uso terapêutico de antimicrobianos na produção leiteira é devido à mastite, considerada a doença que mais afeta a cadeia produtiva do leite por conta da prevalência e ocorrência, e por ser extremamente comum, sobretudo na forma subclínica. Como estratégia para erradicação de microrganismos contagiosos, um plano de cinco pontos de controle da mastite foi desenvolvido por volta de 1960, pelo Instituto Nacional de Pesquisa sobre Laticínios na Inglaterra. Esse plano tinha como foco a manutenção da máquina de ordenha, a desinfecção dos tetos após a ordenha, o tratamento de casos clínicos, a terapia para vaca seca e a política de abate dos animais infectados. Uma extensão destas práticas de manejo foi adotada pelo Conselho Nacional de Mastite dos Estados Unidos, passando por várias modificações até se tornar o conhecido, e atualmente utilizado, Programa de Controle de Mastite (Anexo A) sendo adotado ao redor do mundo e considerado fundamental para o gerenciamento da saúde do úbere (HILLERTON; BOOTH, 2018).

Com a progressão da resistência bacteriana, estas estratégias de controle e prevenção tem se mostrado ineficientes em conter o avanço dos casos de mastite.

---

[3] Parte desta revisão será publicada na revista ***Spei Domus***
eISSN: 2382-4247
ISSN: 1794-7928
Website: https://revistas.ucc.edu.co/index.php/sp

Os antimicrobianos têm sido amplamente usados no período seco para a prevenção de novos casos, mas também para tratamento de animais que desenvolveram a doença durante a lactação. De acordo com OLIVER; MURINDA, (2012) esta prática tem aumentado o risco de resíduos no leite e favorecido o surgimento de genes relacionados à resistência aos antimicrobianos. Por ser um problema de saúde pública, há uma crescente preocupação com o uso consciente de antimicrobianos na produção animal. Uma técnica que tem sido amplamente difundida nos últimos anos é a cultura na fazenda, uma vez que ela pode identificar os patógenos de forma rápida e assertiva, possibilitando a tomada de decisão como a escolha das estratégias de tratamento que potencializam a taxa de cura (LAGO et al., 2011), ou em casos que é prevista a recuperação espontânea do animal (DODD; NEAVE; KINGWILL, 1964), pode-se evitar o tratamento, reduzindo o uso de antimicrobianos.

2.2 Resistência aos antimicrobianos

Resistência bacteriana é a capacidade de um microrganismo de resistir à atividade inibitória de um antimicrobiano em um nível de suscetibilidade considerado normal, esse processo ocorre em cepa anteriormente suscetível devido à adaptação prolongada do microrganismo ao antimicrobiano, resultado da evolução da cepa para sobreviver determinadas condições (LOO; LAI; MANSOR, 2019). Segundo estes autores, a resistência pode ser adquirida por meio de mutação do gene ou transferência horizontal de genes de outro microrganismo, que inclui conjugação, transformação e transdução. A capacidade de alguns patógenos, como o *S. aureus,* de entrarem em diferentes populações de espécies hospedeiras é uma grande ameaça à saúde pública e à segurança dos alimentos (RICHARDSON et al., 2018). Estes autores ressaltam que os genes associados à resistência antimicrobiana estão distribuídos entre hospedeiros humanos e animais, refletindo práticas distintas de uso de antibióticos na medicina e na agricultura. Na produção animal, os bovinos se apresentam como os principais reservatórios de MRSA (*S. aureus* resistente à meticilina) devido às interações horizontais bem-sucedidas com os humanos (RICHARDSON et al., 2018).

Dentre as inúmeras espécies de bactérias que causam mastite, apenas algumas são prevalentes e constituem um problema real (RAINARD et al., 2018). Um dos microrganismos mais importantes é o *S. aureus* que responde por cerca de

um terço dos casos de mastite severa ou subclínica (LI et al., 2017). Casos de resistência e multirresistência já foram relatados para cepas de *S. aureus* recuperadas em casos de mastite clínica e subclínica contra ampicilina, enrofloxacina, eritromicina, penicilina, pirlimicina e tetraciclina, sendo que algumas cepas apresentaram resistência a múltiplos medicamentos (OLIVEIRA; LANGONI; HULLAND, 2012). A multirresistência bacteriana afeta o sucesso do tratamento (PHOPHI; PETZER; QEKWANA, 2019), tornando-se um problema de saúde pública, pois afeta diretamente a saúde humana. Por outro lado, embora menos prevalente, *E. coli* é um importante agente causador de mastite clínica, especialmente na forma aguda, doença comum e geralmente fatal em vacas leiteiras devido à endotoxemia e coagulação intravascular disseminada (GUERRA et al., 2019; HERRY et al., 2017).

O conceito de saúde única prevê a segurança dos alimentos tendo uma interface entre a saúde humana, do animal e do ambiente, principalmente sob a perceptiva de produção e segurança de alimentos lácteos, uma vez que grande parte das bactérias envolvidas tem potencial zoonótico (GARCIA; OSBURNB; CULLOR, 2019). *S. aureus* tem se mostrado resistente à meticilina (MRSA) desde a década de 1960, logo após a introdução dessa classe de penicilinas na prática clínica humana, e sendo uma das principais causas de infecções bacterianas nos serviços de saúde (LEE et al., 2018). Devido à sua toxicidade, a meticilina foi substituída por penicilinas mais estáveis como a oxacilina, mas mesmo assim, o termo MRSA continua sendo utilizado e em meados dos anos 2000 começou a ser associado à produção animal (LEE et al., 2018). Segundo estes autores, esse patógeno tem uma variação geográfica notável, variando de baixa prevalência na Escandinávia até a mais alta prevalência na Ásia e, principalmente, na América do Sul (Fig. 1). Essa variação se deve a fatores como diferenças nas práticas de controle da infecção entre os países e características dos patógenos em circulação, sendo que o sucesso desta bactéria é devido à extensa gama de fatores de virulência associados a ela (LEE et al., 2018). No Brasil, o controle do uso de antimicrobianos na pecuária ainda não é restritivo como na União Europeia, por isso há uma grande responsabilidade do setor pecuário em reduzir o uso de antimicrobianos no sistema produtivo.

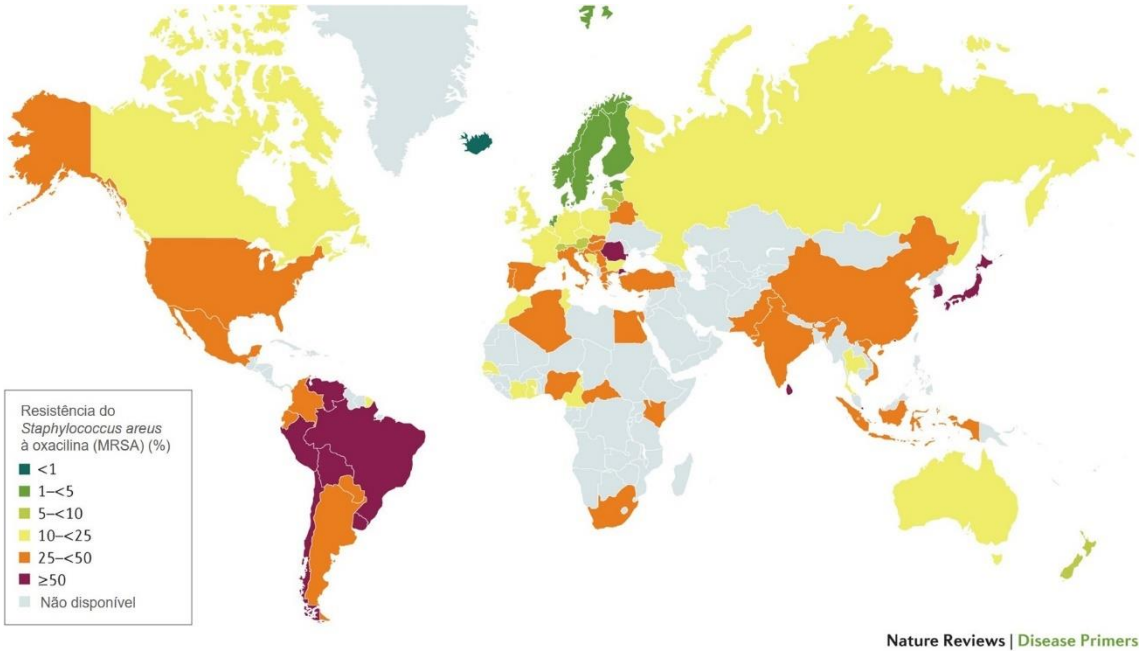


Figura 1. Porcentagem de isolados de *Staphylococcus aureus* resistentes à oxacilina em humanos. Os dados foram retirados do Centro de Dinâmica de Doenças, Economia e Políticas (CDDEP). Países que apresentam resistência acima de 50% na América Latina são listados como: Bolívia, Brasil, Chile, Peru, Uruguai e Venezuela.
Fonte: Adaptado de LEE et al., (2018).

2.3 *Imunossupressão periparturiente*

A mastite é causada por uma série de fatores ligados a três pilares principais: o animal, o patógeno e o ambiente em que eles estão inseridos. Segundo DERAKHSHANI et al. (2018), os fatores intrínsecos ao animal são genótipo, capacidade da resposta imune, ordem de parição, estádio de lactação e características anatômicas do úbere, enquanto os fatores ligados aos patógenos envolvem interações microbianas, fatores de virulência e desencadeamento de respostas pró e anti-inflamatórias. Por sua vez, os fatores ambientais estão relacionados às práticas de manejo como condições das instalações, higiene na ordenha, manejo nutricional, fatores estressantes e uso de antimicrobianos.

A aproximação do parto gera declínio na função imune do animal devido a mudanças fisiológicas, como a liberação de hormônios de estresse (BURTON; ERSKINE, 2003; SORDILLO; STREICHER, 2002), pelo fato de o animal passar de não-lactante para lactante e por conta de manejos como reagrupamento de lotes e, principalmente, a ordenha. Estes fatores fazem parte do período de transição, que engloba as três semanas que antecedem o parto e três semanas após o início da lactação (DRACKLEY, 1999). A Fig. 2 apresenta um esquema da suscetibilidade às

infecções intramamárias (IIM) em relação às mudanças na fisiologia e no ambiente da vaca antes e durante a primeira lactação.

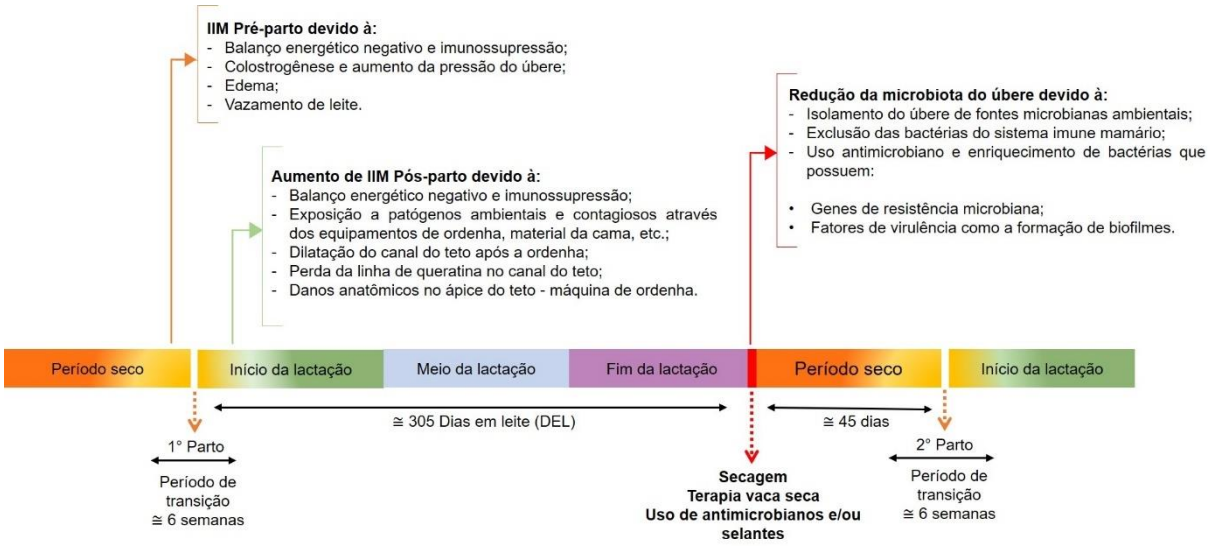


Figura 2.Flutuações especulativas na suscetibilidade das IIM e diversidade da microbiota do úbere.
Fonte: Adaptado de DERAKHSHANI et al. (2018).

A inflamação da glândula mamária é desenvolvida por alterações metabólicas, fisiológicas, lesões traumáticas e, frequentemente, por infecções bacterianas. E outras causas como o acúmulo de leite dentro do úbere quando a data do parto se aproxima, resultando no aumento da pressão intramamária (OVIEDO-BOYSO et al., 2007; PAULRUD, 2005). A primeira linha de defesa contra patógenos é barreira física representado pelo tampão de queratina (no ápice do teto), o músculo do esfíncter e o canal do teto (PAULRUD, 2005; SORDILLO; STREICHER, 2002). Durante o período seco, este tampão é considerado o primordial para evitar a penetração de microrganismos, mas ao aproximar-se da data do parto, a glândula mamária inicia o processo de colostrogênese; o acúmulo de leite aumenta a pressão intramamária, dilatando o canal do teto e liberando secreção, comprometendo a barreira contra IIM (SORDILLO; STREICHER, 2002). Após a ordenha o canal do teto permanece relaxado, aumentando as chances de invasão bacteriana, sendo usual a adoção da estratégia de ofertar alimentação aos animais no intuito que permaneçam em estação até o completo fechamento do esfíncter, evitando assim, entrada de patógenos, item 2. do Programa de Controle de Mastite (Anexo A).

Microrganismos ambientais e contagiosos invadem o úbere através do canal do teto, ultrapassando as barreiras físicas de proteção, e alcançam o interior do úbere. Os neutrófilos (também chamados neutrófilos leucócitos polimorfonucleares) são recrutados dos vasos sanguíneos para o lúmen alveolar através de um processo chamado diapedese e desempenham um papel fundamental contra a IIM (ver item 2.4.2). Além dos neutrófilos, macrófagos e linfócitos são recrutados para o sítio da infecção, e juntamente com as células epiteliais dos alvéolos danificados, originam as células somáticas do leite. A contagem de células somáticas (CCS) é um indicador de saúde do úbere (SURIYASATHAPORN et al., 2006). O desenvolvimento deste escore permite quantificar a relação linear entre mastite subclínica e redução da produção de leite (RUEGG, 2017).

A terapia vaca seca consiste na infusão de antimicrobianos diretamente na glândula mamária a fim de expor a bactéria ao princípio ativo, aumentando a chance de cura e prevenindo novas IIM. A tendência é que no Brasil este procedimento seja banido, assim como em países europeus. De acordo com DERAKHSHANI et al. (2018) esta prática gera disbiose no possível microbioma mamário (Fig. 2), o que pode interferir no aumento da resistência e fatores de virulência de bactérias patogênicas, além de eliminar algumas bactérias benéficas para o equilíbrio do microbioma. Apesar de ser recomendada pelos especialistas por longas décadas, segundo GREEN; BRADLEY (2013), a terapia vaca seca não é uma prática sustentável, podendo ser substituída por selantes de teto. Na era pós antibiótico, o que se busca é a prevenção e não a cura, evitando assim perdas futuras e a resistência gerada, sucessivamente, a cada surgimento de novas classes de antimicrobianos.

2.4 Classificação e caracterização da mastite

A mastite é classificada quanto aos agentes etiológicos em contagiosa e ambiental, e quanto à sua patogenia em subclínica e clínica que apresenta 3 graus de severidade (Fig. 3). Há uma forte relação inversa entre o recrutamento de neutrófilos e o resultado de uma infecção intramamária, vacas com cura espontânea geralmente têm uma resposta rápida e maciça característica da mastite aguda, enquanto animais com resposta lenta sofrem com infecções crônicas (BURTON; ERSKINE, 2003). Essa reposta é devido às características intrínsecas dos

patógenos, como exemplo clássico utilizamos *E. coli*, principal causador de mastite tóxica aguda e *S. aureus,* responsável pelo desenvolvimento de uma infecção crônica e subclínica (Fig. 3).

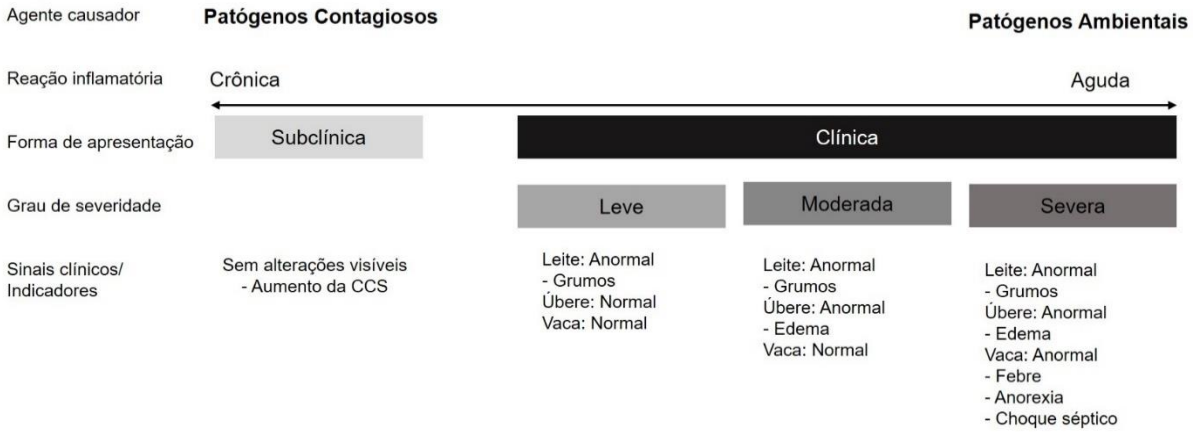


Figura 3. Classificação da mastite quando à etiologia e patogenia associados aos sinais clínicos.
Fonte: Panazzolo, 2020.

Os patógenos contagiosos são transmitidos entre animais, dos contaminados para os sadios, ou entre os quartos do mesmo animal pelo equipamento ou manipulação durante a ordenha. Por esse motivo há uma preocupação com a transmissão horizontal entre humanos e animais (RICHARDSON et al., 2018), e o esforço em eliminar o animal, que não responde ao tratamento, do rebanho (RAINARD, 2005), item 8. do Programa de Controle de Mastite (Anexo A). Eles são responsáveis principalmente por infecções crônicas e subclínicas que se manifestam pelo aumento da CCS no leite. Esse grupo inclui: *Streptococcus agalactiae, Staphylococcus aureus, Corynebacterium bovis* e *Mycoplasma spp* (BURTON; ERSKINE, 2003; OVIEDO-BOYSO *et al.*, 2007).

Os patógenos ambientais estão relacionados às instalações e condições de higiene ao qual o animal está inserido, e fazem parte deste grupo as enterobactérias como a *Escherichia coli* e *Klebsiella spp,* além de bactérias gram-positivas com o *Streptococcus dysgalactiae* e o *Streptococcus uberis.* (OVIEDO-BOYSO et al., 2007; RUEGG, 2012). O plano de controle de mastite não é eficiente em controlar estes patógenos, que geralmente surgem em estações chuvosas relacionados às questões de falta de higiene, principalmente associados à qualidade da cama e manejo durante a ordenha.

Como consequência direta da mastite ocorrem gastos com teste de diagnóstico (em caso de mastite subclínica), serviço de veterinário e medicamentos, descarte de leite (com presença de resíduos) e aumento da mão-de-obra (para manejar os animais infectados para o final da linha de ordenha). Os custos indiretos se relacionam com a perda de produção futura, comprometimento da reprodução, descarte precoce de animais (abate prematuro) e reposição de animais que foram abatidos (HEIKKILÄ et al., 2018; RAINARD, 2005; ROLLIN; DHUYVETTER; OVERTON, 2015; RUEGG, 2012).

A extensão da perda econômica é muito variável entre os países, dependendo de fatores como preço do leite, custos de tratamento e custos de reposição (HALASA et al., 2007). Esta perda também está relacionada com a forma que cada microrganismo interage com a glândula mamária. HEIKKILÄ et al. (2018) relatam que a perda é específica ao patógeno e à patogenia da doença, sendo que na forma clínica por *E. coli* é possível observar perdas diárias de 3,5 kg e que, ao final de 305 dias em leite (DEL) somam uma perda de 10,6% na produção acumulada. Em relação a *S. aureus,* que provoca principalmente a forma subclínica, foram detectadas perdas de 7,1% (2,3 kg/dia), não diferindo da perda por mastite clínica que foi de 2,2 kg/dia (HEIKKILÄ et al., 2018).

2.4.1 Fatores de virulência

Staphylococcus aureus é um agente oportunista com reconhecida capacidade de evasão do sistema imunológioco, certamente devido aos inúmeros fatores de virulência de que dispõe para se adaptar às condições e seguir infectando o hospedeiro (LEE et al., 2018). É considerado o mais virulento e difícil de eliminar do rebanho, pois causa infecção subclínica de longa duração (RAINARD et al., 2018; REYHER et al., 2012). Devido à baixa taxa de cura, o alto custo do tratamento e à necessidade de eliminar o reservatório de infecção para evitar a disseminação no rebanho, as vacas são abatidas precocemente (RAINARD, 2005; ROLLIN; DHUYVETTER; OVERTON, 2015; RUEGG, 2012). Dentre os motivos do insucesso do tratamento na mastite associada ao *S. aureus* é a capacidade desta bactéria em formar biofilme no interior da glândula mamária, fator que impede o fluxo adequado e o acúmulo de antibiótico no local alvo, consequentemente a destruição bacteriana é interrompida (RAZA et al., 2013; SAGLAM et al., 2017).

O processo de estabelecimento de biofilme é conhecido por ter etapas definidas: a fixação inicial e formação de microcolônia; maturação de bactérias aderidas em um biofilme diferenciado; e destacamento e dispersão das células planctônicas dos biofilmes (COSTERTON; STEWART; GREENBERG, 1999). A fixação de *S. aureus* no epitélio e acúmulo em multicamadas é direcionada por genes específicos, destaca-se a produção de adesinas intercelulares através dos genes *icaA*, *icaD*, and *bap* tendo como consequência a formação de biofilme (BUDRI et al., 2015). SILVA et al. (2016) consideram que muitos compostos secundários de plantas podem agir tendo como alvo os fatores de virulência, como por exemplo, alguns flavonoides são capazes de regular negativamente genes *icaA* e *icaD*, associados à adesão e acúmulo bacteriano no biofilme.

Essa mudança de estilo de vida planctônio para microcolônia, favorece a sobrevivência em ambientes hostis e aumenta a resistência bacteriana no úbere. Segundo MELO et al., (2013), quando 94 cepas de *S. aureus* foram coletadas de rebanhos leiteiros 98,9% dos isolados apresentaram genes *icaA* e *icaD*, consequentemente produziram forte fixação do biofilme *in vitro.* Geralmente cepas que são multirresistentes a antibióticos produzem biofilme mais forte e podem persistir na fazenda por mais de 3 anos (KIM et al., 2019). Estes mesmos autores destacam que mais de 60% dos quartos mamários apresentavam CCS maior que 200.000 células/mL, evidenciando as perdas devido à mastite subclínica e à dificuldade de eliminação dos estafilococos do rebanho.

De acordo com LEE et al., (2018), *S. aureus* tem a habilidade de apresentar resistência a qualquer classe de antibióticos pouco tempo depois do início da utilização do princípio ativo. A capacidade de formar biofilme lhe confere proteção, resistindo à resposta imune e aos antibióticos. A falha do sistema imunológico em eliminar o patógeno por causa do biofilme causa danos ao tecido alveolar, aumentando a CCS no leite, prolongando a duração da infecção e falha na cicatrização (SHARMA; JEONG, 2013). Os estafilococos entram na glândula mamária através do canal do teto, utilizando o leite como nutriente para multiplicar-se e como veículo para disseminar-se nos ductos e alvéolos do tecido secretor da glândula mamária, colonizando e lesionando o epitélio (Fig. 4), fator que desencadeia a resposta inflamatória (RAINARD, 2005). De acordo com os mesmos autores, o fraco sucesso da antibioticoterapia e a falta de vacinas estimulam a eliminação dos animais infectados a fim de controlar a propagação da infecção.

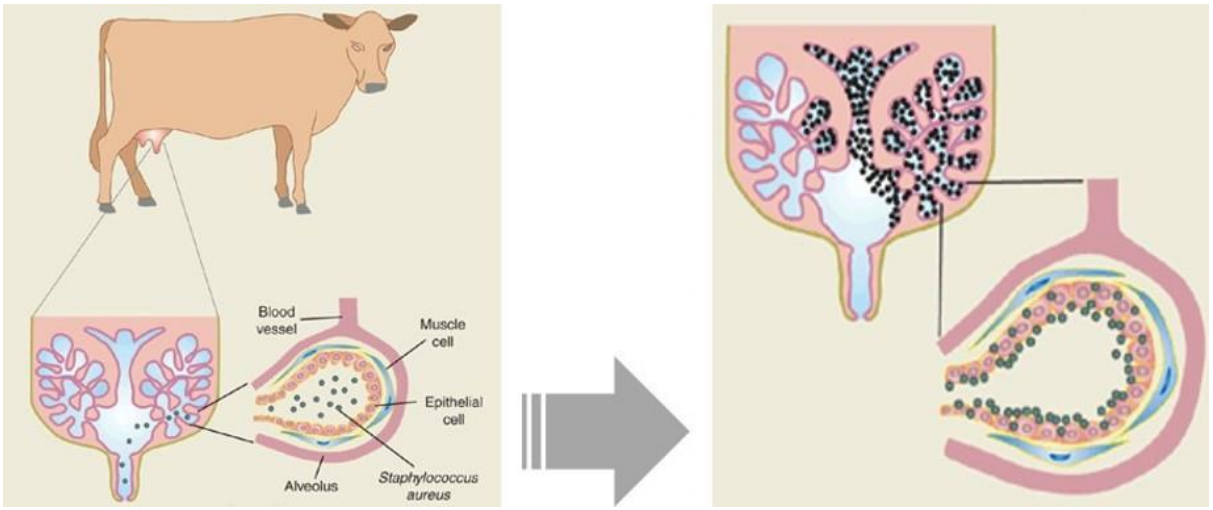


Figura 4.Colonização dos alvéolos mamários por *S. aureus*.
Fonte: Adaptado de Rainard (2005).

Patógenos ambientais têm muitos fatores de virulência que favorecem o desenvolvimento de mastite (GUERRA et al., 2019). As cepas patogênicas de *E. coli* utilizam mecanismo semelhante a outros patógenos que consiste na colonização de um local da mucosa, evasão da defesa do hospedeiro, multiplicação e dano ao tecido. Elas são capazes de produzir adesinas que além de interargir com as células hospedeiras, formam estruturas protéicas que se projetam para fora da superfície celular e identificam receptores nas células alvo (KAPER; NATARO; MOBLEY, 2004). *E. coli* é capaz de formar biofilme, estimulado por um fator estressor, como a subdosagem de antibióticos (COSTA et al., 2012). No entanto, seu efeito mais poderoso é a rápida multiplicação e a liberação de parte de suas membranas que estimulam fortemente o sistema imunológico do hospedeiro, o que pode desencadear um choque séptico (GUERRA et al., 2019). Esse fato deve-se à membrana celular conter lipopolissacarídeos (LPS), específicos de bactérias gram-negativas liberados quando a bactéria se multiplica ou é fagocitada, e que estimula fortemente a reação do sistema imunológico.

2.4.2 Imunologia da glândula mamária

Durante a fase inicial da infecção, após a diapedese, predomina a resposta imune inata mediada pelos macrófagos, neutrófilos, células NK, células dendríticas e fatores solúveis como a lactoferrina, sistema complemento, lisozima e enzima lactoperoxidase. Se o agente não for eliminado pelas células da imunidade

inata é necessária a ativação da imunidade específica, onde o agente patogênico é reconhecido pelas células apresentadoras de antígenos e apresentado via MHC às diferentes populações linfoides (RIOLLET; RAINARD; POUTREL, 2000; SORDILLO; STREICHER, 2002). Um número elevado de neutrófilos viáveis é benéfico para a defesa da glândula mamária, uma vez que agem contra as bactérias impedindo a infecção. Eles liberam citocinas pró-inflamatórias (ex: interleucinas (IL), interferons (IFN) e fator de necrose tumoral (TNF)) que possuem efeitos locais e sistêmicos. A IL-1 e o TNF-a estimulam a secreção de IL-8, importante mediador da migração de neutrófilos, por ativar as células endoteliais a expressarem mais selectina E e P, fazendo com que os neutrófilos se liguem mais firmemente ao endotélio, aumentando o fluxo destas células para a o sítio da infecção (BURTON; ERSKINE, 2003). Segundo estes autores, vacas com cortisol circulante (na ocasião do parto) reduzem a diapedese, assim as bactérias proliferam livremente aumentando a chance de mastite. Esta migração de neutrófilos do sangue para o foco da infecção é essencial para a defesa da glândula mamária e a rapidez do recrutamento, e a quantidade de neutrófilos recrutada, são determinantes para o resultado da infecção (PAAPE *et al.*, 2002; RIOLLET; RAINARD; POUTREL, 2000).

A elevada produção de citocinas ativa os macrófagos e os neutrófilos tornando-os capazes de realizar um mecanismo chamado “explosão respiratória”, aumentando a sua capacidade de eliminar bactérias através da liberação de espécies reativas do oxigênio (ERO): peróxido de hidrogênio, superóxido e radicais de hidroxila. Porém, estes oxidantes não destroem somente as bactérias, mas também o tecido da área circundante (PAAPE *et al.*, 2002). O aumento da atividade de enzimas endógenas como a catalase, superóxido dismutase e glutationa auxiliam no equilíbrio redox do ambiente, eliminando espécies reativas. Quando este equilíbrio é comprometido, ocorre um processo chamado estresse oxidativo (ELLAH, 2013; HALLIWELL; GUTTERIDGE, 2015). Se a resposta inflamatória aguda permitir a eliminação das bactérias, cessa o recrutamento do neutrófilos e a CCS retorna aos níveis saudáveis, normalmente abaixo de 200.000 células/mL de leite. Mas, se as bactérias sobrevivem a inflamação e a migração dos neutrófilos continuam causando danos ao parênquima mamário e reduzindo a produção de leite; é o caso das infecções por *S. aureus,* que são crônicas e subclínicas (RIOLLET; RAINARD; POUTREL, 2000).

