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FARMACOBOTÂNICA, COMPOSIÇÃO QUÍMICA E ATIVIDADES BIOLÓGICAS DO PICÃO-PRETO (*Bidens subalternans* DC.)

BOA VISTA, RR

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Dissertação apresentada ao Programa de Pós-graduação em Recursos Naturais – PRONAT, da Universidade Federal de Roraima, como pré-requisito para obtenção do título de Mestre em Ciências Ambientais (Recursos Naturais). Área de concentração: Manejo e conservação de bacias hidrográfica. Linha de Pesquisa: Bioprospecção.

Orientador: Prof. Dr. Marcos José Salgado Vital

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Dissertação apresentada como pré-requisito para conclusão do Curso de Mestrado em Ciências Ambientais (Recursos Naturais) da Universidade Federal de Roraima, defendida em 18 de dezembro de 2023 e avaliada pela seguinte Banca Examinadora:

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"À memória eterna de minha mãe, cujo amor e encorajamento moldaram cada passo deste percurso. Seu sonho de me ver no mestrado permanece vivo nesta realização."

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“Em todas as coisas da natureza
existe algo de maravilhoso!”
(Aristóteles)

RESUMO

Bidens subalternans, pertencente à família Asteraceae, é uma planta herbácea, popularmente conhecida como picão-preto. É nativa do Brasil e amplamente distribuída. Reconhecida por sua invasão em áreas agrícolas e adaptação a ambientes adversos, faz parte do Complexo *Bidens*, apresentando desafios na identificação precisa devido às semelhanças morfológicas com outras espécies do gênero, como *B. pilosa* e *B. alba*. Apesar de seu destaque como planta invasora em estudos agronômicos, a literatura sobre *B. subalternans* é limitada. Raros relatos etnobotânicos mencionam seu uso em tratamentos para hepatite, inflamações, diabetes e cicatrização. Este estudo teve como objetivo investigar a atividade antimicrobiana e antioxidante *in vitro* dos extratos etanólicos das partes aéreas de *B. subalternans* coletadas após cultivo em ambiente natural e controlado. Além disso, foram realizadas a prospecção química dos extratos etanólicos, análise do perfil químico por APCI, a avaliação da toxicidade em relação à *Artemia salina* e estudo farmacobotânico da lâmina foliar. A atividade antimicrobiana foi avaliada utilizando o método de disco-difusão, enquanto a atividade antioxidante foi determinada por meio dos ensaios com os radicais DPPH e ABTS, seguidos pela quantificação dos compostos fenólicos totais utilizando o método Folin-Ciocalteau. Os resultados demonstraram que os extratos de *B. subalternans* apresentaram atividade antibacteriana contra *Staphylococcus aureus (ATCC25923), Enterococcus faecalis (ATCC00531), Bacillus cereus (ATCC9634), Listeria monocytogenes (ATCC7644), Escherichia coli (ATCC10536),* com valores de concentração inibitória mínima (CIM) variando de 300 a 500 μg.mL-1. No que diz respeito aos compostos fenólicos, os extratos mostraram teores significativos de fenólicos totais, com valores de 1.149 mgEAG.g^{-1} para o extrato proveniente do ambiente e 140,6 mgEAG.g^{-1} para o extrato cultivado. No ensaio DPPH, os valores de IC50 dos dois extratos variaram de 85,8 a 30,57 mg.mL^{-1}, com percentuais de inibição de 69,1% e 12,5%, respectivamente. Os resultados relacionados ao radical ABTS também foram promissores, com valores de 1.969,0 μMTrolox.g-1 e uma taxa de inibição de 99,2% para o extrato da planta do ambiente, e valores de 1.855,1 μMTrolox.g-1 e 93,57% de inibição para o extrato da planta cultivada. A análise dos extratos revelou a presença de diversas classes de metabólitos secundários, como saponinas, taninos, substâncias fenólicas, fenóis e flavonóis. A análise do perfil químico por APCI-MS identificou compostos como ácido clorogênico, ácido cafeico, rutina e tricin hexoside, entre outros. No estudo farmacobotânico, foram observados os aspectos anatômicos típicos das folhas da espécie *B. subalternans*, incluindo estômatos anomocíticos e anisocíticos, parênquima paliçádico e lacunoso, e feixes vasculares bem desenvolvidos. Também foram observados compostos fenólicos, mucilagens e gotículas lipídicas no mesofilo e nos tricomas. A presença constatada de metabólitos secundários com atividade antioxidante e antibacteriana indicam o potencial terapêutico de *B. subalternans*. Considerando que esses resultados podem decorrer da sinergia entre as substâncias, a realização de estudos de isolamento dos metabólitos secundários e avaliações farmacológicas se mostram essenciais. Essas etapas adicionais contribuirão significativamente para uma exploração mais aprofundada do potencial terapêutico e da segurança associados a esses extratos. Esses estudos, pioneiros para a espécie podem desempenhar um papel crucial em pesquisas futuras, especialmente no que diz respeito ao desenvolvimento da bioeconomia no Estado de Roraima.

