UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Ludmilla Louise Cerqueira Maia Prates

Análise do extrato e constituintes químicos de *Erythrina verna* Vell. sobre o crescimento, in vitro, de estreptococos do grupo mutans e toxicidade aguda em modelo experimental murino

Montes Claros – MG

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Orientador: Profa. Dra. Mariléia Chaves Andrade

Coorientador: Prof. Dr. Sérgio Avelino Mota Nobre

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RESUMO

Erythrina verna Vell. (EV) é uma planta nativa brasileira comumente utilizada na medicina popular, principalmente como tranquilizante e também no tratamento de infecções. Embora composições fitoterápicas sejam comercializadas com esta espécie, poucos estudos sobre o perfil químico e toxicológico têm sido relatados. O objetivo do estudo foi avaliar a atividade biológica e toxicológica do extrato da casca de *Erythrina verna* Vell.. O perfil fitoquímico do extrato de *E. verna* (EVE) foi determinado por cromatografia gasosa acoplada à espectrometria de massas, e a Concentração Inibitória Mínima e a Concentração Bactericida Mínima do extrato butanóico da casca de EV e constituintes químicos foram identificados contra isolados de estreptococcus do grupo mutans (EGM). Além disso, analisamos o efeito sinérgico dos compostos ativos no extrato. A toxicidade aguda foi avaliada de acordo com o protocolo da Organização para Cooperação e Desenvolvimento Econômico (OCDE 423). Uma única dose do extrato nas concentrações de 5, 50, 300 e 2000 $mgKg^{-1}$ (n=3/grupo) foi administrada por via oral em camundongos Swiss. Os animais foram monitorados por 14 dias juntamente com a avaliação dos sinais clínicos, bioquímicos e histopatológicos, bem como a presença de mortalidade. O EVE apresentou metabólitos secundários e substâncias com atividade antimicrobiana, ácido benzoico e ácido azeláico. Dos três compostos isolados de EVE, o ácido esteárico apresentou a maior atividade bactericida contra os microrganismos testados. No entanto, comparando a atividade bactericida do extrato bruto e do constituinte químico isolado, o EVE apresentou atividade antibacteriana mais eficaz, enquanto os da cavidade oral (C17VA, C12VA e C04PD) apresentaram maior resistência ao extrato e ao ácido esteárico comparado com as cepas padrão (ATCC 25175 e ATCC 27392). O EVE demonstrou efeito sinérgico quando associado à clorexidina, com redução de cerca de 98% da população bacteriana em todos os microrganismos testados. Já a toxicidade aguda não produziu mortalidade ou alterações comportamentais e hematológicas, porém, houve alterações nos níveis de HDL significativamente elevados quando comparados aos animais do grupo controle. O exame histológico do baço, coração e rim mostrou alterações na arquitetura tecidual com focos de infiltrados inflamatórios a partir da concentração de 50 $mgKg^{-1}$. O extrato da casca de *E. verna* pode ser uma alternativa no tratamento de doenças causadas por estreptococos do grupo mutans, principalmente se associado à clorexidina. Entretanto o uso oral do extrato da casca de *E. verna* deve ser realizado com cautela e em baixas doses.

Palavras-Chave: *Erythrina verna* Vell. estreptococos do grupo mutans, fitoterapia, toxicidade aguda.

ABSTRACT

Erythrina verna Vell. (EV) is a native Brazilian plant commonly used in folk medicine, mainly as a tranquilizer and also in the treatment of infections. Although herbal compositions are commercialized with this species, few studies on the chemical and toxicological profile have been reported. The aim of the study was to evaluate the biological and toxicological activity of the bark extract of *Erythrina verna* Vell.. The phytochemical profile of the *E. verna* extract (EVE) was determined by gas chromatography coupled to mass spectrometry, and the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the butanoic extract of EV bark and chemical constituents were identified against isolates from mutans group streptococci (MGS). In addition, we analyzed the synergistic effect of the active compounds in the extract. Acute toxicity was assessed according to the Organization for Economic Co-operation and Development protocol (OECD 423). A single dose of the extract at concentrations of 5, 50, 300 and 2000 $mgKg^{-1}$ (n=3/group) was orally administered to Swiss mice. The animals were monitored for 14 days along with the evaluation of clinical, biochemical and histopathological signs, as well as the presence of mortality. EVE showed secondary metabolites and substances with antimicrobial activity, benzoic acid and azelaic acid. Of the three compounds isolated from EVE, stearic acid showed the highest bactericidal activity against the microorganisms tested. However, comparing the bactericidal activity of the crude extract and the isolated chemical constituent, EVE showed more effective antibacterial activity, while those from the oral cavity (C17VA, C12VA and C04PD) showed greater resistance to the extract and to stearic acid compared to the standard strains (ATCC 25175 and ATCC 27392). EVE demonstrated a synergistic effect when associated with chlorhexidine, with a reduction of about 98% of the bacterial population in all tested microorganisms. On the other hand, acute toxicity did not produce mortality or behavioral and hematological changes, however, there were changes in HDL levels significantly elevated when compared to animals in the control group. The histological examination of the spleen, heart and kidney showed changes in tissue architecture with foci of inflammatory infiltrates from a concentration of 50 $mgKg^{-1}$. E. verna bark extract can be an alternative in the treatment of diseases caused by mutans group streptococci, especially if associated with chlorhexidine. However, the oral use of *E. verna* bark extract should be performed with caution and in low doses.

Keywords: *Erythrina verna* Vell. mutans group streptococci, phytotherapy, acute toxicity.

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SUMÁRIO

1. INTRODUÇÃO

O uso dos recursos naturais pela humanidade, com finalidade profilática, curativa e/ou paliativa, é relatado desde a antiguidade. Antes do século XX, extratos de plantas, animais, microrganismos e minerais representavam os únicos produtos disponíveis para o tratamento das doenças [1].

As plantas constituem a principal fonte para o desenvolvimento de produtos utilizados na medicina tradicional. Seu uso é bastante difundido na cultura popular devido sua facilidade de aquisição e eficácia no tratamento de enfermidades [2,3]. A Organização mundial da Saúde (OMS) apoia o uso adequado de medicamentos à base de espécies vegetais, principalmente para os cuidados primários a saúde, além de incentivar pesquisas científicas nesta área [4]. Com isso houve um aumento no interesse de pesquisadores na investigação do potencial terapêutico, morfologia, composição química e propriedades farmacológicas de várias espécies de plantas [5-7].

O potencial terapêutico das plantas medicinais é justificado pela presença de componentes bioativos que apresentam muitas atividades biológicas comprovadas [8]. Dentre os componentes bioativos das plantas destaca-se os metabolitos secundários, que são substância determinantes na adaptação às condições ambientais e proteção das espécies vegetais [9].

Um dos efeitos terapêuticos dos metabólitos secundários de elevada importância e interesse farmacêutico é a atividade antimicrobiana. Devido à preocupação com a falta de eficácia dos agentes antimicrobianos para patologias diversas, associada aos efeitos colaterais e resistência microbiana confirmada nos últimos anos [10,11].

Os estreptococos do grupo mutans são microrganismos presente na cavidade oral e está relacionado com o processo cariogênico, a partir da formação de biofilme. Um dos fatores que determina o poder de virulência desses microrganismos é sua capacidade de produção de biofilme [12]. Em estudo realizado pelo nosso grupo de pesquisa observou-se que a seleção de isolados de estreptococos do grupo mutans pela sua capacidade de produção de biofilme está diretamente ligado a resistências desses microrganismos a antibiótico e extrato vegetais [13]. A cárie é uma doença multifatorial que acomente um grande número de pessoas no mundo, sendo que seu tratamento acarreta em gastos elevado os orgão públicos e ao individuo acometido, com isso a busca por novas alternativas que auxiliem no tratamento e prevenção da cárie são vista com bons olhos na atualidade [14]. Nesse cenário as plantas é

uma alternativa de baixo custo, fácil acesso e de ampla aceitação pela população. Além disso, o Brasil destaca-se como uma das regiões de maior biodiversidade vegetal [15].

A espécie *Erythrina verna* Vell. é utilizada como antidepressiva, sedativa, calmante, ansiolítica, antimicrobiano, tônico hepático e anti-inflamatória [16-18]. Apesar de seu uso está bem difundido na medicina popular essa espécie carece de estudos que amplie o seu leque de ações farmacológicas e descreva seus efeitos tóxicos aos seres vivos.

Apesar do uso tradicional das plantas medicinais e da crença da população que os produtos naturais são inócuos e menos tóxicos, o seu uso deve ser feito com cautela e amparado por estudos que avaliam a toxicidade das substâncias químicas das plantas ao organismo vivo [19].

Portanto o presente trabalho investiga a composição química, a atividade antimicrobiana e toxicológica de *Erythrina verna* a fim de validar as indicações terapêuticas empíricas referentes a esta planta.

2. OBJETIVOS

2.1 Objetivo Geral

Investigar a composição química e mensura os potenciais efeitos biológicos e toxicológicos do extrato da casca de *Erythrina verna* Vell..

2.2 Objetivos Específicos

i. Mensurar a Concentração Inibitória Mínima do extrato da casca de *Erythrina verna* Vell. e seus componentes sobre estreptococos do grupo mutans.

ii. Prover a caracterização fitoquímica do extrato de *Erythrina verna* Vell..

iii. Avaliar o efeito sinérgico do extrato de *Erythrina verna* Vell. associado com a clorexidina.

iv. Estabelecer a dose letal 50% (DL50) do extrato da casca de *Erythrina verna* Vell., por meio do teste de toxicidade aguda em um modelo murino experimental.

v. Analisar os parâmetros clínicos, bioquímicos e histopatológicos após o teste de toxicidade aguda em um modelo murino experimental.

3. REFERENCIAL TEÓRICO

3.1 Aspectos históricos das Plantas Medicinais

Ao longo do tempo, o homem tem utilizado dos recursos oferecidos pela natureza no tratamento de um largo espectro de doenças. Um dos primeiros relatos de documentação escrita sobre a utilização das plantas medicinais é a obra chinesa Pen Ts'ao (A Grande Fitoterapia), há 2800 anos a.C.. Na história egípcia os papiros documentavam o uso das plantas como remédio pelos médicos, o Papiro de Ebers, de 1550 a.C. documenta mais de 700 medicamentos, no qual são mencionadas fórmulas com espécies vegetais que são usadas até hoje [20]. Outro documento importante é a Matéria Médica Chinesa, que foi extensamente documentada ao longo dos séculos, com primeiro registro em 1100 a.C., contendo indicações de tratamento à base de fitoterápicos para 52 doenças [8,19]. Outras contribuições do uso dos produtos naturais foram relatadas por civilizações do Egito, China e Grécia datados de 500 a.C. [22,23].

As primeiras informações sobre a toxicidade das plantas foram relatadas por Dioscorides, na obra De Materia Medica (sobre as formas de curar), onde descrevia informações a respeito das dosagens sugeridas e os possíveis efeitos tóxicos de cerca de 600 espécies vegetais. Durante os séculos V a XI os conhecimentos das plantas medicinais foram mantidos na cultura europeia, através da tradução de várias obras de Galeno, Dioscorides e Hipócrates [20].

No Brasil, a história da utilização de plantas no tratamento de doenças apresenta influências marcantes das culturas indígenas, africanas e europeia. Os índios foram os pioneiros nessa abordagem no território brasileiro e detinham o conhecimento sobre as ações benéficas causadas pelo uso das plantas. Os seus conhecimentos são transmitidos de gerações, através dos pajés. Já os africanos trouxeram consigo plantas que eram utilizadas em rituais religiosos, e por suas propriedades farmacológicas, empiricamente descobertas. Os colonizadores europeus introduziram no Brasil as boticas, locais onde eram

comercializados os medicamentos trazidos da Europa. A união dessas três vertentes foi a base do conhecimento das plantas medicinais no Brasil [24-26].

No início do século XX com o desenvolvimento da síntese química ocorreu a introdução dos fármacos sintéticos fazendo com que a terapia com plantas medicinais passasse a ser negligenciada. A medicina tradicional passou a ser substituída pelos medicamentos industrializados [27]. Entretanto no final do século XX houve uma mudança no pensamento e hábito das pessoas com a retomada do uso das plantas medicinais no lugar dos medicamentos sintéticos. Favorecido pelo fato de os fármacos sintéticos apresentarem alto custo, diversas reações adversas, a cultura de que o natural é melhor e as ideias de desenvolvimento sustentável [28,29].

3.2 Plantas medicinais

As plantas tem sido uma fonte exemplar de medicamentos e tem atraído a atenção dos cientistas de todo o mundo devido as suas ações farmacológicas e seus efeitos colaterais mínimos. Inicialmente as plantas foram usadas na sua forma natural (chás, emplastros, infusões e outros) com o decorrer dos anos, precisamente no início do século XIX elas se tornaram fontes de matérias-primas para a síntese de fármacos e atualmente são peças-chave para o desenvolvimento de medicamentos [30].

Para a Organização Mundial da Saúde (OMS), planta medicinal é “todo e qualquer vegetal que possui, em um ou mais órgãos, substâncias que podem ser utilizadas para fins terapêuticos ou que sejam precursores de fármacos semissintéticos”. Na Declaração de Alma-Ata (1978) a OMS relata que cerca de 80% da população de países em desenvolvimento utilizam plantas medicinais para os cuidados básicos com a saúde [4].

As plantas medicinais têm contribuído significativamente para a atenção primária à saúde das pessoas em todo o mundo. Aumentos populacionais, uso inadequados de medicamentos, alto custo de tratamentos, efeitos colaterais de várias drogas sintéticas e desenvolvimento de resistência a doenças infecciosas levaram a o uso crescente de materiais vegetais como fonte de medicamentos para uma ampla variedade de doenças humanas. Devido a esses motivos, associado ao vasto conhecimento tradicional sobre o uso de plantas

medicinais e a facilidade da obtenção do material vegetal percebe-se um crescimento na utilização destas plantas em populações de países em desenvolvimento [31,32].

As plantas medicinais e seus derivados fazem parte dos recursos terapêuticos da Medicina Tradicional (MT) e/ou Medicina Complementar/Alternativa (MCA). Esses tipos de medicinas representam diferentes recursos que complementam ou substituem as terapias convencionais [1][33]. A estratégia da Organização Mundial da Saúde (OMS), 2014-2023, visa fortalecer o papel da medicina tradicional, enfatizando a importância de promover e incluir a utilização de plantas medicinais nos sistemas de saúde de seus países membros [2][34].

No Brasil apesar do uso das plantas medicinais ser uma prática antiga e seu conhecimento repassado ao longo dos anos, a introdução desse tipo de tratamento nos Cuidados Primários a Saúde é recente. O primeiro fitomedicamento totalmente pesquisado e desenvolvido no Brasil foi o Acheflan (2005) a partir da planta erva-baleeira ou maria-milagrosa (*Cordia verbenacea*) e possui afeito anti-inflamatório de uso tópico [35]. O que acarretou no aumentando de interesse dos pesquisadores e órgãos governamentais na área da fitoterapia. Um marco importante ocorreu em 22 de junho de 2006 com a promulgação do decreto presidencial nº 5.813 na qual, foi criada a Política Nacional de Plantas Medicinais e Fitoterápicos (PNPMF), objetivando a garantia da eficácia e segurança frente ao uso das plantas medicinais. No mesmo ano ocorreu implantação das Práticas Integrativas e Complementares (PICS), tratamentos que utilizam recursos terapêuticos baseados em conhecimentos tradicionais para prevenir e curar doenças. Continuado pela publicação do Programa Nacional de Plantas Medicinais e Fitoterápicos em 2008. Já em 2010, por meio da portaria n° 886 foi criada e implementada no Sistema Único de Saúde (SUS), a primeira assistência farmacêutica baseada na utilização de plantas medicinais, o Programa Farmácia Viva, que tem como objetivo a produção de fitoterápicos acessíveis à população [36]. A implementação da fitoterapia no SUS representa o resgate de uma prática milenar, onde se integram o conhecimento científico, o conhecimento popular e tradicional e seus fundamentos sobre o adoecimento e as formas de tratá-los. O Ministerio da Saúde através da resolução, RDC nº 26/2014, define as categorias de medicamento fitoterápico e produto tradicional fitoterápico e estabelece os requisitos mínimos para o registro e renovação de registro de medicamento fitoterápico, e para o registro, renovação de registro e notificação de produto tradicional fitoterápico [37]. Publicado pela Anvisa o Memento Fitoterápico da

Farmacopeia Brasileira (MFFB) e aprovado pela RDC nº84/2016 tem como objetivo de orientar a prescrição de plantas medicinais e fitoterápicos [38]. Essas resoluções são importante para estabelecer regras que garantam a segurança no uso de produtos fitoterápicos, principalmente pela notoriedade que as plantas medicinais vem ganhando no cuidado com a saúde no Brasil.