2.5 Compostos secundários e nutrição animal

Em condições de pastejo, com adequada disponibilidade de forragem, os ruminantes exercem sua seletividade sobre plantas ou parte delas que podem beneficiá-los em termos de bem-estar e saúde (PROVENZA et al., 2003; PROVENZA; VILLALBA, 2010; VILLALBA et al., 2014). Esses autores sugerem que compostos secundários ativos de plantas podem afetar os sistemas digestivo e imunológico, podendo fazer parte de uma estratégia preventiva dos animais para resistir a doenças, tornando-se uma alternativa para a saúde de longo prazo, bem como estratégias econômicas, ecológicas e socialmente eficazes para produção sustentável.

As plantas produzem compostos do metabolismo primário: carboidratos, proteínas e lipídeos, nutrientes que servem de base para a alimentação animal. Mas também produzem compostos do metabolismo secundário, que podem ser classificados, de acordo com a via biossintética do ácido chiquímico e do mevalônico, em três grupos principais: terpenos, compostos fenólicos e alcaloides (PERES, 2015). Eles não são essenciais para a sobrevivência celular das plantas, mas desempenham um papel importante na defesa do organismo contra fatores bióticos e abióticos (PAGARE et al., 2015). Geralmente, compostos do metabolismo secundário não tem função nutricional para os mamíferos, mas nas plantas uma das principais tarefas é atuar contra infecções fúngicas e bacterianas que podem afetar seu desenvolvimento (PAGARE et al., 2015; PERES, 2015). Muitos desses compostos têm baseado estudos para uso como substituintes aos antimicrobianos sintéticos utilizados comercialmente. Uma vez ingeridos pelos animais, esses compostos são metabolicamente eliminados de diferentes maneiras via urina, fezes e/ou leite.

Estudos com vacas leiteiras mostram que elas podem ingerir plantas ricas em compostos secundários, como os terpenos (LEJONKLEV et al., 2013; POULOPOULOU et al., 2012; VIALLON et al., 2000), compostos fenólicos (OLAGARAY; BRADFORD, 2019; TEDESCO et al., 2004) e alcaloides (DE NIJS et al., 2017; HOOGENBOOM et al., 2011). Desde que eles podem ser transferidos do trato gastrointestinal para a glândula mamária e, portanto, para o leite (LEJONKLEV et al., 2013) é possível que eles possam interferir diretamente no crescimento bacteriano dentro da glândula mamária (TORNAMBÉ et al., 2008) e/ou ter atividade

sobre o sistema imunológico, uma vez que grande parte destes compostos, principalmente flavonoides, apresentam atividade antioxidante.

O período de transição é uma fase estressante para vacas leiteiras (DRACKLEY, 1999) e predispõe a muitas doenças, como por exemplo mastite, retenção de placenta, metrite, hipocalcemia, cetose, etc. Segundo SPEARS; WEISS, (2008), esse fato se deve ao surgimento do estresse oxidativo, enquanto as células do sistema imunológico são estimuladas a produzirem EROs, ao mesmo tempo são sensíveis à peroxidação, deste modo as defesas do organismo ficam comprometidas. Vitamina E, caroteno, selênio, cobre, cromo e zinco estão envolvidos no sistema antioxidante e sua deficiência causa depressão no sistema imunológico (SPEARS; WEISS, 2008). Forragens verdes são fontes ricas de compostos com ação antioxidante, por outro lado volumosos conservados são deficientes e uma estratégia para manter a saúde de vacas leiteiras confinadas é realizar a suplementação destes compostos na dieta, como aditivos. Os aditivos alimentares não possuem função nutricional, porém tem a capacidade de modular o metabolismo animal.

Espécies de solanáceas têm sido utilizadas na produção animal com o intuito de alcançar atividades imunomoduladoras. As raízes de *Solanum nigrum* utilizadas na alimentação de camarões, previamente desafiados com uma bactéria gram-negativa, demonstraram ter efeito imunoestimulante aumentando a resistência à doença. Este efeito foi devido ao aumento da explosão respiratória e da atividade da enzima superóxido dismutase (HARIKRISHNAN et al., 2011). Também na área da aquacultura, frações das folhas de *Solanum trilobatum* solúveis em água e hexano foram injetadas em tilápias, para avaliar mecanismos imunológicos. Houve significativa resistência contra a bactéria *Aeromonas hydrophila*, além de reforçar parâmetros imunológicos inespecíficos (DIVYAGNANESWARI; CHRISTYBAPITA; MICHAEL, 2007).

De acordo com CHOUBEY et al., (2015), a maior dose de um suplemento contendo *S. nigrum* na dieta de cabras aumentou a atividade antioxidante das enzimas glutationa peroxidase, glutationa transferase e catalase, além de reduzir o cortisol sanguíneo de maneira dose dependente. A espécie *Solanum glaucophyllum* é rica em vitamina D, composto que desempenha papel fundamental na homeostase do cálcio no sangue (ISHII et al., 2015). Segundo os mesmos autores, ao suplementar vacas com cerca de 36 g de folhas de *S. glaucophyllum,*

aproximadamente 3 dias antes do parto, esta espécie de solanácea demonstrou eficiência em reduzir os casos de hipocalcemia no pós-parto. Esse efeito foi devido à presença de vitamina D3 que quando suplementada em alta concentração manteve o nível sérico de cálcio após o parto.

2.6 Importância e caracterização das Solanáceas

Nos últimos anos, a influência dos radicais livres sobre o desenvolvimento de doenças tem despertado o interesse pela nutracêutica. Essa área combina a alimentação com a prevenção de doenças, os chamados alimentos funcionais que tem como foco melhorar o estado de saúde em humanos (PERES, 2015). Dentre eles, o aumento da popularidade do *Lycium barbarum* tem sido relacionado a atividades imunomoduladoras, através de atividades antioxidantes e anti-inflamatórias (WANG et al., 2017). Grande parte das plantas produzem compostos do metabolismo secundário, e muitos deles previnem doenças devido à atividade antioxidante que apresentam, como por exemplo os carotenóides e os flavonóides (PERES, 2015). As solanáceas são reconhecidas como uma família que inclui espécies de grande valor econômico, alimentício e terapêutico (BOHS, 2007), sendo que *Solanum melongena* é um dos vegetais com maior atividade antioxidante conhecida (HONG; LEE; KIM, 2018). As solanáceas são tradicionalmente usadas quanto as suas propriedades medicinais, alucinógenas e venenosas, devido à presença de alcaloides (KUTCHAN, 1995). No Quadro 1 a seguir, são distribuídas filogeneticamente algumas espécies de solanáceas de alto valor alimentício e/ou seus princípios ativos amplamente conhecidos e utilizados mundialmente.

Quadro 1. Filogenia da Família Solanaceae

Sub-Família	**Clado**	**Espécie**
Solanoideae		*Coffea arábica* (Café - cafeína)
	Ipomoeae	*Ipomoeae purpurea* *Ipomoeae batatas* (Batata-doce) *Ipomea cairica*
	Nicotineae	*Nicotiana tormetosiformis* *Nicotiana undulata* *Nicotiana tabacum* (Tabaco - nicotina) *Nicotiana sylvestris*
	Lycieae	*Grabowskia duplicata* *Lycium cestroides* *Lycium barbarum* (Goji berry)
	Hyoscyameae	***Atropa beladona*** (Atropina, beladonina e hiosciamina) *Hyoscyamus niger (*Escopolamina e hiosciamina)
	Datureae	*Datura stramonium (*Escopolamina)

Continuação: Filogenia da Família Solanaceae

Solanoideae	*Capsiceae* *Liciantes*	*Capsicum frutensces* (Pimenta - capsaicina) *Capsicum annuum* (Pimentão)
	Physaleae *Sub: Physalinae* *Iochrominae* *Withaninae*	*Physalis peruviana* *Physalis angulata* *Withania somnífera* *Iochroma loxense*
	Solaneae	*Solanum dulcamara* ***Solanum nigrum*** (Solanina e solamargina) *Solanum bulbocastanum* *Solanum tuberosum* (Batata – solanina e chaconina) *Solanum commersonii* *Solanum pennellii* *Solanum neorickii* *Solanum chilense* *Solanum peruvianum* *Solanum habrochaites* *Solanum galapagense* *Solanum pimpinellifolium* *Solanum lycopersicum* (Tomate - tomatidina) *Solanum melongena* (Beringela) *Solanum torvum* *Solanum wrightii*

Fonte: Adaptado de (AMIRYOUSEFI; HYVÖNEN; POCZAI, 2018; HIDAYAT et al., 2016; KUTCHAN, 1995; OLMSTEAD et al., 2008; PERES, 2015).

Muitos equívocos ocorrem quando se trata de solanáceas, certamente devido à semelhança de algumas espécies em relação aos frutos, pequenos e negros (Fig. 05). *Atropa belladona* apresenta alto potencial farmacológico devido seu principal composto, alcaloide atropina. *Solanum nigrum* foi uma denominação atribuída às solanáceas que possuíam frutos negros, portanto várias espécies que possuem os frutos negros ainda são erroneamente denominadas dessa forma. Para uma revisão aprofundada sobre *Black Nightshades* do Velho Mundo visitar SÄRKINEN et al., (2018). De acordo com estes autores, estas espécies são endêmicas da Europa e Ásia, porém devido à semelhança botânica podem ser facilmente confundidas com espécies encontradas no continente americano.

Figura 5. Frutos negros das espécies: *Atropa belladona* (A) e *Solanum nigrum* (B).
Fonte: Jardim Botânico da Universidade de Trás-os-Montes e Alto Douro.
Acesso: https://jb.utad.pt/ em 10/09/2020.

Dentro da família das solanáceas, o gênero *Solanum* é o que mais tem representantes e se distribui amplamente no Rio Grande do Sul com mais de 60 espécies nativas, divididas em três subgêneros: *Solanum*, *Leptostemonum* e *Bassovia* (que se divide em duas seções) de acordo com o Quadro 2 a seguir.

Quadro 2. Filogenia do gênero *Solanum* no Rio Grande do Sul

Sub-Gênero	Seção	Espécie
Solanum		*S. adscendens* *S. alatirameum* ***S. americanum*** *S. amygdalifolium* *S. arenarium* *S. campaniforme* *S. cassioides* *S. chacoense* *S. chenopodioides* *S. commersonii* *S. compressum* *S. concinnum* *S. delicatulum* *S. flaccidum* *S. gemellum* *S. granulosoleprosum* *S. hirtellum* *S. inodorum* *S. laxum* *S. mauritianum* *S. megalochiton* *S. nigrescens* *S. odoriferum*

Continuação: Filogenia do gênero *Solanum* no RS

		S. pabstii *S. paranense* *S. pseudocapsicum* *S. pseudoquina* *S. ramulosum* *S. reitzii* *S. sanctae-catharinae* *S. sarachoides* *S. subsylvestris* *S. trachytrichium* *S. viscosissimum*
Leptostemonum		*S. aculeatissimum* *S. affine* *S. aparadense* *S. atropurpureum* *S. bistellatum* *S. capsicoides* *S. guaraniticum* *S. hasslerianum* *S. palinacanthum* *S. paniculatum* *S. platense* *S. reflexum* *S. reineckii* *S. sisymbriifolium* *S. vaillantii* *S. variabile* *S. viarum* *S. wacketii*
Bassovia	*Cyphomandropsis* (espécies provindas de *Solanum*)	*S. fusiforme* *S. glaucophyllum* *S. iraniense* *S. johannae* *S. pelagicum*
	Pachyphylla (espécies provindas de *Cyphomandra,* 1995*)*	***S. corymbiflorum*** *S. diploconos* *S. sciadostylis*

Fonte: Adaptado de (BOHS, 1995; MENTZ et al., 2007; SOARES; MENTZ, 2006).

2.6.1 Solanum corymbiflorum

Solanum corymbiflorum (Sendtn.) Bohs (syn. *Cyphomandra corymbiflora)*, popularmente conhecida como “baga-de-veado, cuião-de-veado”, é uma espécie arbustiva e ocorre na floresta da metade norte do estado do Rio Grande do Sul, sendo observada em Floresta Ombrófila Densa, Floresta Ombrófila Mista e Floresta Estacional Decidual da região do Alto Uruguai (Fig. 06). Ela se distribui pelo bioma Mata Atlântica, relatada nos estados de Rio Grande do Sul, Santa Catarina e

Paraná, geralmente cresce em clareiras de florestas primárias ou locais de vegetação secundária, como margem de estradas, trilhas e pastagens (BOHS, 1989). Faz parte de diversas formações vegetais, tanto campestres quanto florestais, sendo comumente encontrada na borda de florestas e locais alterados, como margens de estradas (SOARES; MENTZ, 2006).

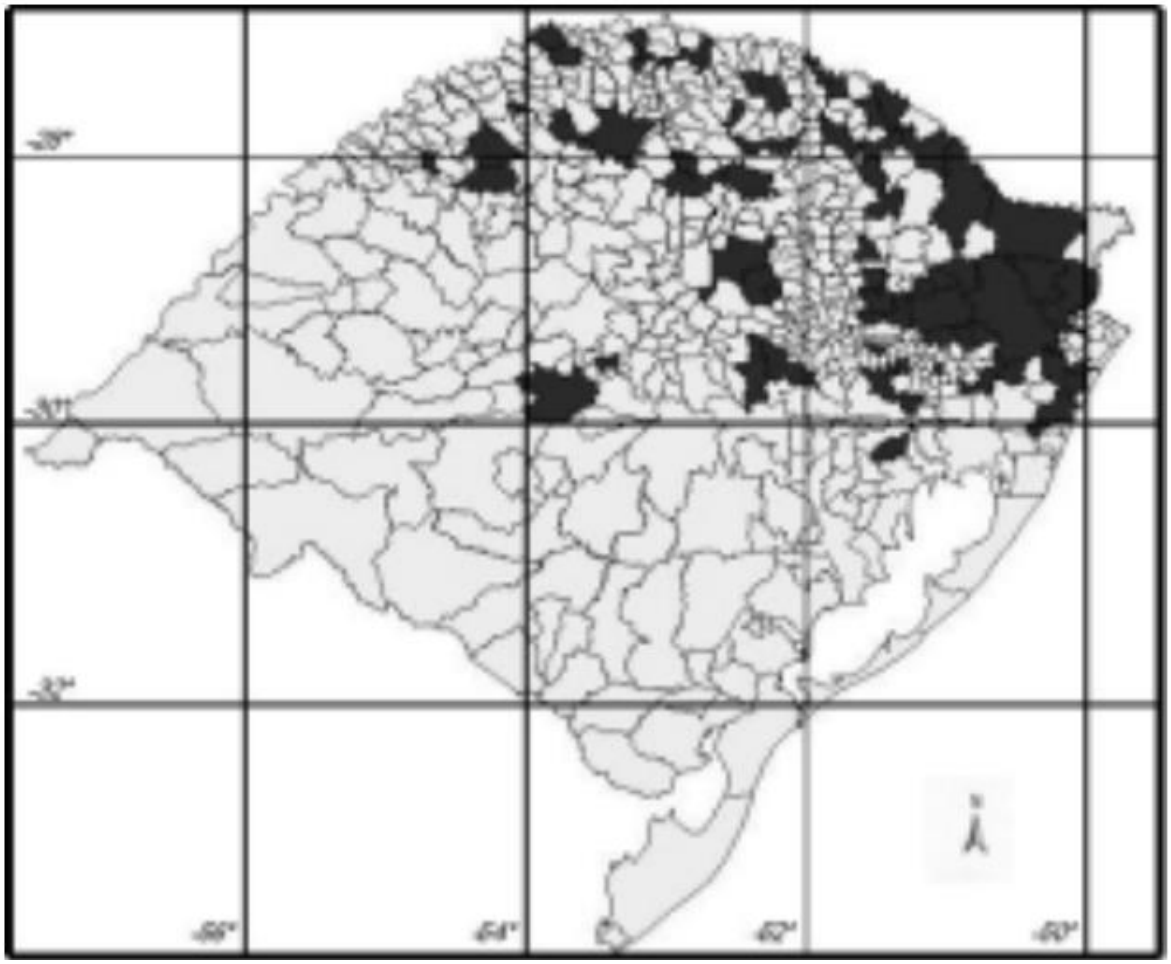


Figura 6. Mapa de ocorrência de *Solanum corymbiflorum* no Rio Grande do Sul
Fonte: (SOARES; MENTZ, 2006).

Ela se caracteriza como arbustos ou arvoretas de 0,8 a 1,5 m de altura, são inermes (sem acúleos ou espinhos). As folhas são membranáceas com a superfície recoberta por tricomas (SOARES; MENTZ, 2006). A floração e frutificação ocorrem duas vezes ao ano, geralmente é possível encontrar flores em maio e novembro (Fig. 7).

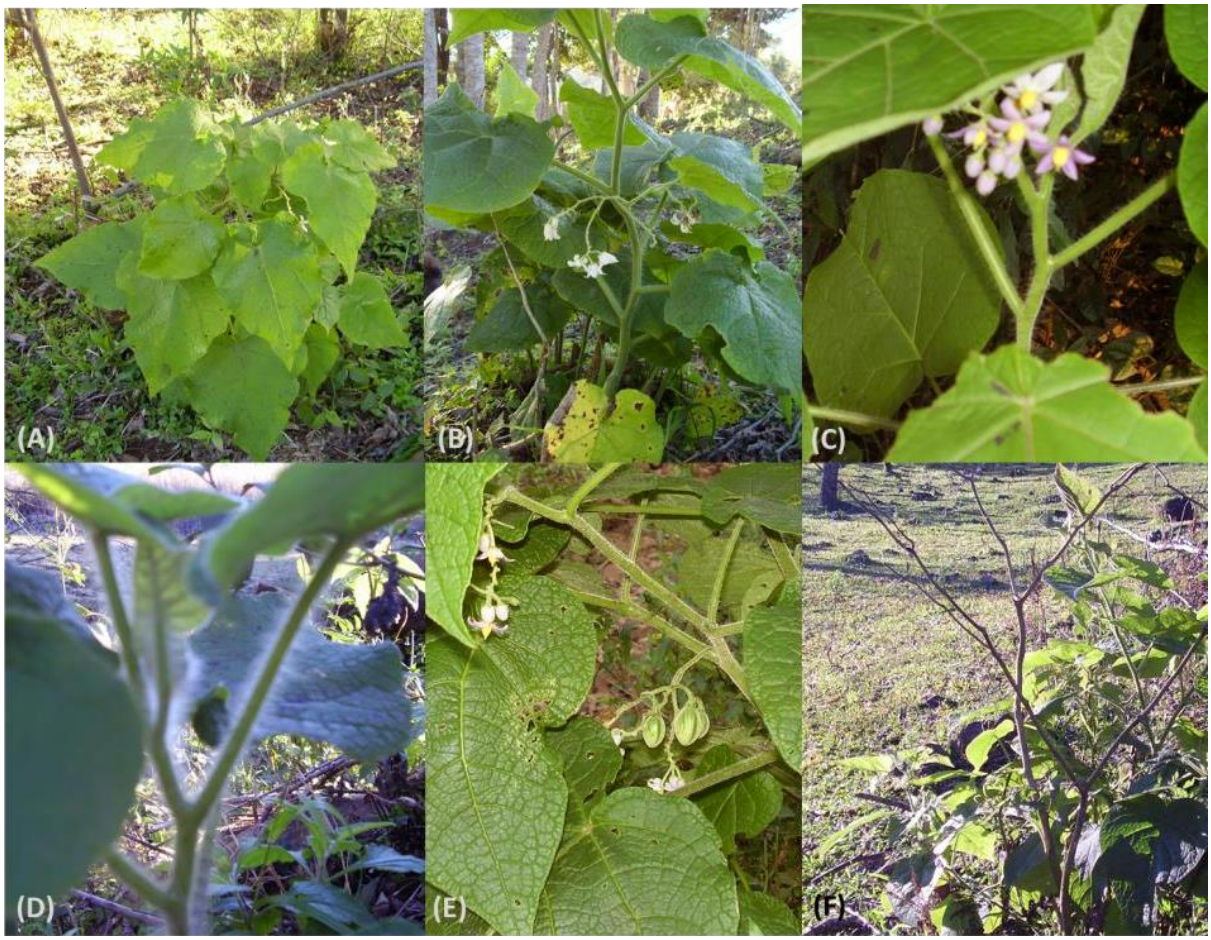


Figura 7. *Solanum corymbiflorum* localizada na região no noroeste do RS.
(A) Estrutura da planta (B) Flores de cor branca (C) Flores de cor lilás (D) Tricomas em planta jovem (E) Frutificação (F) Senescência e brotação de novos galhos.
Fonte: Panazzolo, 2020.

Segundo Keller (2003), a seleção das plantas que possuíam ou não propriedades medicinais era determinada por atributos como forma, cor ou características marcantes, esse método era chamado de “Doutrina da assinatura ou semelhança”. Atualmente, cerca de 40% das plantas medicinais utilizadas por povos tradicionais (comunidades indígenas) seguem esta doutrina. A planta *S. corymbiflorum* tem seu uso associado ao tratamento de inflamações e seu nome ‘hierba del sapo’ está relacionado à capacidade dos sapos de inflarem quando expostos ao perigo (KELLER, 2003). Segundo Keller and Prance (2012), esta espécie se estende pela Argentina, Paraguai e Brasil usada por diversas tribos para muitas finalidades conforme o Quadro 3, onde se observa que um dos propósitos de uso é o tratamento da mastite por membros da comunidade indígena Guarani (KELLER; PRANCE, 2012). De acordo com Keller (2003) colônias de imigrantes brasileiros vivem no entorno dessa comunidade e se dedicam à agricultura primária.

Quadro 3. *Solanum corymbiflorum* de acordo com seu nome e uso tradicional

Idioma/Dialeto	**Nomes**	**Aplicações**
Português	Baga de bugre (baya de indio) Baga de veado (baya de venado) Bordão de veado (corazón de venado)	Alcoolismo Sarna Picadas de carrapato Furúnculos Dor de cabeça **Mastite** Taquicardia Lumbago Otite Retenção de líquidos
Espanhol	Tomate de monte Pata de cuervo	
TupíGuaraní	Oriva (frutal frondosa)	
Guaraní Ava Chiripa	Manduchu	
Guaraní Mbya	Yryvuka'a (hierba Del jote) Kururuka'a (hierba del sapo)	

Fonte: Adaptado de KELLER e PRANCE (2012).

Devido à proximidade geográfica e à distribuição botânica no bioma adjacente à região nordeste do Rio Grande do Sul, a prática foi adotada pelos agricultores locais que, usando princípios de etnoveterinária, fornecem a planta na alimentação de vacas leiteiras acometidas com sintomas clínicos de mastite: "*Toda planta é colhida, seca na sombra e fornecida por 4 ou 5 dias na dieta de vacas que apresentam sinais clínicos de mastite. Aproximadamente 50 gramas de matéria seca por dia"* (*comunicação pessoal*). Estudo realizado recentemente por Piana et al. (2016), comprova a eficiência do extrato das folhas como potente anti-inflamatório tópico, sendo capaz de inibir o edema causado por uma irritação na pele, através da redução da atividade da mieloperoxidase da mesma forma que o controle dexametasona.

2.6.2 Solanum americanum

Solanum americanum Mill. está distribuída globalmente nas áreas de trópicos e subtrópicos; provavelmente nativo do continente americano, mas com poucas evidências sobre sua origem (SÄRKINEN et al., 2018). É muito comum nas Américas Central, do Norte e do Sul, coloniza solos perturbados e é encontrada em áreas abertas, ao longo de estradas, clareiras e nas costas de praias, desde o nível do mar até 2.000 metros acima dele (KNAPP et al., 2019; MENTZ et al., 2007). No Brasil, é conhecida como "maria-preta, maria-pretinha" (SILVA et al., 2017). Em muitos lugares, é considerado sinônimo de *Solanum nigrum* Linn, que é nativa da Eurásia. Segundo SÄRKINEN et al., (2018), análises moleculares mostram que é possível diferenciar estas espécies através de contagem de cromossomos, mas sua

identificação visual é relativamente difícil (Fig. 8 e 9). *S. nigrum* é uma espécie autoaloplóide e pode ser derivada de um cruzamento entre *S. villosum* (tetraplóide) e *S. americanum* (diplóide). Outra característica que estes autores citam para distinguir *S. nigrum* de *S. americanum* é o tamanho sementes e o número destas por baga.

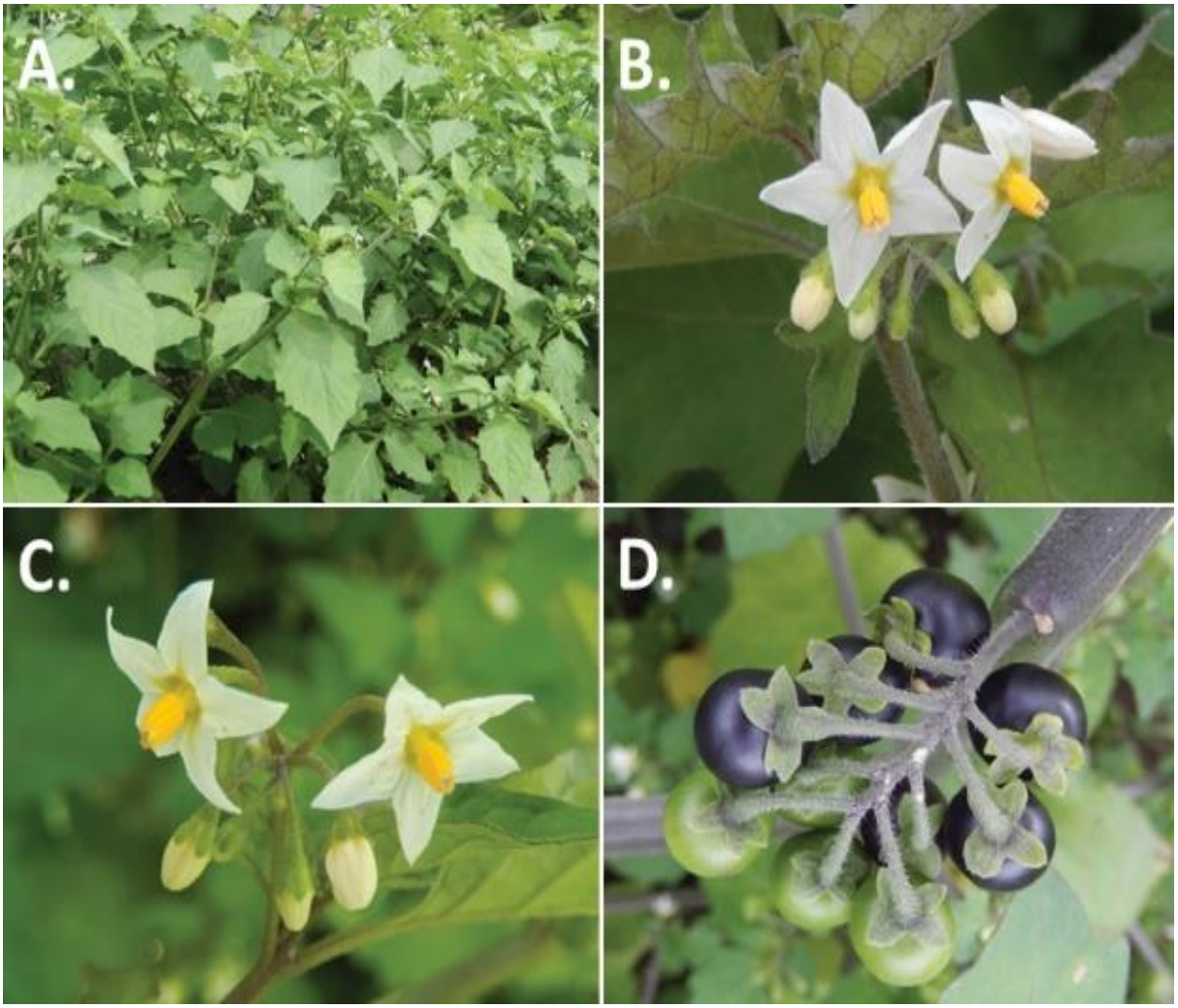


Figura 8. *Solanum nigrum* endêmica da Eurásia.
Quanto ao seu (A) hábito de crescimento, (B) inflorescência (indumento denso) (C) inflorescência (indumento esparso) (D) frutos pretos totalmente maduros, lóbulos de cálice permanecendo deprimidos ou ligeiramente espalhados.
Fonte: Adaptado de (KNAPP et al., 2019).