Palavra-chave: Extratos etanólicos. Amazônia. Histoquímica. Atividade antioxidante. Atividade Antibacteriana. Roraima.

ABSTRACT

Bidens subalternans, belonging to the Asteraceae family, is a herbaceous plant, popularly known as picão-preto. It is native to Brazil and widely distributed. Recognized for its invasion into agricultural areas and adaptation to adverse environments, it is part of the Bidens Complex, presenting challenges in accurate identification due to morphological similarities with other species of the genus, such as *B. pilosa* and *B. alba*. Despite its prominence as an invasive plant in agronomic studies, the literature on *B. subalternans* is limited. Rare ethnobotanical reports mention its use in treatments for hepatitis, inflammation, diabetes and wound healing. This study aimed to investigate the in vitro antimicrobial and antioxidant activity of ethanolic extracts from the aerial parts of *B. subalternans* collected after cultivation in a natural and controlled environment. In addition, chemical prospecting of ethanolic extracts, chemical profile analysis by APCI, toxicity assessment in relation to *Artemia salina* and pharmacobotanical study of the leaf blade were carried out. Antimicrobial activity was evaluated using the disk diffusion method, while antioxidant activity was determined using DPPH and ABTS radical assays, followed by quantification of total phenolic compounds using the Folin-Ciocalteau method. The results demonstrated that *B. subalternans* extracts presented antibacterial activity against *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC00531), *Bacillus cereus* (ATCC9634), *Listeria monocytogenes* (ATCC7644), *Escherichia coli* (ATCC10536), with minimum inhibitory concentration values (MIC) ranging from 300 to 500 μg.mL-1. With regard to phenolic compounds, the extracts showed significant levels of total phenolics, with values of 1,149 mgEAG.g-1 for the extract from the environment and 140.6 mgEAG.g-1 for the cultivated extract. In the DPPH assay, the IC50 values of the two extracts ranged from 85.8 to 30.57 mg.mL-1, with inhibition percentages of 69.1% and 12.5%, respectively. The results related to the ABTS radical were also promising, with values of 1,969.0 μMTrolox.g-1 and an inhibition rate of 99.2% for the environmental plant extract, and values of 1,855.1 μMTrolox.g-1 and 93.57% inhibition for the cultivated plant extract. Analysis of the extracts revealed the presence of several classes of secondary metabolites, such as saponins, tannins, phenolic substances, phenols and flavonols. Analysis of the chemical profile by APCI-MS identified compounds such as chlorogenic acid, caffeic acid, rutin and tricin hexoside, among others. In the pharmacobotanical study, the typical anatomical aspects of the leaves of the species *B. subalternans* were observed, including anomocytic and anisocytic stomata, palisade and lacunous parenchyma, and well-developed vascular bundles. Phenolic compounds, mucilages and lipid droplets were also observed in the mesophyll and trichomes. The presence of secondary metabolites with antioxidant and antibacterial activity indicates the therapeutic potential of *B. subalternans*. Considering that these results may result from synergy between the substances, carrying out isolation studies of secondary metabolites and pharmacological evaluations are essential. These additional steps will significantly contribute to further exploration of the therapeutic potential and safety associated with these extracts. These pioneering studies for the species can play a crucial role in future research, especially with regard to the development of the bioeconomy in the State of Roraima.