As ações terapêuticas realizada pelas plantas medicinais devem-se aos compostos que apresentam ampla diversidade em termos de estruturas, propriedades físico-químicas e biológicas [39]. Esses compostos são encontrados em diferentes partes da planta (raízes, caules, folhas, frutos, flores e semente), sendo eles responsáveis pelas respostas fisiológicas em organismos vivos [40].

Dentre os compostos produzidos pelas plantas os metabólitos secundários são os mais utilizados na síntese de novos fármacos, visto que estes metabólitos, geralmente apresentam estruturas complexas, exercendo funções fisiológicas importantes para a adaptação das plantas ao meio ambiente em que estão inseridas contribuindo com a interação em diferentes ecossistemas [41]. Essas substâncias são encontradas em concentrações relativamente baixas em determinados grupos de plantas, conferindo-lhes a resistência necessária para sobrevivência contra a ação de patógenos (bactérias, fungos e vírus), predadores ou mesmo outras plantas (atividade antigerminativa ou tóxicas) [42].

Os metabólitos secundários de plantas são classificados em três grandes grupos de acordo com sua rota biossintética: compostos fenólicos, alcaloides e terpênicos. Estas moléculas são utilizadas pela indústria farmacêutica para a produção de medicamentos, cosméticos ou nutracêuticos [41, 33-45].

Alguns desses compostos conferem aos extratos vegetais e suas frações propriedades que possibilita o seu uso com fins medicinais. Várias espécies vegetais já possuem suas propriedades descritas, dentre as quais se destacam: *Achillea millefolium* - Antitérmica, analgésica, expectorante, antidiarréica, anti-hipertensiva, antiinflamatória, hemostática e cicatrizante [46,47], *Achyrocline satureioides* - antiespasmódica, carminativa, sedativa [48,49], *Valeriana officinalis* - Sedativa, hipotensora, anticonvulsivante, antiespasmódica [50], *Passiflora incarnata* - Sedativa, ansiolítica, hipnótica suave, miorrelaxante e espasmolítica, estabilizadora do sistema nervosos central [51,52], *Trifollium pratensis* - estrogênica, antioxidante, utilizada na Síndrome Climatérica para controle dos fogachos e sudorese [53], *Vernonia polyanthes* - expectorante empregada nas tosses, gripes, bronquites e pneumonias

[54,55], *Tabebuia avellanedae* - Lapachol possui atividade antiinflamatória, antimalárica, antibacteriana, antifúngica, antiparasitária e imunomoduladora [50], *Sesamum indicum* - Indicado para tonteiras, visão turva, acúfenos e constipação intestinal [56], *Scutellaria baicalensis* - Antibacteriana, antitérmica, detoxicante, antinflamatória, antitumoral, anti-HIV-1, antioxidante, diurética, hipotensora, antialérgica. Indicada em infecções das vias aéreas superiores, como amigdalites e laringo-faringites, escarlatina, hepatites virais, nefrites e anexites [57,58], *Salvia officinalis* - Carminativa, antisudorífica, antioxidante, hipoglicemiante, estrogênica, antibacteriana, anticolinesterásica, ativadora da memória [59,60]. Entretanto apenas 20% das espécies vegetais tiveram suas ações farmacológicas descritas cientificamente. Essa medicina natural desperta o interesse dos pesquisadores, que buscam descobrir e/ou obter novas drogas contribuindo para o aumento do arsenal de ações farmacológicas atribuídas as plantas medicinais. Muitas dessas pesquisas partem das observações populares sobre o uso e eficácia das plantas medicinais em populações tradicionais. Dentre as ações farmacológicas a antimicrobiana vem ganhando destaque no meio cientifico, pelo fato da falta de eficácia dos agentes antimicrobianos para patologias diversas associada a resistência microbiana, despertam a atenção na busca de drogas mais efetivas. A resistência bacteriana vem sendo considerada como um crescente problema de saúde pública mundial, uma vez que devido a seleção de microrganismos patogênicos resistentes houve uma redução do número de antibióticos válidos disponíveis [61,62]. Com isso o uso de antimicrobianos de origem natural torna-se uma alternativa eficaz e econômica.

3.3 Erythrina

O gênero *Erythrina* pertence à família botânica *Fabaceae* com cerca de 130 espécies de regiões tropicais e subtropicais do mundo. É amplamente distribuído no sul e sudeste do Brasil, norte da Argentina e Paraguai, sul da Bolívia e Peru [63]. São árvores de porte médio com flores grandes vermelhas ou alaranjadas, nas quais deriva o nome *Erythrina*, já que *erythros* (grego) significa vermelho, em alusão à cor das flores de diversas espécies deste gênero. Ocorrem numa ampla variedade de habitats, desde matas tropicais até bosques de altitude. No Brasil são encontradas cerca de onze (11) espécies de *Erythrina*, no território: *E. verna, E. falcata, E. domuinguezii, E. amazônica, E. velutina, E. crista-galli, E. fucas, E. poeppigiana, E. speciosa, E. similis, E. ulei* [64].

Sabe-se que essas espécies produzem alcaloides [65-69], terpenos [70] e flavonoides [67,68,70-75] e suas cascas e folhas de caule são comumente usadas para fazer chás (infusão ou decocção) que se acredita que exibem propriedades tranquilizantes e anti- ansiedade

[76,77]. Com base em seu uso comum na medicina popular, a maioria das pesquisas sobre o gênero envolveu o isolamento e a caracterização de seus constituintes alcaloides. Desde a primeira pesquisa fitoquímica do alcaloide de *Erythrina* na década de 1930 [78], o número total desse composto só aumentou, sendo hoje relatados aproximadamente 110 alcaloides em plantas do gênero *Erythrina* [79]. A triagem de bioensaio desses alcaloides mostrou atividade ansiolítica [80], sono induzido [81], atividade anticonvulsivante [82], antagonismo dos receptores nicotínicos neuronais da acetilcolina [83], leishmanicida e anticatarato [84].

Outra classe de substância fitoquímica estudada no gênero *Erythrina* são os flavonoides. Estudos relatam que esses compostos podem modular as vias de sinalização de proteínas e lipídio [85-89]. Além disso, os polifenóis têm sido associados a um risco reduzido de desenvolvimento de demência [90].

3.4 Erythrina verna

Erythrina verna Vell. é uma árvore com aproximadamente 20 metros de altura, com espinhos ao longo dos troncos, perde todas as folhas na época da floração, produz pequenos frutos do tipo vagem, contendo entre uma e três sementes. Ocorre naturalmente no Cerrado, Amazônia e Mata Atlântica, preferencialmente nas encostas e matas abertas. É conhecida popularmente como mulungu, flor-de-coral, suina-suínã, corticeira, canivete, amansa-senhor, capa-homem, tiririceiro, árvore de coral entre outros. A espécie *Erythrina verna* Vell. é uma das mais utilizada para fins medicinais, tendo as seguintes ações farmacológicas descritas: antidepressiva, sedativa, calmante, ansiolítica, antibacteriana, e anti-inflamatória [14-16]. É também utilizada popularmente contra ansiedade, estresse, febre, bronquite, dor no estômagoe e asma [16, 91,92].

Alguns medicamentos em associação com *Erythrina verna* são descritos na literatura como comprimidos contendo *E. verna*, *Crataegus oxyacantha* L. e *Passiflora alata*. Também são encontradas cápsulas ou soluções contendo extrato seco de *E. verna*, *Passiflora alata*, *Adonis vernalis* e *Leptolobium elegans*. Esses medicamentos são usados no tratamento de ansiedade e como calmante [92-94].

As ações farmacológicas da *E. verna* são provenientes dos compostos químicos presente nas diversas partes vegetais desta espécie. Ela é rica em alcaloides (erisotrina, eritratidina, eritratidinona, 11-hidroxieritratidinona e epieritratidinona, mas também apresenta tripterpenos (lupeol e eritrodiol), flavonoides (faseolina e homohesperidina), fenóis (faseolidina) e fitoesteróides (betasistosterol e estigmasteroel) [94-96].

Em estudo realizado por De Lima e colaboradores (2006) foram descritos os metabolitos flavanonas, flavanoides, flavanois e xantonas encontrados no extrato etanólico das cascas [97]. Já Proença e colaboradores (2012) relataram a presença de alcaloides, flavonoides, taninos, triterpenos e esteroides após análise por cromatografia em camada delgada das cascas de *E. verna* [98]. De Bona e colaboradores (2012) relatam açúcares redutores, fenóis, taninos, flavonoides, alcaloides, derivados de cumarina, esteróides e triterpenóides, saponina e depsídeos e depsidonas em extrato hidroalcoólico das folhas [99].

A *Erythrina verna* mais especificamente as suas cascas estão inseridas na primeira edição da Farmacopeia Brasileira. Também está incluída no Programa Nacional de Plantas Medicinais e Fitoterápicos, uma lista contendo as plantas medicinais com potencial para gerar produtos de interesse ao SUS (Relação Nacional de Plantas Medicinais de Interesse aos SUS-RENISUS), publicado pelo Ministério da Saúde [100,101].

Entretanto é importante destacar que são escassas as informações bibliográficas sobre a espécie *E. verna*, restringindo à investigação da atividade farmacológica e caracterização de alcaloides.

3.5 Estreptococos do grupo mutans

Dentre as ações farmacológicas atribuídas a espécie *Erythrina verna* a atividade antimicrobiana é um importante fator estudado no meio cientifico, apoiado por relatos de populações tradicionais sobre seu uso em infecções na cavidade bucal. Dentre os microrganismos presentes na cavidade oral os estreptococos do grupo mutans ganham destaque, uma vez que estão relacionados aos casos de infecções periodontais e periapicais [102].

Entre as patologias infecciosas mais comum na cavidade oral a cárie é a que apresenta maior evidência, por se tratar de uma doença multifatorial, transmissível e dieta dependente, caracterizada pela desmineralização localizada do tecido dentário (esmalte, dentina e cemento). Apesar dos avanços na redução de incidência, continua sendo um processo com elevado índice de prevalência em seres humanos [103,104]. O processo de formação de cárie dentária tem sido intensivamente estudado [105], sendo caracterizado pela interação de três fatores primordiais: hospedeiro, dieta e microbiota, onde bactérias acidogênicas e acidúricas,

destacando os estreptococos do grupo mutans, particularmente, *Streptococcus mutans*, interagem com outros microrganismos no biofilme sobre a superfície do dente ocasionando a erosão ou à desmineralização do tecido dentário mais externo através de ácidos, em especial o lático, produzido na fermentação de carboidratos realizada pelos microrganismos que constitui o biofilme dentário [106].

Os estreptococos do grupo mutans (EGM), são bactérias gram, que foram descobertos no início do século XX, e descritos como "mutantes" por sua morfologia celular ser mais achatada do que outros estreptococos [107]. Essa bactéria é capaz de sintetizar polissacarídeos extracelulares insolúveis, por meio de enzimas glucosiltransferase (GTFs) o que promove o acúmulo e permanência do microrganismo no dente, possui alta capacidade de metabolizar diversos carboidratos presentes na dieta produzindo ácidos orgânicos que desmineralizam o esmalte do dente, o que lhe confere caráter acidogênico e a habilidade de sobreviver em meio ácido (aciduricidade) [108,109]. Além da acidogenicidade e da aciduricidade, a capacidade de sintetizar glucanos, na presença de sacarose, representa importante fator de virulência associado ao EGM [105,110].

A enzima glucosiltransferase (GTF) secretada pelo EGM hidrolisa a sacarose em glicose e frutose e polimeriza as moléculas de glicose liberadas, formando polissacarídeos extracelulares (PEC) denominados glucanos. Diversos tipos de glucanos são produzidos, os quais variam na solubilidade em água, onde GTF B, responsável pela produção de glucanos insolúveis em água; GTF C, responsável pela produção de glucanos solúveis e insolúveis; e GTF D, responsável, exclusivamente, pela produção de glucanos solúveis e também na proporção das ligações entre as moléculas de glicose, as quais podem ser do tipo α-(1-3) ou do tipo α-(1-6). Os glucanos insolúveis em água são aqueles onde prevalecem as ligações do tipo α-(1-3), e são os mais importantes na formação de uma matriz extracelular "pegajosa" insolúvel, essencial para o acúmulo de *S. mutans* no biofilme dentário dental [101,102].

3.6 Cárie

A cárie dentária é uma doença multifatorial, transmissível e dieta dependente, caracterizada pela desmineralização localizada do tecido dentário (esmalte, dentina e cemento). Apesar dos avanços na redução de incidência, continua sendo um processo com elevado índice de prevalência em seres humanos [113,114]. O processo de formação de cárie dentária tem sido intensivamente estudado [115,116], sendo caracterizado pela interação de três fatores

primordiais: hospedeiro, dieta e microbiota, onde bactérias acidogênicas e acidúricas que interagem com outros micro-organismos no biofilme sobre a superfície do dente ocasionam a erosão ou à desmineralização do tecido dentário mais externo através de ácidos, em especial o lático, produzido na fermentação de carboidratos realizada pelos micro-organismos que constitui o biofilme dentário [117, 121].

Com relação à suscetibilidade à cárie, o indivíduo pode apresentar fatores extrínsecos relacionados e estrutura sociocultural e fatores intrínsecos como fluxo, composição e capacidade tampão da saliva, aspectos hereditários e imunológicos difíceis de serem controlados, já os dentes possuem graus de mineralização do esmalte com maior ou menor resistência aos ácidos. No entanto não existe dente suficientemente resistente ao processo cariogênico. Sendo o fator suscetibilidade de grande importância para as estratégias de prevenção [122].

A cárie, apesar de apresentar significante declínio em algumas populações, continua sendo um importante problema de saúde pública. De acordo com a Organização Mundial da Saúde (OMS) a cárie é considerada uma das doenças mais comum no mundo e afeta quase 100% dos adultos, 80% dos adolescentes e cerca de 60% a 90% das crianças em idade escolar, o que representa um alto custo em despesas públicas em países desenvolvidos (em torno de 5% a 10% da despesa total com saúde), já os países em desenvolvimento esse valor excede a capacidade financeira desses países e muitas das pessoas acometidas por essa doença não são assistidas [123].

Os principais grupos de bactérias que produzem ácido lático são *Streptococcus* do grupo *mutans* e *Lactobacillus*. Esses grupos de bactérias, atuando em conjunto ou isoladamente, são os principais causadores da cárie dentária [124,125]. Os microrganismos que ganham mais destaque no processo cariogênico são os *Streptococcus* do grupo *mutans*, que são cerca de 60% dos microrganismos que compõem o biofilme dentário [126].

O controle desses microrganismos na prevenção da cárie dentária se dá principalmente pelos métodos de remoção mecânica e controle químico. Outro fator que contribui para diminuição do índice de carie é a mudanças de hábitos como a redução da frequência do consumo de açúcares que contribui para um equilíbrio ecológico do biofilme [127]. Com relação as substâncias químicas empregadas nesse controle destacam-se os antibióticos, antissépticos, enzimas e fluoretos. A clorexidina é o antisséptico mais utilizado para prevenir o acúmulo de biofilme, sendo também utilizado como referência em estudos testes de eficiência de agente antimicrobiano. Apesar de baixa toxicidade dessa substancia, já existem relatos de feitos colaterais, dos quais podemos citar: pigmentação extrínseca dos dentes, gosto metálico na boca, descamação de mucosa, náuseas e vômitos, sensibilidade oral, além de reações alérgicas [128].

A busca por novos processos e/ou substâncias químicas que ajude no controle do processo cariogênico se faz cada vez mais necessário, principalmente em comunidades que não tem acesso aos tratamentos odontológicos convencionais. Nesse cenário as plantas medicinais é uma importante alternativa, visto que já são relatados na literatura o uso de 132 espécies de plantas na odontologia, com destaque para a romã (*Punica granatum*) [129]. Dentre as espécies utilizada na medicina tradicional a *Erythrina verna* pode ser considerada uma alternativa para reduzir doenças como a cárie devido relatos do uso em comunidades tradicionais no tratamento de infecções orais e pela ação antimicrobiana da espécie.

3.7 Segurança no uso de plantas medicinais

As plantas medicinais contêm substâncias bioativas que podem ser benéficas ou nocivas à saúde humana, dependendo da dose empregada [130]. As pessoas creem erroneamente que produtos naturais são "ausentes de produtos químicos ou tóxicos" que seu uso não trará danos ou perigos à sua saúde, ignorando possíveis efeitos secundários e tóxicos de espécies utilizadas habitualmente [131]. A busca por extratos ou plantas com novas ações farmacológicas deve seguir critérios para assegurar a eficácia e diminuir os efeitos adversos causados pelo uso desses produtos naturais.

Dentre as formas de uso das plantas como fonte terapêutica incluem-se os chás, os extratos brutos ou suas frações padronizadas em preparações farmacêuticas e os compostos isolados, usados diretamente como drogas ou precursores em processos de síntese. Independente do uso considerado, fatores como qualidade, segurança e eficácia são requisitos indispensáveis [132,133].