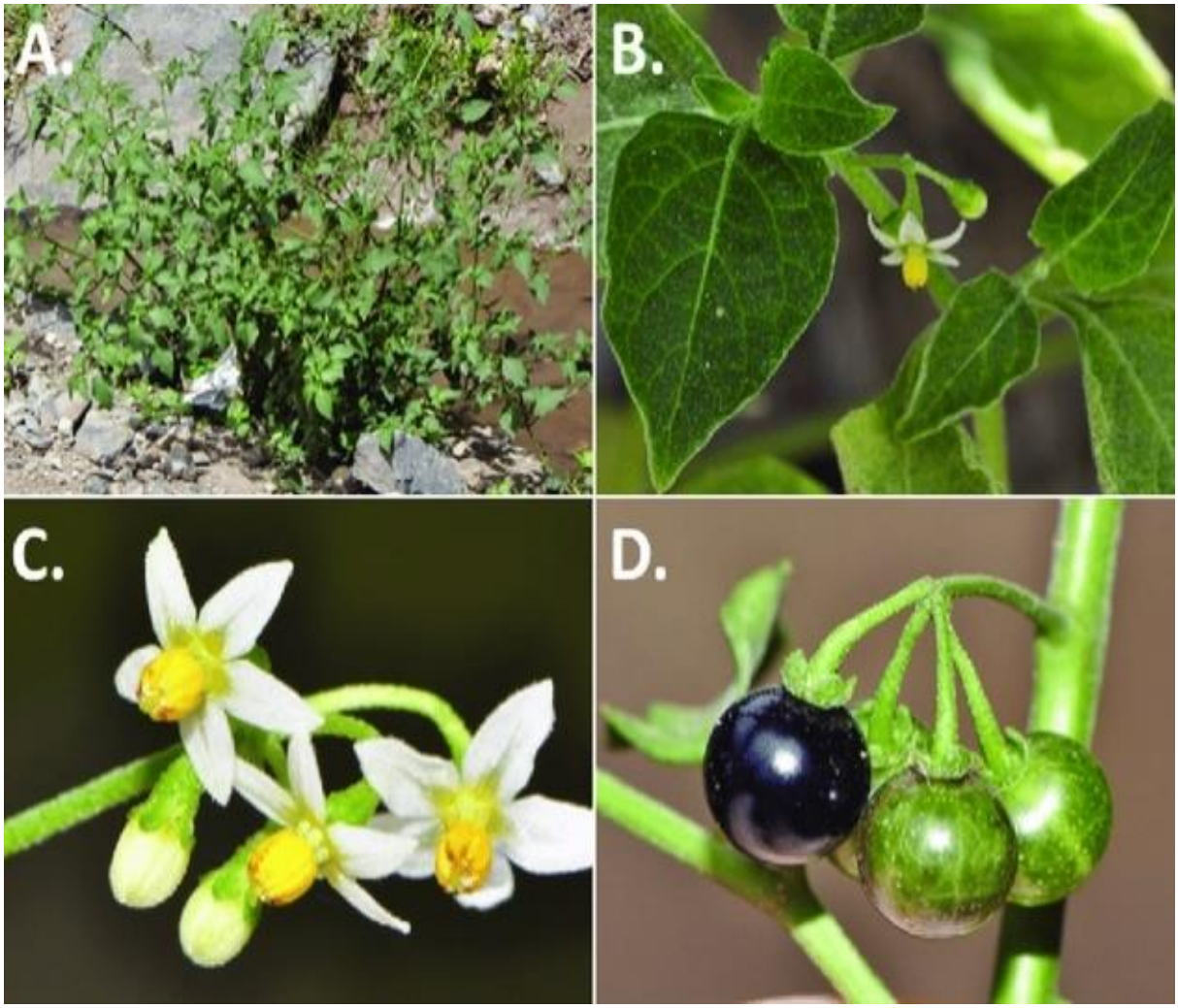


Figura 9. *Solanum americanum* distribuída em todo continente americano.
Quanto ao seu (A) hábito de crescimento, (B) folhas e inflorescências jovens (C) botões e flores (D) frutos pretos maduros e brilhantes com lóbulos de cálice refletidos.
Fonte: Adaptado de (KNAPP et al., 2019).

Solanum americanum é naturalmente distribuída na vegetação secundária do Rio Grande do Sul (MENTZ et al., 2007) e considerada uma erva daninha invasora de lavouras, principalmente na horticultura (BIANCO; CARVALHO; BIANCO, 2010). Está presente dentro das propriedades leiteiras fazendo parte da vegetação nos piquetes de pastejo dos animais (Fig. 10). Dessa forma, *S. americanum* é uma espécie que cresce espontaneamente em pastagens sendo consumida por vacas leiteiras em pastejo (*comunicação pessoal*). Enquanto *S. corymbiflorum* é suplementada na dieta de animais que apresentam sintomas clínicos de mastite, como inchaço, vermelhidão e alterações físico-químicas do leite (*comunicação pessoal*).

Figura 10. *Solanum americanum* localizada na região sul do RS.
(A) Estrutura da planta com frutos maduros (B) Flores brancas – em beira de cercas e estradas (C) Dentro dos piquetes de vacas leiteiras (D) Planta consumida durante pastejo.
Fonte: Panazzolo, 2020.

3. Hipótese e objetivos

Os compostos presentes nas folhas das espécies *S. americanum* e *S. corymbiflorum* apresentam ação antibacteriana e antioxidante.

Objetivo geral:

Verificar se os extratos metanólico, alcaloídico e aquoso de folhas das espécies *Solanum corymbiflorum* e *Solanum americanum* apresentam atividade antimicrobiana e antioxidante.

Objetivos específicos:

1. Verificar a capacidade dos extratos em inibir o crescimento de *S. aureus* e *E. coli*;
2. Avaliar a capacidade dos extratos em inibir a formação do biofilme de *S. aureus* e *E. coli*;
3. Avaliar a atividade antioxidante dos extratos.
4. Realizar a caracterização fitoquímica dos extratos através do ensaio bio-guiado.

CAPÍTULO II

Ethno-veterinary use of Solanaceae species as a tool to reduce a virulence factor in dairy cow mastitis[4]

[4] Este capítulo é apresentado de acordo com as normas de publicação do ***Journal of Ethnopharmacology*.**
Fator de impacto: 3.115
Fator de impacto (5 anos): 3.671
Website: https://www.journals.elsevier.com/journal-of-ethnopharmacology

Ethno-veterinary use of Solanaceae species as a tool to reduce a virulence factor in dairy cow mastitis

Dejani Maíra Panazzolo[a], Vivian Fischer[a*], José Ângelo Zuanazzi[b], Elen de Oliveira Alves[b], Alexandre José Macedo[c], Rodrigo Campos[c], Flávia Brust[c], Sidnei Moura[d]

[a] *Animal Science Research Program, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

[b] *Laboratory of Pharmacognosy, Department of Raw Material Production, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

[c] *Biotechnology Center, Universidade Federal of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

[d] *Laboratory of Biotechnology of Natural and Synthetics Products, University of Caxias do Sul, Rio Grande do Sul, Brazil*

∗ Corresponding author at: Faculty of Agronomy, Federal University of Rio Grande do Sul. Av. Bento Gonçalves, 7712, 91540-000, Porto Alegre, Brazil. Tel.: +55 51 999835081; fax: +55 51 33086048.

E-mail address: vivinha.fischer@hotmail.com (V. Fischer)

ABSTRACT

Ethnopharmacological relevance: Solanum corymbiflorum (Sendtn.) Bohs is naturally distributed in the range of native vegetation in the southern states of Brazil and Argentina, popularly known as “baga-de-veado”. As an ethnoveterinary practice by local’s native tribes, the dried plant is mixed into the diet offered to dairy cows showing clinical mastitis. *Solanum americanum Mill.* is known as “maria-pretinha” and naturally occurs in the boundaries of paddocks being grazed by dairy cows in the south of Brazil.

Aim of the study: Perform a bio-guided search of antibacterial compounds in the leaves of *Solanum corymbiflorum* (SC) and *Solanum americanum* (SA), evaluating the bacterial growth and biofilm formation of *Staphylococcus aureus* and *Escherichia coli*, and perform chemical characterization of the promising extract.

Materials and methods: Three forms of extraction were performed on each plant’s leaves: aqueous (AQU), alkaloid (ALK), and methanolic (MET), that were further evaluated at two initial concentrations (10 and 250 *μ*g/mL). The biological tests on the bacteria planktonic growth and adhesion ability of bacteria to forming biofilm in extracts presence were evaluated by broth microdilution and violet crystal staining methods, respectively. The absorbance data (ABS), measured by optical density, were subjected to analysis of variance (ANOVA) and Dunnett’s test was used as a means comparison test, adopting a 5% significance level. The chemical characterization was determined by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and high-resolution spectrometry mass (HRMS).

Results: AQU extract of SA stimulated the planktonic growth of *S. aureus* and *E. coli.* MET extract of SC stimulated biofilm formation for *S. aureus* but reduced this ability for *E. coli.* ALK extracts of both species were able to in inhibit the biofilm formation for *S. aureus*. Based on the correspondence data on exact mass, isotopic ratio, fragmentation pathway, and UV spectrum, it is possible to suggest the presence of the *N-trans*-feruloyltyramine (m/z 314.1382) in ALK extract of SA, an alkaloid already reported in Solanum species.

Conclusion: The ALK extracts of the leaves of *Solanum corymbiflorum* and *Solanum americanum* inhibited biofilm formation of *S. aureus*. This effect can be considered beneficial as it can reduce the main virulence factor that affects milk production in

cases of mastitis and may explain its use following the traditional associated knowledge in dairy cows.

Keywords: alkaloids, antibacterial, antibiofilm, *Solanum corymbiflorum*, *Solanum americanum,* Traditional Associated Knowledge

1. Introduction

Solanum corymbiflorum (Sendtn.) Bohs (syn. *Cyphomandra corymbiflora)*, popularly known as “baga-de-veado, cuião-de-veado, cordão-de-veado” is naturally distributed in the southern states of Brazil and border areas with Argentina (Bohs, 1989; Keller and Prance, 2012; Mentz et al., 2007), growing in clearings of primary forests, places of secondary vegetation or countryside formations, on forest edges and altered locations such as margins of roadsides, trails, and pastures (Bohs, 1989; Soares and Mentz, 2006). Local communities of indigenous tribes in the Misiones Province of Argentine use SC in the mastitis treatment (Keller and Prance, 2012). The use of medicinal plants in the associated traditional knowledge is a powerful tool to search for active compounds in plants (Mubarack et al., 2011). This practice has been adopted by local smallholders’ dairy farms that use traditional knowledge to treat their herds. The plant is harvested, dried in the shade, and further mixed with food and fed for 4 to 5 days to cows showing clinical signs of mastitis (*personal communication*).

Solanum americanum Mill. is globally distributed in the tropics and subtropics areas; possibly native in the American continent, but with little evidence about its origin (Särkinen et al., 2018). It is very common in Central, North and South America, colonizes disturbed soil and it is found in open areas, along with roadsides, tree openings and on the back of beaches, from the sea level to 2,000 m above sea level (KNAPP et al., 2019; MENTZ et al., 2007). In southern Brazil, it is known as “maria-preta, maria-pretinha” widely distributed in the Rio Grande do Sul state and generally considered a weed (Mentz et al., 2007; Silva et al., 2017), occurring in paddocks where it is consumed by grazing dairy cows (*personal communication*).

A previous study with SC found anti-edematogenic activity, corroborating the traditional use of skin diseases. They attributed this effect to the presence of phytochemicals such as polyphenols, flavonoids and alkaloids present in this

species. It is worth to notice the high content of phenolic compounds, especially the acids chlorogenic, caffeic, rosmarinic, gallic and rutin (Piana et al., 2016). Moreover, some species of Solanaceae are known to be rich sources of alkaloids, compounds with a wide variety of biological activities. In studies with SA, these phytochemical classes were also reported, especially the phenolic acids, flavonoids and alkaloids (Silva et al., 2017; Vagula et al., 2018).

Mastitis is an inflammation of the mammary gland caused by microbial infection and it is characterized by physical, chemical, and bacteriological changes in milk and by pathologic changes in the glandular tissue (Zhao and Lacasse, 2008). Among the numerous bacteria that cause mastitis, only a few species are prevalent and constitute a real issue (Rainard et al., 2018). One of the most important microorganisms causing mastitis is *Staphylococcus aureus* that accounts for about one-third of the cases of severe or subclinical mastitis (Li et al., 2017). Among the reasons for the failure of treatment in *S. aureus*-associated mastitis is the abscess formation surrounded by thick fibrous capsules in the mammary gland, that can prevent the adequate flow and accumulation of antibiotic on-target site, and thus, bacterial destroying is interrupted (Raza et al., 2013; Saglam et al., 2017). The failure of the immune system to eliminate the pathogen because of the biofilm can damage the alveolar tissue, increasing the somatic cell count (SCC) present in the milk, extending the duration of the infection and healing failure (Sharma and Jeong, 2013).

Antibiotics have been widely used in dairy production to improve performance and control diseases caused by gram-positive and gram-negative bacteria, especially during the dry-off period to prevent mastitis (Garciaa et al., 2019). These practices have increased the risk of residues in milk favoring bacterial resistance to antimicrobials (Oliver and Murinda, 2012). The resistance of *S. aureus* recovered in cases of clinical and subclinical mastitis against ampicillin, enrofloxacin, erythromycin, penicillin, pirlimycin, and tetracycline have been reported, including having an isolate that presented resistance to multiple drugs (Oliveira et al., 2012). Multiple drug resistance affects treatment success (Phophi et al., 2019) and may become a public health problem. On the other hand, *E. coli* is an important agent causing clinical mastitis, especially in an acute form, a common and usually fatal disease in lactating dairy cows due to endotoxemia and disseminated intravascular coagulation (Guerra et al., 2019; Herry et al., 2017).

The ethnoveterinary practice may be an effective approach for tackling problems like mastitis, and prospection of plants used by the traditional knowledge may identify those more promising species, leading to the identification of active fractions or molecules with antimicrobial properties (Doss et al., 2012; Kalayou et al., 2012). Usually, traditional knowledge uses the dried plant material directly, administering it into the animal's feed (Mubarack et al., 2011). Plant natural products added to the animal diet are called phytogenic additives and may enhance health status of farm animals (Lillehoj et al., 2018). But, the effects of SC and SA, when ingested by ruminants, are still unknown. Therefore, the aim of this study was to conduct a bio-guided assay testing the aqueous, methanolic and alkaloid extracts of *Solanum corymbiflorum* and *Solanum americanum* on bacteria planktonic growth and biofilm formation of *S. aureus* and *E. coli.* This study also aimed to characterize the compounds in the extract with the most promising results.

2. Material and methods

2.1 Plant material: source, sampling, and identification

The leaves of SC and SA were harvested at the edge of secondary vegetation and in dairy cows' paddocks located in the northwest of Rio Grande do Sul (27°47'12" S, 53°00'57" W), Brazil, between October and November 2017. The specimens were identified and deposited as exsiccates in the Herbarium of the Institute of Natural Sciences (ICN – UFRGS) under the registration number: *Solanum americanum* Mill: ICN 195408 and *Solanum corymbiflorum* Bohs: ICN 195409. The authors registered the species according to the new Brazilian legislation about the access to the biodiversity - Law 13.123/15 and Decree 8772/16 (Silva and Oliveira, 2018) in the National System of Genetic Heritage and Associated Traditional Knowledge under registration number A382EE. Approximately 2 kg of fresh leaves were dried at room temperature (± 25 °C) in a ventilated place, ground in particle size of 2 mm and stored in paper bags on Pharmacognosy's laboratory of the UFRGS.

2.2 Extraction methods and chemical characterization

Three extraction methods were used for each specimen: SC and SA following the AQU, MET and ALK extraction methods used in the Pharmacognosy's laboratory. The MET and ALK extracts were subjected to a TLC analysis to search alkaloids. Then, the ALK extract was subjected to liquid chromatography and mass spectrometry.

Aqueous extraction (AQU): A ratio of 2.85 g of ground leaves for every 100 mL of distilled water was used (0.2:10 w/v). The sample was extracted with warm water (40°C) for 30 min until it reached room temperature. The solution was filtered using a filter paper, frozen, lyophilized (Savant Micro Modulyo, United States) to obtain the AQU extract, and stored at -20°C until use (Li et al., 2008).

Methanolic extraction (MET): Methanolic extraction followed the method described by Scopel et al. (2010). Briefly, extractive solutions were made using a ratio of 1 g of ground leaves mixed into 20 ml of methanol (1:20 w/v). Extraction proceeded twice with turbolysis (Ultra Turrax Marconi – speed 3 for 3 min equivalent to 9000 RPM), ultrasound (Ultrasound bath cleaner at 132 w power – for 10 minutes), and maceration overnight (Table 1). Total methanolic extract (100 ml) was filtered using filter paper. Methanol was evaporated from the sample using a rotary evaporator (Buchi rotavapor V-710, Switzerland) at 40°C under a maximal pressure of 340 mbars. The dried extract was resuspended in ethyl ether and washed with Mili-Q® water to remove chlorophyll, and frozen (-20°C) for later lyophilization (Savant Micro Modulyo, United States).

Table 1. Process of methanolic extraction

Quantity and process	Running time	Total of methanol
2 × 20 mL => Turbolysis	3 min	40 mL
2 × 20 mL => Ultrasound	10 min	40 mL
1 × 20 mL => Maceration	24 h (overnight)	20 mL

Alkaloid extraction (ALK): The extraction of total alkaloids was performed according to Reis et al. (2019), mixing 10 g of the ground dried leaves and 100 ml of 10% HCl (v/v) on an ultrasound water bath at 50°C (twice for 15 min). The samples were gravity filtered and the supernatant washed with dichloromethane (3 × 100 mL). The aqueous fraction was alkalinized with 25% ammonium hydroxide (v/v) until pH 9. This alkaline aqueous solution was partitioned with dichloromethane (3 × 100 mL). The organic residue was filtered over anhydrous sodium sulfate (to remove the aqueous residue) and the solvent was evaporated under reduced pressure to recover the alkaloid rich residue.

Thin layer chromatography analysis (TLC): The extracts of SC were subjected to TLC in the silica gel plate ($60GF_{254}$). MET extract used a solution of ethyl acetate – methanol – H_2O (100: 13.5: 10 v / v) and ALK extract used dichloromethane: methanol (9.8: 0.2 v / v) as the eluent system. The samples were evaluated at 254 and 365 nm (Hildebert Wagner and Bladt, 1996).

High-performance liquid chromatographic analysis (HPLC): The ALK extracts were resuspended in methanol 2 mL, filtered through a 0.45 µm membrane (Millipore®, Bedford, UK), and injected on a Waters Alliance e2695 with a diode arrangement detector (PDA Waters 2998). Collecting 20 spectra per second over a range of 210 to 400 nm, and using the software Empower 3 HPLC (Waters®) for data acquisition. The chromatographic column employed was the reverse phase C18 (Luna Phenomenex® 100A, 5µm, 250 × 4.60 mm) coupled to a reversed-phase pre-column (Security Guard Cartridges™ Fusion Phenomenex®; 4.0 × 3.0 mm) on a linear elution gradient. For the development of an adequate chromatographic system, several conditions of sample preparation and composition of the mobile phase were tested. The gradual elution system used was composed of water: trifluoroacetic acid (TFA) 0.01% (mobile phase A, v/v), and acetonitrile: TFA 0.08% (mobile phase B, v/v); the flow rate used was 0.7 mL min^{-1} and 45 min of analysis. The gradient started with solvent A at 95% (0–7 min), 75% (2 min); 65% (10 min), 63% (6 min); 40% (10 min); 10% (4 min) and returned to A at 95% kept constant for the next minute to allow the spine to balance before the next run.

High-resolution electrospray ionization mass spectrometry (HRMS-ESI): The ALK extracts were resuspended in methanol for analysis in positive and negative electrospray ionization (ESI) modes, according to Rosales et al. (2019). The individual solutions were infused directly into the ESI source via a syringe pump (Harvard Apparatus, Hamilton, Reno, Nevada), at a flow rate of 180 µL min^{-1}. The ESI mass spectrometry (ESI(+)-MS) and tandem ESI(+)-MS/MS were acquired using a hybrid high-resolution and high-accuracy micrOTOF-Q mass spectrometer (Bruker Scientific®, Billerica, USA) under the following conditions: capillary and cone voltages were set to +3500 and +40 V, respectively, with a desolvation temperature of 200 °C. The collision-induced dissociations energy (CID) for the ESI (+) MS-MS was optimized in automatic mode for each component. Diagnostic ions in different fractions were identified by the comparison of their ESI (+)-MS/MS dissociation patterns with those of compounds identified in previous studies. For data acquisition

and processing, time-of-flight (TOF) control and data analysis software (Bruker Scientific®) were used. The data were collected in the 100–1000 *m/z* range, at the speed of two scans per second, providing 50.000 FWHM resolution, at 200 *m/z*. For exceptionally reliable identification of the target compound by ESI–Q–TOF, the accepted accuracy threshold for confirmation of elemental compositions was established as ≤ 5 ppm (Brenton and Godfrey, 2010).

2.3 Biological assays

Planktonic bacterial growth assay: The AQU, MET and ALK extracts of SC and SA were diluted in 5% of dimethyl sulphoxide (DMSO). The AQU extract of SC did not dilute in DMSO; therefore, no data were obtained for this treatment. The strains of *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC25904 – a strong biofilm-producing) were cultured in Mueller Hinton agar plates and incubated at 37°C overnight. Planktonic susceptibility test was performed by broth microdilution assay using a 96-well polystyrene flat-bottom microtiter plate (COSTAR 3599; Corning, NY, USA). Each well had a volume of 80 *μ*L of bacterial suspensions (1.0 McFarland) + 40 *μ*L of tryptone soy broth (TSB) (Oxoid Ltd., England) + 80 *μ*L of extracts (10 or 250 *μ*g/mL) or 80 *μ*L of Milli-Q® water for positive control. The results of planktonic susceptibility were measured as absorbance data by the optical density (OD) with reading at 600 nm (Epock – Biotek Spectrophotometer) and shown as the difference at the beginning (time 0) and at the end of the incubation (24 hours) (Antunes et al., 2010).

Biofilm formation assay: Following the incubation period (37°C for 24 h) the content of the wells was removed and washed three times with sterile saline. The remaining attached bacteria were heat-fixed at 60 °C for 1 h. The planktonic bacterial cells adherent in a layer biofilm formed was stained with 0.4% crystal violet for 15 min at room temperature. The stain bound to the cells was solubilized with 99.5% ethanol (Sigma–Aldrich Co., USA) and absorbance was measured at 570 nm (Spectramax M2e Multimode Microplate Reader, Molecular Devices, USA). The assays were performed in triplicate for each isolate and repeated independently three times to minimize the variability in absorbance measurements. The positive control was considered to represent 100% of bacterial adhesion for biofilm formation. Values

lesser than 100% represent inhibition of biofilm formation in comparison to the control (Antunes et al., 2010; Trentin et al., 2011).

2.4 Statistical analysis

Absorbance (ABS) data for the studied variables: bacterial planktonic growth and biofilm formation were tested for normality (Shapiro-Wilk test), homogeneity of variances (Levene's test) and subsequently subjected to analysis of variance (ANOVA) according to a completely randomized design (CRD) with 11 treatments (Fig. 1) performed in triplicate (average of three wells) with three replications. Variables that did not follow the normal distribution underwent a logarithmic transformation. Statistical model: $\gamma_{ij} = \mu + \tau_j + \varepsilon_{ij}$, where μ is a common parameter for all treatments known as the global mean and τ_j is a characteristic parameter of the treatment known as the treatment effect. ε_{ij} is a random error component that incorporates all other sources of experiment variability such as the difference between experimental units (test material/equipment). Dunnett's test was used as the means comparison test of all treatments with the positive control. Differences were adopted when $P \leq 0.05$, all analyzes were performed by SAS 9.4 procedures.

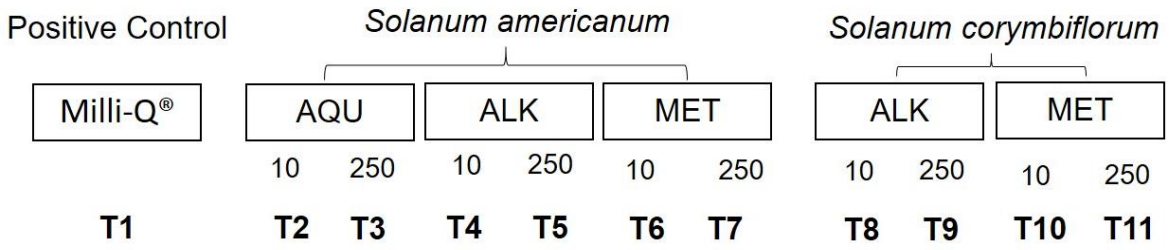


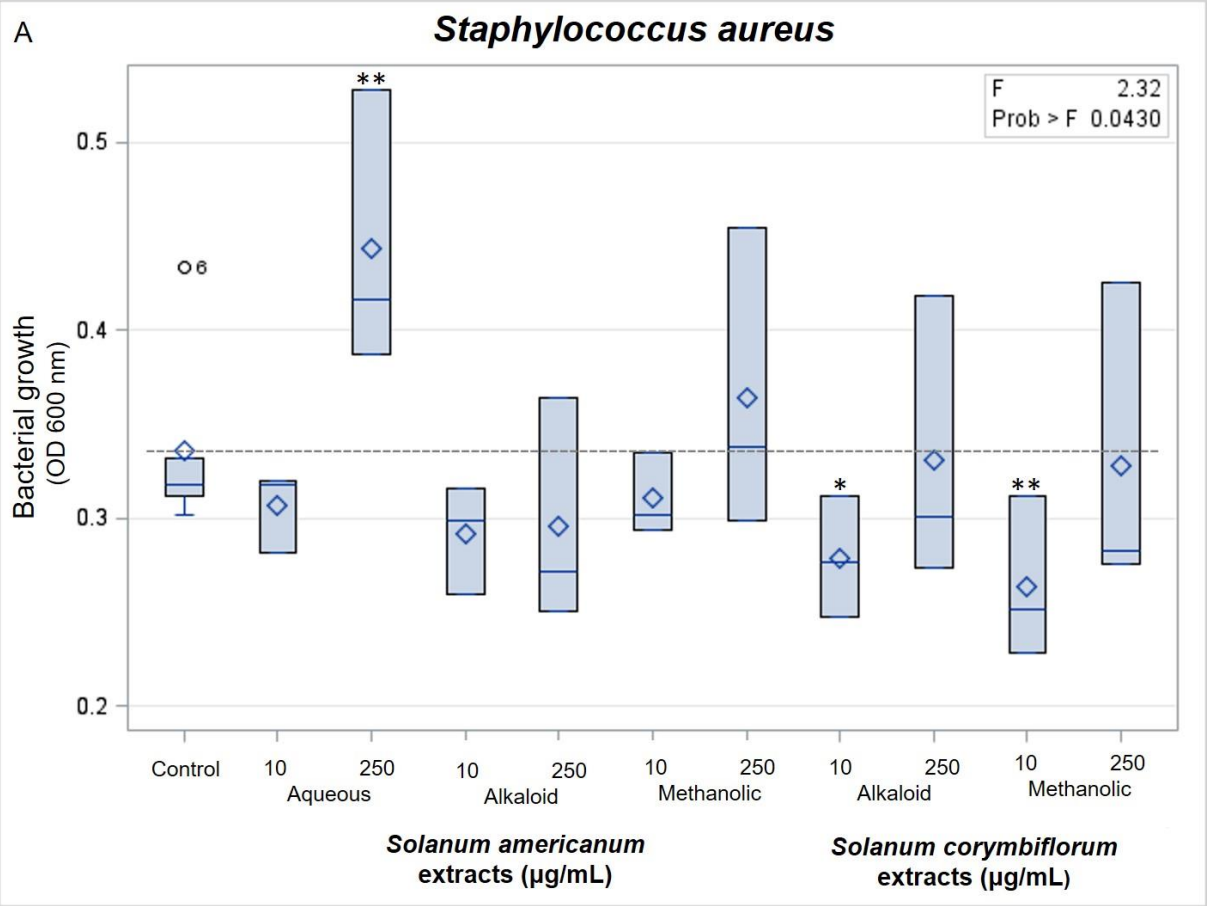
Figure 1. Treatments description subjected to statistical analysis. The concentrations used for each extract were 10 or 250 µg/mL.

3. Results

3.1 *Effect of extracts on bacterial growth*

Significant differences were found for bacterial planktonic growth (Table 01 in Supplementary Material). In a descriptive analysis, several treatments inhibited the *S. aureus* planktonic growth (Fig. 2A), while many of them stimulated bacterial growth for *E. coli* (Fig. 2B). Except for the AQU extract of SA that stimulated the planktonic growth of both bacteria, *S. aureus* ($P \leq 0.05$ = 0.0432), and *E. coli* ($P \leq 0.05$ = 0.0117) in 250 *µ*g/mL (Fig. 2A and B). It was not possible to obtain the results for the AQU extract of SC because the sample did not dilute in the DMSO. MET extract of SC

reduced the growth of *S. aureus* in 10 µg/mL (*P≤0.05* = 0.0216) but stimulated the planktonic growth of *E. coli* at the higher concentration (*P≤0.05* = 0.0058). Trends in ALK extract of SC and MET extract of SA are shown in Table 1 of the Supplementary Material.



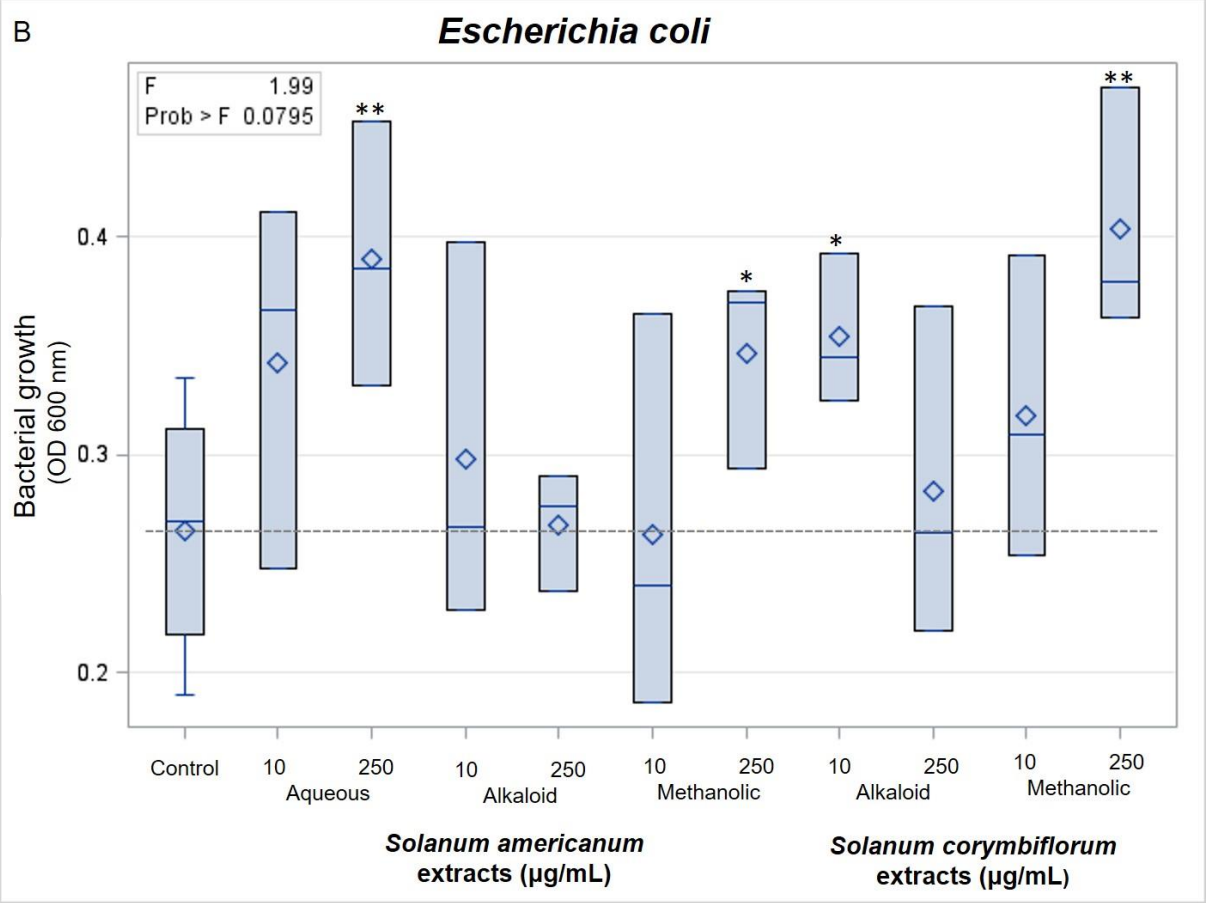


Figure 2. Effect of AQU, ALK, and MET extracts of SA and SC leaves on bacterial growth of *S. aureus* (A) and *E. coli* (B) strains.
Each bar represents the range of variation within the treatment, the line represents the median and diamond the mean. The dashed line represents the control's mean compared with treatments means (one-way ANOVA followed by post hoc bilateral Dunnett's test). Where *** = highly significant difference (*P≤0.001*), ** = significant difference (*P≤0.05*) and * = trends (*0.05<P<0.10*).