Keywords: Ethanolic extracts. Amazon. Histochemistry. Antioxidant activity. Antibacterial activity. Roraima.

SUMÁRIO

1 INTRODUÇÃO

A utilização de produtos naturais, particularmente da flora, com fins medicinais, nasceu com a humanidade. Indícios do uso de plantas medicinais foram encontrados nas civilizações mais antigas, sendo considerada uma das práticas mais remotas utilizadas pelo homem para cura, prevenção e tratamento de doenças, servindo como importante fonte de compostos biologicamente ativos (ANDRADE; CARDOSO; BASTOS, 2007).

O Brasil possui uma grande diversidade de espécies vegetais, inúmeras utilizadas como medicinais perfaz uma grande riqueza florística. A maioria dessas plantas é utilizada com base no conhecimento popular, muitas vezes sem a confirmação científica de suas propriedades farmacológicas e toxicológicas, por não terem sido investigadas ou comprovadas em testes pré-clínicos e clínicos (TUROLLA; NASCIMENTO, 2006).

A família Asteraceae Bercht. & J. Presl é o maior grupo de angiospermas, abrangendo aproximadamente 33.000 espécies, agrupadas em 1911 gêneros, 13 subfamílias e 43 tribos (BESSADA; BARREIRA; OLIVEIRA, 2015; SMITH; RICHARDSON, 2011), sendo 280 gêneros e mais de 2.000 espécies ocorrentes no Brasil (ROQUE; TELES; NAKAJIMA, 2017). Possui distribuição cosmopolita em todos os continentes, exceto na Antártida, sendo amplamente representada em regiões temperadas e semiáridas dos trópicos e subtrópicos (ROQUE; BAUTISTA, 2008).

Muitas espécies de Asteraceae têm sido utilizadas como plantas medicinais, dentre os registros do uso popular encontra-se principalmente a ação contra febre, dores de dente, picada de cobra, diurética, anti-inflamatório, diabetes, antimicrobiana (KATINAS; FUNK, 2020). Estudos fitoquímicos indicam a presença de polifenóis, sesquiterpenos, ácidos graxos e orgânicos em espécies da familias, que têm sido associados ao sucesso do tratamento de doenças cardiovasculares, câncer, infecções microbianas e virais, inflamação e outras doenças (MORALES et al., 2014). São destaques as pesquisas relacionadas à riqueza de substâncias químicas direcionadas à tratamentos de enfermidades crônicas e ativação de processos imunológicos, na utilização como Plantas Alimentícias Não Convencionais (PANCs), bem como na indústria fitocosmética (ROQUE; TELES; NAKAJIMA, 2017).

Dentre as tribos da família Asteraceae, destaca-se Coreopsideae Lindl, que é colocada em aliança com a tribo Heliantheae (BALDWIN, 2009). Coreopsideae tem distribuição pantropical e compreende aproximadamente 24 gêneros (sendo Bidens L. e

Coreopsis L. os maiores) e aproximadamente 600 espécies (CRAWFORD et al., 2009; PANERO, 2007). Esta tribo é caracterizada pela presença de antocloros: pigmento de cor amarela do grupo das flavonas que ocorrem nas flores (BOHM; STUSSY, 2001; CRAWFORD; STUESSY, 1981).

Entre as várias espécies pertencentes à tribo Coreopsideae, destaca-se o gênero Bidens, que tem sido objeto de minuciosas investigações. Seu nome deriva do latim "bis" (dois) e "edens" (dente), uma referência sugestiva aos frutos da planta, notadamente caracterizados por apresentarem cerdas e farpas. Compreendendo aproximadamente de 150 a 250 espécies distintas, esse gênero tem uma ampla distribuição em habitats ruderais, proliferando de maneira proeminente em regiões subtropicais, tropicais e temperadas da América do Norte e América do Sul (LUCCHETTI et al., 2009). Este gênero abrange uma diversidade de espécies com usos etnofarmacológicos variados. Algumas delas são empregadas como emenagogos, afrodisíacos, antifebris e analgésicos para complicações relacionadas a ouvidos e vias urinárias, bem como para auxiliar na eliminação de cálculos renais e beneficiar a saúde hepática (JAGER; HUTCHINGS; VAN STADEN, 1996; MORTON, 1981). Outras têm sido submetidas a investigações no contexto do combate a tumores de mama, esplenoesclerose, dores de garganta, questões gástricas e asma (HARTWELL, 1971; REDL; DAVIS; BAUER, 1993).