Estudos são extremamente importantes, pois o uso de produtos derivados de plantas pode levar a diversos agravos à saúde, como reações alérgicas, reações tóxicas, efeitos adversos e efeitos mutagênicos [134], já que muitas plantas que possuem poder curativo podem apresentar substâncias tóxicas ou composição química variável [135,136]. Para avaliar as condições de uso, faz-se necessário o estudo das plantas comprovando os efeitos terapêuticos, administrando diferentes doses e também direcionando as pesquisas com o intuito de promover o uso com segurança. Para tanto não basta comprovar apenas o efeito terapêutico, é necessário comprovar a inocuidade avaliando os riscos e usos através de

estudos de toxicidade, levando em consideração o perfil bioquímico, hematológico e patológico do animal em teste. Existem inúmeros estudos científicos que comprovam que algumas plantas são tóxicas e mesmo assim são empregadas pela população, em algumas situações, sem restrições [137].

No desenvolvimento de fitoterápicos são necessários alguns dados sobre identificação botânica, autenticidade, pureza, integridade, análise das substâncias ativas, forma de exploração dos recursos naturais, controle em processo e métodos utilizados, testes de estabilidade, farmacologia e toxicologia pré-clínica e clínica, entre outros requisitos [138].

Do ponto de vista toxicológico, é preciso considerar que uma planta medicinal ou um possível fitoterápico, possa apresentar não somente efeitos imediatos, o que é facilmente correlacionado após a ingestão, mas também efeitos não proeminentes após determinado período acarretando hepatotoxicidade e nefrotoxicidade [139,142]. A legislação brasileira (Resolução 196/96 - CNS) regulamenta as pesquisas pré-clínicas e clínicas para registro de medicamentos, para a utilização de determinada planta medicinal como medicamento, tendo visto a comprovação científica das análises farmacológicas e toxicológicas [143].

Estudos toxicológicos referente a espécie *Erythrina verna* são escassos na literatura. Existem relatos de que as sementes de *E. verna* apresentam certa toxicidade, podendo levar à problemas desde que existam outros fatores de risco associados, tais como o uso concomitante de outros medicamentos [65]. Por esse motivo também é importante verificar a composição química e os feitos tóxicos das demais partes vegetais da espécie em questão.

4. PRODUTOS TÉCNICO-CIENTÍFICOS GERADOS

Produto 1: Antimicrobial and Synergistic Activity of *Erythrina verna* Extract Against Isolates of Prominent Dental Cavity Bacteria of the mutans group streptococci

Artigo formatado segundo as normas para publicação do periódico Brazilian Journal Microbiology.

ABSTRACT

Erythrina verna Vell. (EV) popularly known as 'mulungu' is used in Brazilian folk medicine as a tranquilizer and also in the treatment of infections. This work aimed to evaluate the antimicrobial and synergistic activity of the EV extract and its chemical constituents against the mutans group streptococci (MGS) isolates. The phytochemical profile of *E. verna* extract (EVE) was determined by gas chromatography coupled with mass spectrometry, and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the butanoic extract of the EV bark and chemical constituents were identified against MGS isolates. Furthermore, we analyzed the synergistic effect of the active compounds in the extract. The EVE showed secondary metabolites and substances with antimicrobial activity, benzoic acid and azelaic acid. Of the three compounds isolated from EVE, stearic acid showed the highest bactericidal activity against the microorganisms tested. However, comparing the bactericidal activity of the crude extract and the chemical constituent isolated, the EVE showed more effective antibacterial activity, while those from the oral cavity (C17VA, C12VA and C04PD) showed greater resistance to the extract and stearic acid than the standard. strains did (ATCC 25175 and ATCC 27392). EVE demonstrated a synergistic effect when associated with chlorhexidine, with a reduction of about 98% bacterial population in all tested microorganisms. *E. verna* bark extract can be an alternative in the treatment of diseases caused by MGS, especially if associated with chlorhexidine.

Keywords: *Erythrina verna* Vell., mutans group streptococci, antimicrobial activity, medicinal plant, synergism.

INTRODUCTION

The mutans of group streptococci (MGS) comprises gram-positive bacteria (GPB) that colonize the surface of teeth, participating in the formation of the oral biofilm [1]. The *Streptococcus mutans* species is the most prominent for being an important etiological agent of dental cavities. Dental cavity is a highly prevalent and costly disease, with higher prevalence in children in early childhood and low socioeconomic status [2,3].

Biofilm formation occurs through an orderly and dynamic process where there is a need for the fixation and proliferation of bacteria on the surfaces of the teeth (reversible and irreversible bacterial adhesion), maturation

and dispersion [4]. The most used control is the mechanical method, mainly through brushing, but thismethod has some limitations and for this reason it is associated with chemical methods for the disruption of this cariogenic biofilm [5-6]. Although the association with a chemical method is often effective, the development of resistance by microorganisms to antimicrobials and usual synthetic compounds has led researchers from different areas to seek alternatives in natural products used in traditional medicine to combat the most diverse varieties of oral pathologies [7-10]. Plants are important sources of natural therapeutic products, many of which constitute a model for the synthesis of many drugs. Studies report that plant extracts and phytochemical compounds have been used more frequently for medicinal purposes [11-16].

The genus *Erythrina* belongs to the Fabaceae family and is distributed across tropical and subtropical regions of the northern and southern hemispheres. The generic name *Erythrina* comes from the Greek "*erythros*" which means red, due to the color of the flowers of the species in the genus. Among the 110 species of the genus, 70 are native to America. In Brazil, 11 species of this genus have been cataloged [17]. The species *Erythrina verna* (EV) is frequently used in Brazilian folk medicine as an anxiolytic, antidepressant, and in the treatment of insomnia. The use of *Erythrina verna* bark in the treatment of oral infections is a cultural knowledge passed on for several generations in some communities in Minas Gerais, however there are no reports of this practice in the literature and neither are there any scientific studies that prove this pharmacological action.

Popular observations on the use and efficacy of medicinal plants contribute significantly to the dissemination of their pharmacological actions. This natural medicine arouses the interest of researchers, who seek to discover and/or obtain new drugs, either in their entire form or isolated compounds. The therapeutic potential of medicinal plants is justified by the presence of bioactive components that have many proven biological activities [18,19] Among the bioactive components of plants, secondary metabolites stand out, which are crucial substances in adapting to environmental conditions and protecting plant species. Furthermore, these compounds serve as attractants for pollinators and act as agents of competition between plants and of symbiosis between plants and microorganisms [20,21]. The pharmacological potential and popular reports encourage research with *E. verna*, since the investigation of the chemical constitution of the species is essential for the association between its bioactive compounds and respective pharmacological properties.

MATERIAL AND METHOD

Botanical material

The bark of *E. verna* was collected in the Vale do Arapuim community, municipality of Varzelândia, Minas Gerais, Brazil (15.76107°S and 043.85611°W The harvest was under registration A701FE6 on SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge. The species was identified by Dr. Santos D'Angelo Neto, and the voucher specimen was deposited and registered in the herbarium of the State University of Montes Claros under the registration 5802. The validation of the species name was done through www.theplantlist.org.

Extract preparation

For the preparation of the extract, the collected EV inner bark underwent an asepsis process, followed by drying in an oven with forced air circulation at 50 °C, crushing and spraying in an industrial blender and knife mill with subsequent sieving until dusting [22]. Cold extraction was carried out using butanol as an extractor. For this procedure, 50g of powdered plant material were weighed in an amber flask, to which 500 ml of the extractor were added. The flask was kept at room temperature (27°C) for five days, with daily homogenizations. We performed rough filtration using gauze to retain the larger fragments and, subsequently, vacuum filtration to separate the smaller particles. Soxhlet extractions were performed using the solvents dichloromethane and ethyl acetate, distinctly, in the same mass-volume ratio applied for the butanoic extract (10% m/v). The extracts were dried in a forced circulation oven (model FANEM, 502) at 60 °C.

Phytochemical characterization

Preliminary phytochemical analysis of the butanoic extract from the EV bark was conducted according to the methodology described by Matos [23]. The extract was analyzed through chemical reactions of coloration and/or precipitation considering the main classes of secondary metabolites: tannins, flavonoids, steroids and triterpenoids, saponins, alkaloids, coumarin, phenolic compounds, and reducing sugars.

CG-MS Analyses

The characterization of the compounds was carried out by gas chromatography coupled to a mass spectrometer (GC-MS). Chromatographic analyses were performed using a gas chromatograph (Agilent Technologies, GC 7890A), gas chromatography-mass spectrometry (GC-MS) and DB-5 capillary column (Agilent Technologies, 30 m long x 0.25 mm internal diameter x 0.25 µm film thickness). Helium (99.9999% purity) was used as the carrier gas at a rate of 1.0 $mL.min^{-1}$. Sample preparation and analysis followed the manufacturer's instructions. The procedure was performed in triplicate. Identification of the components of each extract was performed using the mass spectra database (NIST 2.0) and scientific literature.

Antimicrobial Activity and Bacterial Strains

S. mutans ATCC 25175 and *S. sobrinus* ATCC 27392 from international reference collections of the American Type Culture Collection (ATCC) were used in this study. We also used strains (C12VA, C17VA, and C04PD) belonging to the Laboratory of Epidemiology and Biocontrol of Microorganisms and previously isolated from biofilms from the oral cavity of school-age children. These isolates belong to a study carried out previously by our research group, in which isolates of streptococci from the mutans group were selected based on the biofilm production capacity (virulence factor), where the C12VA isolate has a high, average C04PD and o C17VA low biofilm production capacity []. The microorganisms were cultivated in brain heart infusion agar (BHI – Oxoid England) at 37 ºC for 24 h. The suspension of each strain was prepared in sterile saline solution (NaCl at 0.85% m/v) and calibrated using a spectrophotometer as recommended by the Clinical and Laboratory Standards Institute (CLSI) [24].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC of the butanoic extract of *Erythrina verna* and active compounds of EV was measured using the adapted macrodilution method [24]. The extract and chemical constituents were analytically weighed and then solubilized in dimethylsulfoxide (DMSO). Each of the aforementioned bacterial strains was subjected to an antibiogram containing six concentrations (3000, 1500, 750, 375, 187.5, and 93.75 μgmL^{-1}) of the dry extract-DMSO solutions. Chlorhexidine (CLX - 0.12% v/v) was used as a reference control substance in addition to the treatments: Controls of sterility of the culture broth, controls of each active compound in the extract, and controls of the viability of the inoculum used. Each treatment was performed in triplicate. Test tubes were incubated at 37°C for 24 hours. 10 µL aliquots were removed from each tube and spread onto Petri dishes with BHI agar for subsequent direct colony counting. MBC was defined as the lowest concentration of the extract component capable of causing the death of 99.9% of bacterial cells, in relation to the count obtained in the control treatment of inoculum viability. The plates were incubated at 37 °C for 24h, followed by an assessment of the number of colonies in each replicate [25]. The absence of bacterial growth after 24h of incubation determined the MBC.

Synergistic Activity

Different combinations of EVE and chlorhexidine were tested the two against the standard strains *S. mutans* (ATCC 25175) and *S. sobrinus* (ATCC 27392) using the adapted checkerboard method [26]. The tests were performed in triplicate. The concentrations of the agents used started with the 8 times the MBC value of the extract (375 $\mu g.mL^{-1}$) and chlorhexidine (2,0 $\mu g.mL^{-1}$) were serially diluted in steps of six times, referring to 1X, (1/2)X, (1/4)X, (1/8)X, (1/16)X and (1/32)X. The 96-well plate microdilution method was used, following the standard guidelines [24]. Each bacterial strain was inoculated into BHI broth at 37 °C until reaching standard turbidity on a 0.5 McFarland scale. Then 50 µL of one substance and 50 µL of the other substance were added to the well together with 100 µL of the inoculated broth. The interpretation of the synergistic effect of each tested substance was determined by calculating the Inhibitory Fraction Index (IFI) using the following formula:

$$IFI\ of\ substance\ (A) = \frac{Combined\ MBC}{MBC\ of\ substance\ A\ alone}$$

$$IFI\ of\ substance\ (B) = \frac{Combined\ MBC}{MBC\ of\ substance\ B\ alone}$$

$$\Sigma\, IFI = IFI\ (A) + IFI\ (B)$$

The results of combinations of antimicrobial agents were classified according to the criteria proposed by Lee, Jang and Cha as synergism (IFI ≤ 0.5), indifference (IFI > 0.5< 4), and antagonism (IFI ≥ 4) [27].

Data analysis

Statistical analysis was performed in GraphPad Prism software (version 7.0, San Diego, California, USA), with 95% ($p<0.05$) confidence. Data were given as mean ± standard error (SE). Normality was checked by the Shapiro Wilk test. Statistical significance of values for the different groups was estimated by two-way ANOVA (number of bacterial cells) and t-test (synergism), followed by post-test Bonferroni control multiple comparisons.

RESULTS

The phytochemical analysis of EVE detected classes of flavonoids, tannins, phenolic compounds, triterpenes, steroids, and reducing sugars (Suppl. 1). Saponins and coumarins were not detected by the phytochemical analysis of EVE. Moreover, the extracts with the ethyl acetate and dichloromethane solvent, and butanoic extract had 13, 19, and 17 compounds identified by GC-MS analysis out of the 32, 47 and 55 components present in the samples, respectively (Suppl.2). Several fatty acids were detected, among which palmitic acid (hexadecanoic acid), stearic acid (octadecanoic acid), and linoleic acid (9Z, 12Z)-octadeca-9,12-dienoic acid) were the only ones common in the three extracts.

The butanoic extract of *E. verna* was used for antimicrobial activity assessment because it has greater bactericidal potential against MGS isolates than do the extracts with the solvents ethyl acetate and dichloromethane, a result previously observed in a work by our group research (unpublished data). Palmitic acid, linoleic acid, and stearic acid, which were present in the three extracts, were also used in the antimicrobial activity assessment (Table 1).

The identical substances found in the chromatographic analysis, in the different types of extracts, were tested for their inhibitory (MIC) and bactericidal (MBC) capacity. As to MIC and MBC evaluations, we observed that palmitic acid inhibited exclusively the isolate C17VA at concentrations equal to 375 μgmL^{-1}. Linoleic acid did not present bactericidal properties against the tested microorganisms. Interestingly, stearic acid inhibited growth of all tested isolates, with a MIC value of 375 μgmL^{-1} for *Streptococcus mutans*, *Streptococcus sobrinus*, C17VA, and C12VA, and 1500 μgmL^{-1} for C04PD. MBC value for *Streptococcus mutans*, *Streptococcus sobrinus*, C17VA and C12VA was of 750 μgmL^{-1} and for CO4PD was of 3000 μgmL^{-1}. The butanoic extract also inhibited all tested isolates, with a MIC of 187.5 $\mu gmL^{-1}$for *Streptococcus mutans* and *Streptococcus sobrinus*; 375 μgmL^{-1} for C12VA, C17VA and C04PD. MBC for *Streptococcus mutans* and *Streptococcus sobrinus* was of 375μgmL^{-1} and 750 $\mu g\ mL^{-1}$for C17VA, C12VA, and C04PD (Table 1).

The synergistic effect was evaluated by the checkerboard method, where the MIC and MBC of EVE and CLX were analyzed separately and also the combination of EVE+ CLX against MGS isolates. From the assay it was observed that for the tested isolates there was a synergistic action between EVE and CLX (EVE+CLX). For *S. mutans*, C12VA and C04PD showed an IFI of 0.375, while for *S. sobrinus* and C17VA an IFI of 0.25 (Table 2). The minimum bactericidal concentration of EVE for *S. mutans*, C12VA and C04PD was 375 $\mu g\ mL^{-1}$ when combined with CLX, MBC reduced to 93.75 $\mu g\ mL^{-1}$ (4 times lower), whereas for the isolates *S. sobrinus* and

C17VA this reduction is 8 times, going from 375 µg mL^{-1} to 46.875 µg mL^{-1} of MBC. Regarding chlorhexidine for all isolates, the combination of EVE+ CLX reduced MBC from 2.0 µg mL^{-1} to 0.25 µg mL^{-1}. The count of EVE+CLX colony forming units ($2,92x10^2$) had a reduction of more than 98% of CFUs compared to the control ($1,28x10^8$) (Figure 1)

DISCUSSION

Secondary metabolites are agents of interaction between plants and other organisms, and these compounds are generally responsible for the pharmacological action of medicinal plants. Phytochemical screening is important as it enables the identification of phytochemical constituents and indicates the relevant secondary metabolite groups in a plant species. Phytochemical prospection of EVE demonstrated the presence of flavonoids, tannins, phenolic compounds, triterpenes, steroids, and reducing sugars. These results corroborate those reported by Proença et al. who observed the presence of flavonoids, tannins, triterpenes, and steroids through thin-layer chromatography analysis of *E. verna* barks [28]. De Lima et al. reported the presence of flavonoids in the ethanol extract of *E. verna* barks [29]. Coumarins and saponins, which are substances present in this species, were not detected, but this is justified by the fact that they are found in other parts of the plant [16]. The constituents identified in the phytochemical analysis corroborate the pharmacological properties of this plant species, however, the detailed identification of compounds performed by GC-MS contributes with additional information about pharmacological roles of this species.