3.2 Effect of plant extracts on biofilm's formation

Significant differences were found for biofilm formation for these gram-positive and negative strains (Table 2 in Supplementary Material). In a descriptive analysis, some extracts inhibited the biofilm formation for *S. aureus* and *E. coli*. (Fig. 3A and 3B). ALK extracts of both species, SA (*P≤0.05* = 0.0034) and SC (*P≤0.05* = 0.0208), inhibited the biofilm formation for *S. aureus* at 250 *µ*g/mL (Fig. 3A), but had no effect on *E. coli* (Fig. 3B). MET extract of SC significantly stimulated the biofilm formation of *S. aureus* at the higher concentration *(P≤0.001* = 0.0011*)* but inhibited for *E. coli.* (*P≤0.05* = 0.0145). AQU extracts also inhibited the biofilm formation for *E. coli* in both concentrations, 10 (*P≤0.05* = 0.0451) and 250 *µ*g/mL (*P≤0.001* = 0.0006), without effects on *S. aureus*. Trends in MET extract of SA and SC are shown in Table 2 of the Supplementary Material.

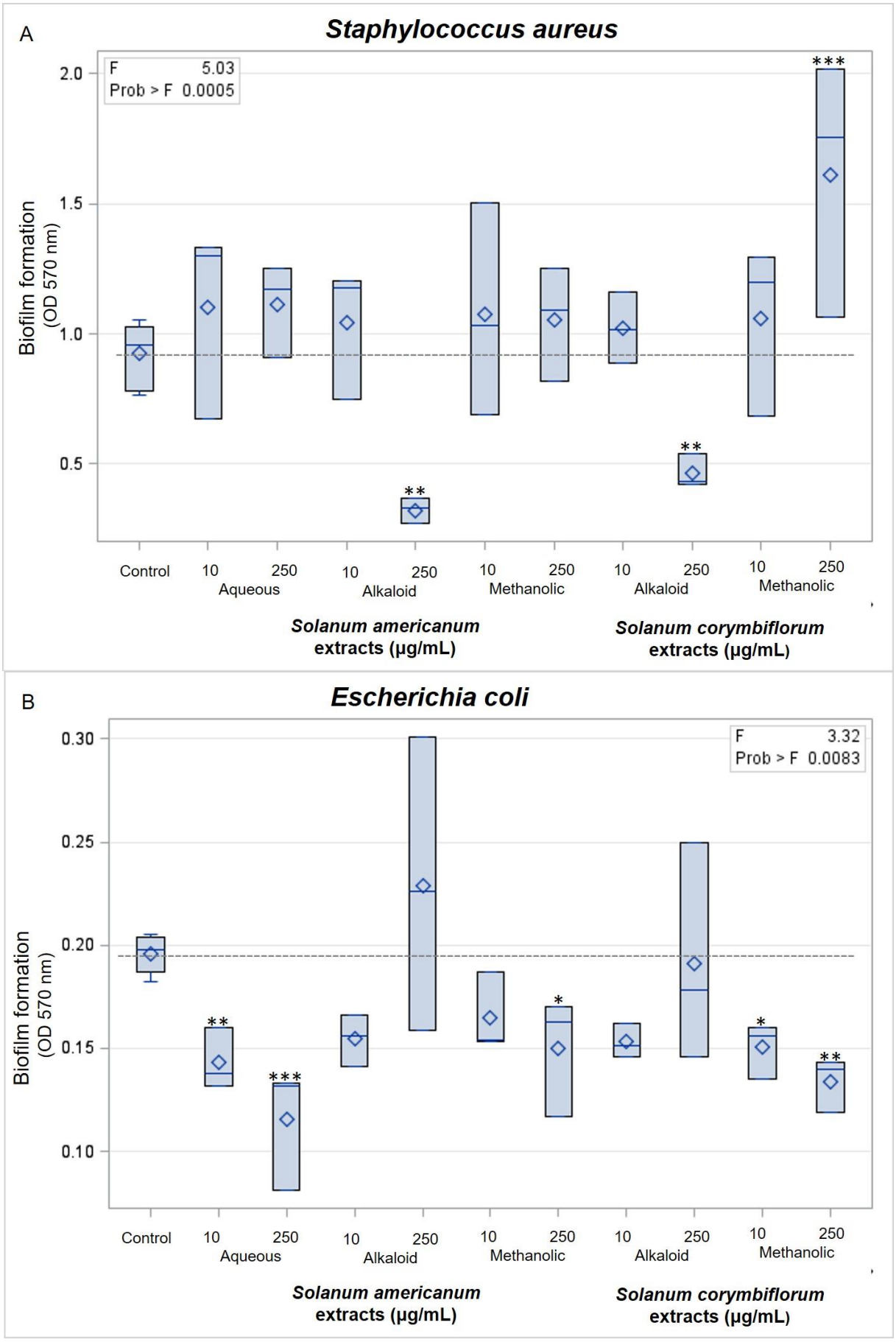

A
Staphylococcus aureus
F 5.03
Prob > F 0.0005
Biofilm formation (OD 570 nm)
2.0
1.5
1.0
0.5
***
**
**
Control
10
250
Aqueous
10
250
Alkaloid
10
250
Methanolic
10
250
Alkaloid
10
250
Methanolic
Solanum americanum extracts (µg/mL)
Solanum corymbiflorum extracts (µg/mL)
B
Escherichia coli
F 3.32
Prob > F 0.0083
Biofilm formation (OD 570 nm)
0.30
0.25
0.20
0.15
0.10
**
***
*
*
**
Control
10
250
Aqueous
10
250
Alkaloid
10
250
Methanolic
10
250
Alkaloid
10
250
Methanolic
Solanum americanum extracts (µg/mL)
Solanum corymbiflorum extracts (µg/mL)

Figure 3. Effect of AQ, ALK, and MET extracts of SA and SC leaves on biofilm formation of *S. aureus* (A) and *E. coli* (B) strains
Each bar represents the range of variation within the treatment, the line represents the median and diamond the mean. The dashed line represents the control's mean compared with treatments means (one-way ANOVA followed by post hoc bilateral Dunnett's test). Where *** = highly significant difference (P≤0.001), ** = significant difference (P≤0.05) and * = trends (0.05<P<0.10).

3.3 Chemical characterization: TLC, HPLC and HRMS

A preliminary phytochemical analysis to detect the major components of the extracts was carried out. TCL was used in the MET and ALK (Fig. 4) extract of SC leaves. Fig. 4 B and C shows spots characteristic of phenolic compounds, but not reactive to alkaloids (Fig. 4 D). Fig. 4 E and F, ALK extract showed an orange color specific to detect the presence of alkaloids (Hildebert Wagner and Bladt, 1996).

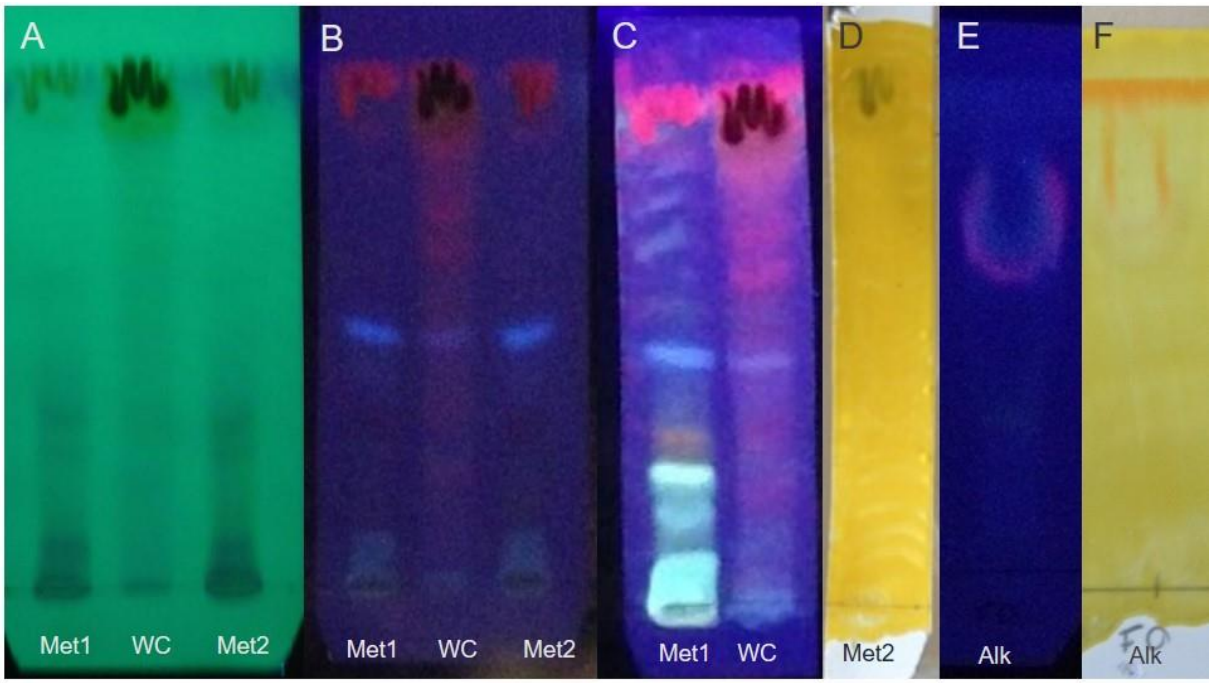


Figure 4. TLC of MET and ALK extracts of SC leaves.
A) TLC plate with 3 lanes with the same sample of the MET extract at 254 nm. The central lane with the presence of chlorophyll (WC) and the first (Met1) and the last (Met2) band after removing the chlorophyll with petroleum ether. B) Light blue spots in 365 nm. C) Spots with PEG 400 reagent in 365 nm. D) Dragendorff's reagent. E) ALK extract spots in 365 nm. F) Dragendorff's reagent.

In the Fig. 5 A and B, the chromatographic profiles of the ALK extract contain the main peaks for SA and SC, as well as the UV spectra corresponding for these peaks. Although the retention time of the two samples was similar, only peak 2 showed wavelength of λ_{max} 239 in both species. The peak 6 of SA shows absorption at wavelengths of λ_{max} 220, 290, and 317 nm. The peaks 8, 9 and 10 of the chromatograms of SA (Fig. 5 A) presented spectra with characteristics evidencing

absorption in a longer wavelength (Fig. 1 in Supplementary Material), which may characterize the presence of flavonoids in the extract, while no peaks were observed in SC at 400 nm wavelength (Fig. 2 in Supplementary Material). Chromatograms for SA and SC were obtained at wavelengths between 220 and 400 nm (Fig. 1 and 2 in Supplementary Material) with most peaks showing better absorption at 254 nm, a characteristic attributed to alkaloids (Sangster and Stuart, 1965).

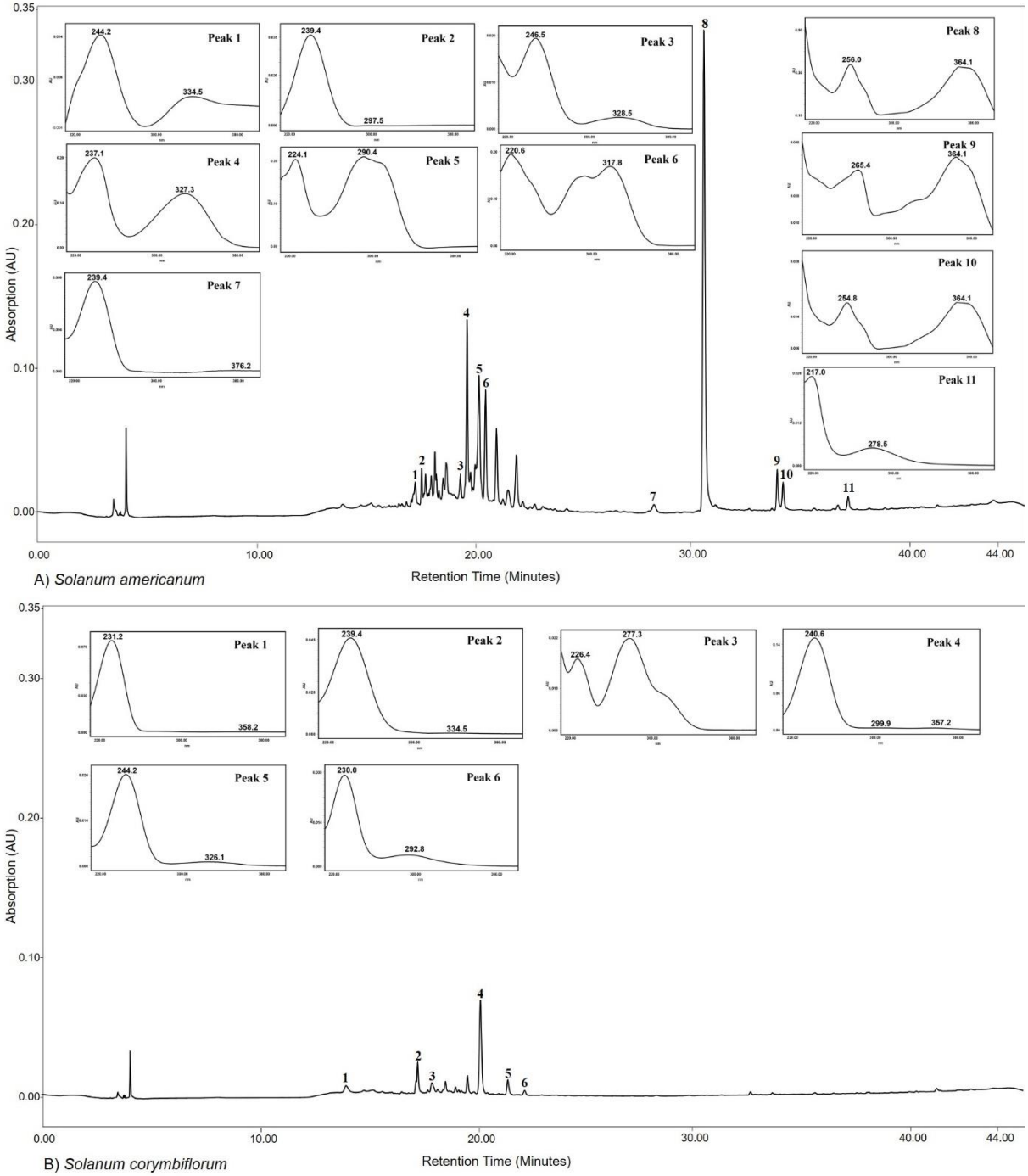


Figure 5. Chromatographic profiles by HPLC-DAD of ALK extract of SA and SC.
Above the chromatogram are shown the UV spectra corresponding to the main peaks, at 254 nm.

Table 04 (Supplementary material) shows some compounds with one possible indication and the Fig. 3 (Supplementary material) shows the full mass spectrum HRMS carried-out from the ALK extracts of SA and SC in positive and negative ionization mode. The search for compounds carried out, by comparison, showed the molecular ion at *m/z* 314.1382 founded in this spectrum was comparable with the exact mass, isotopic ratio, and fragmentation pathway (Fig. 6) suggesting the presence of the compound *N-trans*-feruloyl tyramine.

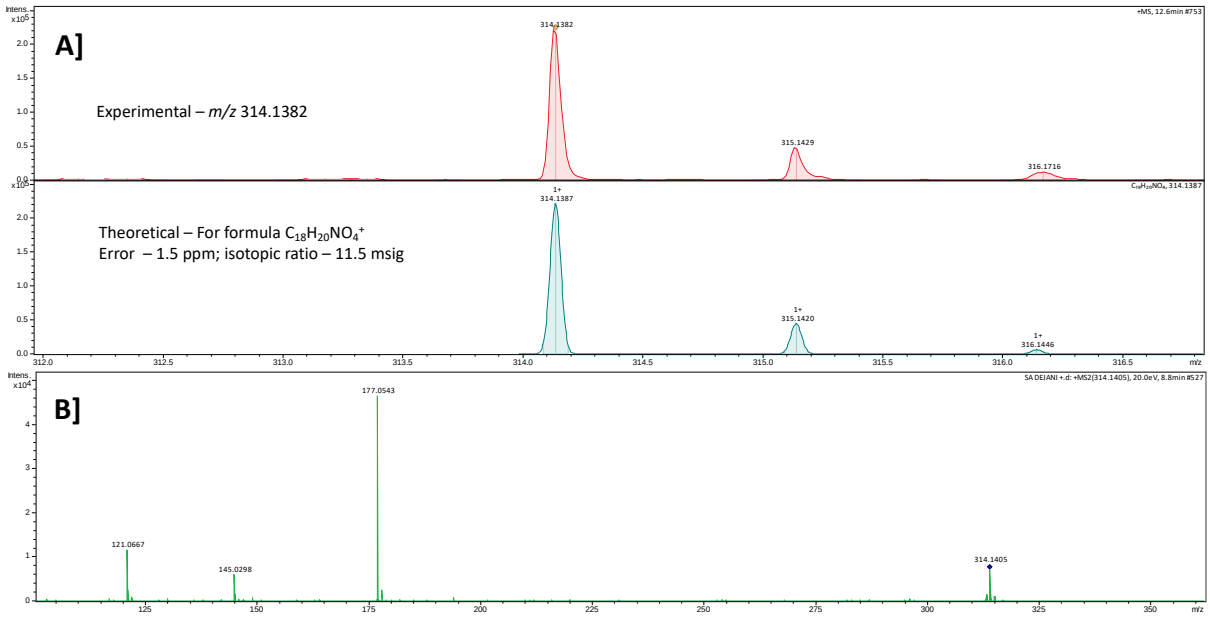


Figure 6. ESI (+) MS-MS of *N-trans*-feruloyl tyramine in ALK extract of SA.
In A] the comparison between the *m/z* exact of experimental and theoretical for elementary formula $C_{18}H_{20}NO_4^+$; in B] the pattern fragmentation for *m/z* 314.

4. Discussion

The application of antibiotics for many years, with underuse, overuse, and misuse of many active compounds, increased the selective pressure resulting in the development of generations of resistant microbes (Davies, 1996). According to these authors, gene exchange is a universal property of bacteria, and in this respect, the importance given to horizontal transference has increased, as they can carry genes for pathogenicity. In dairy production, the treatment of mastitis represents the greatest use of antibiotics, representing a high cost of the productive system (Zafalon et al., 2007), in addition to the fact that cattle are the main reservoirs of methicillin-resistant *S. aureus*, due to successful horizontal interactions with humans (Richardson et al., 2018).

For this reason, research on natural plant products in the animal production sector has grown, to the detriment of the use of synthetic chemical compounds. The different extraction methods used in raw material may affect differently microorganisms because the solvent can to carry distinct compounds (Tiwari et al., 2011). Our result showed this fact as extracts at low and high concentrations presented distinct effects on growth of *S. aureus* and *E. coli*. For example, only the high dose of the AQU extract of SA significantly stimulated the planktonic growth of *S. aureus* and *E. coli*. This activity might be related to the capability of water to extract starch (Tiwari et al., 2011) supplying nutrients which favored the growth gram-positive and negative species. On the other side, MET extract of SC in the lowest concentration inhibited the planktonic growth for *S. aureus*, without consistent inhibitory effect when 250 *µ*g/mL was tested (Fig. 2A). The same effect tended to occur with ALK extract of SC. Generally, a linear effect could be expected, with increasing dose, increased growth inhibition would occur (Abbas et al., 2014).

We also verified the distinct effects of extracts according to the Solanum species. For example, MET extract of SA had no effect on *S. aureus* and *E. coli* growth compared with the control. But the MET extract of SC inhibited growth of *S. aureus* while it stimulated for *E. coli* (Fig. 2B). Stimulated growth also tended to occur with MET extract of SA and ALK extract of SC. The fact that these extracts stimulate the planktonic growth of *E. coli* can be dangerous since your most powerful effect is the rapid multiplication and the release of part of its membranes. This process strongly stimulates the host's immune system and which can trigger a septic shock leading to the animal at death (Guerra et al., 2019; Herry et al., 2017).

If it causes the death of the bacteria resistance in the strains can be induced, this factor accompanies the schedule for the development of new drugs. According to Davies, (1996), the explain is: "If resistance is biochemically possible, it will occur." Antivirulence therapy is an alternative approach to control these infections reducing their severity and allowing clearance by the host's immune system (Cegelski et al., 2008). Plant natural products have been increasingly reported as effective alternatives to the current antivirulence agents, like alkaloids and flavonoids that act regulating bacteria's communication and inhibiting the release of adhesins, responsible for biofilm attachment (Silva et al., 2016). According to the same authors, the most recognized bacterial virulence factors, developed to evade the

immune system and the action of antibiotics, are quorum sensing (QS), biofilms, motility, toxins, pigments, enzymes, and surfactants.

The first step in the formation of biofilm is the adhesion of planktonic bacteria, that is, free-living to an abiotic or biotic surface (Costerton et al., 1999), and in the case of dairy cows, it occurs in the cells of the mammary epithelium. Federman et al. (2016) found that citrus oil can inhibit adhesion of *S. aureus in vitro*, however, they did not discuss the importance of this fact. According to Silva et al. (2016), inhibition of bacterial adhesion seems to be the most promising strategy since bacteria in the planktonic lifestyle are likely to be more susceptible. Biofilms have been reported as possessing susceptibilities towards antibiotics that are 100 to 1000 times less than equivalent populations of planktonic bacteria (Gilbert et al., 2002). This means that not only are biofilms frequently encountered but they are more difficult to eliminate, and ineffective treatments are more likely to be deployed against them (Gilbert et al., 2002). Our results evidence that the ALK extracts of SA and SC were able to inhibit biofilm formation *S. aureus* at 250 *μ*g/mL (Fig. 3A). According to Patel et al. (2013), gram-positive bacteria are more susceptible than gram-negative bacteria to plant extracts. Some factors of virulence appear to be fundamental to many pathogens, and drugs targeting these elements should exhibit broader spectrum activity. However, many antivirulent drugs should be designed to target specific pathogens and virulence factors unique to their pathogenic cascades (Cegelski et al., 2008; Mertenat et al., 2020).

Methanol is a universal solvent due to the ability to remove most compounds, including flavonoids and alkaloids present in the leaf extract (Daji et al., 2018). The hypothesis is that the low proportion of alkaloids on methanolic extract interacted with the flavonoids, with possible counteract effects, and were not effective in inhibiting the biofilm formation. Indeed they might have functioned as a stressful factor for *S. aureus* stimulating the adhesion of the biofilm to prevent the destruction of planktonic cells, as it occurs with sub-lethal doses of antibiotics (Aka and Haji, 2015; Costa et al., 2012; Song et al., 2016). This might explain why the MET extract of SC reduced the growth of planktonic bacteria in 10 *μ*g/mL (Fig. 2A) and increased biofilm formation at 250 *μ*g/mL (Fig. 3A).

Staphylococcus aureus is a contagious pathogen, considered the most virulent and difficult to eliminate from the herd because it causes a long-lasting subclinical infection in dairy herds (Rainard et al., 2018; Reyher et al., 2012). Due to the low

healing rate, high treatment cost, and the need to eliminate the infection reservoir to prevent spread into the herd, cows are slaughtered early (Rainard, 2005; Rollin et al., 2015; Ruegg, 2012). *S. aureus* can attach to the mammary gland epithelium, being able to proliferate, and accumulate cells in multiple layers, that favors survival in hostile environments and increases resistance, persisting inside the cow's udder (Melo et al., 2013). According the same authors, when 94 strains of *S. aureus* were collected from dairy herds, 98.9% of them produced strong biofilm's adherence and presented *icaA* and *icaD* genes. The *icaA*, *icaD*, and *bap* genes are responsible for the production of intercellular adhesins (Budri et al., 2015). According to Kim et al. (2019), strains that are multidrug-resistant produce stronger biofilms and can persist for on-farm over 3 years.

Silva et al. (2016) reported that the use of solanaceous alkaloids as tomatidine and capsaicin on *S. aureus* can block or reduce the expression of several genes, such as *hla*, responsible to produce alpha toxin, also known as alpha hemolysin. The release of enzymes is another virulence factor presented by several bacteria, mainly by *S. aureus*. Hemolysin provokes acute mastitis and is associated with pathological changes observed during the course of staphylococci infections, such as the formation of abscesses, hemorrhagic, and necrotic lesions (Da Silva et al., 2012). Some flavonoids were able to down regulate *icaA* and *icaD genes*, which are associated with adhesion and bacterial accumulation in biofilm (Silva et al., 2016).

AQU extract of SA and MET extract of SC in both concentrations, like that MET extract of SA in the higher concentration tended to inhibit biofilm formation for *E. coli* strain. But, considering that these same extracts also stimulated planktonic growth (Fig. 2B and 3B), and, generally, spontaneous cure of the animal occurs in the case of *E. coli* mastitis, we focused the present study in the effect of ALK extracts on *S. aureus*, considered a major problem of the farms.

Considering the previous reports about the Solanum *ssp.* (Cai et al., 2010; Chen et al., 2013; Kanada et al., 2012; Silva et al., 2017; Syu et al., 2001), the results obtained in the present study may suggest the presence of *N-trans*-feruloyltyramine (*m/z* 314.1382). The indication of the compound was based on a match of data, such as accurate mass (Wang et al., 2017), fragmentation pathway (Jiao et al., 2018; Kang et al., 2018; Nishioka et al., 2014; Wang et al., 2017) and UV spectrum (Etalo et al., 2013; Jiang et al., 2015). The precursor íon (m/z) and the

fragmentation pathway (ms/ms) can be evidenced in Fig. 7, as well as the full spectrum in the positive mode of ALK extract of SA (Fig. 6). The peak 6 of SA chromatogram (Fig. 4 A) shows an absorption very similar with a standard λ_{max} 228, 294 and 319 nm used to identify *N-trans*-feruloyltyramine in *Solanum lycopersicum* (Etalo et al., 2013) and in *Arcangelisia gusanlung* λ_{max} 220, 293 and 319 nm (Jiang et al., 2015). Therefore, we consider the above information as a positive although indirect indication of its presence in the ALK extract of SA.

A study with rats indicates that when supplying 20 mg/kg of *N-trans*-feruloyltyramine orally, much of this compound is metabolized and some metabolites may appear in the feces, but part of it remained unchanged in the urine (Xu et al., 2018). This fact supports the hypothesis that it can be eliminated together with milk secretion and act directly on bacteria within the mammary gland. *N-trans*-feruloyltyramine can also positively influence the immune system, acting as an antioxidant and anti-inflammatory, through inhibition of nitric oxide (NO), prostaglandins, and reactive oxygen species (ROS) in the face of induction with LPS, without presenting toxicity to the macrophages of rats (Jiang et al., 2015). This ability to inhibit the release of NO and ROS may reduce cellular damage within the mammary gland. This characteristic is interesting from the point of view of animal production, as it may reduce somatic cells in milk (CCS), decreasing the negative effects on milk production and composition.

5. Conclusion

The ALK extracts of SA and SC showed promising potential to be used in the treatment or prevent of mastitis caused by *S. aureus* due to its action reducing the biofilm formation. This can make planktonic bacteria more susceptible to the action of the antimicrobials or to the action of the immune system. We do not encourage the use of these Solanaceae for mastitis caused *by E. coli*, as the same extracts that reduce biofilm formation also increase planktonic growth, which may aggravate the acute and systemic condition of the disease. Our results can corroborate the traditional knowledge associated with local smallholder dairy farmers who use it to treat mastitis.

Ethical considerations

This study was approved by the post-graduation Commission and Ethic committee in Use of Animals (CEUA – UFRGS), number 37824, as part of thesis project: Potential use of plants of *Solanum* genus on diseases of dairy herds - Anti-inflammatory and antimicrobial characteristics.

Acknowledgements

The authors would like to thank Lilian Mentz, Márcia Vignoli (*Solanum americanum*) and Edson Soares (*Solanum corymbiflorum*) of Botanical Department of Federal University of Rio Grande do Sul for providing the identification of species and to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the grants.

Author's contributions: The idea for the paper was conceived by Dejani Maíra Panazzolo and Vivian Fischer. The experiments were designed by Dejani Maíra Panazzolo, Vivian Fischer, José Ângelo Zuanazzi and Alexandre José Macedo. The experiments were performed Dejani Maíra Panazzolo, Elen Oliveira, Rodrigo Campos and Flavia Brust. The data were analyzed by Dejani Maíra Panazzolo and Sidnei Moura. The paper was written by Dejani Maíra Panazzolo and reviewed by Vivian Fischer.