A total of 49 compounds was identified by GC-MS, of which only four compounds found in significant amounts were present in all extracts, namely: palmitic acid, stearic acid, linoleic acid, and glycerol. He et al. observed the presence of many fatty acids, including palmitic, stearic, linoleic, cerotic, myristic, margaric, and pentadecanoic acid, which represent important substances in the development and maintenance of plants [30]. Among the other substances identified in *Erythrina verna* extracts, benzoic and azelaic acid have antimicrobial activity [31,32]. The flavonoid trithydroxyflavonone was also identified, which belongs to a group of compounds with pharmacological actions already well described in the literature as anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antidiabetogenic, antithrombotic, antiviral, among others [33,34]. Despite alkaloids being the main substances reported in the literature in various parts of *Erithryna verna,* using the phytochemical methods of Mayer, Dranderndorff and Bouchardart, we did not detected alkaloids [16,35,36]. This was confirmed by chromatography. Some factors associated with such discrepancy may be associated with variations such as type of solvent (polarity difference between organic solvents), methodology used in the extraction process, inherent plant physiology, environmental conditions including climate, nutrition, geography, season and time of collection, soil conditions (pH and nutrients), interactions between plant/plant, plant/insects and plant/microorganisms [37-40].

The need to overcome defenses acquired by MGS against traditionally used drugs drives the search for new extracts and/or active substances. The present work is a pioneer in testing the antimicrobial action of EV against MGS isolates. The genus *Erythrina* represents a group of plants that have in its composition bioactive

compounds with antimicrobial activity [41,42]. In this study, we observed that both the plant extract and its acid constituent showed bactericidal and bacteriostatic action with different degrees of activity (MIC and MBC) against MSG bacteria. There is still no consensus among authors on the parameters of antibacterial activity for extracts and pure compounds. According to Kuete and Efferth, where antibacterial activity parameters are provided for extracts as: significant (MIC < 100 µg mL^{-1}), moderate (100 < MIC ≤ 625 µg mL^{-1}) or weak (MIC > 625 µg mL^{-1}) [43]. As for Tamokou and collaborators, it is estimated that they are very active if MIC values < 100 µg mL^{-1}, significantly active if 100 ≤ MIC ≤ 512 µg mL^{-1}, moderately active if 512 < MIC ≤ 2048 µg mL^{-1} and little active if MIC > 2048 µg mL^{-1} [44]. As for Aligiannis and collaborators, the extract is considered a strong inhibitor MIC ≤ 500 µg mL^{-1}, moderate inhibitor ≥500 µg mL^{-1}MIC ≤1500 µg $mL^{-1}$and weak inhibitor: MIC ≤1500 µg mL^{-1} [45]. Considering the Tamokou line, the butanolic extract of *E. verna* is considered to be significantly active, since it has an MIC equal to 187.5 µg mL^{-1} compared to *S. mutans* and *S. sobrinus*. And moderately active for the other strains (C17VA, C04PD and C12VA). With regard to stearic and palmitic acids, because they are pure substances, they are considered low or insignificant, since they have an MIC equal to 375 µg mL^{-1}, following criteria adopted by Kuete and Efferth for pure substances (or isolated): significant (MIC < 10 µg mL^{-1}), moderate (10 < MIC ≤ 100 µg mL^{-1}) and low or insignificant (MIC > 100 µg mL^{-1}) [43]. However, despite not falling within the parameters established above, stearic acid showed bactericidal and bacteriostatic action in all tested isolates and may present antimicrobial action when associated with other substances of the *E. verna* extract. Another factor observed was that the isolates from the oral cavity (C12VA, C17VA, and C04PD) were more resistant than were the standard strains (ATCC 25175 and ATCC 27 392) when subjected to the action of EVE and stearic acid. This phenomenon can be explained by the fact that, in order to multiply and/or colonize an ecological site, all cellular organisms produce some type of inhibitory substance as part of their self-defense mechanism. The oral cavity is an environment where complex interactions occur between microorganisms with intense competition between them; therefore, the production of compounds, such as lytic enzymes, organic acids, toxins, and peptides (bacteriocins), allow these organisms to compete or defend themselves against other living beings to guarantee their survival, factors that may explain the greater resistance of the C12VA, C17VA, and C04PD isolates to the tested substances [46].

Studies on the antimicrobial action of plant extracts demonstrate that antimicrobial effects usually occur due to structural and functional damage to the plasma membrane, action on the enzyme system and genetic material of bacteria [47,48]. Comparing the results obtained using the butanoic extract from the inner bark of *E. verna* and the identified fatty acids, we observed that the extract has superior antimicrobial potential against MGS. Some studies have shown that medicinal plant extracts exhibit more pronounced biological activities than their isolated constituents [49,50]. The complex chemical composition of extracts favors joint action on multiple cellular and molecular targets, leading to measurable biological effects. Crude extracts from plants can often have more effective antimicrobial action against pathogens due to synergism between bioactive constituents [42].

The synergistic action was observed in the present work between extract and chlorhexidine (Table 2). The combination of EVE with CLX has provided a reduction in the minimum bactericidal concentration of 8 times

for *S. mutans*, C12VA and C04PD and 16 times for *S. sobrinus* and C17VA. Regarding chlorhexidine, its association with EVE resulted in a 16 time reduction in the value of MBC for the tested isolates. Synergistic interactions are observed when the effect produced by a combination of substances is greater than what might be expected based on the individual contribution of its components [14]. The combination of active substances has shown promise in the treatment of complex diseases, as such association allows for simultaneous action on several targets, ensuring greater efficacy and less chance of drug resistance [51].

The synergic effect observed in this study allowed to reduce the concentration from one of the main antibiotic used against MSG. This probably happened by the formation of a new structural complex that allowed greater affinity with components of the bacterial cell structure, even in reduced concentration [52].

Therefore, the results have shown that the butanolic extract of *E. verna* bark can become an important adjuvant of known antibacterial drugs, being key to helping to mitigate the emergence of multi-resistant microorganisms and assist in the treatment of diseases caused by MGS.

CONCLUSION

The present study validates the potential antimicrobial effect of *Erythrina verna* extracts against different strains of the *mutans Streptococci* group. Our results showed that the crude EV bark extract has more effective antimicrobial activity against MSG than its tested isolated constituents. The association of EVE with CLX had a synergistic effect at different concentrations, in addition to providing a significant reduction in the MBC values of both. Thus, the data obtained support the use of this extract in the treatment of diseases caused by MSG and establish its capacity as a probable basis for alternative antibiotic resistance compounds.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

ACCB, LLCMP, MCA and SAMN conceived the project, DPSL, GMP, LLCMP and VHDG performed the experiments. LLCMP, MCA and SAMN wrote the manuscript. ACCB, MCA and SAMN contributed to the critical revision of the manuscript. LLCMP, MCA and VHDG edited the manuscript and helped to perform the

statistical analysis of the data. All the authors discussed the results and commented on the manuscript. The authors read and approved the final manuscript.

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Table 1. Minimum Inhibitory and Bactericidal Concentrations capable of reducing populations of the *Streptococcus mutans* group to levels less than or equal to 90%, depending on the different substances tested.

	Streptococcus strains of the mutans group									
	ATCC 25175		**ATCC 27392**		**C1[illegible]VA**		**C0[illegible]PD**		**C1[illegible]VA**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
E.verna Extract	187.5	375	187.5	375	375	750	375	750	375	750
Palmitic acid	WI	WI	WI	WI	375	1500	WI	WI	WI	WI
Stearic acid	375	750	375	750	375	750	1500	3000	375	750
Linoleic acid	WI	WI	WI	WI	WI	WI	WI	WI	WI	WI
CLX	0.5	2.0	0.25	2.0	0.25	2.0	0.5	2.0	0.5	2.0

CLX= chlorhexidine, MIC = Minimum Inhibitory Concentration (µg/mL), MBC = Minimum Bactericidal Concentration, capacity to reduce ≥90% cell count (µg/mL), WI = Without Inhibition

Table 2. Synergistic effect of *E. verna* extract with chlorhexidine on *Streptococcus mutans* group.

Strain	Substance tested	MBC (µg mL^{-1}) combined/ alone	IFI	∑IFI	Effect
S. mutans ATCC 25175	EVE	93.75/375	0.25	0.375	Synergistic
	CLX	0,25/2	0.125		
S. sobrinus ATCC 27392	EVE	46.875/375	0.125	0.25	Synergistic
	CLX	0.25/2	0.125		
C12VA	EVE	93.75/375	0.25	0.375	Synergistic
	CLX	0.25/2	0.125		
C17VA	EVE	46.875/375	0.125	0.25	Synergistic
	CLX	0.25/2	0.125		
C04PD	EVE	93.75/375	0.25	0.375	Synergistic
	CLX	0.25/2	0.125		

IFI: Inhibitory Fraction Index; (EVE): *E. verna* extract; (CLX): chlorhexidine; MBC: minimum bactericidal concentration.

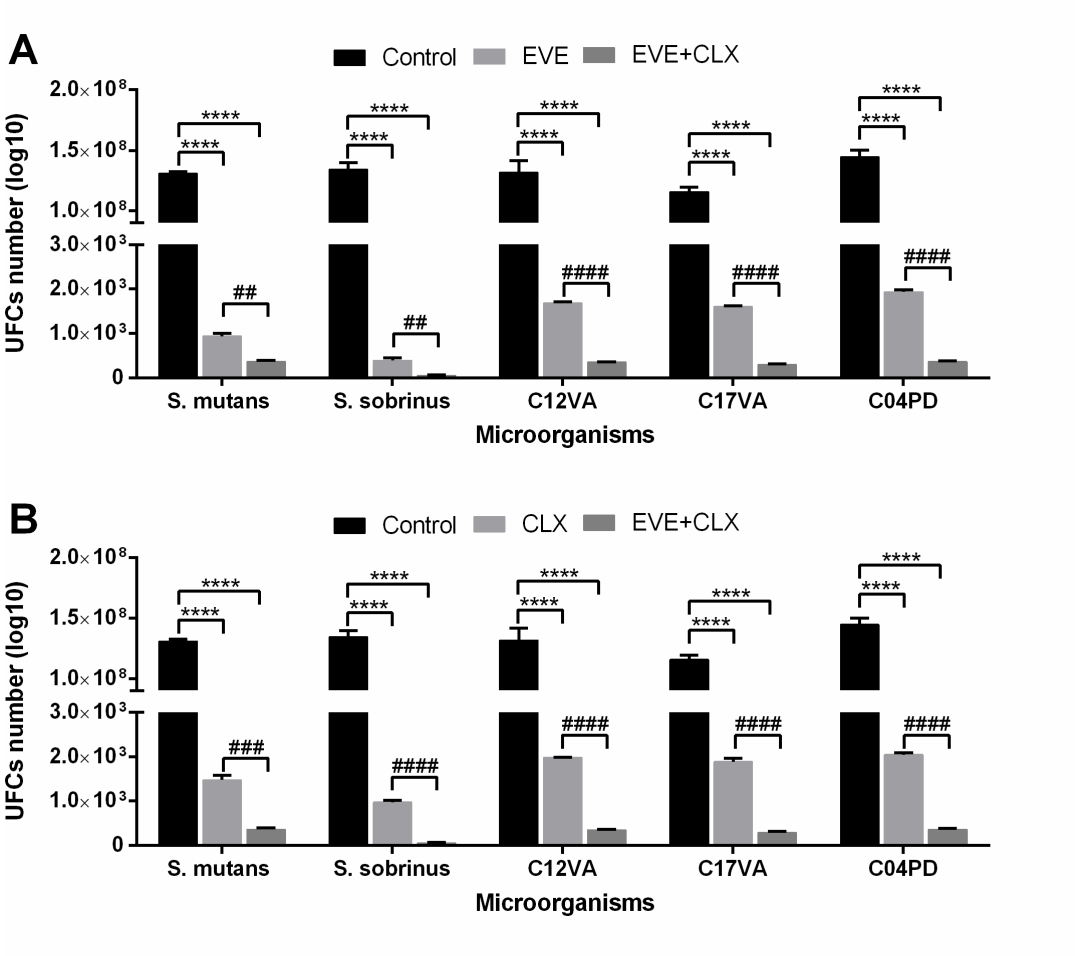

Figure 1- A-Effect of *E. verna* butanolic extract and the association of the extract with chlorhexidine and B-Effect of chlorhexidine and the association of the extract with chlorhexidine on the number of colonies forming units of the microorganisms *S. mutans*, *S. sobrinus*, C12VA, C17VA and C04PD in relation to the control. Values expressed as mean ± SEM. Two-way ANOVA followed by Bonferroni multiple comparison test. **** $p < 0.0001$ when compared to the control group. Unpaired t-test. ## $p<0.01$, ### $p<0.001$ and #### $p<0.0001$.

Produto 2: Safety evaluation of acute oral intake of *Erythrina Verna* Vell. extract (Fabaceae): a toxicological approach to clinical, biochemical, and histopathological parameters in an experimental murine model

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ABSTRACT

Erythrina verna Vell. (Fabaceae) is a Brazilian native plant commonly used in folk medicine, mainly as an anxiolytic. Although herbal compositions are marketed with this species, few studies on the toxicity profile have been reported. The aim of the study was to evaluate the safety of acute oral intake of the butanolic extract of *Erythrina verna* on clinical, biochemical, and histopathological parameters in an experimental murine model. Acute toxicity did not produce mortality or behavioral and hematological changes, however, there were changes significantly elevated HDL levels when compared to animals in the control group. Histological examination of the spleen, heart, and kidney showed degenerative characteristics with foci of inflammatory infiltrates starting at the 50 $mgKg^{-1}$ concentration. The results of the study suggest that the oral use of *Erythrina verna* bark extract should be performed with caution and at low doses.

Keywords: Acute toxicity; *Erythrina verna* Vell.; Mice; Phytotherapy.

INTRODUCTION

Since ancient times, natural products have been used in the treatment of several pathologies (Sousa et al. 2020), highlighting the medicinal plants, mainly due to the

easy access and low cost. According to the World Health Organization (WHO), about 80% of the world population uses natural products to treat diseases, and in many developing countries medicinal plants are the only way to access health (WHO 2002, Junior et al. 2005). Since the '70s, WHO has encouraged the implementation of public policies for the use of Traditional and Complementary/Alternative Medicine in member States (OMS 2006). In Brazil, the use of medicinal plants was reinforced with the publication, in 2006, of the National Policy for Integrative and Complementary Practices (PNPIC), in SUS (Unified Health System). Incorporating in Primary Health Care the use of the following alternative practices: homeopathy, medicinal plants and phytotherapy, traditional Chinese medicine/acupuncture, anthroposophic medicine, and social thermalism-crenotherapy (BRASIL 2006, BRASIL 2012).

These measures contribute to natural products gaining greater prominence and visibility; however, one must consider that the use of medicinal plants based on traditional knowledge is not enough to validate them as effective and safe drugs (BRASIL 2008), requiring scientific research of their real effects on the body, knowledge of the active principle and toxicity analysis. As with any other formulation with a medicinal application, it is necessary to guarantee the parameters of quality, effectiveness, efficiency, and safety (BRASIL 2004).

The incorrect use of plants, plant extracts, or isolated active constituents may cause several health problems, such as allergic and toxic reactions and mutagenic effects, once they present variable chemical composition, and may trigger adverse reactions due to the components themselves or by the presence of contaminants (BRASIL 2004). Studies in the area of toxicology and pharmacology of these natural products should be carried out, according to the recommendations of the legislation in force, to ensure safety and efficacy for their use (Brandão et al. 2008, Zöllner and Schwarz 2013). *In vitro* and *in vivo* research are necessary for the quality of phytotherapeutic products, considering that there is little information available regarding the toxic potential of a great part of the plants described as medicinal (Schmidt 2006). Many plant species are commonly used by the population but lack scientific studies that add to the knowledge of popular use information to ensure the safety and efficacy of its

use, such as how to prepare, how to use, appropriate dose, correct identification of the plant and knowledge of which part of the plant should be used.

In this scenario, the species *Erythrina verna has* few studies on its safe and rational use, although it is widely used in traditional medicine and is even an ingredient of phytotherapeutic formulations marketed in our country. The species *E. verna* is native to Brazil and is distributed throughout the central-western, northeastern, northern, and southeastern regions of the country (Lorenzi 1992). Due to its popular use, *E. verna* was listed among the medicinal species with the potential to generate products for SUS in the RENISUS list (National List of Plants of Interest to SUS) (BRASIL 2022), highlighting the following pharmacological actions attributed to this species the use for insomnia (Vasconcelos et al. 2011, Guaratini et al. 2014), as a tranquilizer (Flausino 2007, Patocka 2009), antidepressant (Faggion et al. 2011, De Oliveira et al. 2012), hypnotic (Faggion et al. 2011, Rosa et al. 2012), pain in general (Faggion et al. 2011, Vasconcelos et al. 2011), anti-inflammatory (Vasconcelos et al. 2011, Rosa et al. 2012), antimicrobial (De Lima et al. 2006), anticonvulsant (Faggion et al. 2011, Rosa et al. 2012), antipyretic (Oliveira 2009) and in the treatment of central nervous system disorder (Vasconcelos et al. 2007, De Oliveira et al. 2012).