REFERENCES

Abbas, K., Niaz, U., Hussain, T., Saeed, M.A., Javaid, Z., Idrees, A., Rasool, S., 2014. Antimicrobial activity of fruits of Solanum nigrum and Solanum xanthocarpum. Acta Pol. Pharm. - Drug Res. 71, 415–421.

Aka, S.T., Haji, S.H., 2015. Sub-MIC of antibiotics induced biofilm formation of Pseudomonas aeruginosa in the presence of chlorhexidine. Brazilian J. Microbiol. 46, 149–154. https://doi.org/10.1590/S1517-838246120140218

Antunes, A.L.S., Trentin, D.S., Bonfanti, J.W., Pinto, C.C.F., Perez, L.R.R., Macedo, A.J., Barth, A.L., 2010. Application of a feasible method for determination of biofilm antimicrobial susceptibility in staphylococci. Apmis 118, 873–877. https://doi.org/10.1111/j.1600-0463.2010.02681.x

Bohs, L., 1989. Ethnobotany of the genus Cyphomandra (Solanaceae). Econ. Bot. 43, 143–163. https://doi.org/10.1007/BF02859855

Brenton, A.G., Godfrey, A.R., 2010. Accurate mass measurement: Terminology and treatment of data. J. Am. Soc. Mass Spectrom. 21, 1821–1835.

https://doi.org/10.1016/j.jasms.2010.06.006

Budri, P.E., Silva, N.C.C., Bonsaglia, E.C.R., Fernandes, A., Araújo, J.P., Doyama, J.T., Gonçalves, J.L., Santos, M. V., Fitzgerald-Hughes, D., Rall, V.L.M., 2015. Effect of essential oils of Syzygium aromaticum and Cinnamomum zeylanicum and their major components on biofilm production in Staphylococcus aureus strains isolated from milk of cows with mastitis. J. Dairy Sci. 98, 5899–5904. https://doi.org/10.3168/jds.2015-9442

Cai, X.F., Chin, Y.W., Oh, S.R., Kwon, O.K., Ahn, K.S., Lee, H.K., 2010. Anti-inflammatory constituents from solanum nigrum. Bull. Korean Chem. Soc. 31, 199–201. https://doi.org/10.5012/bkcs.2010.31.01.199

Cegelski, L., Marshall, G.R., Eldridge, G.R., Hultgren, S.J., 2008. The biology and future prospects of antivirulence therapies. Nat. Rev. Microbiol. 6, 17–27. https://doi.org/10.1038/jid.2014.371

Chen, Y.C., Lee, H.Z., Chen, H.C., Wen, C.L., Kuo, Y.H., Wang, G.J., 2013. Anti-inflammatory components from the Root of Solanum Erianthum. Int. J. Mol. Sci. 14, 12581–12592. https://doi.org/10.3390/ijms140612581

Costa, J.C.M., Espeschit, I. de F., Pieri, F.A., Benjamin, L. dos A., Moreira, M.A.S., 2012. Increased production of biofilms by Escherichia coli in the presence of enrofloxacin. Vet. Microbiol. 160, 488–490. https://doi.org/10.1016/j.vetmic.2012.05.036

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: A common cause of persistent infections. Science (80-.). https://doi.org/10.1126/science.284.5418.1318

Da Silva, E.R., Silva, T.R.M., Pereira, A.M.G., Machado, A.C., Santoro, K.R., 2012. Produção de hemolisinas por Staphylococcus aureus isolados de casos de mastite bovina subclínica. Acta Vet. Bras. 6, 118–123.

Daji, G., Steenkamp, P., Madala, N., Dlamini, B., 2018. Phytochemical Composition of Solanum retroflexum Analysed with the Aid of Ultra-Performance Liquid Chromatography Hyphenated to Quadrupole-Time-of-Flight Mass Spectrometry (UPLC-qTOF-MS) . J. Food Qual. 2018, 1–8. https://doi.org/10.1155/2018/3678795

Davies, J., 1996. Origins and evolution of antibiotic resistance. Microbiologia. https://doi.org/10.1128/mmbr.00016-10

Doss, A., Muhamed Mubarack, H., Vijayasanthi, M., Venkataswamy, R., 2012. In-vitro antibacterial activity of certain wild medicinal plants against bovine mastitis isolated contagious pathogens. Asian J. Pharm. Clin. Res. 5, 90–93.

Etalo, D.W., Stulemeijer, I.J.E., Peter van Esse, H., de Vos, R.C.H., Bouwmeester, H.J., Joosten, M.H.A.J., 2013. System-Wide hypersensitive response-associated transcriptome and metabolome reprogramming in tomato. Plant Physiol. 162, 1599–1617. https://doi.org/10.1104/pp.113.217471

Federman, C., Joo, J., Almario, J.A., Salaheen, S., Biswas, D., 2016. Citrus-derived oil inhibits Staphylococcus aureus growth and alters its interactions with bovine mammary cells. J. Dairy Sci. 99, 3667–3674. https://doi.org/10.3168/jds.2015-

10538

Garciaa, S.N., Osburnb, B.I., Cullor, J.S., 2019. A one health perspective on dairy production and dairy food safety. One Heal. https://doi.org/https://doi.org/10.1016/j.onehlt.2019.100086

Gilbert, P., Allison, D.G., McBain, A.J., 2002. Biofilms in vitro and in vivo: Do singular mechanisms imply cross-resistance? J. Appl. Microbiol. Symp. Suppl. 92, 98–110. https://doi.org/10.1046/j.1365-2672.92.5s1.5.x

Guerra, S.T., De Paula, C.L., Bolaños, C.A.D., Hernandes, R.T., Ribeiro, M.G., 2019. Virulence factors os Escherichia coli: An overview of human and animal infections with emphasis in bovine mastitis. Semin. Agrar. https://doi.org/10.5433/1679-0359.2019v40n5p2087

Herry, V., Gitton, C., Tabouret, G., Répérant, M., Forge, L., Tasca, C., Gilbert, F.B., Guitton, E., Barc, C., Staub, C., Smith, D.G.E., Germon, P., Foucras, G., Rainard, P., 2017. Local immunization impacts the response of dairy cows to Escherichia coli mastitis. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-03724-7

Hildebert Wagner, Bladt, S., 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas, 2nd ed. Springer Dordrecht Heidelberg London New York, Berlin. https://doi.org/10.1016/S0003-2670(00)85252-6

Jiang, Y., Yu, L., Wang, M.H., 2015. N-trans-feruloyltyramine inhibits LPS-induced NO and PGE2 production in RAW 264.7 macrophages: Involvement of AP-1 and MAP kinase signalling pathways. Chem. Biol. Interact. 235, 56–62. https://doi.org/10.1016/j.cbi.2015.03.029

Jiao, Q.S., Xu, L.L., Zhang, J.Y., Wang, Z.J., Jiang, Y.Y., Liu, B., 2018. Rapid Characterization and Identification of Non-Diterpenoid Constituents in Tinospora sinensis by HPLC-LTQ-Orbitrap MS n. Molecules 23. https://doi.org/10.3390/molecules23020274

Kalayou, S., Haileselassie, M., Gebre-egziabher, G., Tiku'e, T., Sahle, S., Taddele, H., Ghezu, M., 2012. In-vitro antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. Asian Pac. J. Trop. Biomed. 2, 516–522. https://doi.org/10.1016/S2221-1691(12)60088-4

Kanada, R.M., Simionato, J.I., Arruda, R.F. de, Santin, S.M. de O., Maria Conceição de Souza, Silva, C.C. da, 2012. N-trans-feruloyltyramine and flavonol glycosides from the leaves of Solanum sordidum. Brazilian J. Pharmacogn. 22, 502–506. https://doi.org/10.1590/S0102

Kang, C., Zhao, D., Kang, L., Wang, S., Lv, C., Zhou, L., Jiang, J.Y., Yang, W., Li, J., Huang, L.Q., Guo, L., 2018. Elucidation of characteristic sulfur-fumigated markers and chemical transformation mechanism for quality control of achyranthes bidentate blume using metabolome and sulfur dioxide residue analysis. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.00790

Keller, H.A., Prance, G.T., 2012. Etnobotánica de las especies de Solanum, Subgénero Bassovia, sección Pachyphylla (Solanaceae) de missiones, Argentina. Bonplandia 21, 45–54.

Kim, S.J., Moon, D.C., Park, S.C., Kang, H.Y., Na, S.H., Lim, S.K., 2019. Antimicrobial resistance and genetic characterization of coagulase-negative staphylococci from bovine mastitis milk samples in Korea. J. Dairy Sci. 102, 11439–11448. https://doi.org/10.3168/jds.2019-17028

Knapp, S., Barboza, G.E., Bohs, L., Särkinen, T., 2019. A revision of the Morelloid Clade of Solanum L. (Solanaceae) in North and Central America and the Caribbean. PhytoKeys 123, 1–144. https://doi.org/10.3897/phytokeys.123.31738

Li, J., Li, Q., Feng, T., Li, K., 2008. Aqueous extract of Solanum nigrum inhibit growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells. Fitoterapia 79, 548–556. https://doi.org/10.1016/j.fitote.2008.06.010

Li, T., Lu, H., Wang, X., Gao, Q., Dai, Y., Shang, J., Li, M., 2017. Molecular characteristics of Staphylococcus aureus causing bovine mastitis between 2014 and 2015. Front. Cell. Infect. Microbiol. 7, 1–10. https://doi.org/10.3389/fcimb.2017.00127

Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez-Miyakawa, M.E., Chi, F., Cravens, R.L., Oh, S., Gay, C.G., 2018. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet. Res. 49, 1–18. https://doi.org/10.1186/s13567-018-0562-6

Melo, P. de C., Menezes Ferreira, L., Nader Filho, A., Francisco Zafalon, L., Godoy Vicente, H.I., de Souza, V., 2013. Comparison of methods for the detection of biofilm formation by Staphylococcus aureus isolated from bovine subclinical mastitis. Brazilian J. Microbiol. 44, 119–124. https://doi.org/10.1590/S1517-83822013005000031

Mentz, L.A., Vendruscolo, G.S., Soares, E.L.D.C., Vignoli-Silva, M., 2007. Solanaceae nativas no Rio Grande do Sul, Brasil – Listagem II : Solanum L . Rev. Bras. Biociências 5, 1059–1061.

Mertenat, D., Cero, M.D., Vogl, C.R., Ivemeyer, S., Meier, B., Maeschli, A., Hamburger, M., Walkenhorst, M., 2020. Ethnoveterinary knowledge of farmers in bilingual regions of Switzerland – is there potential to extend veterinary options to reduce antimicrobial use? J. Ethnopharmacol. 246, 112184. https://doi.org/10.1016/j.jep.2019.112184

Mubarack, H.M., Doss, A., Dhanabalan, R., Venkataswamy, R., 2011. Activity of some selected medicinal plant extracts against bovine mastitis pathogens. J. Anim. Vet. Adv. https://doi.org/10.3923/javaa.2011.738.741

Nishioka, T., Kasama, T., Kinumi, T., Makabe, H., Matsuda, F., Miura, D., Miyashita, M., Nakamura, T., Tanaka, K., Yamamoto, A., 2014. Winners of CASMI2013: Automated Tools and Challenge Data. Mass Spectrom. 3, S0039–S0039. https://doi.org/10.5702/massspectrometry.s0039

Oliveira, L., Langoni, H., Hulland, C., 2012. Recovered From Clinical and Subclinical Cases of Bovine Mastitis. J. Dairy Sci. 95, 1913–1920.

Oliver, S.P., Murinda, S.E., 2012. Antimicrobial resistance of mastitis pathogens. Vet. Clin. North Am. - Food Anim. Pract. 28, 165–185. https://doi.org/10.1016/j.cvfa.2012.03.005

Patel, R., Patel, Y., Joshi, C., Kunjadia, A., 2013. Herbal Plants: A potential agent to cure infectious mastitis in bovine animals. Int. J. Phytomedicine 5, 362–366.

Phophi, L., Petzer, I.M., Qekwana, D.N., 2019. Antimicrobial resistance patterns and biofilm formation of coagulase-negative Staphylococcus species isolated from subclinical mastitis cow milk samples submitted to the Onderstepoort Milk Laboratory. BMC Vet. Res. 15, 420. https://doi.org/10.1186/s12917-019-2175-3

Piana, M., Camponogara, C., Boligon, A.A., Machado, M.M., De Brum, T.F., Oliveira, S.M., De Freitas Bauermann, L., 2016. Topical anti-inflammatory activity of Solanum corymbiflorum leaves. J. Ethnopharmacol. 179, 16–21. https://doi.org/10.1016/j.jep.2015.12.036

Rainard, P., 2005. Tackling mastitis in dairy cows. Nat. Biotechnol. 23, 430–432. https://doi.org/10.1038/nbt0405-430

Rainard, P., Foucras, G., Fitzgerald, J.R., Watts, J.L., Koop, G., Middleton, J.R., 2018. Knowledge gaps and research priorities in Staphylococcus aureus mastitis control. Transbound. Emerg. Dis. 65, 149–165. https://doi.org/10.1111/tbed.12698

Raza, A., Muhammad, G., Sharif, S., Atta, A., 2013. Biofilm Producing *Staphylococcus aureus* and Bovine Mastitis: A Review. Mol. Microbiol. Res. https://doi.org/10.5376/mmr.2013.03.0001

Reis, A., Magne, K., Massot, S., Tallini, L.R., Scopel, M., Bastida, J., Ratet, P., Zuanazzi, J.A.S., 2019. Amaryllidaceae alkaloids: identification and partial characterization of montanine production in Rhodophiala bifida plant. Sci. Rep. 9, 27–31. https://doi.org/10.1038/s41598-019-44746-7

Reyher, K.K., Haine, D., Dohoo, I.R., Revie, C.W., 2012. Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new intramammary infections with major mastitis pathogens-A systematic review and meta-analysis. J. Dairy Sci. 95, 6483–6502. https://doi.org/10.3168/jds.2012-5594

Richardson, E.J., Bacigalupe, R., Harrison, E.M., Weinert, L.A., Lycett, S., Vrieling, M., Robb, K., Hoskisson, P.A., Holden, M.T.G., Feil, E.J., Paterson, G.K., Tong, S.Y.C., Shittu, A., van Wamel, W., Aanensen, D.M., Parkhill, J., Peacock, S.J., Corander, J., Holmes, M., Fitzgerald, J.R., 2018. Gene exchange drives the ecological success of a multi-host bacterial pathogen. Nat. Ecol. Evol. 2, 1468–1478. https://doi.org/10.1038/s41559-018-0617-0

Rollin, E., Dhuyvetter, K.C., Overton, M.W., 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Prev. Vet. Med. 122, 257–264. https://doi.org/10.1016/j.prevetmed.2015.11.006

Rosales, P.F., Marinho, F.F., Gower, A., Chiarello, M., Canci, B., Roesch-Ely, M., Paula, F.R., Moura, S., 2019. Bio-guided search of active indole alkaloids from Tabernaemontana catharinensis: Antitumour activity, toxicity in silico and molecular modelling studies. Bioorg. Chem. https://doi.org/10.1016/j.bioorg.2018.12.016

Ruegg, P.L., 2012. New Perspectives in Udder Health Management. Vet. Clin. North Am. - Food Anim. Pract. 28, 149–163. https://doi.org/10.1016/j.cvfa.2012.03.001

Saglam, A.G., Sahin, M., Çelik, E., Çelebi, Ö., Akça, D., Otlu, S., 2017. The role of staphylococci in subclinical mastitis of cows and lytic phage isolation against to Staphylococcus aureus. Vet. World 10, 1481–1485. https://doi.org/10.14202/vetworld.2017.1481-1485

Sangster, A.W., Stuart, K.L., 1965. Ultraviolet Spectra of Alkaloids. Chem. Rev. 65, 69–130. https://doi.org/10.1021/cr60233a003

Särkinen, T., Poczai, P., Barboza, G.E., van der Weerden, G.M., Baden, M., Knapp, S., 2018. A revision of the Old World Black Nightshades (Morelloid clade of Solanum L., Solanaceae). PhytoKeys 223, 1–223. https://doi.org/10.3897/phytokeys.106.21991

Scopel, M., Mentz, L.A., Henriques, A.T., 2010. Comparative analysis of sambucus nigra and sambucus australis flowers: Development and validation of an HPLC method for raw material quantification and preliminary stability study. Planta Med. 76, 1026–1031. https://doi.org/10.1055/s-0029-1240850

Sharma, N., Jeong, D.K., 2013. Stem cell research: A novel boulevard towards improved bovine mastitis management. Int. J. Biol. Sci. 9, 818–829. https://doi.org/10.7150/ijbs.6901

Silva, M. da, Oliveira, D.R. de, 2018. The new Brazilian legislation on access to the biodiversity (Law 13,123/15 and Decree 8772/16). Brazilian J. Microbiol. 49, 1–4. https://doi.org/10.1016/j.bjm.2017.12.001

Silva, E.L., Almeida-Lafetá, R.C., Borges, R.M., Staerk, D., 2017. Dual high-resolution inhibition profiling and HPLC-HRMS-SPE-NMR analysis for identification of α-glucosidase and radical scavenging inhibitors in Solanum americanum Mill. Fitoterapia 118, 42–48. https://doi.org/10.1016/j.fitote.2017.02.002

Silva, L.N., Zimmer, K.R., Macedo, A.J., Trentin, D.S., 2016. Plant Natural Products Targeting Bacterial Virulence Factors. Chem. Rev. 116, 9162–9236. https://doi.org/10.1021/acs.chemrev.6b00184

Soares, E.L. de C., Mentz, L.A., 2006. As Espécies De Solanum Subgênero Bassovia Seção Pachyphylla (= Cyphomandra Mart. Ex Sendtn. - Solanaceae) No Rio Grande Do Sul, Brasil. Pesqui. Botânicas 57, 231–254.

Song, T., Duperthuy, M., Wai, S.N., 2016. Sub-optimal treatment of bacterial biofilms. Antibiotics 5, 1–18. https://doi.org/10.3390/antibiotics5020023

Syu, W.J., Don, M.J., Lee, G.H., Sun, C.M., 2001. Cytotoxic and novel compounds from Solanum indicum. J. Nat. Prod. 64, 1232–1233. https://doi.org/10.1021/np010186v

Tao, Y., Jiang, Y., Li, W., Cai, B., 2016. Zeolite based solid-phase extraction coupled with UPLC-Q-TOF-MS for rapid analysis of acetylcholinesterase binders from crude extract of: Corydalis yanhusuo. RSC Adv. 6, 98476–98486. https://doi.org/10.1039/c6ra24585d

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction - A review. Int. Pharm. Sci. 1, 98–106.

Trentin, D.D.S., Giordani, R.B., Zimmer, K.R., Da Silva, A.G., Da Silva, M.V., Correia,

M.T.D.S., Baumvol, I.J.R., MacEdo, A.J., 2011. Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against Staphylococcus epidermidis planktonic and biofilm lifestyles. J. Ethnopharmacol. 137, 327–335. https://doi.org/10.1016/j.jep.2011.05.030

Vagula, J.M., Rocha, B.A., Silva, A.R., Narain, N., Bersani-Amado, C.A., Junior, O.O.S., Visentainer, J. V., 2018. Analysis of Solanum Americanum Mill. by Ultrafast Liquid Chromatography with Diode Array and Time-Of-flight Mass Spectrometry Detection with Evaluation of Anti-Inflammatory Properties in Rodent Models. Anal. Lett. 51, 1973–1985. https://doi.org/10.1080/00032719.2017.1399413

Wang, S., Suh, J.H., Zheng, X., Wang, Y., Ho, C.T., 2017. Identification and quantification of potential anti-inflammatory hydroxycinnamic acid amides from wolfberry. J. Agric. Food Chem. 65, 364–372. https://doi.org/10.1021/acs.jafc.6b05136

Zafalon, L.F., Nader Filho, A., Oliveira, J. V., Resende, F.D., 2007. Mastite subclínica causada por Staphylococcus aureus: Custo-benefício da antibioticoterapia de vacas em lactação. Arq. Bras. Med. Vet. e Zootec. 59, 577–585. https://doi.org/10.1590/s0102-09352007000300005

Zhao, X., Lacasse, P., 2008. Mammary tissue damage during bovine mastitis: causes and control. J. Anim. Sci. 86, 57–65. https://doi.org/10.2527/jas.2007-0302

Supplementary material

Tables

Table 1. Descriptive statistics with means, standard deviation (SD), coefficient of variation (CV) and significance level of each treatment on the bacterial planktonic growth variable.

	Staphylococcus aureus				***Escherichia coli***			
	Mean	SD	CV	*P>F*	Mean	SD	CV	*P>F*
Control	0.335	0.048	14.49	-	0.265	0.054	20.68	-
SAMET10	0.310	0.021	7.00	0.5422	0.263	0.091	34.82	0.9684
SAMET250	0.364	0.081	22.30	0.5873	0.346	0.045	13.10	**0.0903***
SAALK10	0.291	0.028	9.84	0.2195	0.298	0.088	29.75	0.4853
SAALK250	0.295	0.060	20.47	0.1967	0.267	0.027	10.26	0.9627
SAAQU10	0.306	0.021	6.97	0.4703	0.342	0.084	24.75	0.1065
SAAQU250	0.443	0.074	16.78	**0.0432****	0.390	0.060	15.52	**0.0117****
SCMET10	0.263	0.043	16.46	**0.0216****	0.318	0.069	21.82	0.2604
SCMET250	0.328	0.084	25.63	0.6676	0.404	0.056	14.09	**0.0058****
SCALK10	0.278	0.032	11.67	**0.0914***	0.354	0.034	9.86	**0.0642***
SCALK250	0.331	0.076	23.12	0.7722	0.283	0.076	26.94	0.6955

SA = *Solanum americanum*; SC = *Solanum corymbiflorum;* MET = methanolic; AQU = aqueous; ALK = alkaloid

ANOVA followed by Dunnett's test. Where *** = highly significant difference (*P≤0.001*), ** = significant difference (*P≤0.05*) and * = trends (*0.05<P<0.10*).

Table 2. Descriptive statistics with means, standard deviation, coefficient of variation and significance level of each treatment on the biofilm formation variable.

	Staphylococcus aureus				***Escherichia coli***			
	Mean	SD	CV	*P>F*	Mean	SD	CV	*P>F*
Control	0.924	0.124	13.48	-	0.188	0.018	9.96	-
SAMET10	1.076	0.410	38.12	0.4237	0.164	0.019	11.74	0.3080
SAMET250	1.055	0.218	20.72	0.4890	0.150	0.028	19.19	**0.0786***
SAALK10	1.044	0.254	24.33	0.5272	0.154	0.012	8.15	0.1394
SAALK250	0.322	0.048	15.12	**0.0034****	0.228	0.071	31.06	0.2100
SAAQU10	1.102	0.372	33.78	0.3479	0.143	0.014	10.28	**0.0451****
SAAQU250	1.111	0.177	15.96	0.3254	0.115	0.029	25.78	**0.0006*****
SCMET10	1.058	0.327	30.94	0.4803	0.150	0.013	8.93	**0.0952***
SCMET250	1.612	0.493	30.61	**0.0011*****	0.134	0.013	9.75	**0.0145****
SCALK10	1.023	0.135	13.20	0.6018	0.153	0.008	5.34	0.1258
SCALK250	0.465	0.066	14.21	**0.0208****	0.191	0.053	27.83	0.9731

SA = *Solanum americanum*; SC = *Solanum corymbiflorum;* MET = methanolic; AQU = aqueous; ALK = alkaloid

ANOVA followed by Dunnett's test. Where *** = highly significant difference (*P≤0.001*), ** = significant difference (*P≤0.05*) and * = trends (*0.05<P<0.10*).

Table 3. Value of precursor ion, possible elementar composition, error, isotopic ratio, collision energy-dissociation and fragmentation pathway of the alkaloid extracts of S. americanum (SA) and S. corymbiflorum (SC) on positive and negative mode ESI QTOF-MS.

Entry	Precursor íon (*m/z*)	Elementar composition	Error (ppm)	Isotopic ratio (mSig)	CID (eV)	Fragmentation MS/MS (I%)	Reference/Compound
S. americanum							
1	274.2740	$C_{16}H_{36}NO_2^+$	0.1	60.0	15.0	274.2713 (100) **256.2621 (39)** 230.2463 (8) 212.2329 (4)	(Tao et al., 2016)
2	**314.1382**	**$C_{18}H_{20}NO_4^+$**	**1.5**	**11.5**	**15.0**	**177.0544 (100)** **121.0666 (13)** **315.1429 (11)** **145.0270 (12)**	(Wang et al., 2017) *N-trans*-feruloyl tyramine
3	338.3404	$C_{22}H_{44}NO^+$	4.1	82.3	15.0	338.3404 (100) **321.3137 (21)** **303.3018 (16)** **130.0809 (13)** **97.1012 (5)** **115.1148 (4)** **149.1308 (3)**	(Tao et al., 2016) Unknown
4	479.2700	$C_{32}H_{35}N_2O_2^+$	-1.4	44.0	30.0	443.2742 (100) 281.1582 (72) 317.1819 (54) 425.2697 (48)	Unknown
5	282.1221	$C_{11}H_{16}N_5O_4^-$	-4.8	10.9	35.0	119.0512 (100) 162.0603 (16) 132.0562 (13) 174.0585 (6) 145.0298 (5)	Unknown
6	591.2484	$C_{23}H_{35}N_2O_6^-$	3.7	36.0	37.7	205.0545 (100) 223.0644 (43) 367.1133 (38) 591.2484 (12)	Unknown
S. corymbiflorum							
1	284.3311	$C_{19}H_{42}N^+$	0.4	27.1	35.0	284.3311 (100) 102.1295 (40) 285.3345 (26) 105.0705 (12) 240.2333 (7)	Unknown
2	339.2042	$C_{16}H_{27}N_4O_4^-$	-1.3	25.6	40.0	183.0160 (100) 112.9864 (70) 339.2042 (56) 184.0160 (14) 340.2121 (10)	Unknown
3	325.1868	$C_{14}H_{29}O_8^-$	1.2	37.6	35.0	325.1864 (100) 183.0153 (89) 326.1878 (20) 184.0159 (11) 216.0151 (10)	Unknown
4	144.0450	$C_9H_6NO^-$	3.6	17.4	35	144.04050 (100) 126.0328 (40) 116.0508 (45) 117.0350 (24) 115.0392 (17)	Unknown

Figures

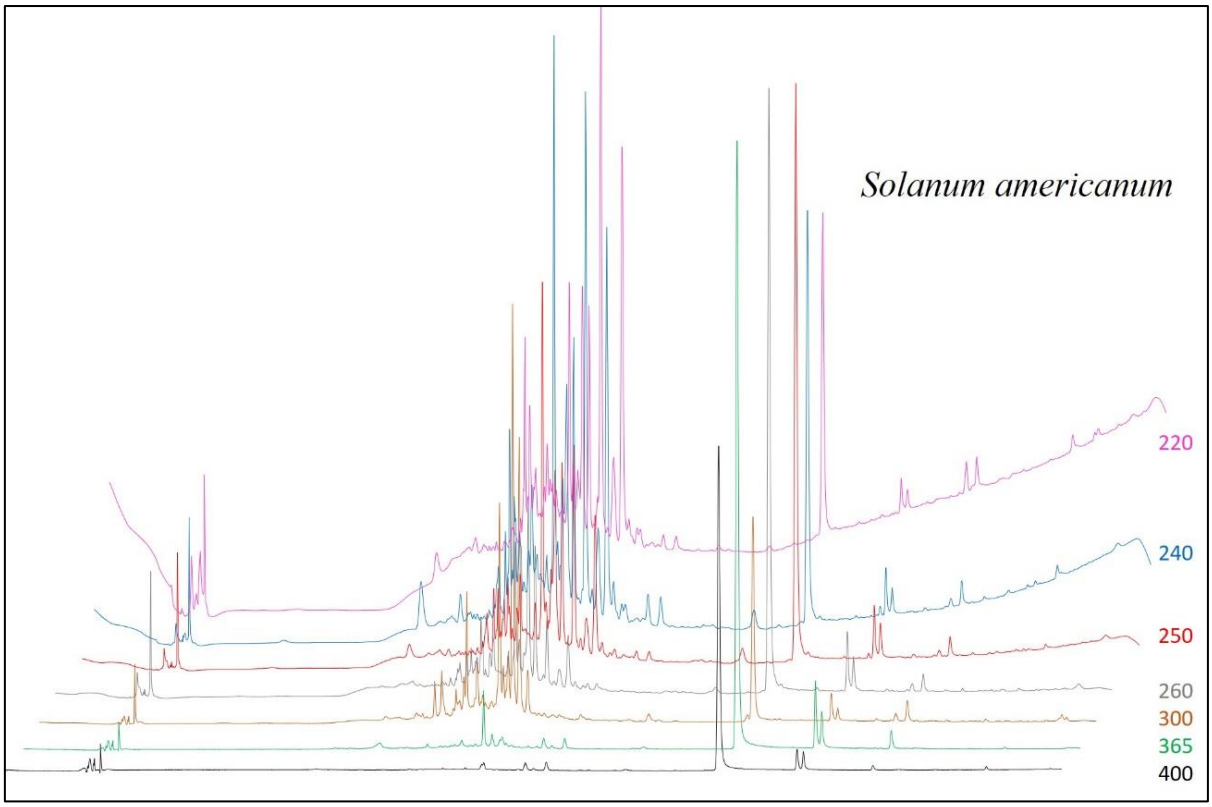


Figure 1. Chromatographic profiles of the ALK extract of SA compared in an overlay on different wavelengths by HPLC – DAD