Despite some reports in the literature about the toxic effects of plant parts of the species *E. verna*, there is a great lack of studies that determine the levels of toxicity and the identification of possible histological changes caused by this plant in microscopic structures of biological tissues. Thus, this work aimed to evaluate the toxicity profile of the oral ingestion of the butanolic extract of *E. verna* on clinical, biochemical, and histopathological parameters in an experimental murine model.

1. METHODS

2.1 Botanical material

The bark of *E. verna* was collected in the Vale do Arapuim community, municipality of Varzelândia, Minas Gerais, Brazil (15.76107°S and 043.85611°W). The harvest was registered under registration A701FE6 on SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge). The species was identified by Dr. Santos D'Angelo Neto, and the voucher specimen was deposited and registered in the herbarium of the State University of Montes Claros under the registration 5802. The validation of the species name was done through www.theplantlist.org.

2.2 Extract Preparation

For the extract preparation, the collected bark of *E. verna was* aseptically dried in running water and sterile distilled water (ADE) in an oven (model 502-2-A/ FANEM) with forced air circulation at 40°C, crushed, and pulverized in an industrial blender and knife mill, and then sieved until a fine powder material was obtained (Yenesew et al. 2005). For the cold butanolic extract, 50 g of the powdered plant material was weighed into an amber flask and 500 mL of the solvent (butanol) was added. The flask was then stored for five days, with daily homogenization. After this period, the extract was filtered with gauze to retain the larger fragments. Subsequently, vacuum filtration was performed to retain the smaller particles. The extracts were dried in a forced circulation oven at 50°C.

2.3 GC-MS Analyses

The characterization of the compounds was carried out by gas chromatography coupled to a mass spectrometer (GC-MS). Chromatographic analyses were performed using a gas chromatograph (Agilent Technologies, GC 7890A), gas chromatography-mass spectrometry (GC-MS) and DB-5 capillary column (Agilent Technologies, 30 m long x 0.25 mm internal diameter x 0.25 µm film thickness). Helium (99.9999% purity) was used as the carrier gas at a rate of 1.0 $mL.min^{-1}$. Sample preparation and analysis followed the manufacturer's instructions. The procedure was performed in triplicate. Identification of the components of

each extract was performed using the mass spectra database (NIST 2.0) and scientific literature.

2.4 Animals

Swiss mice (*Mus musculus*), females (nulliparous and non-pregnant), 6 to 8 weeks old and an average weight of 34 – 35 g, from the animal house of the State University of Montes Claros (Unimontes) were used for the present assay. The animals were kept under standard conditions of temperature (22° C ± 3° C), relative humidity (30-70%), and illumination (12h- light/dark cycle). They received water and diet *ad libitum throughout the* experiments. The management of the animals, from the beginning to the end of the study, was ethically conducted according to the experimental protocol approved by the Ethics Committee on Animal Experimentation and Welfare of the State University of Montes Claros (CEEBEA- Unimontes), process (227/2021).

2.5 Acute Toxicity Study

The experimental protocol was designed following the OECD (*Organization for Economic Cooperation and Development*) *Guideline* 423 (acute toxicity) guidelines (OECD, 2001). A total of 12 female mice were randomly divided into four groups (n=3 mice), which received treatment with butanolic extract of *E. verna*, here after referred to as EV. The extracts were administered in suspension, by gavage technique in a single dose at concentrations of 5, 50, 300, and 2000 $mgKg^{-1}$ in a fixed volume of 0.34 mL. The control group received only the vehicle and freshwater. After dose administration, all groups of animals were observed for 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h after dosing and then daily for a period of 14 days. Bodyweight, food consumption, and water intake were recorded. The experimental design is depicted in Figure 1.

2.6 Macroscopic Analysis (General Signs) of Toxicity

To facilitate monitoring, the animals were marked on various parts of the body. Daily, mice were observed for effects on locomotion, behavior (agitation, drowsiness, decreased activity, paw licking, avoidance reaction, and aggressiveness), respiration, cyanosis, piloerection, tail erection (straub), diarrhea, tremors, convulsions, and death

(Iwamoto et al. 2015, Litcheld and Wilcoxon 1949). The toxicity analysis was done by determining the lethal dose (LD50), statistically calculated from observations of mortality after exposure to doses/concentrations of the test substance, to verify the relationship between the dose administered and the toxicological response obtained.

2.7 Microscopic Toxicity Analysis

At the end of the treatment, the animals were fasted for 12 hours and anesthetized with an intraperitoneal injection of a solution of xylazine and ketamine of maximum 0.10mL/100g of body mass, in the proportion of 8.75mL of ketamine and 1.25 mL of xylazine. Blood samples were collected from abdominal laparotomy, followed by exsanguination via the inferior vena cava. One part of the blood was added in tubes containing EDTA (for evaluation of hematological parameters), and another part in dry tubes (for evaluation of biochemical parameters). The animals were necropsied for macroscopic evaluation of organ morphology. The organs kidney, liver, lung, spleen, and heart were carefully removed, dissected, and had their weight measured on analytical scales (Vijavalakshmi et al. 2000).

2.8 Histopathological analysis

Portions of each organ were fixed in 10% neutral buffered formalin solution and subjected to the histological process of progressive dehydration in alcohol, diaphanization, and clarification with xylene and paraffin embedding. The sections (5µm thick) were stained with hematoxylin and eosin (H&E). Evaluations were performed using a fluorescence microscope (Olympus, Center Valley, PA, USA). The analysis was performed blinded by a pathologist.

2.9 Statistical analysis.

Statistical analysis was performed in GraphPad Prism software (version 7.0, San Diego, California, USA), with 95% ($p<0.05$) confidence. Data were given as mean ± standard error (SE). Normality was checked by the Shapiro Wilk test. Statistical significance of values for the different groups was estimated by one-way ANOVA (organs weight, hemogram, and biochemical profile) and two-way ANOVA (body weight and intake), followed by post-test Bonferroni control multiple comparisons.

2. RESULTS

3.1 Identification of chemical compounds by GC/MS

Twelve substances present in the crude extract of *E. verna* (Table S1).

3.2 Ingestion of E. verna extract does not produce clinical signs of toxicity in animals.

The intragastric administration of a single dose of the butanolic extract of *E. verna* at concentrations of 5, 50, 300 and 2000 $mgKg^{-1}$ in *Swiss* mice did not produce mortality or signs of toxicity during the 14-day observation period. At concentrations of 300 $mgkg^{-1}$ and 2000 $mgKg^{-1}$ of the EV butanolic extract, reduced mobility (no response to stimuli) was observed after 30 minutes of gavage and recovered after 4 hours of treatment. No significant changes were observed in body weight over time, water consumption and food intake during the 14 days of acute oral administration of *E. verna* extract compared to the control group (Figure 2). Acute administration of the EV butanolic extract did not produce death in 50% of the mice at the maximum dose of 2000 $mgkg^{-1}$. Therefore, the LD50 was higher than 2000 $mgKg^{-1}$.

3.3 Evaluation of body weight, relative organ weight, food, and water intake

The 14-day treatment did not result in significant changes in the weight (g) of the animals (Figure 2), also without reflection on the relative weight of the organs in the treated groups compared to the control group (Table 3). Regarding food intake, there was no change in feed consumption by animals in any group. However, animals that received EV extract at a concentration of 50 $mgkg^{-1}$ ingested more water than the control animals ($p<0.005$).

3.4 Oral administration of E. verna does not produce hematological changes

Regarding the hematological parameters, no significant changes were seen in the animals treated with the different doses of EV when compared to the control, as verified in Table 1.

3.5 Acute administration of E. verna butanolic extract does not produce signs of toxicity on biochemical parameters

Biochemical parameters were evaluated after 14 days of oral administration of *E. verna* extract and are shown in Table 2. EV administration of 50 $mgKg^{-1}$ resulted in a significant increase in glutamic oxaloacetic transaminase (TGO) levels. In turn, animals treated with the concentrations of 300 and 2000 $mgKg^{-1}$ showed significantly increased levels of HDL (high-density lipoprotein) when compared to animals in the control group. For the other parameters evaluated, no significant differences were observed when compared to the control group.

3.6 Acute consumption of E. verna extract causes dose-dependent histopathological changes in experimental animals

Acute administration of the butanolic extract of *E. verna* produced dose-dependent histological changes in the liver, kidney, lung, and spleen (Figure 3).

The impact on renal tissue was more intense since changes were observed in the groups treated with the butanolic extract of *E. verna* at all concentrations, with intensity positively dose-related and associated with the presence of inflammatory process and glomerular alteration.

The histological analysis of the liver and heart shows that the animals treated with the extract did not show any changes in the organs compared to the control group. In the spleen, the concentration of 5 $mgKg^{-1}$ did not present changes, but distinct consequences were evidenced with the increase of the dose: with 50 mg/Kg we observed punctual necrotic processes. At the dose of 300 $mgKg^{-1}$, there was an increase in the necrotic process with relevant macrophage infiltration, and these signs were intensified at the dose of 2000 $mgKg^{-1}$. In the lung, no changes caused by the extract were observed.

DISCUSSION

The species *E. verna* is traditionally used in folk medicine, mainly to treat insomnia, nervous system disorders and as a sedative (Vasconcelos et al. 2011, Guaratini et al. 2014, Rosa et al. 2012). Although this plant is widely used in traditional medicine in macerates (oral and topical use), there is a gap in the scientific literature about its oral toxicity. Therefore, the present study adds information on the toxicological profile of the butanolic extract of the bark of *E. verna*, performing an oral toxicity test in mice for 14 days.

In toxicological analysis, the main criterion for assessing acute toxicity by a substance (LD50) is mortality (Asare et al. 2012). No deaths were observed after single-dose administration of the extract. The chemical substance can be classified into three categories according to toxicity in rats: very toxic, a substance with LD50 less than 25 $mgKg^{-1}$; toxic, substances with DL50 between 25 and 200 $mgKg^{-1}$; and harmful with DL50 between 200 and 2000 $mgKg^{-1}$. According to OECD 423 (OECD, 2001), the bark extract of *E. verna* has low acute toxicity and should be included in category 5 with an estimated LD50 greater than 2000 $mgKg^{-1}$. A study by Proença and colleagues (2012) evaluated the LD50 for the hydroalcoholic extract, from the barks of *E. verna*, administered as a single oral dose in Swiss mice, and no death was reported in the animals during the observation period (14 days).

De Bona and collaborators (2012), in a study with extract of the leaves and/or inflorescences of *E. verna*, when administered as a single dose intraperitoneally in Swiss mice, observed an LD50 of 1.37 gkg^{-1} of the inflorescence extract, and for the leaf extract, there was no death of the animals up to 48 hours of observation. The genus *Erythrina*, despite presenting several pharmacological actions widespread by popular knowledge, must be used with caution, since depending on the plant part or species used it can have a toxic effect. A study with aqueous extract of *E. velutina* leaves did not observe mortality or adverse symptoms up to a dose of 5 gKg^{-1} in rats orally (Silva 2008).

However, hydroalcoholic extract of *E. velutina* inflorescence has a median lethal dose (LD50) equal to 1.37 gKg^{-1} (Leite et al. 2006). Lollato et al. (2010), evaluating extracts of *E. speciosa* did not observe mortality or adverse effects up to a dose of

2000 $mgKg^{-1}$ in mice. The species *E. falcata* presents an intermediate dose between 3.75 gkg^{-1} and 5.0 gkg^{-1} (Cerutti et al. 2000) and *E. senegalensis* presents a dose of 450 $mgKg^{-1}$ (Saidu et al. 2000).

Another important factor to analyze the toxicity of a substance is behavioral changes, and in our study, the extract analyzed did not affect the behavior of animals, except motility, which was affected during the first 4 hours after the extract administration. The *E. verna* extract sedative action may explain the reduced motility observed. Pereira and Machado (2008) in a study with *E. verna* showed that acute intraperitoneal administration of *E. verna* bark hydroalcoholic extract provided an anxiolytic effect, comparable to clonazepam.

Changes in body weight without metabolic regulation may be an indicator of toxicity (Yi-Chen et al. 2018). In our study evaluating acute toxicity, oral administration of the extract did not cause significant changes in the animals' body weight, in relation to the relative weight of the evaluated organs, only the spleen showed a significant difference (5, 300 and 2000 $mgKg^{-1}$) when compared to animals in the control group.

Because hematological parameters usually indicate a pathological state before macroscopic changes, this evaluation is of great importance as they are highly sensitive indicators of drug-induced toxicity (Marinho et al. 2022). In the present study, single-dose administration of the butanolic extract of the bark of *E. verna* did not result in hematological changes in most parameters. There was a change in hemoglobin at concentrations of 300 and 2000 $mgkg^{-1}$. However, it should be considered that hematological and biochemical changes occur relatively slowly, and the experimental time and administration profile may not be sufficient to identify all possible changes (Ferreira et al. 2014).

In the present study, the administration of a single dose of butanolic extract from the bark of *Erythrina verna* did not result in hematological changes in most parameters, there was a change in hemoglobin at concentrations of 300 and 2000 $mgkg^{-1}$.

Interestingly, the concentrations of 300 and 2000 $mgKg^{-1}$ showed an increase in the HDL parameter when compared to the control, demonstrating a hypolipemic property

of *E. verna*. This increase may represent a beneficial action to the body, since HDL, high-density lipoproteins, are responsible for removing excess free cholesterol from the periphery, driving it to the liver, and promoting metabolization and secretion into bile, which is known as reverse cholesterol transport (Lemos et al. 2006). What needs to be clarified is which active ingredient is inducing this increase and whether it might not be a counterregulatory mechanism of homeostatic processes altered by *E.* verna. For the other biochemical variables, blood glucose, total cholesterol, triglycerides, TGP, and albumin, the results showed that the levels were within the normal range (Table 2).

Few histological studies of *E. verna* extract are reported in the literature, despite the importance of understanding the microscopic events underlying the macroscopic changes that occur in organs following *E. verna* administration. This evaluation is of utmost relevance to ensure reliability and safety of use. And interestingly, it was in these analyses that we demonstrated the most impacts of the tested substance on the animals' bodies. Histopathological changes in the kidneys were observed in the acute toxicity test in animals that received any test concentrations of the extract. The kidney is an organ that performs three main functions, including the elimination of toxic substances that are produced during metabolism, the regulation of hemostasis of the internal fluid medium, and the production of hormones that can be used to assess renal status (Oh and Hustead 2011). Several metabolites present in medicinal plants, such as flavonoids, can be toxic to the kidneys, renal tubular changes have been described following exposure to these compounds (Wang et al. 2019). In the present study, the kidney presented changes in tissue architecture from the concentration of 50 $mgKg^{-1}$ when compared to the control (arrow). In the concentration of 50 $mgKg^{-1}$ there was loss of distal tubule architecture, 300 $mgKg^{-1}$ presence of swelling, with compressed Bowman's Capsule and decrease in the lumen of the distal tubules and 2000 $mgKg^{-1}$ presence of inflammatory process.

In the histological analysis of the heart, it was observed that the EV extract from the concentration of 5 $mgKg^{-1}$ presented inflammatory infiltrates. Cardiotoxicity is a fundamental parameter to be observed in phytotherapy since the substances present in natural products can cause heart failure and lead to death (Thrall et al. 2015). The

spleen presented alterations in those treated with EV extract, at concentrations of 50, 300 and 2000 $mgKg^{-1}$, presence of giant cells (proportional to dose dependent) and the high number of macrophages observed in the histological analysis indicates a mononuclear inflammatory process determined by innate immunity, perhaps because little-selective antigenic stimuli coming from natural products induce less aggressive inflammatory responses to the organism. Further studies with more analysis points will demonstrate details of this inflammatory process, delimiting its intensity, cellular activation *status*, and the resolving aspects mediated by cytokine profiles.

Toxicological studies are necessary to ensure safety in the use of natural products since several toxic substances of chemical active principles are described in the literature (Atchou et al., 2021; Auti and Kulkarni, 2019; Figueredo et al., 2018). This study showed that *E. verna* extract does not produce mortality in mice by a single dose, however, punctual alterations, dose-dependent, observed in different tissues signal and reinforce caution for its oral use. Moreover, we highlight that the study performed by OECD 423 presents itself as toxicity screening, making it possible to explore target organs with a larger number of animals and for a prolonged period of drug administration to visualize significant differences. Thus, we reinforce the importance of this study to support the selection of the dose for future studies of chronic toxicity tests, as well as the safe use of this species by the population.

3. CONCLUSION

The present toxicity study found that the acute oral lethal dose of butanolic extract of *E. verna* is higher than 2000 $mgKg^{-1}$. However, even single doses should be used with caution, since these doses of 5, 50, 300, and 2000 $mgKg^{-1}$ resulted in histological changes in the spleen, kidney, and heart of mice. Because *E. verna* is already used in Brazilian popular medicine for the treatment of insomnia, nervous system disorders, and as an anxiolytic and sedative, these results provide primary but valuable data regarding the toxicity profile of this species that can guide future studies on the action of bioactive compounds and their actions on target organs.

LIST OF ABBREVIATIONS

ALT – alanine aminotransferase; ANOVA – analysis of variance; AST– aspartate aminotransferase; BW – body weight; CEEBEA – ethics committee on animal experimentation and welfare; AP – alkaline phosphatase; HDL – high-density lipoprotein; H&E – haematoxylin and eosin; SisGen – Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado; SE – standard error; EV – *Erythrina verna*; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; MCV = Mean corpuscular volume.

DECLARATION OF INTERESTS

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

ETHICS APPROVAL

All applicable institutional and/or national guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

All authors do not have conflicts of interest.

AUTHOR CONTRIBUTIONS

ACCB, LLCMP, MCA and SAMN conceived the project, DPSL, RVF, BVC, GMP, LLCMP and VHDG performed the experiments. LLCMP, MCA and SAMN wrote the manuscript. ACCB, MCA and SAMN contributed to the critical revision of the manuscript. LLCMP, MCA and VHDG edited the manuscript and helped to perform the statistical analysis of the data. All the authors discussed the results and commented on the manuscript. The authors read and approved the final manuscript.

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Figure legends

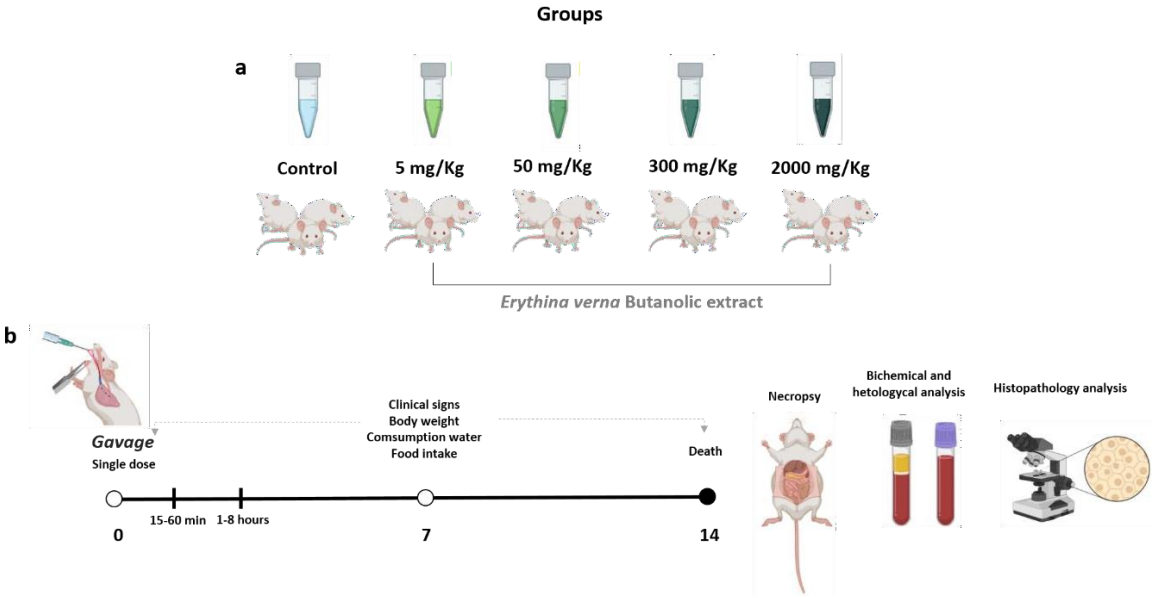


Figure 1. Summary methodological scheme of the procedures performed in the study.

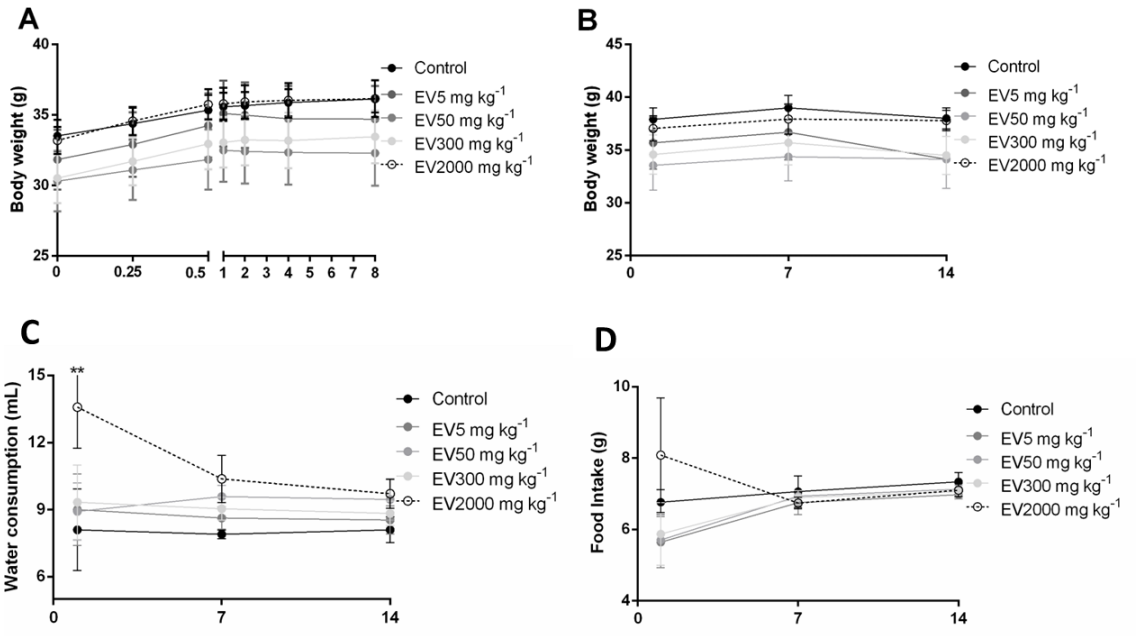


Figure 2. A and B- Effect of the butanolic extract of *E. verna* on the body weight of female Swiss mice treated in a single dose at concentrations 5, 50, 300 and 2000 mgKg^{-1} in relation to the control. C and D – Values obtained in the consumption of water (C) and feed (D) evaluated for 14 days, in female mice, in the control group and treated at different doses with

E. verna extract. Values expressed as mean ± SEM. One-way ANOVA followed by Bonferroni multiple comparison test. ** p <0.1 when compared to the control group, n = 3 for each group.

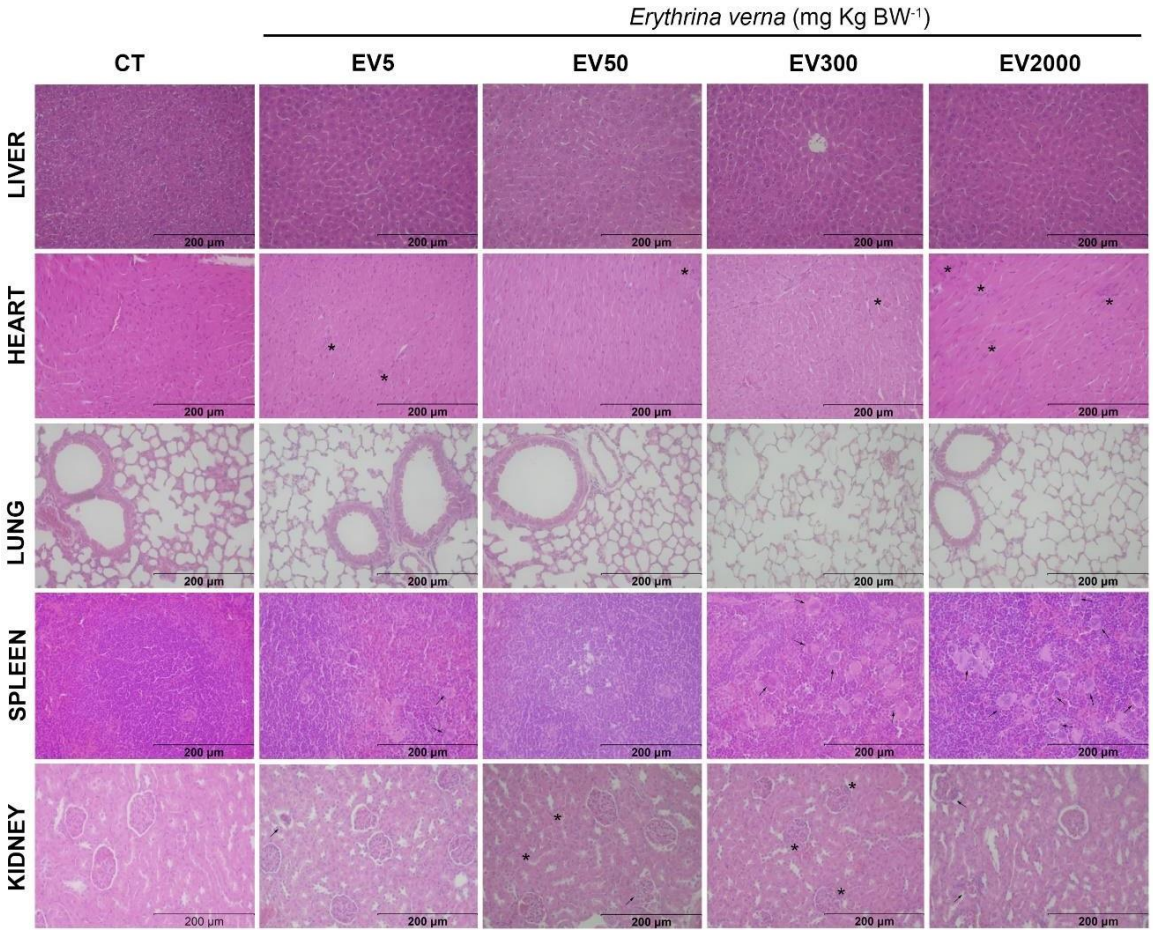


Figure 3. Photomicrograph of the liver, kidney, spleen and lung of female mice control and treated with 50, 300 and 2000 mgKg^{-1} of butanolic extract of *Erythrina verna* in the acute oral toxicity test after 14 days of treatment. Size bar for comparison 200 µm. H& E (20x). * Inflammatory infiltrate.

Table 1

Parameters	Control	*Erythrina verna*			
		5 mgKg^{-1}	**50 mgKg^{-1}**	**300 mgKg^{-1}**	**2.000 mgKg^{-1}**
Erytrocytes (millions/µL)	8.62 ± 0.11	8.89 ± 0.34	9.05 ± 0.42	9.07 ± 0.19	9.65 ± 1.46
Hemoglobin (gm/dL)	14.5 ± 0.81	15.6 ± 0.32	15.2 ± 0.42	16.6 ± 0.26**	15.9 ±0.61*
Hematocrit (%)	47.47 ± 0.38	47. ± 1.51	51.9 ± 3.06	51.9 ±1.37	50.9 ± 1.90
MCV (fL)	55.06 ±0.49	53.8 ± 1.45	57.3 ± 1.51	57.3 ± 2.48	53.4 ± 6.79
MCH (pg)	16.78 ± 0.78	17.6 ± 0.81	16.9 ± 1.24	18.3 ± 0.13	16.8 ± 3.02
MCHC (g/dL)	30.46 ± 1.47	32.7 ± 0.89	29.4 ± 2.37	32.0 ± 1.30	31.2 ± 1.66
Platelets	970.33 ± 167.81	1,938.33 ± 185.73	1,749.33 ±101.05	2,663.33 ± 873.79	1,918.33 ± 811.20
Total leucocytes	4200.00 ± 624.5	3833.33 ± 251.7	3033.3 ± 208.2	4366.7 ± 950.4	5066.7 ± 1887.7
Segmented neutrophils (%)	41.33 ± 6.11	38.3 ± 7.64	26.7 ± 2.89	36.7 ± 5.77	44.0 ± 21.0
Lynphocytes (%)	56.67 ± 5.77	61.7 ± 7.64	73.3 ± 2.89	63.0 ± 6.08	55.0 ± 21.8
Eosinophil (%)	2.00 ± 2.0	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.58	1.00 ± 1.00
Monocytes (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0,00	0.00 ± 0.00

MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume. Values expressed as mean ± SEM. One-way ANOVA followed by Bonferroni multiple comparison test. * $p < 0.05$, ** and $p < 0.1$ when compared to the control group, n = 3 for each group.

Table 2

Parameters	Controle	Erythrina verna			
		5 $mgKg^{-1}$	**50 $mgKg^{-1}$**	**300 $mgKg^{-1}$**	**2.000 $mgKg^{-1}$**
Colesterol Total (mg/dl)	96.7 ± 16.17	131.3 ± 6.11	129.0 ± 31.0	138.3 ± 48.69	67.3 ± 19.43
Glicose (mg/dl)	170.3 ± 74.3	189.3 ± 18.2	157.3 ± 4.16	131.3 ± 48.8	134.0 ± 26.5
AST (U/L)	158.0 ± 42.1	213.3 ± 15.5	217.3 ± 26.0	224.0 ± 57.7	224.0 ± 52.3
Triglicerideos (mg/dL)	131.3 ± 40.3	92.7 ± 5.77	65.0 ± 6.25***	98.3 ± 5.86	133.3 ± 37.0
ALT (U/L)	29.7 ± 11.0	38.3 ± 6.80	33.0 ± 10.1	37.7 ± 11.6	32.7 ± 1.20
Albumina (g/dl)	3.57 ± 0.38	4.67 ± 0.64	4.50 ± 0.30	4.07 ± 0.95	4.73 ± 0.23
HDL (mg/dl)	28.7 ± 5.87	36.3 ± 3.06	38.5 ± 5.16	58.1 ± 9.43***	53.7 ± 3.84**

HDL = high density lipoprotein; AST = aspartate aminotransferase; ALT = alanine aminotransferase. Values are mean ± SEM. (n = 3). n significantly different from control, $p < 0.05$ using the Kruskal-Wallis test, followed by the post-Dunn test. Values expressed as mean ± SEM. One-way ANOVA followed by Bonferroni multiple comparison test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group., n = 3 for each group.

Table 3.

Organs	Control	Erythrina verna			
		5 mgKg^{-1}	**50 mgKg^{-1}**	**300 mgKg^{-1}**	**2.000 mgKg^{-1}**
Spleen (g/BW)	0.0043 ± 0.0004	0.0044 ± 0.0005	0.0039 ± 0.0014*	0.0038 ± 0.0012**	0.0043 ± 0.0006*
Heart (g/BW)	0.0045 ±0.0005	0.0048 ± 0.001	0.0054 ± 0.0019	0.0047 ± 0.0007	0.0045 ± 0.0005
Liver (g/BW)	0.0540 ± 0.0056	0.0474 ± 0.0012	0.0440 ± 0.0038	0.0429 ± 0.0019	0.0437 ± 0.0019
Lung (g/BW)	0.0063 ± 0.0003	0.0099 ± 0.0020	0.0064 ± 0.0013	0.0096 ± 0.0012	0.0067 ± 0.0006
Kidney (g/BW)	0.0112 ± 0.0005	0.0121 ± 0.0006	0.0108 ± 0.0008	0.0114 ± 0.0002	0.0123 ± 0.0009

BW- Body Weight. One-way ANOVA followed by Bonferroni multiple comparison test * $p < 0.05$, ** $p < 0.1$ when compared to the control group.

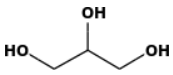
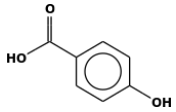
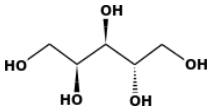
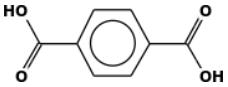
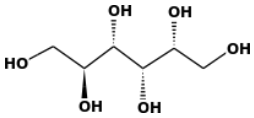
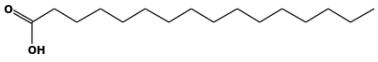
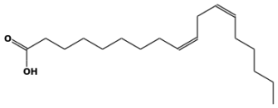
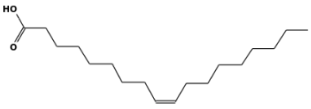
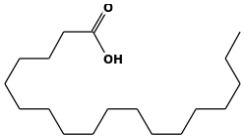
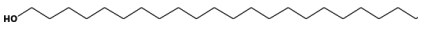
Table 1. Hematological parameters of Swiss mice treated with butanolic extract of *Erythrina verna* bark in the acute toxicity study.