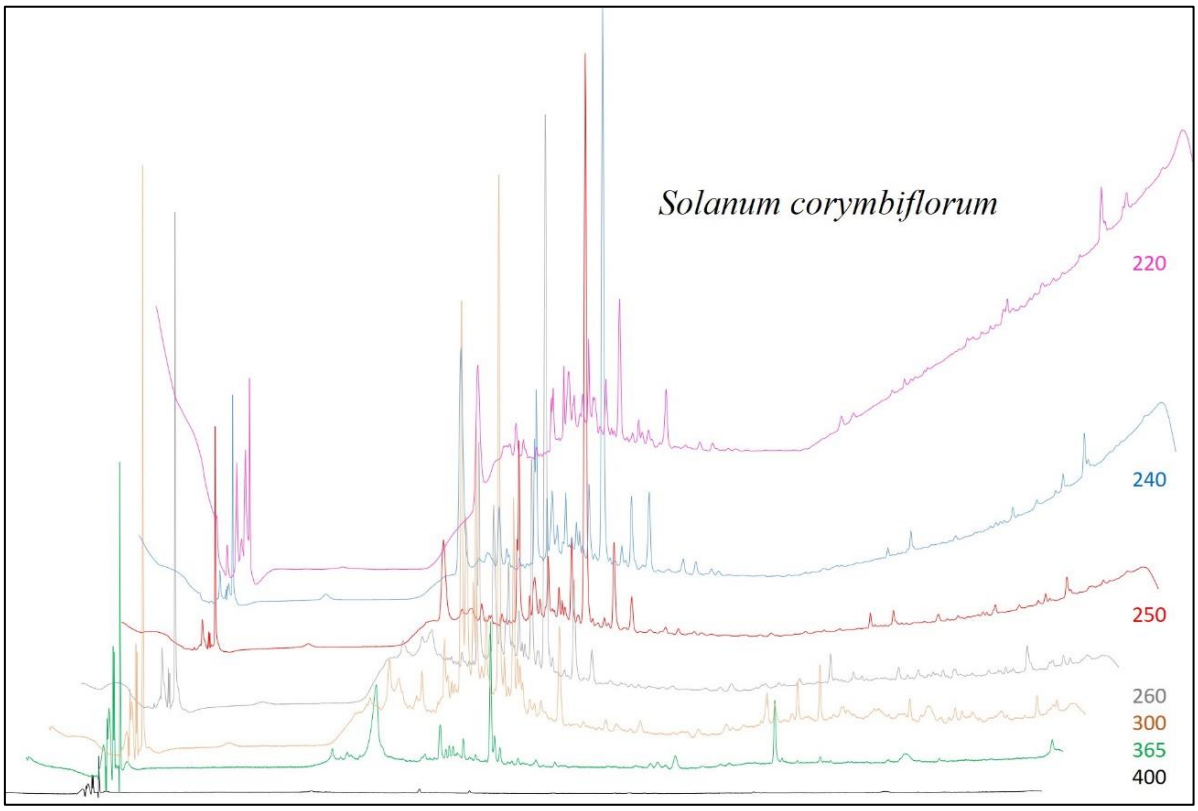


Figure 2. Chromatographic profiles of the ALK extract of SC compared in an overlay on different wavelengths by HPLC – DAD

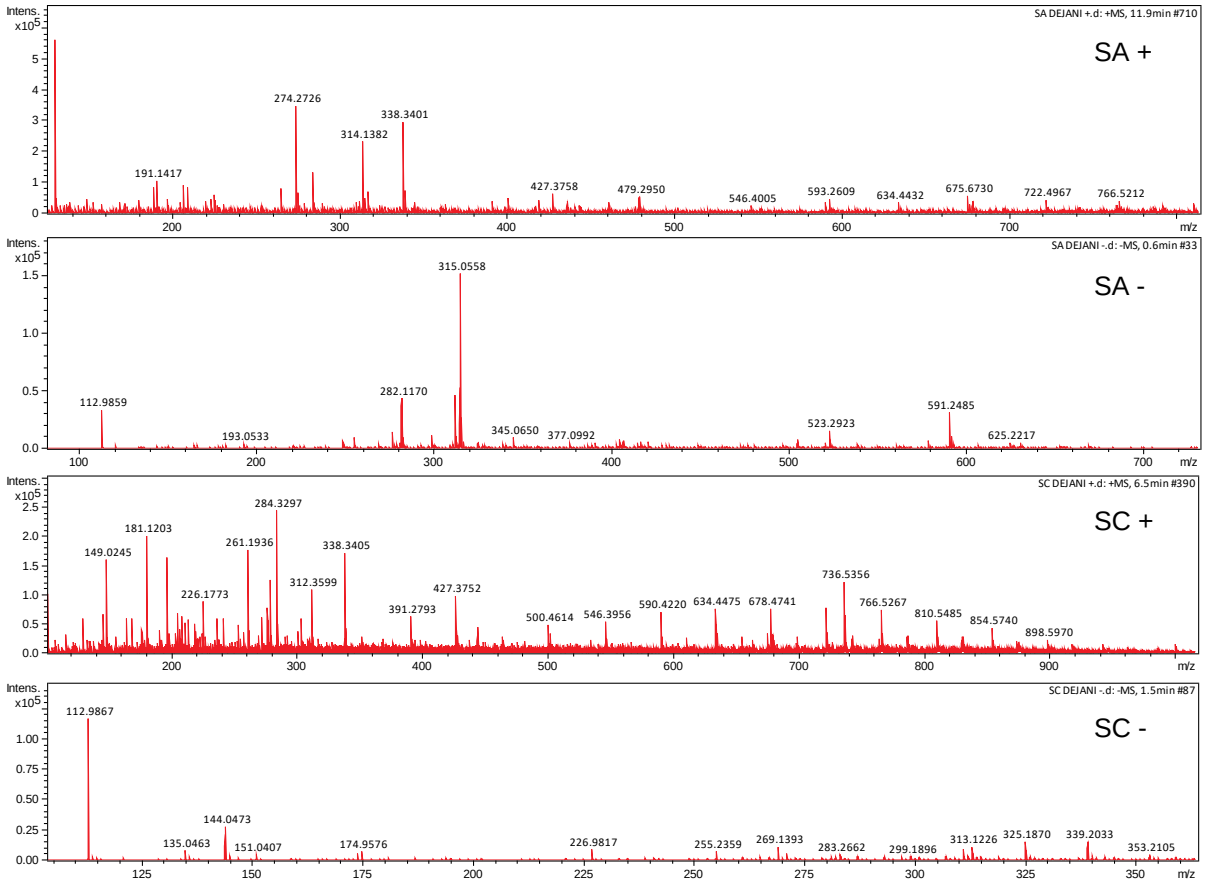


Figure 3. Typical spectra of HRMS for ALK extracts of *Solanum americanum* and *Solanum corymbiflorum*.
Base peak chromatogram (BPC) under positive and negative ionization mode.

CAPÍTULO III

Antioxidant activity of Solanaceae species naturally distributed in dairy farms[5]

[5] Este capítulo é apresentado de acordo com as normas de publicação do ***Journal of Ethnopharmacology*.**
Fator de impacto: 3.115
Fator de impacto (5 anos): 3.671
Website: https://www.journals.elsevier.com/journal-of-ethnopharmacology

Antioxidant activity of Solanaceae species naturally distributed in dairy farms

Dejani Maíra Panazzolo[a], Vivian Fischer[a*], José Ângelo Zuanazzi[b], Elen de Oliveira Alves[b], Krissie D. Soares [b], Miriam A. Apel[b]

[a] *Animal Science Research Program, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

[b] *Laboratory of Pharmacognosy, Department of Raw Material Production, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

∗ Corresponding author at: Faculty of Agronomy, Federal University of Rio Grande do Sul. Av. Bento Gonçalves, 7712, 91540-000, Porto Alegre, Brazil. Tel.: +55 51 999835081; fax: +55 51 33086048.

E-mail address: vivinha.fischer@hotmail.com (V. Fischer)

ABSTRACT

Ethnopharmacological relevance: Solanum corymbiflorum (Sendtn.) Bohs is native in the southern states of Brazil and in Argentina, where indigenous tribes offer it as a food for dairy cows showing clinical signs of mastitis. *Solanum americanum Mill.* is naturally distributed in range pastures and it is consumed by grazing dairy cows although considered as a weed by dairy farmers in the south of Brazil.

Aim of the study: Perform a bio-guided search for the antioxidant activity of the compounds present in the leaves of *Solanum corymbiflorum* (SC) and *Solanum americanum* (SA).

Materials and methods: Three forms of extraction were performed using each plant's leaves: aqueous (AQU), alkaloid (ALK), and methanolic (MET). These extracts were evaluated for their ability to scavenge free radicals through the DPPH radical in the concentrations of 250, 125, 62.5, 31.2, 15.6, and 7.8 *µ*g/mL, and its kinetic behavior in the first 30 min of reaction. The absorbance data (ABS), measured by optical density, were transformed to antioxidant activity (AA%) and subjected to analysis of variance (ANOVA) followed by Tukey's test, adopting $P \leq 0.05$.

Results: MET extract of SA presented antioxidant activity like BHT in the concentrations of 125 and 62.5 *µ*g/mL. AQU and ALK extracts of SA and MET extract of SC presented more than 50 % of antioxidant activity at 250 *µ*g/mL. kinetic behavior shows that MET extract of SA was more efficient than BHT in quenching DPPH radical at 62.5 *µ*g/mL.

Conclusion: MET extracts of SC and SA, as well as AQU and ALK extracts of SA show strong ability of scavenging free radicals tested *in vitro*. Further studies are necessary to test if these extracts present antioxidant activity *in vivo* and may contribute to reduce the clinical symptoms of mastitis, a fact that may be associated with traditional use in dairy cows.

Keywords: antioxidant, ethnoveterinary practices, Solanaceae*, Solanum americanum, Solanum corymbiflorum*, Traditional Associated Knowledge

1. Introduction

Solanum corymbiflorum (Sendtn.) Bohs (syn. *Cyphomandra corymbiflora)*, popularly known as “baga-de-veado, cordão-de-veado” is endemic in the south region of Brazil and Argentina (Bohs, 1989; Keller and Prance, 2012; Mentz et al., 2007). In the local communities’ medicine practices, indigenous tribes of the Misiones Province of Argentine use in the inflammation treatment, e.g leaves can be applied as a hot poultice and friction are used to relieve low back pain, headaches, scabies, tick bite, boils, mastitis, otitis and feet inflammation due to fluid retention (Keller and Prance, 2012). According Keller, (2003) colonies of Brazilian immigrants live in the vicinity of this community and are dedicated to primary agriculture. For this reason, smallholders’ dairy farmers in the south region of Brazil use this specie to treat dairy cows with mastitis symptoms (*personal communication*).

Solanum americanum Mill., known as “maria-pretinha” (Silva et al., 2017), is also found in the natural range in the south region of Brazil. In many places is considered a synonym for *Solanum nigrum* Linn*,* (for relevant details about differences see SÄRKINEN et al., 2018). *S. nigrum* (SN) has been used in northwestern Pakistan, its leaves applied on the skin to cure eczema and edible fruits to cure fever (Sher et al., 2015). Li et al. (2008) reported that SN aqueous extract stimulated the host immune system by increasing the size of the spleen and thymus, the number of CD4+ T lymphocytes as well as the ratio of CD4+/CD8+ T lymphocyte. *S. americanum* is widely distributed in the Rio Grande do Sul state (Mentz et al., 2007), and usually considered as a weed, naturally occurring in paddocks where it is consumed by grazing dairy cows (*personal communication*).

Despite its use based on the claimed traditional knowledge, the effects of SC and SA when ingested by ruminants are still not studied. On the other hand, radical scavenging activity and anti-inflammatory properties of these species have been reported when the extracts of leaves or fruits are applied directly to the wounds and inflamed tissues (Piana et al., 2016b, 2016a; Silva et al., 2017; Vagula et al., 2018). To the best of our knowledge, there is no information about the antioxidant effects comparing these extracts obtained of leaves of *S. americanum* and *S. corymbiflorum*. Therefore, the aim of this study was to conduct a bio-guided assay to test the free radical scavenging capacity and the kinetic reaction of the aqueous, methanolic and alkaloid extracts of *Solanum corymbiflorum* and *Solanum americanum.*

2. Material and methods

2.1 Plant material: source, sampling, and identification

The leaves of SC and SA were harvested at the edge of secondary vegetation and in dairy cows' paddocks located in the northwest of Rio Grande do Sul (27°47'12" S, 53°00'57" W), Brazil, between October and November 2017. The specimens were identified and deposited as exsiccates in the Herbarium of the Institute of Natural Sciences (ICN – UFRGS) under the registration number: *Solanum americanum* Mill: ICN 195408 and *Solanum corymbiflorum* Bohs: ICN 195409. The authors registered the species according to the new Brazilian legislation about the access to the biodiversity - Law 13.123/15 and Decree 8772/16 (Silva and Oliveira, 2018) in the National System of Genetic Heritage and Associated Traditional Knowledge under registration number A382EE. Approximately 2 kg of fresh leaves were dried at room temperature (± 25 °C) in a ventilated place, ground in particle size of 2 mm and stored in paper bags on Pharmacognosy's laboratory of the UFRGS.

2.2 *Extraction and chemical characterization*

Three extraction methods were used for each specimen, SC and SA, following the AQU, MET and ALK extraction methods used in the Pharmacognosy's laboratory. The MET and ALK extracts were subjected to a TLC analysis to search alkaloids. Then, the ALK extract was subjected to liquid chromatography and mass spectrometry.

Aqueous extraction (AQU): A ratio of 2.85 g of ground leaves to 100 mL of distilled water was used (0.2:10 w/v). The sample was extracted with warm water (40°C) for 30 min until it reached room temperature. The solution was filtered on a filter paper, frozen, lyophilized (Savant Micro Modulyo, United States) to obtain the AQU extract, and stored at -20°C until use.

Methanolic extraction (MET): Methanolic extraction followed the method described by Scopel et al., (2010). Briefly, extractive solutions were made using a ratio of 1 g of ground leaves mixed into 20 ml of methanol (1:20 w/v). Extraction proceeded twice with turbolysis (Ultra Turrax Marconi – speed 3 for 3 min equivalent to 9000 RPM), ultrasound (Ultrasound bath cleaner at 132 w power – for 10 minutes), and maceration overnight (Table 01). Total methanolic extract (100 ml) was filtered using filter paper. Methanol was evaporated from the sample using a rotary evaporator (Buchi rotavapor V-710, Switzerland) at 40°C under a maximal pressure of 340

mbars. The dried extract was resuspended in ethyl ether and washed with Mili-Q® water to remove chlorophyll, and frozen (-20°C) for later lyophilization (Savant Micro Modulyo, United States).

Table 1. Process of methanolic extraction.

Quantity and process	Running time	Total of methanol
2 × 20 mL => Turbolysis	3 min	40 mL
2 × 20 mL => Ultrasound	10 min	40 mL
1 × 20 mL => Maceration	24 h (overnight)	20 mL

Alkaloid extraction (ALK): The extraction of total alkaloids was performed according to Reis et al. (2019), mixing 10 g of the ground dried leaves and 100 ml of 10% HCl (v/v) on an ultrasound water bath at 50°C (twice for 15 min). The samples were gravity filtered and the supernatant washed with dichloromethane (3 × 100 mL). The aqueous fraction was alkalinized with 25% ammonium hydroxide (v/v) until pH 9. This alkaline aqueous solution was partitioned with dichloromethane (3 × 100 mL). The organic residue was filtered over anhydrous sodium sulfate (to remove the aqueous residue) and the solvent was evaporated under reduced pressure to recover the alkaloid rich residue.

2.3 Antioxidant activity

DPPH radical scavenging activity: The free radical scavenging capacity of the extracts of SA and SC was evaluated in the presence of DPPH radical (2,2-diphenyl-1-picrylhydrazyl radical). The sample stock solution of each extract was diluted to a final concentration of 250 μg mL^{-1} in methanol (MeOH). A total of 300 μL of the sample was added to 2.700 μL of a 0.3 mM DPPH methanol solution in a cuvette and placed on the spectrophotometer Agilent. Absorbance data (ABS) were obtained by optical density measured at 517 nm every 30 seconds, for the first 5 minutes, then every 5 minutes for up to 30 minutes (Xie and Schaich, 2014). The radical scavenging ability was transformed into antioxidant activity (AA%), calculated as follows: (AA %) = 100 – [(ABS sample – ABS blank) x 100/ ABS control], were: ABS sample = is the absorbance of extracts; ABS blank = is the absorbance of pure

MeOH, and ABS control = is the absorbance of MeOH in DPPH solution (Nascimento et al., 2011). MeOH was used as blank, while DPPH solution plus MeOH was used as the negative control. The positive control was BHT (Butylated Hydroxytoluene) in the same concentrations of the sample. After calculating the scavenging capacity, a serial dilution was performed adjusted to the 250, 125, 62.5, 31.12, 15.62, and 7.81 *μ*g/mL, based on previous concentrations on antibacterial studies with these plants extracts. All tests were done at room temperature.

2.4 Statistical analysis

ABS data were obtained in triplicate, expressed as mean ± SD, and transformed into antioxidant activity percentage. AA % data did not follow the normal distribution and were transformed into new data set log using the formula: $z = x + y$, were z = tAA (transformed AA); x = repetition and y = AA. Data were tested for normality by Shapiro-Wilk ($P \geq 0.05$ = 0.9782) and homogeneity of variances by Levene's test ($P > 0.05$ = 0.9334) and subjected to analysis of variance (Procedure ANOVA of SAS®). Tukey's test was used to compare means and significant differences were declared when $P \leq 0.05$.

3. Results

AQU and ALK extracts of SA as well as the MET extracts of both species showed antioxidant activity higher than 50% at 250 *μ*g/mL. It is worth to notice that 31.1 *μ*g/mL of BHT and 62.5 *μ*g/mL of MET extract of SA reached 90% antioxidant activity (Table 2).

Table 2. Percentage of radical scavenging of *Solanum corymbiflorum* and *Solanum americanum* extracts in different concentrations.

*μ*g/mL	Antioxidant activity (%)						
	BHT	*Solanum corymbiflorum*			*Solanum americanum*		
		Aqueous	Methanolic	Alkaloid	Aqueous	Methanolic	Alkaloid
250	93.58	18.34	58.7	12.5	83.13	90.81	53.04
125	93.34	5.65	24.24	-	-	91.78	46.17
62.5	91.29	-	14.48	-	36.77	91.45	-
31.1	91.96	-	-	-	11.4	29.98	13.04
15.6	83.28	-	-	-	-	17.73	-
7.81	69.51	-	-	-	-	11.03	-

Antioxidant activity did not differ significantly between BHT and MET of SA extract at 125 and 62.5 *µ*g/mL (Fig. 1). Highest antioxidant activity was observed at 250 *µ*g/mL of BHT, but the MET extract of SA also showed strong ability to eliminate the DPPH radical compared to a consolidated commercial antioxidant (BHT).

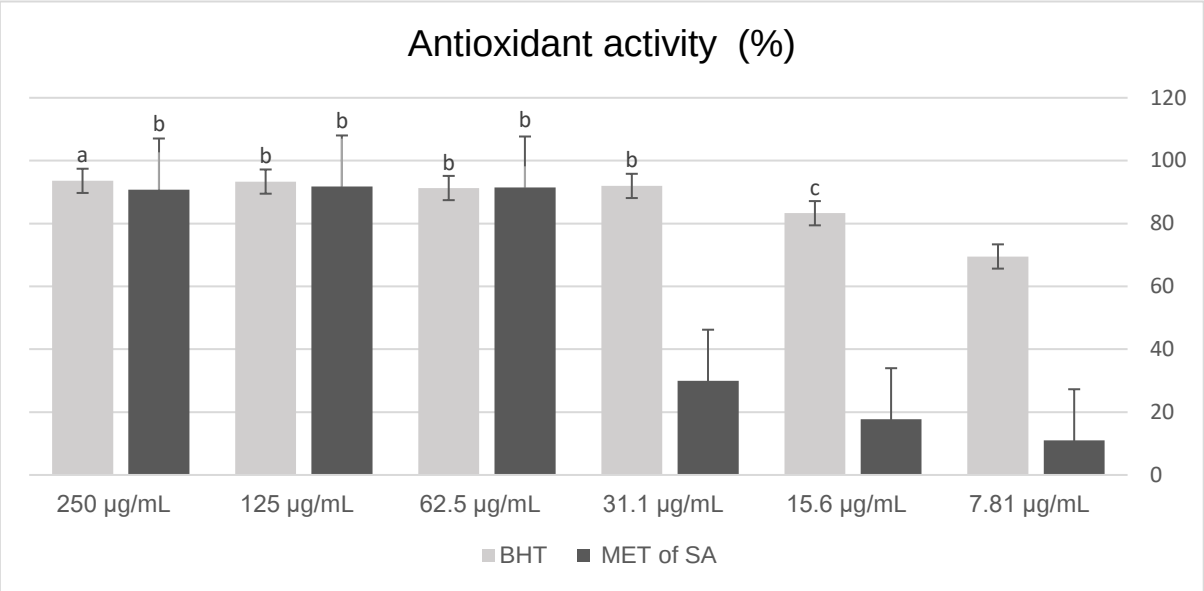


Figure 1. Antioxidant activity of BHT compared to methanolic extract of *Solanum americanum*. [a, b] Means with the same letter are not significantly different (*P<0.05*) on ANOVA followed by Tukey's test. The bars show the standard error.

The evaluation of kinetic behavior in a short term (performed every 5 minutes, for 30 min) indicates that BHT antioxidant activity occurs in a dose-dependent way, where the percentage of radical scavenging activity is directly proportional to the reaction time (Fig.2A). The kinetic effect of MET extract of SA indicated fast and strong extinction of DPPH radical with concentrations equal or greater to 62.5 *µ*g/mL as the sample reached the plateau around 5 minutes after starting the reaction (Fig. 2B). BHT and MET extract of SA showed differences in kinetic behavior within the same concentration (Fig.2C). MET extract of SA started quenching around 60% of DPPH radical and reached almost 90% of activity 5 minutes after the beginning of the reaction, while BHT started with 20% of activity and reached 90% after 20 minutes. In Fig. 2D, AQU and ALK extracts of SA and MET extract of SC at 250 *µ*g/mL concentration showed more than 50% at the end of 30 minutes.

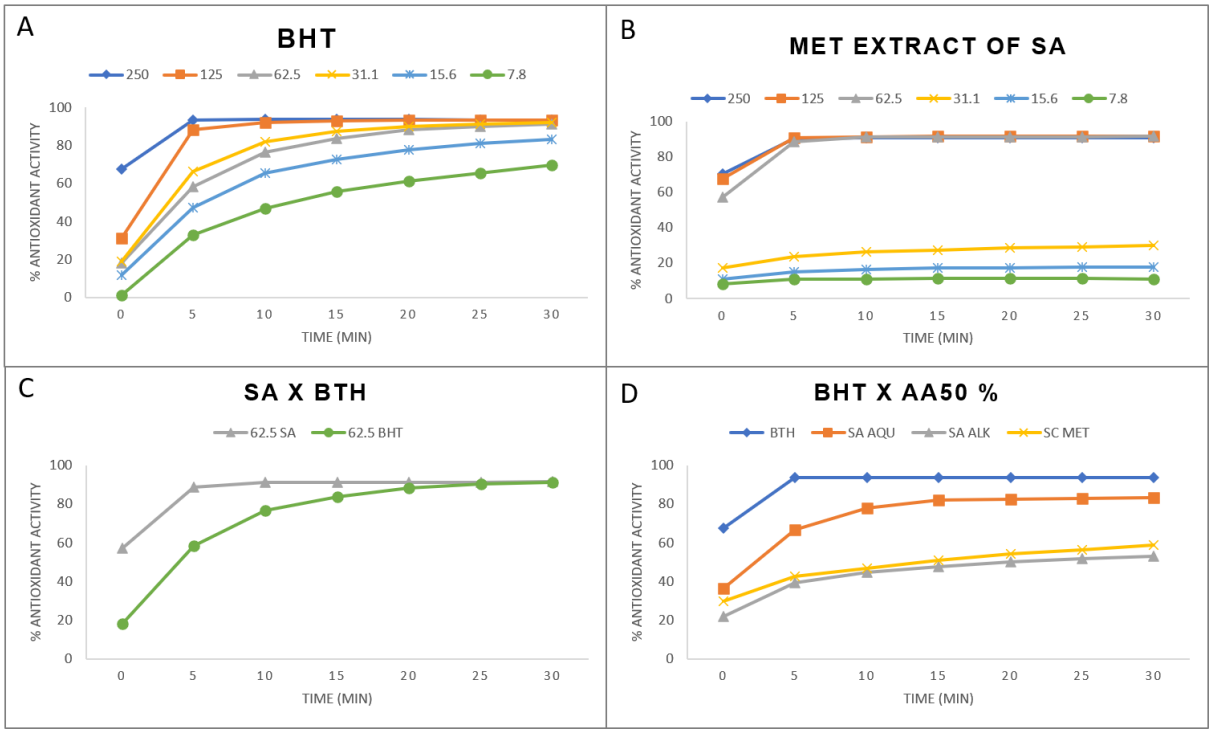


Figure 2. Kinetic behavior of BHT and extracts of *Solanum americanum* and *Solanum corymbiflorum* on quenching DPPH radical.
A) BHT effect in all concentrations. B) Effect of MET extract of SA in all concentrations. C) MET extract of SA and BHT in 62.5 *µ*g/mL. D) Extracts that showed more than 50% AA at 250 *µ*g/mL.

4. Discussion

This study aimed to evaluate the antioxidant capacity of two species of Solanaceae consumed by dairy cows. The main contribution of this study is to show that the extracts of both species SA and SC present *in vitro* antioxidant capacity. The strong activity of the MET extract of SA for scavenging DPPH radical might be related to the ability of methanol (MeOH) to extract polyphenols during the extraction processes (Tiwari et al., 2011). Phenolic compounds are recognized for having a hydroxyl group (-OH) that inhibits reactive oxygen species (ROS), that interrupts the lipid peroxidation chain reaction (Halliwell and Gutteridge, 2015). Polyphenols may exert anti-inflammatory effects notably by radical scavenging activities, regulation of cellular activities in inflammatory cells, modulation of the actives of enzymes, and on the production of the pro-inflammatory cytokines, that help to prevent diseases in humans (Hussain et al., 2016) and animals (Celi, 2010).

Piana et al. (2016b) reported good scavenging radical activity of the ethanolic extract of SC leaves, where the SC_{50} value (scavenging concentration required to quench the DPPH radical in 50%) was 23.94 mg/mL, like ascorbic acid and quercetin standards. These authors attributed this effect to a high content of total polyphenols,

flavonoids and alkaloids found in this specie (Piana et al., 2016a). In the present study, we found similar effects but at a higher dose, as the methanolic extract was efficient at 250 *µ*g/mL. Piana et al. (2016b) also verified anti-edematogenic and anti-inflammatory effects against topical dermatitis in a croton oil induced model by the inhibition of myeloperoxidase activity (Piana et al., 2016b). Vagula et al. (2018) reported that aqueous and chloroform extracts of ripe fruits of *S. americanum* Mill presented significant anti-inflammatory activity. These studies confirm the popular use of SA and SC for reducing the edema formation and the cell infiltrates when applied onto the injured tissue. In the other hand, our study considers the possibility of adding these compounds into the diet, as the traditional knowledge already have been recommending, to help reduce inflammation effects in the mammary gland.

Mastitis is an inflammatory reaction of the mammary gland to bacterial infections or trauma. To combat the infection, the immune system sends phagocytes to the infection site, and through cytokines, they become activated and increase the “respiratory burst” releasing toxic free radicals to bacteria. However, these free radicals also reach the alveolar tissue causing damage in the alveoli of the mammary gland tissue, compromising the secretion of milk. During the early lactation period, both clinical and subclinical mastitis are associated with the release of free radicals, increasing total oxidant capacity, and decreased total antioxidant capacity in milk (Ellah, 2013). *In vitro* and *in vivo* studies indicate that the use of antioxidants and other protective compounds in mastitis control programs is worth investigating, because they may aid in alleviating damage to secretory cells and, thus, reduce subsequent milk loss (Zhao and Lacasse, 2008).

The antioxidant capacity varied according to the Solanum species and extract, verified by the good antioxidant activity on all extracts of SA only the MET extract presented high antioxidant activity in both species. However, more important than the percentage of antioxidant activity at a given time, the speed with which these molecules can quench free radicals is more meaningful. It is worth to notice that DPPH is a stable radical that persists for hours in solution, and thus it may not reflect the effects in living tissues (e.g. mammary gland tissue), where most active radicals such as hydroxyl radicals, superoxide anion, nitric oxide, and lipid peroxyl have lifetimes in the order of milliseconds to seconds. In this case, the number of reactive groups of the antioxidant compound is less important than the speed with which each group can quench the radicals present (Xie and Schaich, 2014).

Antioxidants can quench DPPH radicals by electron and hydrogen transfer: if the reaction is extremely fast in the order of few seconds, free radical stabilization occurred mostly by electron transfer, while when it is slower it might indicate the transfer mechanism of hydrogen atoms (Xie and Schaich, 2014). Vagula et al. (2018) showed that SA has caffeic acid, which has a fast reaction pattern (Xie and Schaich, 2014), and may explain the antioxidant activity exhibited by MET (90%), AQU (83%), and ALK (53%) extracts of SA for quenching radicals. On the other side, the leaves of SC have chlorogenic, caffeic, rosmarinic and gallic acids (Piana et al., 2016b) that have fast and medium reaction patterns (Xie and Schaich, 2014), that might be related to less expressive antioxidant activity than SA.

The detection of N-*trans*-feruloyltyramine in the ALK extract of SA is (*data not are shown*) may positively influence the immune system, inhibiting the production of nitric oxide (NO), prostaglandin E2 (PGE2) and reactive oxygen species (ROS) in lipopolysaccharide-stimulated cells (Jiang et al., 2015). As grazing animals consume these plants distributed in wide range of natural vegetation, we speculate that several secondary compounds may be eliminated through the milk (de Nijs et al., 2017; Hoogenboom et al., 2011; Lejonklev et al., 2013; Viallon et al., 2000). Before their elimination, these compounds of plant's secondary metabolism may act protecting mammary gland tissue from the action of free radicals. Further studies are needed to confirm whether free-range animals consume these plants during grazing or following supplementation and in amounts necessary to allow antioxidant effects to be detected in the mammary gland.

5. Conclusion

MET extracts of *S. americanum* and *S. corymbiflorum* as well as AQU and ALK extracts of *S. americanum* show strong scavenging free radicals' activity in *in vitro* conditions. Our results corroborate the traditional knowledge associated with local smallholder dairy farmers who use it to alleviate clinical symptoms and treat mastitis. Both *S. corymbiflorum* and *S. americanum* present potential anti-inflammatory effects.