Table 2. Biochemical parameters of Swiss mice treated with butanolic extract of *Erythrina verna* bark in the acute toxicity study.

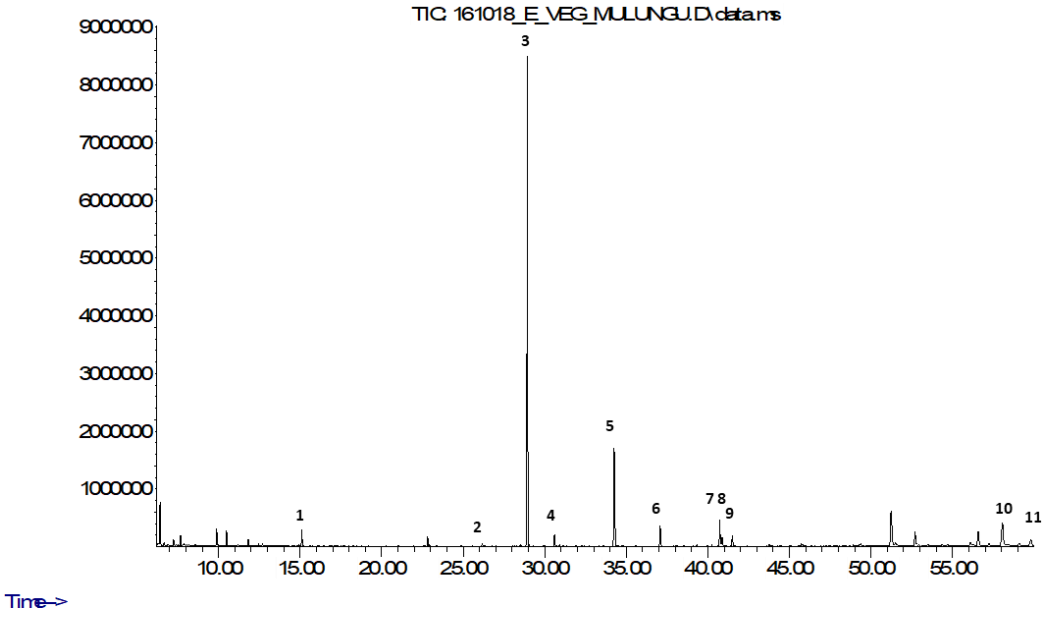
Table 3. Effect of the butanolic extract of *Erythrina Verna* bark on the relative weight of organs in *Swiss* mice treated for 14 days with different doses.

Supplementary material

Table S1. Chemical constituents identified by GC/MS from crude extract of *Erythrina verna*.

Peak	RT (min)	Area	%	Compound	Chemical structure	Reference
1	15.10	902164	1.43	Glycerol		[NIST]
2	26.16	7447225	0.28	4-Hydroxybenzoic Acid		[NIST]
3	28.94	1474834	50.16	Arabitol		[NIST]
4	30.59	261211587	1.16	Terephthalic acid		[NIST]
5	34.25	6054125	11.91	Glucitol		[NIST]
6	37.06	62028244	2.30	Hexadecanoic acid		[NIST]
7	40.72	11983991	2.51	(Z,Z)-9,12-Octadecadienoic acid		[NIST]
8	40.86	13087589	0.98	(Z)-Octadec-9-enoic acid		[NIST]
9	41.49	5112903	1.21	Octadecanoic Acid		[NIST]
10	56.57	15895056	3.05	Hexacosan-1-ol		[NIST]
11	59.79	7624170	1.46	Hexacosanoic Acid		[NIST]

RT: retention time; %: relative area


Abundance
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5. CONSIDERAÇÕES FINAIS

Os resultados permitem concluir que o extrato da casca de *Erythrina verna* apresenta potencial efeito antimicrobiano contra diferentes cepas de estreptococos do grupo mutans. Além disso o extrato bruto de EV foi mais eficiente na inibição dos microrganismos testados comparado com seus compostos isolados. O uso do extrato de EV associado com a clorexidina possui efeito sinérgico, uma vez que reduz significativamente o número de unidades formadoras de colônia. Com relação a toxicidade constatou que a dose letal oral aguda do extrato butanólico de *E. verna* é superior a 2000 $mgKg^{-1}$. Os dados obtidos suportam o uso deste extrato no tratamento de doenças causadas por estreptococos do grupo mutans e estabelecem sua capacidade como provável base para compostos alternativos de resistência a antibióticos. No entanto, mesmo doses únicas devem ser usadas com cautela, pois doses de 5, 50, 300 e 2.000 $mgKg^{-1}$ resultaram em alterações histológicas no baço, rim e coração de camundongos.

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ANEXOS

ANEXO A – Parecer do Comitê de Ética e Pesquisa

Universidade Estadual de Montes Claros
Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes
CEEBEA

Unimontes

CERTIFICADO

Certificamos que o protocolo nº **227**, relativo ao projeto intitulado *"Erythrina mulungu* (Mart. ex Benth): Estudo fitoquímico, atividade biológica, toxicológica e mutagênica de seus constituintes" - Coordenador: Prof. Dr. Sérgio Avelino Mota Nobre está de acordo com os princípios éticos na experimentação animal, adotados pela Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes, e encontra-se **APROVADO**.

A quantidade total de animais pelo CEEBEA para este projeto foi de 27 animais.
Este certificado é válido por cinco anos após sua aprovação.

Montes Claros, 06 de Agosto de 2021.

Profª Drª Antonia de Maria Filha Ribeiro
Coordenadora do CEEBEA/UNIMONTES

Profª Antonia de Maria F. Ribeiro
Coordenadora da
CEEBEA da Unimontes

Ao retornarmos as atividades presencias todos os documentos como CD, protocolo, projeto impresso, memorando ou Ata do departamento deverão ser entregue a CEEBEA/Pró-Reitoria de Pesquisa.

ANEXO B – Normas do periódico Brazilian Journal Microbiology.

Brazilian Journal of MicrobiologySubmission Guidelines

Last update: March 2021

The official journal of the Brazilian Society of Microbiology

Online ISSN: 1678-4405

About the journal: https://www.springer.com/journal/42770/

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Table of Contents

Type of Articles

The Brazilian Journal of Microbiology accepts submissions of the following article types:

- Research Papers: report results of original research, which has not been published elsewhere.
- Short communications: a short communication should report new and significant findings. Submit form is the same way as research paper. They receive the same review, they are not published more rapidly than research paper.
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Your manuscript must be written clearly, in comprehensible and linguistically correct English. Manuscripts written in poor English will not be accepted. Please check the section "English Language Support" how to get assistance.

Sections

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- Food Microbiology: Applications of microorganisms (bacteria and fungi) for food production. Food borne diseases, food spoilage, and microbial ecology in foods.
- Bacterial and Fungal Pathogenesis: The genetic, biochemical, and structural basis of bacterial pathogenesis.
- Clinical Microbiology: Studies of medically-important bacteria, fungi and virus.
- Environmental Microbiology: Ecology of natural microbial assemblages, microbial diversity of natural environments such as water, soil, sediments and higher organisms. Microbial interactions. Biodegradation, Bioremediation, and Environmental considerations for genetically engineered microorganisms.

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- Bacterial, Fungal and Virus Molecular Biology: Fungal and bacterial genetics, molecular biology, gene regulation, DNA replication and repair, genomics, proteomics, transcriptomics.

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• Contributed new methods or models
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When applicable, also include trial registration number and date of registration

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Consent to participate (include appropriate statements) Consent for publication (include appropriate statements)Availability of data and material (data transparency) Code availability (software application or custom code)

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  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8
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- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

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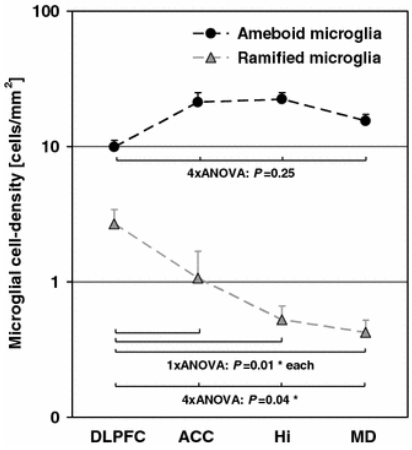

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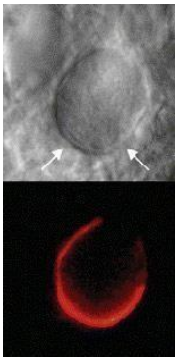

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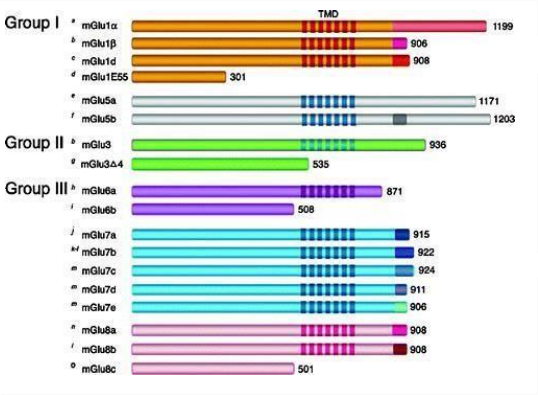

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Research Data Policy

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Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the

Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled “Compliance with Ethical Standards” when submitting a paper:

Disclosure of Potential Conflicts of Interest

- Research grants from funding agencies (please give the research funder and the grant number)
- Honoraria for speaking at symposia
- Financial support for attending symposia
- Financial support for educational programs
- Employment or consultation
- Support from a project sponsor
- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

- Position on advisory board or board of directors or other type of management relationships
- Multiple affiliations
- Financial relationships, for example equity ownership or investment interest
- Intellectual property rights (e.g. patents, copyrights and royalties from such rights)
- Holdings of spouse and/or children that may have financial interest in the work

In addition, interests that go beyond financial interests and compensation (non-financial interests) that may be important to readers should be disclosed. These may include but are not limited to personal relationships or competing interests directly or indirectly tied to this research, or professional interests or personal beliefs that may influence your research.

The corresponding author collects the conflict of interest disclosure forms from all authors. In author collaborations where formal agreements for representation allow it, it is sufficient for the corresponding author to sign the disclosure form on behalf of all authors. Examples of forms can be found

here:

The corresponding author will include a summary statement in the text of the manuscript in a separate section before the reference list, that reflects what is recorded in the potential conflict of interest disclosure form(s).

See below examples of disclosures:

Funding: This study was funded by X (grant number X).

Conflict of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is a member of committeeZ.

If no conflict exists, the authors should state:

Conflict of Interest: The authors declare that they have no conflict of interest.

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or

comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed inthe manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on Informed Consent.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: *Filip1*$^{tm1a(KOMP)Wtsi}$ RRID:MMRRC_055641-UCD

Cell Line: RST307 cell line RRID:CVCL_C321

Antibody: Luciferase antibody DSHB Cat# LUC-3, RRID:AB_2722109

Plasmid: mRuby3 plasmid RRID:Addgene_104005

Software: ImageJ Version 1.2.4 RRID:SCR_003070

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register anew resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectivelyassigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as “A health intervention is an actperformed for, with or on behalf of a person or population whose purpose is to assess, improve,
maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For
example www.clinicaltrials.gov or any of the primary registries that participate in the WHO InternationalClinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered’ should be included as the last line of the manuscript abstract.

Purely observational trials will not require registration.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

- Randomised trials (CONSORT) and Study protocols (SPIRIT)
- Observational studies (STROBE)
- Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)
- Diagnostic/prognostic studies (STARD) and (TRIPOD)
- Case reports (CARE)
- Clinical practice guidelines (AGREE) and (RIGHT)
- Qualitative research (SRQR) and (COREQ)
- Animal pre-clinical studies (ARRIVE)
- Quality improvement studies (SQUIRE)
- Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and included on a title page that is separate from the manuscript with a section entitled "Declarations" when submitting a paper. Having all statements in one place allows for a consistent and unified review of the information by the Editor-in-Chief and/or peer reviewers and may speed up the handling of the paper. Declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements. Please use the following template title page for providing the statements.

Once and if the paper is accepted for publication, the production department will put the respective statements in a distinctly identified section clearly visible for readers.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

• Provide “Ethics approval” as a heading (see template)

Examples of ethics approval obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No..).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was grantedby the Ethics Committee of University B (Date.../No).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of C (Ethics approval number: ...).

Examples of a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospectivenature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval.An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples no ethical approval required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethicalapproval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, whichprovides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional

Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent or guardian if the participant is incapable) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that theprior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if thedeceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by theeditor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and included on a title page that is separate from the manuscript with a section entitled "Declarations" when submitting a paper. Having all statements in one place allows for a consistent and unified review of the information by the Editor-in-Chief and/or peer reviewers and may speed up the handling of the paper. Declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements. Please use the template Title Page for providing the statements.

Once and if the paper is accepted for publication, the production department will put the respective statements in a distinctly identified section clearly visible for readers.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Provide “Consent to participate” as a heading

Sample statements consent to participate:

- Informed consent was obtained from all individual participants included in the study.
- Informed consent was obtained from legal guardians.
- Written informed consent was obtained from the parents.
- Verbal informed consent was obtained prior to the interview.
- The patient has consented to the submission of the case report for submission to the journal.

Provide “Consent to publish” as a heading

- The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.
- The participant has consented to the submission of the case report to the journal.
- Patients signed informed consent regarding publishing their data and photographs.
- Sample statements if identifying information about participants is available in the article:
- Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.
- Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.
- If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.
- Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

- Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

Research involving animals

Experimental research on vertebrates or any regulated invertebrates must comply with institutional, national, or international guidelines, and where available should have been approved by an appropriate ethics committee. The Basel Declaration outlines fundamental principles to adhere to when conducting research in animals and the International Council for Laboratory Animal Science (ICLAS) has also published ethical guidelines.

A statement detailing compliance with relevant guidelines (e.g. Guide for the Care and Use of LaboratoryAnimals and Directive 2010/63/EU in Europe) and/or ethical approval (including the name of the ethics committee and the reference number where appropriate) must be included in the manuscript.

For experimental studies involving client-owned animals, authors must also document informed consent from the client or owner and adherence to a high standard (best practice) of veterinary care.

Field studies and other non-experimental research on animals must comply with institutional, national, or international guidelines, and where available should have been approved by an appropriate ethics committee. A statement detailing compliance with relevant guidelines and/or appropriate permissions or licenses must be included in the manuscript. We recommend that authors comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Utilization of plants, algae, fungi

This journal values stewardship, transparency, and adhering to governance with regards to collecting and utilizing specimens and conducting experiments and/or field studies. Therefor the journal sets out the following guidelines:

Field studies involving genetically engineered plants must be conducted in accordance with national or local legislation and, if applicable, the manuscript needs to include a statement specifying the appropriate permissions and/or licences.

Authors utilizing genetic plant resources received via local suppliers/collectors, such as species collected from protected areas or endangered species with medical importance, must conduct their experiments following the Nagoya Protocol (as part of the Convention on Biological Diversity).

Authors whose research is focusing on quarantine organisms (i.e. harmful or pest organisms, including plant pathogens) should adhere to national legislation and notify the relevant National Plant Protection Organization of new findings before publication. More information can be found via the International Plant Protection Convention.

In principle, it is recommended that authors comply with:

- The International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and consult the IUCN red list index of threatened species
- Convention on the Trade in Endangered Species of Wild Fauna and Flora

Voucher specimens ensure that the identity of organisms studied in the field or in laboratory experiments can be verified, and ensure that new species concepts can be applied to past research. Voucher specimens documenting all investigated accessions (for population samples at least one specimen per population) are to be deposited in a public herbarium, for example: Index Herbariorum, or other public collection providing access to deposited material. Information on the voucher specimen and who identified it must be included in the manuscript such as Genus name, species name, author, and year of publication.

Names of plants, algae and fungi

Manuscripts containing new taxon names or other nomenclatural acts must follow the guidelines set by the International Code of Nomenclature for algae, fungi, and plants.

Authors describing new fungal taxa should register the names with a recognized repository, such as Mycobank, and request a unique digital identifier which should be included in the published article.

These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work is submitted.

The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

Authorship Principles

2) drafted the work or revised it critically for important intellectual content;

3) approved the version to be published; and

4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

* Based on/adapted from:

ICMJE, Defining the Role of Authors and Contributors,

Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

Disclosures and declarations

All authors are requested to include information regarding sources of funding, financial or non-financialinterests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).

The decision whether such information should be included is not only dependent on the scope of the journal, but also the scope of the article. Work submitted for publication may have implications for public health or general welfare and in those cases it is the responsibility of all authors to include the appropriate disclosures and declarations.

Data transparency

All authors are requested to make sure that all data and materials as well as software application or custom code support their published claims and comply with field standards. Please note that journals may have individual policies on (sharing) research data in concordance with disciplinary norms and expectations. Please check the Instructions for Authors of the Journal that you are submitting to for specific instructions.