Ethical considerations

This study was approved by the post-graduation Commission and Ethic committee

in Use of Animals (CEUA – UFRGS), number 37824, as part of thesis project: Potential use of plants of *Solanum* genus on diseases of dairy herds - Anti-inflammatory and antimicrobial characteristics.

Acknowledgements

The authors would like to thank Lilian Mentz, Márcia Vignoli (*Solanum americanum*) and Edson Soares (*Solanum corymbiflorum*) of Botanical Department of Federal University of Rio Grande do Sul for providing the identification of species, to Brazilian National Research Council (CNPq) and to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the grants.

Author's contributions: The idea for the paper was conceived by Dejani Maíra Panazzolo and Vivian Fischer. The experiments were designed by Dejani Maíra Panazzolo, Vivian Fischer, José Ângelo Zuanazzi and Mirian Apel. The experiments were performed Dejani Maíra Panazzolo, Elen Oliveira and Krissie Soares. The data were analyzed, paper written by Dejani Maíra Panazzolo, and reviewed by Vivian Fischer.

REFERENCES

Bohs, L., 1989. Ethnobotany of the genus Cyphomandra (Solanaceae). Econ. Bot. 43, 143–163. https://doi.org/10.1007/BF02859855

Cai, X.F., Chin, Y.W., Oh, S.R., Kwon, O.K., Ahn, K.S., Lee, H.K., 2010. Anti-inflammatory constituents from solanum nigrum. Bull. Korean Chem. Soc. 31, 199–201. https://doi.org/10.5012/bkcs.2010.31.01.199

Celi, P., 2010. The role of oxidative stress in small ruminants' health and production. Rev. Bras. Zootec. 39, 348–363. https://doi.org/10.1590/S1516-35982010001300038

de Nijs, M., Mulder, P.P.J., Klijnstra, M.D., Driehuis, F., Hoogenboom, R.L.A.P., 2017. Fate of pyrrolizidine alkaloids during processing of milk of cows treated with ragwort. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 34, 2212–2219. https://doi.org/10.1080/19440049.2017.1364432

Ellah, M.R.., 2013. Role of Free Radicals and Antioxidants in Mastitis. Adv. Vet. Res. 3, 1–7.

Halliwell, B., Gutteridge, J.M.C., 2015. Free Radicals in Biology and Medicine, Fifth. ed. Oxford University Press, New York. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001

Hoogenboom, L.A.P., Mulder, P.P.J., Zeilmaker, M.J., van den Top, H.J., Remmelink, G.J., Brandon, E.F.A., Klijnstra, M., Meijer, G.A.L., Schothorst, R., van Egmond, H.P., 2011. Carry-over of pyrrolizidine alkaloids from feed to milk in dairy cows. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 28, 359–372. https://doi.org/10.1080/19440049.2010.547521

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M.C.B., Rahu, N., 2016. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? Oxid. Med. Cell. Longev. https://doi.org/10.1155/2016/7432797

Jiang, Y., Yu, L., Wang, M.H., 2015. N-trans-feruloyltyramine inhibits LPS-induced NO and PGE2 production in RAW 264.7 macrophages: Involvement of AP-1 and MAP kinase signalling pathways. Chem. Biol. Interact. 235, 56–62. https://doi.org/10.1016/j.cbi.2015.03.029

Keller, H.A., 2003. La doctrina de la signatura en una comunidad Mbya Guaraní de San Pedro, Misiones, Argentina. Comun. Científicas y Tecnológicas 1–4.

Keller, H.A., Prance, G.T., 2012. Etnobotánica de las especies de Solanum, Subgénero Bassovia, sección Pachyphylla (Solanaceae) de missiones, Argentina. Bonplandia 21, 45–54.

Lejonklev, J., Løkke, m. m., Larsen, m. K., Mortensen, G., Petersen, m. a., Weisbjerg, m. r., 2013. Transfer of terpenes from essential oils into cow milk. J. Dairy Sci. 96, 4235–4241. https://doi.org/10.3168/jds.2012-6502

Li, J., Li, Q., Feng, T., Li, K., 2008. Aqueous extract of Solanum nigrum inhibit growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells. Fitoterapia 79, 548–556. https://doi.org/10.1016/j.fitote.2008.06.010

Mentz, L.A., Vendruscolo, G.S., Soares, E.L.D.C., Vignoli-Silva, M., 2007. Solanaceae nativas no Rio Grande do Sul, Brasil – Listagem II : Solanum L . Rev. Bras. Biociências 5, 1059–1061.

Nascimento, J., Lage, L., Camargos, C., Amaral, J., Costa, L., Sousa, A., Oliveira, F., 2011. Determinação da atividade antioxidante pelo método DPPH e doseamento de flavonóides totais em extratos de folhas da Bauhinia variegata L. Rev. Bras. Farmácia 92, 327–332.

Piana, M., Boligon, A.A., Stefanello, S.T., Brum, T.F. De, Filippi, C., Mossmann, N.J., Belke, B.V., 2016a. Antioxidant Activity and Phytochemical Composition of Solanum corymbiflorum Fractions (Leaves and Fruits). J. Food Nutr. Res. 4, 736–741. https://doi.org/10.12691/jfnr-4-11-6

Piana, M., Camponogara, C., Boligon, A.A., Machado, M.M., De Brum, T.F., Oliveira, S.M., De Freitas Bauermann, L., 2016b. Topical anti-inflammatory activity of Solanum corymbiflorum leaves. J. Ethnopharmacol. 179, 16–21. https://doi.org/10.1016/j.jep.2015.12.036

Reis, A., Magne, K., Massot, S., Tallini, L.R., Scopel, M., Bastida, J., Ratet, P., Zuanazzi, J.A.S., 2019. Amaryllidaceae alkaloids: identification and partial characterization of montanine production in Rhodophiala bifida plant. Sci. Rep. 9, 27–31. https://doi.org/10.1038/s41598-019-44746-7

Särkinen, T., Poczai, P., Barboza, G.E., van der Weerden, G.M., Baden, M., Knapp, S., 2018. A revision of the Old World Black Nightshades (Morelloid clade of Solanum L., Solanaceae). PhytoKeys 223, 1–223. https://doi.org/10.3897/phytokeys.106.21991

Scopel, M., Mentz, L.A., Henriques, A.T., 2010. Comparative analysis of sambucus nigra and sambucus australis flowers: Development and validation of an HPLC method for raw material quantification and preliminary stability study. Planta Med. 76, 1026–1031. https://doi.org/10.1055/s-0029-1240850

Sher, H., Aldosari, A., Ali, A., De Boer, H.J., 2015. Indigenous knowledge of folk medicines among tribal minorities in Khyber Pakhtunkhwa, northwestern Pakistan. J. Ethnopharmacol. 166, 157–167. https://doi.org/10.1016/j.jep.2015.03.022

Silva, M. da, Oliveira, D.R. de, 2018. The new Brazilian legislation on access to the biodiversity (Law 13,123/15 and Decree 8772/16). Brazilian J. Microbiol. 49, 1–4. https://doi.org/10.1016/j.bjm.2017.12.001

Silva, E.L., Almeida-Lafetá, R.C., Borges, R.M., Staerk, D., 2017. Dual high-resolution inhibition profiling and HPLC-HRMS-SPE-NMR analysis for identification of α-glucosidase and radical scavenging inhibitors in Solanum americanum Mill. Fitoterapia 118, 42–48. https://doi.org/10.1016/j.fitote.2017.02.002

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction - A review. Int. Pharm. Sci. 1, 98–106.

Vagula, J.M., Rocha, B.A., Silva, A.R., Narain, N., Bersani-Amado, C.A., Junior, O.O.S., Visentainer, J. V., 2018. Analysis of Solanum Americanum Mill. by Ultrafast Liquid Chromatography with Diode Array and Time-Of-flight Mass Spectrometry Detection with Evaluation of Anti-Inflammatory Properties in Rodent Models. Anal. Lett. 51, 1973–1985. https://doi.org/10.1080/00032719.2017.1399413

Viallon, C., Martin, B., Verdier-Metz, I., Pradel, P., Garel, J.P., Coulon, J.B., Berdagué, J.L., 2000. Transfer of monoterpenes and sesquiterpenes from forages into milk fat. Lait 80, 635–641. https://doi.org/10.1051/lait:2000150

Xie, J., Schaich, K.M., 2014. Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. J. Agric. Food Chem. 62, 4251–4260. https://doi.org/10.1021/jf500180u

Zhao, X., Lacasse, P., 2008. Mammary tissue damage during bovine mastitis: causes and control. J. Anim. Sci. 86, 57–65. https://doi.org/10.2527/jas.2007-0302

4. Considerações Finais

Esse estudo teve como objetivo evidenciar cientificamente a validade do emprego de *Solanum corymbiflorum,* seguindo o conhecimento tradicional associado de povos indígenas. Assim como testar o potencial de uso de outra solanácea, *Solanum americanum*, distribuída em propriedades leiteiras e naturalmente consumida pelas vacas em pastejo, sobre atividades biológicas intrínsecas à mastite bovina. Esperava-se verificar ação antimicrobiana, com redução do crescimento dos principais patógenos, um gram-positivo e outro gram-negativo, causadores da mastite bovina. A esperada atividade antimicrobiana do extrato de alcaloides não foi observada. Porém, sabendo que os microrganismos, principalmente o *S. aureus* tem a capacidade de desenvolver um fator de virulência sobre qualquer mecanismo que possa causar sua morte, esse resultado não foi considerado negativo.

Com base no ensaio *in vitro* e na análise estatística realizada com dados de distribuição paramétrica, este estudo mostrou que o extrato de alcaloides, das duas espécies de solanáceas, é capaz de inibir a formação de biofilme do *S. aureus.* A questão de prevenir a adesão e o acúmulo em biofilme pode ser relevante para obter sucesso no tratamento de mastite por esse agente, reduzindo os custos de perdas por mastite no futuro. E como consequência, reduzir o uso de antimicrobiano, tanto para medidas profiláticas como para tratamento.

De forma semelhante, o extrato aquoso de SA e o extrato metanólico de SC inibiram a adesão do biofilme de *E. coli*, porém estes mesmos extratos estimularam sua multiplicação bacteriana. Esse fato indica que essas espécies podem não ser eficazes na prevenção do estabelecimento da infecção, gerando dúvidas sobre a relevância na redução da adesão do biofilme.

As solanáceas em geral possuem expressiva capacidade antioxidante, o que foi confirmado pelo resultado obtido com *Solanum americanum*, que apresentou uma capacidade antioxidante similar à de um antioxidante comercial (BHT), em baixas concentrações. Além disso, as solanáceas apresentaram uma velocidade de reação rápida, que pode ser muito relevante para as condições *in vivo*. Todavia, a transposição dos resultados *in vitro* para *in vitro* não pode ser realizada. Portanto, entre as sugestões para a continuidade dessa linha de pesquisa, envolvem estudos *in vivo* sobre a avaliação de toxicidade destes extratos e da atividade biológica sobre a fisiopatologia da mastite.

Além de um estudo fitoquímico aprofundado para elucidação estrutural, quantificação e isolamento de todos os compostos presentes no extrato de alcaloides de ambas as espécies. Estas avaliações poderão apontar doses seguras de compostos ativos a serem fornecidos através da dieta.

REFERÊNCIAS

AMIRYOUSEFI, Ali; HYVÖNEN, Jaakko; POCZAI, Péter. The chloroplast genome sequence of bittersweet (*Solanum dulcamara*): Plastid genome structure evolution in Solanaceae. **PLoS ONE**, San Francisco, v. 13, n. 4, p. 1–23, 2018.

BIANCO, S.; CARVALHO, L. B.; BIANCO, M. S. Growth, and mineral nutrition of *Solanum americanum*. **Planta Daninha**, Viçosa, MG, v. 28, n. 2, p. 293–299, 2010.

BOHS, Lynn. Ethnobotany of the genus Cyphomandra (Solanaceae). **Economic Botany**, Bronx, v. 43, p. 143–163, 1989.

BOHS, Lynn. Transfer of Cyphomandra (Solanaceae) and its species to Solanum. **Taxon**, Ann Arbor, v. 44, n. 4, p. 583–587, 1995. Disponível em: http://www.jstor.org/stable/1223500. Acesso em: 16 out. 2016.

BOHS, Lynn. Phylogeny of the Cyphomandra clade of the genus *Solanum* (Solanaceae) based on ITS sequence data. **Taxon**, Ann Arbor, v. 56, p. 1012–1026, 2007.

BUDRI, P. E. *et al*. Effect of essential oils of *Syzygium aromaticum* and *Cinnamomum zeylanicum* and their major components on biofilm production in *Staphylococcus aureus* strains isolated from milk of cows with mastitis. **Journal of Dairy Science**, Champaign, v. 98, n. 9, p. 5899–5904, 2015. Disponível em: http://dx.doi.org/10.3168/jds.2015-9442. Acesso em: 16 dez. 2019.

BURTON, Jeanne L.; ERSKINE, Ronald J. Immunity, and mastitis: some new ideas for an old disease. **The Veterinary Clinics of North America. Food Animal Practice**, Philadelphia, v. 19, n. 1, p. 1–45, 2003.

CHOUBEY, Mahipal *et al*. Dietary supplementation of a novel phytogenic feed additive: effects on nutrient metabolism, antioxidant status and immune response of goats. **Animal Production Science**, Collingwood, v. 56, n. 10, p. 1612-1621, 2015.

COSTA, João Carlos Miguel *et al*. Increased production of biofilms by *Escherichia coli* in the presence of enrofloxacin. **Veterinary Microbiology**, Amsterdam, v. 160, n. 3/4, p. 488–490, 2012.

COSTERTON, J. W.; STEWART, Philip S.; GREENBERG, E. P. Bacterial biofilms: a common cause of persistent infections. **Science**, Washington, DC, v. 284, n. 5418, p. 1318-1322, 1999.

DE NIJS, Monique *et al*. Fate of pyrrolizidine alkaloids during processing of milk of cows treated with ragwort. **Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment**, London, v. 34, n. 12, p. 2212–2219, 2017. Disponível em: https://doi.org/10.1080/19440049.2017.1364432. Acesso em: 7 maio 2020.

DERAKHSHANI, Hooman *et al*. Invited review: microbiota of the bovine udder: contributing factors and potential implications for udder health and mastitis susceptibility. **Journal of Dairy Science**, Champaign, v. 101, n. 12, p. 10605–10625, 2018.

DIVYAGNANESWARI, M.; CHRISTYBAPITA, D.; MICHAEL, R. Dinakaran. Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf fractions. **Fish and Shellfish Immunology**, London, v. 23, p. 249–259, 2007.

DODD, F. H.; NEAVE, F. K.; KINGWILL, R. G. Control of udder infection by management. **Journal of Dairy Science**, Champaign, v. 47, n. 10, p. 1109–1114, 1964. Disponível em: http://dx.doi.org/10.3168/jds.S0022-0302(64)88858-5. Acesso em: 12 jun. 2020.

DRACKLEY, James K. Biology of dairy cows during the transition period: the final frontier? **Journal of Dairy Science**, Champaign, v. 82, n. 11, p. 2259–2273, 1999. Disponível em: http://dx.doi.org/10.3168/jds.S0022-0302(99)75474-3. Acesso em: 12 set. 2020.

ELLAH, M. R. Role of free radicals and antioxidants in mastitis. **Journal of Advanced Veterinary Research**, Assiut, v. 3, p. 1–7, 2013.

GARCIA, Sara N.; OSBURNB, Bennie I.; CULLOR, James S. A one health perspective on dairy production and dairy food safety. **One Health**, Amsterdam, v. 7, [art.] 100086, June 2019. Disponível em: https://doi.org/10.1016/j.onehlt.2019.100086. Acesso em: 12 jun. 2020.

GREEN, Martin; BRADLEY, Andrew. Mastitis: the changing face of mastitis control. **The Veterinary Record**, London, v. 173, n. 21, p. 517–521, 2013.

GUERRA, Simony Trevizan *et al*. Virulence factors of *Escherichia coli*: an overview of human and animal infections with emphasis in bovine mastitis. **Semina: Ciências Agrárias**, Londrina, v. 40, n. 5, p. 2087-2100, 2019.

HALASA, T. *et al*. Economic effects of bovine mastitis and mastitis management: a review. **The Veterinary Quarterly**, Abingdon, v. 29, n. 1, p. 18–31, 2007.

HALLIWELL, Barry; GUTTERIDGE, John M. C. **Free radicals in biology and medicine**. 5th ed. New York: Oxford University Press, 2015.

HARIKRISHNAN, Ramasamy *et al*. *Solanum nigrum* enhancement of the immune response and disease resistance of tiger shrimp, *Penaeus monodon* against *Vibrio harveyi*. **Aquaculture**, Amsterdam, v. 318, n. 1/2, p. 67–73, 2011. Disponível em: http://dx.doi.org/10.1016/j.aquaculture.2011.05.024. Acesso em: 24 jun. 2015.

HEIKKILÄ, A. M. *et al*. Pathogen-specific production losses in bovine mastitis. **Journal of Dairy Science**, Champaign, v. 101, n. 10, p. 9493–9504, 2018. Disponível em: http://dx.doi.org/10.3168/jds.2018-14824. Acesso em: 15 maio 2020.

HERRY, Vincent *et al*. Local immunization impacts the response of dairy cows to Escherichia coli mastitis. **Scientific Reports**, London, v. 7, [art.] 3441, 2017.

HIDAYAT, Topik *et al*. Molecular phylogenetic screening of *Withania somnifera* relative from Indonesia based on internal transcribed spacer region. **HAYATI Journal of Biosciences**, Bogor, v. 23, n. 2, p. 92–95, 2016. Disponível em: http://dx.doi.org/10.1016/j.hjb.2016.02.002. Acesso em: 29 abr. 2020.

HILLERTON, Eric; BOOTH, James M. The five-point mastitis control plan-a revisory tutorial! *In*: ANNUAL MEETING OF THE NATIONAL MASTITIS COUNCIL, 57., 2018, Tucson, Arizona. **Proceedings** [...]. New Prague: NMC, 2018. p. 3–17.

HONG, Heeok; LEE, Jun Hyeong; KIM, Soo Ki. Phytochemicals and antioxidant capacity of some tropical edible plants. **Asian-Australasian Journal of Animal Sciences**, Seoul, v. 31, n. 10, p. 1677–1684, 2018.

HOOGENBOOM, L. A. P. *et al*. Carry-over of pyrrolizidine alkaloids from feed to milk in dairy cows. **Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment**, London, v. 28, n. 3, p. 359–372, 2011.

ISHII, Junichiro *et al*. Feeding *Solanum glaucophyllum* to preparturient multiparous cows prevents postparturient hypocalcemia. **Animal Science Journal**, Richmond, v. 86, n. 10, p. 869–877, 2015.

KAPER, James B.; NATARO, James P.; MOBLEY, Harry L. T. Pathogenic *Escherichia coli*. **Nature Reviews Microbiology**, London, v. 2, n. 2, p. 123–140, 2004.

KELLER, H. A.; PRANCE, G. T. Etnobotánica de las especies de Solanum, subgénero Bassovia, sección Pachyphylla (Solanaceae) de missiones, Argentina. **Bonplandia**, Corrientes, v. 21, n. 1, p. 45–54, 2012.

KELLER, Héctor A. La doctrina de la signatura en una comunidad Mbya Guaraní de San Pedro, Misiones, Argentina. **Comunicaciones Científicas y tecnológicas**, [Corrientes], p. 1–4, 2003.

KIM, Su Jeong *et al*. Antimicrobial resistance and genetic characterization of coagulase-negative staphylococci from bovine mastitis milk samples in Korea. **Journal of Dairy Science**, Champaign, v. 102, n. 12, p. 11439–11448, 2019. Disponível em: http://dx.doi.org/10.3168/jds.2019-17028. Acesso em: 18 dez. 2019.

KNAPP, Sandra *et al*. A revision of the morelloid clade of Solanum L. (Solanaceae) in North and Central America and the Caribbean. **PhytoKeys**, Sofia, v. 123, p. 1–144, 2019.

KUTCHAN, Toni M. Alkaloid biosynthesis - The basis of metabolic engineering of medicinal plants. **Plant Cell**, Rockville, v. 7, n. 7, p. 1059–1070, 1995. Acesso em: 20 out. 2019.

LAGO, A. *et al*. The selective treatment of clinical mastitis based on on-farm culture results: II. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. **Journal of Dairy Science**, Champaign, v. 94, n. 9, p. 4457–4467, 2011. Disponível em: http://dx.doi.org/10.3168/jds.2010-4047. Acesso em: 12 jun. 2020.

LEE, Andie S. *et al*. Methicillin-resistant *Staphylococcus aureus*. **Nature Reviews Disease Primers**, London, v. 4, n. 1, p. 1–23, 2018.

LEJONKLEV, J. *et al*. Transfer of terpenes from essential oils into cow milk. **Journal of Dairy Science**, Champaign, v. 96, n. 7, p. 4235–4241, 2013. Disponível em: http://dx.doi.org/10.3168/jds.2012-6502. Acesso em: 10 jan. 2015.

LI, Tianming *et al*. Molecular characteristics of *Staphylococcus aureus* causing bovine mastitis between 2014 and 2015. **Frontiers in Cellular and Infection Microbiology**, Lausanne, v. 7, [art.] 127, [p. 1–10], 2017.

LILLEHOJ, Hyun *et al*. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. **Veterinary Research**, Paris, v. 49, n. 1, p. 1–18, 2018. Disponível em: https://doi.org/10.1186/s13567-018-0562-6. Acesso em: 10 nov. 2019.

LOO, Enli; LAI, Kok Song; MANSOR, Rozaihan. Antimicrobial usage and resistance in dairy cattle production. *In*: BEKOE, S. O.; SARAVANAN, M. (ed.). **Veterinary medicine and pharmaceuticals**. London: IntechOpen, 2019. p. 16.

MELO, Poliana de Castro *et al*. Comparison of methods for the detection of biofilm formation by *Staphylococcus aureus* isolated from bovine subclinical mastitis. **Brazilian Journal of Microbiology**, São Paulo, v. 44, n. 1, p. 119–124, 2013.

MENTZ, Lilian Auler *et al*. Solanaceae nativas no Rio Grande do Sul, Brasil – Listagem II: Solanum L. **Revista Brasileira de Biociências**, Porto Alegre, v. 5, p. 1059–1061, 2007.

MUBARACK, H. Muhamed *et al*. Activity of some selected medicinal plant extracts against bovine mastitis pathogens. **Journal of Animal and Veterinary Advances**, Faisalabad, v. 10, n. 6, p. 738 – 741, 2011.

OLAGARAY, K. E.; BRADFORD, B. J. Plant flavonoids to improve productivity of ruminants – A review. **Animal Feed Science and Technology**, Amsterdam, v. 251, n. Mar. 2018, p. 21–36, 2019. Disponível em: https://doi.org/10.1016/j.anifeedsci.2019.02.004. Acesso em: 21 maio 2020.

OLIVEIRA, L.; LANGONI, H.; HULLAND, C. Recovered from clinical and subclinical cases of bovine mastitis. **Journal of Dairy Science**, Champaign, v. 95, p. 1913–1920, 2012.

OLIVER, Stephen P.; MURINDA, Shelton E. Antimicrobial resistance of mastitis pathogens. **The Veterinary Clinics of North America. Food Animal Practice,** Philadelphia, v. 28, n. 2, p. 165–185, 2012. Disponível em: http://dx.doi.org/10.1016/j.cvfa.2012.03.005. Acesso em: 16 out. 2016.

OLMSTEAD, Richard G. *et al*. A molecular phylogeny of the Solanaceae. **Taxon**, Ann Arbor, v. 57, n. 4, p. 1159–1181, 2008.

OVIEDO-BOYSO, Javier *et al*. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. **The Journal of Infection**, Kent, v. 54, p. 399–409, 2007.

PAGARE, Saurabh *et al*. Secondary metabolites of plants and their role: overview. **Current Trends in Biotechnology and Pharmacy**, Gunter, v. 9, n. 3, p. 293–304, 2015.

PAULRUD, C. O. Basic concepts of the bovine teat canal. **Veterinary Research Communications**, Dordrecht, v. 29, p. 215–245, 2005.

PEREIRA, Marcella Nunes; SCUSSEL, Vildes Maria. Resíduos de antimicrobianos em leite bovino: fonte de contaminação, impactos e controle. **Revista de Ciências Agroveterinárias**, Lages, v. 16, n. 2, p. 170–182, 2017.

PERES, Lázaro E. P. **Metabolismo secundário**. [Piracicaba]: Escola Superior de Agricultura" Luiz de Queiroz", 2015. [Apostila]. Disponível em: http://files.farmacia-unig-20160.webnode.com/200000038-10ef411e95/ce5449dfcf0e02f741a5af86c3c5ae9a.pdf. Acesso em: 14 set. 2020.

PHOPHI, Lufuno; PETZER, Inge Marie; QEKWANA, Daniel Nenene. Antimicrobial resistance patterns and biofilm formation of coagulase-negative *Staphylococcus* species isolated from subclinical mastitis cow milk samples submitted to the Onderstepoort Milk Laboratory. **BMC Veterinary Research**, London, v. 15, n. 1, [art.] 420, 2019.

PIANA, Mariana *et al*. Topical anti-inflammatory activity of *Solanum corymbiflorum* leaves. **Journal of Ethnopharmacology**, Lausanne, v. 179, p. 16–21, 2016a.

PIANA, Mariana *et al*. Antioxidant activity and phytochemical composition of *Solanum corymbiflorum* fractions (leaves and fruits). **Journal of Food and Nutrition Research**, Newark, v. 4, n. 11, p. 736–741, 2016b. Disponível em: http://pubs.sciepub.com/jfnr/4/11/6/index.html. Acesso em: 13 jan. 2020.

POULOPOULOU, I. *et al*. Terpenes transfer to milk and cheese after oral administration to sheep fed indoors. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v. 96, n. 2, p. 172–181, 2012.

PROVENZA, F. D. *et al*. Linking herbivore experience, varied diets, and plant biochemical diversity. **Small Ruminant Research**, Amsterdam, v. 49, n. 3, p. 257–274, 2003.

PROVENZA, F. D.; VILLALBA, J. J. The role of natural plant products in modulating the immune system: an adaptable approach for combating disease in grazing animals. **Small Ruminant Research**, Amsterdam, v. 89, n. 2/3, p. 131–139, 2010. Disponível em: http://dx.doi.org/10.1016/j.smallrumres.2009.12.035. Acesso em: 26 nov. 2019.

RAINARD, P. *et al*. Knowledge gaps and research priorities in *Staphylococcus aureus* mastitis control. **Transboundary and Emerging Diseases**, Oxford, v. 65, p. 149–165, 2018. Supl. 1.

RAINARD, Pascal. Tackling mastitis in dairy cows. **Nature Biotechnology**, New York, v. 23, n. 4, p. 430–432, 2005.

RAZA, Ali *et al*. Biofilm producing *Staphylococcus aureus* and bovine mastitis: a review. **Molecular Microbiology Research**, Richmond, v. 3, n. 1, p. 1-8, 2013.

REYHER, K. K. *et al*. Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new intramammary infections with major mastitis pathogens-A systematic review and meta-analysis. **Journal of Dairy Science**, Champaign, v. 95, n. 11, p. 6483–6502, 2012. Disponível em: http://dx.doi.org/10.3168/jds.2012-5594. Acesso em: 3 fev. 2020.

RICHARDSON, Emily J. *et al*. Gene exchange drives the ecological success of a multi-host bacterial pathogen. **Nature Ecology and Evolution**, London, v. 2, n. 9, p. 1468–1478, 2018. Disponível em: http://dx.doi.org/10.1038/s41559-018-0617-0. Acesso em: 13 jun. 2020.

ROLLIN, E.; DHUYVETTER, K. C.; OVERTON, M. W. The cost of clinical mastitis in the first 30 days of lactation: an economic modeling tool. **Preventive Veterinary Medicine**, Amsterdam, v. 122, n. 3, p. 257–264, 2015. Disponível em: http://dx.doi.org/10.1016/j.prevetmed.2015.11.006. Acesso em: 13 jan. 2020.

RUEGG, Pamela L. New perspectives in udder health management. **The Veterinary Clinics of North America. Food Animal Practice**, Philadelphia, v. 28, n. 2, p. 149–163, 2012. Disponível em: http://dx.doi.org/10.1016/j.cvfa.2012.03.001. Acesso em: 16 out. 2016.

RUEGG, Pamela L. A 100-year review: mastitis detection, management, and prevention. **Journal of Dairy Science**, Champaign, v. 100, n. 12, p. 10381–10397, 2017. Disponível em: http://dx.doi.org/10.3168/jds.2017-13023. Acesso em: 13 jan. 2020.

SAGLAM, Aliye Gülmez *et al*. The role of staphylococci in subclinical mastitis of cows and lytic phage isolation against to *Staphylococcus aureus*. **Veterinary World**, Dist. Morbi, v. 10, n. 12, p. 1481–1485, 2017.

SÄRKINEN, Tiina *et al*. A revision of the Old-World Black Nightshades (Morelloid clade of Solanum L., Solanaceae). **PhytoKeys**, Sofia, v. 223, n. 106, p. 1–223, 2018.

SHARMA, Neelesh; JEONG, Dong Kee. Stem cell research: a novel boulevard towards improved bovine mastitis management. **International Journal of Biological Sciences**, Lake Haven, v. 9, n. 8, p. 818–829, 2013.

SILVA, Eder L. *et al*. Dual high-resolution inhibition profiling and HPLC-HRMS-SPE-NMR analysis for identification of α-glucosidase and radical scavenging inhibitors in *Solanum americanum* Mill. **Fitoterapia**, Amsterdam, v. 118, p. 42–48, 2017. Disponível em: http://dx.doi.org/10.1016/j.fitote.2017.02.002. Acesso em: 22 nov. 2019.

SILVA, Laura Nunes *et al*. Plant natural products targeting bacterial virulence factors. **Chemical Reviews**, Washington, DC, v. 116, n. 16, p. 9162–9236, 2016.

SOARES, Edson Luís de Carvalho; MENTZ, Lilian Auler. As espécies de Solanum subgênero Bassovia seção Pachyphylla (= Cyphomandra Mart. Ex Sendtn. - Solanaceae) no Rio Grande do Sul, Brasil. **Pesquisas Botânicas**, São Leopoldo, v. 57, p. 231–254, 2006.

SORDILLO, Lorraine M.; STREICHER, Katie L. Mammary gland immunity and mastitis susceptibility. **Journal of Mammary Gland Biology and Neoplasia**, Dordrecht, v. 7, n. 2, p. 135–146, 2002.

SPEARS, Jerry W.; WEISS, William P. Role of antioxidants and trace elements in health and immunity of transition dairy cows. **The Veterinary Journal**, London, v. 176, n. 1, p. 70–76, 2008. Disponível em: http://dx.doi.org/10.1016/j.tvjl.2007.12.015. Acesso em: 17 set. 2020.

SURIYASATHAPORN, W. *et al*. Higher somatic cell counts resulted in higher malondialdehyde concentrations in raw cows' milk. **International Dairy Journal**, Barking, v. 16, n. 9, p. 1088–1091, 2006.

TEDESCO, D. *et al*. Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. **Journal of Dairy Science**, Champaign, v. 87, n. 7, p. 2239–47, 2004. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/15328238. Acesso em: 28 jan. 2015.

TORNAMBÉ, G. *et al*. Addition of pasture plant essential oil in milk: influence on chemical and sensory properties of milk and cheese. **Journal of Dairy Science**, Champaign, v. 91, n. 1, p. 58–69, 2008.

VIALLON, Christine *et al*. Transfer of monoterpenes and sesquiterpenes from forages into milk fat. **Le Lait**, Les Ulis, v. 80, n. 6, p. 635–641, 2000.

VILLALBA, Juan J. *et al*. Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism, and origins. **Parasite**, Issy-les-Moulineaux, v. 21, [art.] 31, 2014.

WANG, Siyu *et al*. Identification and quantification of potential anti-inflammatory hydroxycinnamic acid amides from wolfberry. **Journal of Agricultural and Food Chemistry**, Washington, DC, v. 65, n. 2, p. 364–372, 2017.

ZHAO, X.; LACASSE, P. Mammary tissue damage during bovine mastitis causes and control. **Journal of Animal Science**, Champaign, v. 86, p. 57–65, 2008.

ANEXOS

Anexo A. Programa de Controle de Mastite.

NMC
A global organization for mastitis control and milk quality

RECOMMENDED MASTITIS CONTROL PROGRAM

605 Columbus Avenue South | New Prague, MN 56071 | Phone 952-758-2146 | Fax 952-758-5813

Name/Farm: _____ Date: _____

1. Establishment of Goals for Udder Health

- ☐ Set realistic targets for average herd somatic cell count (SCC) or linear score and clinical mastitis rate.
- ☐ Review goals on a timely basis, with input from the Herd Udder Health Advisory Team (veterinarian, producer, herd manager, milking personnel and advisors).
- ☐ Prioritize management changes to achieve stated goals.
- ☐ Other: _____

2. Maintenance of a Clean, Dry, Comfortable Environment

- ☐ Ensure proper stall usage by ensuring adequacy of stall size and design and provide adequate space in loose yards for the number of cows housed.
- ☐ Maintain clean, dry, and comfortable stalls and yards through appropriate bedding management.
- ☐ Keep cow traffic areas clean and dry.
- ☐ Ensure ventilation system is functioning properly.
- ☐ Ensure cows at pasture have uncontaminated lying areas.
- ☐ Control detrimental environmental influences (heat stress, frostbite, stray voltage, etc.).
- ☐ Ensure that cows remain standing after milking (provide fresh feed and water).
- ☐ Other: _____

3. Proper Milking Procedures

- ☐ Examine foremilk to facilitate early detection of clinical mastitis and proper milk letdown.
- ☐ Ensure teats are clean and dry before milking.
- ☐ If local regulations allow, apply pre-milking teat disinfectant that completely covers the teat skin and allow it to remain on teats for at least 30 seconds then dry teats using a properly washed and disinfected cloth towel for use on one cow, or a single service paper towel.
- ☐ Wear clean gloves during the milking process to limit spread of contagious pathogens.
- ☐ Attach teat cups squarely and level with the udder within 90 seconds of udder preparation.
- ☐ Adjust cluster during milking to prevent liner slips and squawks.
- ☐ With manual removal, avoid machine stripping and shut off vacuum to the claw before removing cluster.
- ☐ Apply teat disinfectant immediately following teat cup removal, and assure complete coverage of teats.
- ☐ Any teat disinfectant should be selected based on documented efficacy data which can be found on the NMC website (www.nmconline.org).
- ☐ To optimize mastitis control and reduce costs, teat dipping is preferred to spraying as the method of disinfectant application.
- ☐ Milk cows with confirmed contagious intramammary infections last.
- ☐ Other: _____

4. Proper Maintenance and Use of Milking Equipment

- ☐ Install or update equipment to ISO 5707 (International Organization for Standardization, "Milking machine installations–Construction and performance").
- ☐ Service, maintain, and regularly evaluate equipment function according to manufacturer's guidelines, using dynamic evaluation methods and an appropriate record form.
- ☐ Replace liners and other rubber and plastic parts regularly, according to manufacturer's guidelines.
- ☐ Replace broken or cracked inflations and short milk tubes immediately.
- ☐ Thoroughly wash and sanitize equipment after each milking.
- ☐ Other: _____

5. Good Record Keeping

- ☐ For each case of clinical mastitis, record cow identification, date detected, days in milk, quarter(s) affected, number and type of treatments, outcome of treatments (i.e. return to normal milk, time to discard milk) and the causative bacterial pathogen if a sample was cultured on-farm or in a laboratory.
- ☐ Use a computerized or manual record system to manage information, such as individual cow SCC data, on the prevalence and incidence of subclinical mastitis.
- ☐ Other: _____

6. Appropriate Management of Clinical Mastitis During Lactation

- ☐ Develop and implement a herd clinical mastitis treatment protocol with the Herd Udder Health Advisory team.
- ☐ Carefully consider the economic ramifications of therapy decisions.
- ☐ Collect a pre-treatment milk sample aseptically for microbiological culture so that antimicrobial susceptibility tests can be used when appropriate.

- ☐ Use an appropriate therapeutic regimen; use drugs according to the protocol, or as recommended by the health advisors.
- ☐ Prior to infusion, disinfect the teat with a germicide and scrub the teat-end with an alcohol swab.
- ☐ For infusion of intramammary antibiotics, use a single-dose, regulatory approved product by the partial insertion method.
- ☐ Do not treat chronic non-responsive infections.
- ☐ Observe the correct withdrawal period for the antibiotic used, as stated on the label. If extra-label drug use is necessary, follow regulatory guidelines under the supervision of a veterinarian (i.e. in the systemic treatment of coliform mastitis).
- ☐ Always follow recommended drug storage guidelines and observe expiration dates.
- ☐ Clearly identify all treated cows, and record all treatments in a permanent record.
- ☐ When necessary, test milk for inhibitory substances before consignment.
- ☐ Other: _____

7. Effective Dry Cow Management

- ☐ Decrease the energy density of the ration during late lactation to reduce milk production before dry-off.
- ☐ Dry cows off abruptly and dry treat each quarter immediately following the last milking of lactation.
- ☐ Disinfect teats and scrub the teat-end with an alcohol swab before infusing.
- ☐ Treat all quarters of all cows with a commercially available approved [long-acting] dry-cow antibiotic and/or an approved internal teat sealant.
- ☐ Use the partial insertion method of dry treatment infusion.
- ☐ Disinfect teats immediately following infusion using any approved post milking disinfectant teat dip.
- ☐ Provide adequate dry cow nutrition to enhance immune system function.
- ☐ Maintain a clean, dry, comfortable environment for dry cows. Dry cow environmental management is important to minimize exposure to pathogens.
- ☐ In situations of high environmental pathogen exposure, use an internal or external teat sealant for dry cows in addition to any antimicrobial product.
- ☐ In herds with coliform mastitis problems, vaccinate with a core antigen endotoxin vaccine following manufacturer's directions.
- ☐ Clip flanks and udders to remove excess body hair. Udder singeing may be useful to ensure hair removal.
- ☐ Other: _____

8. Maintenance of Biosecurity for Contagious Pathogens and Marketing of Chronically Infected Cows

- ☐ Request bulk tank and individual cow SCC data. For suspect animals, further diagnostic efforts may be indicated to identify cases of subclinical mastitis prior to purchasing cows.
- ☐ If possible, obtain aseptically collected milk samples for bacteriological culture from cows prior to purchase.
- ☐ Isolate recently purchased cows, and milk separately, until there is assurance of the absence of intramammary infection.
- ☐ Segregate cows with a persistently high SCC or linear score (i.e. SCC greater than 200,000 or linear score greater than or equal to 4.0 for several months) and observe response to dry treatment or other recommended therapy.
- ☐ Market or permanently segregate cows persistently infected with *Staphylococcus aureus* or other non-responsive microbial agents (*Mycoplasma, Nocardia, Pseudomonas, or Arcanobacterium pyogenes*).
- ☐ Consider udder health status of first-calf heifers as this can impinge on herd biosecurity.
- ☐ Other: _____

9. Regular Monitoring of Udder Health Status

- ☐ Enroll in an individual cow SCC program or use some other monitor of subclinical infections.
- ☐ Use a sensitive cow-side monitor of inflammation in cows suspected of infection and in high risk periods (i.e. early lactation).
- ☐ Monitor distributions of high SCC cows, and rates of change to elevated SCC.
- ☐ Conduct milk bacteriological culture of clinical cases and high SCC cows regularly.
- ☐ Monitor udder health for the herd using reports from the regional regulatory agency or milk marketing organization and DHI.
- ☐ Calculate clinical mastitis rates and distributions regularly, paying particular attention to infections in heifers.
- ☐ Use SCC and clinical mastitis records to evaluate protocols, and to make treatment and marketing decisions.
- ☐ Other: _____

10. Periodic Review of Mastitis Control Program

- ☐ Obtain objective evaluations from veterinarian, industry field person or extension representative.
- ☐ A step-by-step approach to the review, and a standard evaluation form are useful.
- ☐ Make use of the entire Herd Udder Health Advisory Team: veterinarian, producer, herd manager, milking personnel, and advisors.
- ☐ Other: _____

Anexo B: Normas utilizadas para a preparação dos Capítulos II e III

Guide for Authors: JOURNAL OF ETHNOPHARMACOLOGY
An Interdisciplinary Journal Devoted to Indigenous Drugs

The *Journal of Ethnopharmacology* is dedicated to the exchange of information and understandings about people's use of plants, fungi, animals, microorganisms and minerals and their biological and pharmacological effects based on the principles established through international conventions. Early people, confronted with illness and disease, discovered a wealth of useful therapeutic agents in the plant and animal kingdoms. The empirical knowledge of these medicinal substances and their toxic potential was passed on by oral tradition and sometimes recorded in herbals and other texts on *materia medica*. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine) came into use through the study of indigenous remedies. Chemists continue to use plant-derived drugs (e.g., morphine, taxol, physostigmine, quinidine, emetine) as prototypes in their attempts to develop more effective and less toxic medicinals.

Please note that figures and tables should be embedded in the text as close as possible to where they are initially cited. It is also mandatory to upload separate graphic and table files as these will be required if your manuscript is accepted for publication.

Classification of your paper

Please note that upon submitting your article you will have to select **at least one classification** and **at least three of the given keywords**. You can preview the list of classifications and keywords. This information is needed by the Editors to more quickly process your article. In addition to this, you can submit free keywords as described below under "Keywords".

1:CLASSIFICATIONS
1.01:Anaesthesia
1.02:Anthropological and historical studies in ethnopharmacology
1.03:Cardiovascular system
1.04:Central nervous system
1.05:Clinical studies
1.06:Commentaries
1.07:Ear, nose, and oropharynx
1.08:Endocrine system
1.09:Eye
1.10:Gastro-intestinal system
1.11:Immunological products and vaccines
1.12:Infections
1.13:Malignant disease and immunosuppression
1.14:Musculoskeletal and joint diseases
1.15:Nutrition and blood
1.16:Obstetrics, gynaecology, and urinary-tract disorder

1.17:Quality traditional medicines
1.18:Respiratory system
1.19:Reviews
1.20:Skin
1.21:Toxicology and safety
1.22:Systems biology and omics

2:KEYWORDS

2.004:abortion
2.008:acne
2.012:adaptogenic
2.016:aging
2.020:AIDS
2.024:alkaloids
2.028:allergy
2.032:alzheimer
2.036:anaemia
2.040:analgesic
2.044:angiogenesis
2.045:antinociceptive
2.048:anthelmintic
2.052:anthraquinones
2.056:anthropology
2.060:antiamoebic
2.064:antiarrhytmic
2.068:antifungal
2.072:antihistamine
2.076:antimicrobial
2.080:antimycobacteria
2.084:antioxidant
2.088:antiplatelet
2.092:antiprotozoal
2.096:antispasmodic
2.100:antiviral
2.104:anxiolytic
2.108:aphrodisiac
2.112:apoptosis
2.113:Archeology
2.116:arrow poisons
2.120:arthritis
2.124:asthma
2.128:atherosclerosis
2.132:Ayurveda
2.136:botany
2.137:Bone healing
2.140:bronchodilators
2.144:calcium channels
2.145:biocides
2.148:cancer
2.152:carbohydrates
2.156:carcinogenesis

2.160:cardiovascular
2.164:chemotaxonomy
2.168:chromatography
2.172:clinical trials
2.176:cognitive performance
2.180:contraception
2.184:corticosteroids
2.188:cosmetics
2.192:cough
2.196:coumarins
2.200:cyclooxygenase
2.204:cytotoxicity
2.208:dementia
2.212:dental hygiene
2.216:depression
2.220:dermatosis
2.224:diabetes
2.228:diarrhea
2.232:diterpenoids
2.236:diuretic
2.240:drug metabolism
2.244:drug tolerance
2.248:drug transport
2.252:drugs of abuse
2.256:eczema
2.260:epilepsy
2.264:erectyl disfunction
2.268:essential oil
2.272:ethnozoology
2.276:expectorant
2.284:fertility
2.288:fever
2.292:flavonoids
2.296:gastric ulcer
2.300:gene expression
2.304:genomics
2.308:gout
2.312:hallucinogenic
2.316:hepatotoxic
2.320:historical sources
2.324:HIV
2.328:honey
2.332:hypertension
2.336:hypnotic
2.340:ileum
2.344:immunomodulation
2.348:inflammation
2.352:influenza
2.356:Kampo medicine
2.360:laxative

2.364:leishmaniasis
2.368:leukocytes
2.372:lignans
2.376:lipids
2.380:liver
2.384:lymphocytes
2.388:macrophages
2.392:malaria
2.396:mast cells
2.400:metabolism
2.404:metabolomics
2.408:monoterpenoids
2.412:multidrug resistance
2.416:mutagenesis
2.420:myocardial infarction
2.424:nematodes
2.428:neurodegenerative disorders
2.432:neuroleptic
2.436:neutrophils
2.440:obesity
2.444:ophthalmology
2.448:osteoporosis
2.452:pain
2.456:parasites
2.460:parkinson
2.464:peptides
2.468:phenylpropanoids
2.472:pharmacodynamics
2.476:pharmacokinetics
2.480:phytochemistry
2.488:plant conservation
2.492:polyphenols
2.496:polysaccharides
2.500:prodrug
2.504:propolis
2.508:proteomics
2.512:psoriasis
2.516:quality control
2.520:radical scavenger
2.524:regulatory affairs
2.528:renal pharmacology
2.532:reproductive pharmacology
2.536:saponins
2.540:sedatives
2.544:sesquiterpenoids
2.548:signal transduction
2.552:steroids
2.556:stress
2.560:sweeteners
2.564:synergy

2.568:systems biology
2.572:tannins
2.576:terpenes
2.580:theories of illness
2.588:toxins
2.592:traditional chinese medicine
2.596:transcriptomics
2.600:transport
2.604:triterpenoids
2.608:trypanosomes
2.612:Unani medicine
2.616:vasoconstriction
2.620:venoms
2.624:veterinary ethnopharmacology
2.628:warts
2.630:Bioavailablity
2.632:Biodiversity Convention
2.634:Conservation
2.635:Constipation
2.636:ethics
2.637:Intellectual Property rights
2.638:Iridoids
2.639:Molecular biology
2.640:Statistics
2.641:Traditional medicine Europe, Mediterranean, Near East
2.642:Traditional medicine Asia & Oceania
2.643:Traditional medicine Africa
2.644:Traditional medicine Northern America
2.645:Traditional medicine Meso- and Southern America
2.646:wound healing

The "rules of 5"

The Editors and Editorial Board have developed the “Rules of 5” (Editorial, Volume 103, Issue 3, 20 February 2006, Pages 309-310) for publishing in JEP. We have produced five clear criteria that each author needs to think about before submitting a manuscript and setting the whole process of editing and reviewing at work. The rules should also be useful for the reviewing of papers.

The rules are the following:

1. Out of scope

The paper should report on traditional use or present results on pharmacological or toxicological studies (positive or negative) that are directly related to the traditional use. These data should eventually contribute to evidence-based traditional medicines.

Immediate rejection criteria:

I. Papers that use ethnopharmacology as an excuse to study an activity which is not related to the traditional use are not accepted, e.g. antitumor effect of plant used against diabetes.
II. Testing of extracts or plant parts that have no relation to the traditional use, e.g. pharmacological and phytochemical studies on a series of plants of one genus, of which only a few are actually used traditionally, do not fit in the scope.
III. Papers on health effects of food are not in the scope of the journal.
IV. Studies on pure compounds are not accepted if not clearly related to a plant and its traditional use.
V. At random screening of plants for activity.

2. Too preliminary

A paper must be based on a thorough and extensive study, using proper controls. Immediate rejection criteria:

I. Antimicrobial activity with single dose, or very high concentration, measuring only inhibition zones and no MIC values, no information on type of activity (-cidal or growth inhibition), microorganisms not relevant for use.
II. Single dose studies with very few animals, no dose-response studies.
III. In-vitro assays with single dose or very high concentration.
IV. No proper controls.
V. Repetition of a simple bioassay for yet another extract or plant.

3. In-vitro antioxidant activity

Antioxidant activity is present in all plants. Screening with in-vitro assays thus has little meaning if no clear evidence is given for in-vivo activity.

Immediate rejection criteria:

I. Only chemical in-vitro assays
II. No direct connection with claimed traditional use
III. No positive controls
IV. Isolation of very common antioxidant compounds (e.g.flavonoids)
V. Not at a relevant dose in in-vivo situation

4. Ethnopharmacological and ethnobotanical surveys normally need to report primary (absolute) data reporting how many times a (botanical) drug has been cited for a certain use and application.

Authors must comply with all relevant standards in the field.

Immediate rejection criteria:

I. Species are listed without presenting the primary data including details about their therapeutic use in the region/culture of study.
II. No information about the ethnographic background of the study or about the methods used.

III. No information about identification and documentation of the plants (voucher specimen).
IV. The ethnopharmacological frame of reference/theory that forms the basis of the study is not spelled out, e.g. no information about how disease diagnosis and practices related to specific plant medical uses were observed and verified.
V. No information on the protection of the biodiversity rights of indigenous people or local government

5. Lack of novelty

The study must represent a novel approach to the study of the activity, i.e. not more or less repeating what has already been published with similar results, but e.g. only using another extract of the same plant, or, in case of antimicrobial activity, some other microorganisms.

Immediate rejection criteria:

I. Repetition of well-known data
II. Use of non-specific pharmacological test methods or of phytochemical screening methods
III. Use of pharmacological assays or clinical trials which are not internationally recognized as valid and relevant
IV. Identification of only well-known ubiquitous compounds with little or no relation to activity (e.g. vitamins, sitosterol)
V. List of use of plants in certain area that confirms already known regional practices

Authors are encouraged to submit video material or animation sequences to support and enhance your scientific research. For more information please see the paragraph on video data below.

Types of paper

The *Journal of Ethnopharmacology* will accept the following contributions:

1. Original research articles - whose length is not limited and should include Title, Abstract, Methods and Materials, Results, Discussion, Conclusions, Acknowledgements and References. As a guideline, a full length paper normally occupies no more than 10 printed pages of the journal, including tables and illustrations.
2. Short Communications - whose average length is not more than 4 pages in print (approx. 2000-2300 words, including abstract and references). A maximum of 2 illustrations (figures or tables) is allowed. See paragraph below for description and format.
3. Letters to the Editors.
4. Reviews - Authors intending to write review articles should consult and send an outline to the Reviews Editor (see inside front cover for contact information) before preparing their manuscripts. The organization and subdivision of review articles can be arranged at the author's discretion. Authors should keep in mind that a good

review sets the trend and direction of future research on the subject matter being reviewed. Tables, figures and references are to be arranged in the same way as research articles in the journal. Reviews on topics that address cutting-edge problems are particularly welcome.
Outlines for potential reviews need to include:

- A detailed abstract using the structure provided in the guidelines
- An annotated table of contents
- A short CV of the lead author

5. Book reviews - Books for review should be sent to the Reviews Editor.
6. Commentaries - *invited*, peer-reviewed, critical discussion about crucial aspects of the field but most importantly methodological and conceptual-theoretical developments in the field and should also provide a standard, for example, for pharmacological methods to be used in papers in the *Journal of Ethnopharmacology*. The scientific dialogue differs greatly in the social / cultural and natural sciences, the discussions about the common foundations of the field are ongoing and the papers published should contribute to a transdisciplinary and multidisciplinary discussion. The length should be a maximum of 2-3 printed pages or 2500 words. Please contact the Reviews Editor j.ethnopharmacol@pharmacy.ac.uk with an outline.
7. Conference announcements and news.

Submission checklist

Please click here to download the Submission **Checklist**. This is a mandatory file during submission. Upload the completed checklist and choose the file type as "Checklist".

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

JOURNAL OF ETHNOPHARMACOLOGY CHECKLIST

Have you consulted the author-pack and verified that your submission adheres to the "Rules of 5"? YES

Have you provided a list of all authors which clearly states the contribution of each coauthor to the article? YES

Is the corresponding author’s postal address, telephone numbers and e-mail address provided in full? YES

Have you included a list of all authors’ email addresses? NO

Do you declare that all the listed authors have read and approved the submitted manuscript? NO

Do you declare that this manuscript/data, or parts thereof, has not been submitted for possible publication to another journal or that the work has previously been published elsewhere? YES

Is the title precise, clear and specific (do not use uncommon acronyms)? YES

Have you provided a clear and easily verifiable scientific reference to the traditional or clinical use of the herbal medicine under investigation (inserted directly next to the related traditional use)? YES

Do you declare that the present study was performed according to international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights? YES

Does the activity studied clearly relate to the traditional use? YES

Have you provided full botanical plant names (refer to www.theplantlist.org), including authorities of all plants? (This applies to all plant species mentioned in the manuscript) YES

In case of organisms other than plants, are the full scientific names with authorities provided? Non Applicable

Have you provided details of the voucher specimen number for each plant species studied, and the name of the recognized herbarium where they are stored? YES

Have you provided a proper chemical profile (e.g. TLC, HPLC, GC, MS or NMR) for future reference, particularly where there no voucher specimens were retained? YES

In case of mixtures, are all the ingredients listed by their full scientific names with authorities? NA

In case of single compound study, have you provide a direct comparison of the activity of the plant extract and the pure compound? NA

In case of industrial products, are full details of the batch number, contents, method of extraction and preparation of the final formulation, including quality control data provided? NA

Surveys – have you provided absolute/primary quantitative data on the frequency of plant use as mentioned in the interviews? And is there a critical assessment of the traditional uses considering regional and global uses and known scientific information on the chemistry and biological effects? NA

Antimicrobial activity – did you provide an in depth analysis of the antimicrobial activity including proper MIC values, and information on whether the activity is at a reasonable dose, and whether it is just inhibitory or microbicidal? NO

Quality control – is your study on quality control clearly linked to the claimed activity of the herbal medicine? NA

Is the abstract in the required structured format? (Ethnopharmacological relevance, Materials and methods, Results, Conclusion) YES

Have you provided a graphical abstract according to the authors guidelines? (no explicit animal or organ photographs are allowed) NO

Did you use the correct format for the references? (numerical referencing is not allowed) YES

Have you provided a list of abbreviations? NO

Have you provided a list of compounds studied? YES

For Reviews

Are the conclusions critical and specific defining the current state of the art? YES

Does the MS provide a comprehensive review of the current literature going beyond an overviews of articles indexed in common databases? YES

If pharmacological or clinical studies are reviewed, have you assessed the quality of the studies under review? YES

For reviews of individual species: Have you included a detailed review of local and traditional uses based on primary sources? YES

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• Relevant declarations of interest have been made

• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

Policy and ethics

In the covering letter, the author must also declare that the study was performed according to the international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights. See below for further information. The ethnopharmacological importance of the study must also be explained in the cover letter.

Animal and clinical studies - Investigations using experimental animals must state in the Methods section that the research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC) or the US guidelines (NIH publication #85-23, revised in 1985). Investigations with human subjects must state in the Methods section that the research followed guidelines of the Declaration of Helsinki and Tokyo for humans, and was approved by the institutional human experimentation committee or equivalent, and that informed consent was obtained. The Editors will reject papers if there is any doubt about the suitability of the animal or human procedures used.

Biodiversity rights - Each country has its own rights on its biodiversity. Consequently for studying plants one needs to follow the international, national and institutional rules concerning the biodiversity rights.

Author contributions
For each author the contribution to the publication should be mentioned.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors should complete the declaration of interest statement using this template and upload to the submission system at the Attach/Upload Files step. If there are no interests to declare, please choose: 'Declarations of interest: none' in the template. This statement will be published within the article if accepted. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form,

in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints
Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal

Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct

scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Additional information

Authors who want to submit a manuscript should consult and peruse carefully recent issues of the journal for format and style. Authors must include the following contact details on the title page of their submitted manuscript: full postal address; fax; e-mail. All manuscripts submitted are subject to peer review. The minimum requirements for a manuscript to qualify for peer review are that it has been prepared by strictly following the format and style of the journal as mentioned, that it is written in good English, and that it is complete. Manuscripts that have not fulfilled these requirements will be returned to the author(s).

In addition, you are recommended to adhere to the research standards described in the following articles:

Cos P., Vlietinck A.J., Berghe D.V., et al. (2006) Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. Journal of Ethnopharmacology, 106: 290-302.

Matteucci, E., Giampietro, O. (2008) Proposal open for discussion: defining agreed diagnostic procedures in experimental diabetes research. Journal of Ethnopharmacology,115: 163-172.

Froede, T.SA. and Y.S. Medeiros, Y.S. (2008) Animal models to test drugs with potential antidiabetic activity. Journal of Ethnopharmacology 115: 173-183. Gertsch J. (2009) How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. Journal of Ethnopharmacology, 122: 177-183.

Chan K., et al. (2012) Good practice in reviewing and publishing studies on herbal medicine, with special emphasis on traditional Chinese medicine and Chinese Materia Medica. Journal of Ethnopharmacology 140: 469-475.

Heinrich, M., Edwards. S., Moerman. D.E.. and Leonti. M. (2009), Ethnopharmacological field studies: a critical assessment of their conceptual basis and methods. J. Ethnopharmacol, 124: 1-17.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then

typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Use of word processing software
It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A

combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Glossary
Please supply, as a separate list, the definitions of field-specific terms used in your article.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

The author should divide the abstract with the **headings *Ethnopharmacological relevance*, *Aim of the study* , *Materials and Methods*, *Results*, and *Conclusions***. Click here to see an example.

Graphical abstract
A graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images also in accordance with all technical requirements.

Keywords
After having selected a classification in the submission system, authors must in the same step select 5 keywords. These keywords will help the Editors to categorize your article accurately and process it more quickly. A list of the classifications and set keywords can be found here.

In addition, you can provide a maximum of 6 specific keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Plant names
In the Materials and Methods section there must be a separate heading for describing the material used. That includes official name, local name, English name (if known), GPS position in case of collection in the wild or cultivation, a voucher specimen must be deposited in an official herbarium for possible future comparison. In the text it should be stated that the plant name has been checked with http://www.theplantlist.org mentioning the data of accessing that website.
In case of commercially procured material should mention the source, batch number,

quality control data. Data on chemical characterization (metabolomics, chromatographic methods) should also be presented, in case of known active compounds their quantitative analysis should be presented.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.

• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Please note that figures and tables should be embedded in the text as close as possible to where they are initially cited. It is also mandatory to upload separate graphic and table files as these will be required if your manuscript is accepted for publication.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions
Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with "Unpublished results". "*Personal communication*" will not be accepted as a reference. Citation of a reference as "in press" implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can

properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/journal-of-ethnopharmacology
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference style

Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-

Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data in Brief

You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be

editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee of 600 USD is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

Data statement
To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

Online proof correction

To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also check the status of your submitted article or find out when your accepted article will be published

5. VITA

Dejani Maíra Panazzolo nasceu no dia 24 de junho de 1990 no município de Engenho Velho – RS, (na época distrito de Constantina – RS), filha de Celda Panazzolo e Ademar Panazzolo.

Cursou o ensino fundamental na Escola Estadual de Ensino Médio Gottfried Thomas Westerich no município de Novo Xingu de 2000 a 2004. E o ensino médio na Escola Estadual de Ensino Médio São José no município de Constantina, de 2005 a 2007. Em 2008, ingressou no Curso de Zootecnia na Universidade Federal de Santa Maria/Centro de Educação Norte do Rio Grande do Sul (UFSM/Cesnors). Tendo desenvolvido vários estágios não curriculares durante o curso. Em 2012, desenvolveu o estágio final supervisionado no Grupo de Pesquisa em Nutrição de Ruminantes, sob orientação do Prof. Harold Ospina Patiño (*In memoriam*).

No ano de 2013 e 2014, cursou o Mestrado em Zootecnia pelo Programa de Pós-Graduação em Zootecnia na Universidade Federal do Rio Grande do Sul (UFRGS). Em 2016, iniciou o curso de Doutorado junto ao mesmo Programa, na área de concentração Produção Animal, como discente bolsista pela CAPES.