Role of the Corresponding Author

One author is assigned as Corresponding Author and acts on behalf of all co-authors and ensures that questions related to the accuracy or integrity of any part of the work are appropriately addressed.

The Corresponding Author is responsible for the following requirements:

- ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors;
- managing all communication between the Journal and all co-authors, before and after publication;*

- providing transparency on re-use of material and mention any unpublished material (for example manuscripts in press) included in the manuscript in a cover letter to the Editor;
- making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate (see above).

* The requirement of managing all communication between the journal and all co-authors during submission and proofing may be delegated to a Contact or Submitting Author. In this case please make sure the Corresponding Author is clearly indicated in the manuscript.

Author contributions

Authors must include contribution statements in the work that specifies the contribution of every author in order to promote transparency. These contributions should be listed at the separate title page.

Examples of such statement(s) are shown below:

• Free text:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name], [full name] and [full name]. The first draft of the manuscript waswritten by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Example: CRediT taxonomy:

• Conceptualization: [full name], …; Methodology: [full name], …; Formal analysis and investigation: [full name], …; Writing - original draft preparation: [full name, …]; Writing - review and editing: [full name], …;Funding acquisition: [full name], …; Resources: [full name], …; Supervision: [full name],….

For review articles where discrete statements are less applicable a statement should be included who hadthe idea for the article, who performed the literature search and data analysis, and who drafted and/or critically revised the work.

For articles that are based primarily on the student's dissertation or thesis, it is recommended that thestudent is usually listed as principal author:

A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA ScienceStudent Council 2006

Affiliation

The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may additionally be stated. Addresses will not be updated or changed after publication of the article.

Changes to authorship

Authors are strongly advised to ensure the correct author group, the Corresponding Author, and the order of authors at submission. Changes of authorship by adding or deleting authors, and/or changes in Corresponding Author, and/or changes in the sequence of authors are not accepted after acceptance of a manuscript.

- Please note that author names will be published exactly as they appear on the accepted submission!

Please make sure that the names of all authors are present and correctly spelled, and that addresses and affiliations are current.

Adding and/or deleting authors at revision stage are generally not permitted, but in some cases it may be warranted. Reasons for these changes in authorship should be explained. Approval of the change during revision is at the discretion of the Editor-in-Chief. Please note that journals may have individual policies on adding and/or deleting authors during revision stage.

Author identification

Authors are recommended to use their ORCID ID when submitting an article for consideration or acquirean ORCID ID via the submission process.

Deceased or incapacitated authors

For cases in which a co-author dies or is incapacitated during the writing, submission, or peer-review process, and the co-authors feel it is appropriate to include the author, co-authors should obtain approval from a (legal) representative which could be a direct relative.

Authorship issues or disputes

In the case of an authorship dispute during peer review or after acceptance and publication, the Journal will not be in a position to investigate or adjudicate. Authors will be asked to resolve the dispute themselves. If they are unable the Journal reserves the right to withdraw a manuscript from the editorial process or in case of a published paper raise the issue with the authors' institution(s) and abide by its guidelines.

Confidentiality

Authors should treat all communication with the Journal as confidential which includes correspondence with direct representatives from the Journal such as Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit consent has been received to share information.

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

- Asking a colleague who is a native English speaker to review your manuscript for clarity.
- Visiting the English language tutorial which covers the common mistakes when writing in English.
- Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. Springer authors are entitled to a 10% discount on their first submission to either of these services, simply follow the links below

English Language Support

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English language tutorial

Nature Research Editing Service

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Please note that the use of a language editing service is not a requirement for publication in this journal and does not imply or guarantee that the article will be selected for peer review or accepted.

If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.

ANEXO C – Normas da revista Anais da Academia Brasileira de Ciências

Instruções aos autores

O periódico Anais da Academia Brasileira de Ciências considera para publicação as submissões feitas exclusivamente pelo sistema online de gerenciamento de artigos. Uma vez que seu artigo esteja de acordo com as instruções abaixo, favor acessar o sistema no link https://mc04.manuscriptcentral.com/aabc-scielo.

Por favor, leia estas instruções com atenção e as siga rigorosamente. Desta forma você irá garantir que a avaliação e a publicação de seu artigo sejam o mais eficiente e veloz quanto possível. Os editores reservam-se ao direito de devolver artigos que não estejam de acordo com estas instruções. Apesar de dispormos de uma página de instruções em português, lembramos que só consideramos para submissão, avaliação e publicação os artigos redigidos de forma clara e concisa na língua inglesa.

Objetivo e política editorial

Todos os manuscritos submetidos devem conter pesquisa original que não tenha sido publicada ou esteja sob consideração em outro periódico. O critério primário para aceitação é qualidade científica. Artigos devem evitar o uso excessivo de abreviações ou jargões, além de ser tão inteligíveis quanto possível para o público em geral. Deve ser dada atenção particular às seções Abstract, Introduction e Discussion, as quais devem detalhar a novidade e significância dos dados relatados. Não cumprir com qualquer um dos pontos acima pode causar atraso na publicação ou até mesmo a recusa do artigo.

Textos podem ser publicados em forma de revisão, artigo completo ou como comunicação curta (short communications). Os volumes regulares dos AABC são publicados em março, junho, setembro e dezembro.

Tipos de artigos

Revisões

Revisões são publicadas apenas por meio de convite, tendo ainda que passer pelo processo de revisão por pares. Contudo, uma proposta de revisão pode ser enviada por e-mail para a Assessoria de publicações (aabc@abc.org.br). O e-mail deve conter os tópicos e autores da revisão proposta, bem como o abstract, área dos AABC na qual o artigo se encaixa e a justificativa pela qual este tópico seria de particular interesse à área.

Os AABC permitem que os autores depositem preprints de seus artigos em servidores de preprint tais como, mas não limitados a, ArXiv.org e bioRxiv.org. Contudo, autores devem atualizar os registros informando que o artigo foi aceito/publicado pelos AABC.

Cartas ao editor

Cartas ao editor (Letters to the Editor) estarão sujeitas à edição e revisão, não podendo conter material que tenha sido submetido ou publicado em outro periódico. Cartas que venham a se referir a um artigo publicado nos AABC não podem exceder 250 palavras (não contando com referências) e devem ser recebidas em até 4 semanas após a

publicação online do artigo. Cartas não relacionadas a um artigo publicados pelos AABC não podem exceder 500 palavras (não contando com referências). Uma carta não pode ter mais de dez referências, além de uma figura ou tabela.

Articles

Sempre que possível, artigos devem estar subdivididos nas seguintes partes: 1. Página de rosto; 2. Abstract (em página separada, 200 palavras ou menos, sem abreviações); 3. Introduction; 4. Materials and Methods; 5. Results; 6. Discussion; 7. Acknowledgments, se aplicável; 8. Author contributions (se o artigo tiver mais de um autor); 9. References; 10. Legendas de figuras e tabelas, se aplicável. Artigos de algumas áreas, como por exemplo Ciências Matemáticas, devem seguir seu format padrão. Em alguns casos, pode ser aconselhável omitir a seção (4) e juntar as partes (5) e (6). Quando aplicável, a seção Materials and Methods deve indicar o Comitê de Ética que avaliou os procedimentos para estudos em seres humanos ou as normas seguidas para tratamentos experimentais em animais.

Short communications

Short communications procuram relatar uma importante e concisa contribuição para pesquisa, a qual progrediu para o estágio em que os resultados devem ser tornados públicos para outros pesquisadores do mesmo campo. Uma short communication também deve possuir Abstract (100 palavras ou menos, neste caso), uma pequena introdução (até 200 palavras) e não pode exceder 1500 palavras. Tabelas e Figuras podem ser incluídas no texto, mas este deve ser proporcionalmente reduzido. Este tipo de publicação nos AABC deve conter contribuições extremamente relevantes, sendo um tipo de artigo com alta competição.

Após recebimento e primeira triagem editorial, artigos serão avaliados por pelo menos dois revisores, sendo eles de instituições educacionais e/ou de pesquisa tanto nacionais quanto internacionais, desde que comprovada sua produção científica. Após possíveis correções e sugestões, o artigo pode ser aceito ou recusado, considerando os pareceres recebidos.

Nós utilizamos o programa integrado Crossref Similarity Check para detectar possíveis plágios.

Os AABC não possuem taxas de submissão, avaliação e publicação de artigos.

Preparação de manuscritos

Todas as seções do manuscrito devem possuir espaçamento duplo. Após o aceite, nenhuma mudança será feita no artigo, de modo que as provas de prelo precisem apenas de correções em erros tipográficos. Lembramos que o envio de artigos é feito exclusivamente pelos autores através do nosso sistema de gerenciamento de artigos.

Tamanho do artigo

Os artigos podem ser de qualquer tamanho necessário para a apresentação e discussão concisa dos dados, mas mantendo-se conciso e cuidadosamente preparado tanto em termos de impacto quanto de legibilidade. No entanto, artigos não devem exceder 50 páginas, incluindo todos os itens (figuras, tabelas, referências, etc.),a menos que possua autorização prévia do Editor-Chefe.

Página de rosto

A página de rosto do artigo deve apresentar os seguintes itens: 1. Título do artigo com até 150 caracteres, sem abreviações e com a tentativa de manter o interesse amplo da comunidade científica; 2. Nomes completos de todos os autores. Utilize números sobrescritos para indicar a filiação de cada autor. 3. Endereços profissionais e ORCID de todos os autores, incluindo instituição, departamento, rua, número, CEP, cidade, estado e país; 4. Key words (de 4 a 6 em ordem alfabética e separadas por vírgulas); 5. Running title (versão resumida – e não abreviada - do título com até 50 caracteres, incluindo espaços); 6. Seção dos AABC à qual o artigo pertence; 7. Nome, endereço, telefone e e-mail do autor para correspondência, a quem serão enviadas as mensagens mais relevantes do processo de avaliação. Este autor ou autora deve ser indicado com um asterisco após seu nome.

Não cumprir com qualquer dos requisitos acima fará com que o artigo seja devolvido (unsubmitted) para correções.

Abstract

O abstract deve conter até 200 palavras e apresentar as principais descobertas do artigo, incluindo uma breve introdução, os objetivos do trabalho e uma conclusão baseada nas presentes descobertas. Caso os autores estejam submetendo uma revisão convidada/autorizada, o abstract deve abordar o principal tema da revisão e explicitar a contribuição de tal revisão à área. O abstract não deve possuir títulos nem citações/referências.

Texto do manuscrito

Todo o texto deve ser escrito com espaçamento duplo utilizando a fonte Times New Roman tamanho 12 ou equivalente, desde que mantida a legibilidade. Por favor, organize seu texto nas seguintes partes sempre que possível: 1. Página de rosto; 2. Abstract (em página separada, 200 palavras ou menos, sem abreviações); 3. Introduction; 4. Materials and Methods; 5. Results; 6. Discussion; 7. Acknowledgments, se aplicável; 8. Author contributions (se o artigo tiver mais de um autor); 9. References; 10. Legendas de figuras e tabelas, se aplicável.

Artigos de algumas áreas, como por exemplo Ciências Matemáticas, devem seguir seu formato padrão. Em alguns casos, pode ser aconselhável omitir a seção (4) e juntar as partes (5) e (6). Quando aplicável, a seção Materials and Methods deve indicar o Comitê de Ética que avaliou os procedimentos para estudos em seres humanos ou as normas seguidas para tratamentos experimentais em animais.

Todos os procedimentos devem ser detalhadamente descritos. Utilize inglês norte-americano para escrever o texto. Nomenclaturas da área de Química devem ser fornecidos de acordo com a União Internacional de Química Pura e Aplicada (IUPAC). Cepas de organismos também devem estar identificadas. Informe nomes de fornecedores de reagentes e/ou equipamentos. Utilize unidades e símbolos de acordo com o Bureau International des Poids et Mesures (SI) sempre que possível.

Acknowledgments

Devem ser incluídos ao fim do texto, antes das referências. Agradecimentos pessoais devem preceder nomes de instituições e agências. De forma ideal, notas de rodapé devem

ser evitadas, mas, quando necessário, devem estar numeradas. Agradecimentos a financiamentos, subsídios, bolsas de estudo e dívidas com outros colegas, bem como menções à origem do artigo (como uma tese, por exemplo), devem estar nesta seção. Favor incluir o nome completo da agência de fomento, país e número do projeto (se aplicável).

Abreviações

Devem ser definidas em sua primeira ocorrência no texto, exceto por abreviações padrão e oficiais. Unidades e seus símbolos devem estar em conformidade com as aprovadas pelo Bureau International des Poids et Mesures (SI).

Legendas de figuras

Esta informação deve ser fornecida ao fim do manuscrito, após as referências. Todas as figuras devem conter legenda. A legenda deve possuir uma sentença introdutória que descreve as principais descobertas. Todas as divisões na figura devem ser identificadas com letras minúsculas, quando aplicável (1a, 2a, 2b, 3c, 3d, etc.). Quando for o caso da utilização de barras de erro, favor informar se um número que vem após o símbolo ± é um Standard Error Of Mean (SEM) ou standard deviation of mean (SD). Deve ser informado na legenda se o resultado apresentado representa N experimentos individuais.

Tabelas

Cada tabela deve possuir um pequeno título acima da mesma. Notas abaixo da tabelas também pode ser utilizadas. Tabelas devem ser citadas no artigo em algarismos romanos (Table I, Table II, Tables IV and V, etc.). Tabelas devem ser submetidas separadamente em arquivos editáveis, preferencialmente .doc ou .docx.

Figuras

Só serão aceitas figuras de alta qualidade (mínimo de 300 dpi). Todas as ilustrações serão consideradas figuras, incluindo desenhos, gráficos, mapas, fotografias, esquemas, etc. Seu posicionamento tentativo deve ser indicado, assim como todas as figuras devem ser citadas com seu respectivo número ao longo do texto. Figuras devem ser enviadas de acordo com as seguintes especificações: 1. Desenhos e ilustrações devem estar em formato .PS/.EPS ou .CDR (PostScript ou Corel Draw) e nunca inseridas no texto; 2. Imagens ou figuras em escala de cinza devem estar em formato .TIF e nunca inseridas no texto; 3. Cada figura deve ser enviada em arquivo separado; 4. Figuras devem, a princípio, ser submetidas no tamanho em que espera-se que estejam publicadas no periódico, ou seja, largura de 8cm (uma coluna) ou 16,2cm (duas colunas), com a altura máxima de cada figura e respectiva legenda sendo menor ou igual a 22cm.

As legendas das figuras devem ser enviadas com espaçamento duplo em página separada. Cada dimensão linear dos menores caracteres e símbolos não pode ser menor que 2mm após redução. Figuras coloridas são aceitas tanto como figuras em preto e branco. No entanto, 5 figuras em p/b são sem custo aos autores, enquanto cada figura colorida na versão impressa será cobrada dos autores, com a comunicação sendo feita durante a fase de produção (após o processo de avaliação). De modo a padronizar a contagem e cobrança de figuras preto e branco, tabelas que ocupem dois terços da página ou que tenham mais que 12 colunas ou 24 colunas serão consideradas figuras p/b. Manuscritos de Matemática, Física ou Química podem ser redigidos em TEX, AMS-TEX ou LaTeX, desde que o

arquivo .BIB seja enviado junto. Manuscritos sem fórmulas podem ser enviados em .RTF ou doc/docx para Windows.

Referências

Os autores são responsáveis pela exatidão das referências, bem como suas respectivas citações. Artigos publicados ou ainda 'In press' podem ser incluídos. Comunicações pessoais (Smith, personal communication) devem ser autorizadas por escritos pelos envolvidos. Referências a teses, abstracts de encontros (não publicados em jornais indexados) e manuscritos em preparação ou apenas submetidos, mas não ainda aceitos, devem ser citados no texto no formato (Smith et al., unpublished data) e NÃO devem ser incluídos na lista de referências.

Referências devem ser citadas no texto no formato a seguir sem a aspa simples, 'Smith 2004', 'Smith & Wesson 2005' ou, quando há 3 ou mais autores, 'Smith et al. 2006'. Quando houver dois ou mais artigos cujo nome do primeiro autor e ano de publicação são idênticos, as referências devem ser diferenciadas por letras minúsculas, como em 'Smith 2004a', 'Smith 2004b', etc.

As referências devem ser listadas alfabeticamente de acordo com o nome do primeiro autor, sempre na ordem SOBRENOME XY, sendo X e Y as iniciais. Se há mais de 10 autores na referência, usar SOBRENOME XY ET AL., sem listar os demais autores. Referências devem conter também o título do artigo. Os nomes dos periódicos devem estar abreviados sem itálico, pontos ou vírgulas. Para as abreviações corretas, verifique listas das maiores bases de dados nas quais o periódico está indexado, ou consulte a World List of Scientific Periodicals. A abreviação a ser usada em referências dos Anais da Academia Brasileira de Ciências é An Acad Bras Cienc. Os seguintes exemplos devem servir de guias para sua lista de referências em nossa revista:

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Comprovante de submissão