Estudos têm revelado diversas propriedades terapêuticas das espécies de Bidens. Por exemplo, os poliacetilenos de *B. campylotheca* Schultz-Bip. demonstraram ação anti-inflamatória (REDL et al., 1994), os flavonoides de *B. aurea* (Ait.) Sherff exibiram propriedades antiulcerogênicas (DE LA LASTRA et al., 1994), e pesquisas com *B. pilosa* indicaram atividades antimaláricas (OLIVEIRA et al., 2004), antimicrobianas (HAIDA et al., 2007), antifúngicas (TAGAMI et al., 2009), analgésicas e anti-inflamatórias (FOTSO et al., 2014; SANTOS et al, 2020). Além disso, estudos conduzidos por Dieamant e colaboradores (2015) destacam o potencial de *B. pilosa* como agente antienvelhecimento e reparador da pele. Os resultados obtidos indicam efeitos comparáveis e, em alguns casos, até superiores aos observados com o uso do retinol e ácido retinóico.

Entre as espécies de Bidens encontra-se a *B. subalternans* DC., planta herbácea de substrato terrícola (Figura 1-A), popularmente conhecida como picão-preto, amor-seco, picão-do-campo, pico-pico ou carrapicho-de-pontas. Seu caule é ereto, de secção

quadrangular, liso glabro ou levemente piloso (Figura 1-B), de ramificação dística em toda a sua extensão e a raiz principal é pivotante (KISSMANN; GROTH, 1997). As folhas da referida espécie são pecioladas opostas e, na maioria dos casos, compostas com 3-5 folíolos (Figura 1- B). Produz flores de coloração amarela e aquênios com quatro a cinco aristas de cor marrom escura (Figuras 1 – C, D e E). A formação de sementes é intensa, podendo chegar a 3.000 aquênios por planta, que se prendem facilmente em tecidos e pelos, o que facilita sua dispersão (BOGOSAVLJEVIĆ; ZLATKOVIĆ, 2015; BRINGEL JÚNIOR; REIS-SILVA, 2020).

Figura 1 – *Bidens subalternans*: A – aspecto da planta jovem em estágio vegetativo; B- aspecto do caule e folhas adultas; C e D – Capítulos floridos; E – Sementes com 4 aristas.

Fonte: Autora (2023).

Com considerável adaptabilidade às condições adversas, *B. subalternans* apresenta-se como uma planta espontânea, inúmeras vezes caracterizada como "daninha" nos ambientes agrícolas. Sua ocorrência tem sido registrada em toda a América do Sul, e em países da Oceania (RANDALL, 2007), Ásia (KIM, 2012) e Europa (BOGOSAVLJEVIĆ; ZLATKOVIĆ, 2015).

B. subalternans é nativa do Brasil, comum em áreas antropizadas, campos limpos, florestas ciliares e galerias e sua distribuição geográfica é confirmada nos seguintes estados brasileiros: Amazonas, Bahia, Ceará, Pernambuco, Rio Grande do Norte, Sergipe, Goiás, Mato Grosso do Sul, Mato Grosso, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul e Santa Catarina; com possíveis ocorrências no Pará e na Paraíba (BRINGEL JÚNIOR; REIS-SILVA, 2020). A espécie é considerada competidora em vários tipos de cultivo, reduzindo o rendimento das culturas, pois têm uma grande capacidade de extrair água e nutrientes do solo, além disso, apresentam resistência a alguns agrotóxicos, como o glifosato e o imazatapir (MENDES et al., 2019; TAKANO et al., 2020).

No campo da atividade biológica, estudo conduzido por Gonçalves e colaboradores (2018) investigou o efeito bactericida dos extratos de *B. subalternans* contra bactérias Gram-negativas. Os resultados foram favoráveis, demonstrando ação contra *Escherichia coli*, *Acinetobacter baumannii* e *Enterobacter aerogenes.* Este fato se reveste de importância visto que *E. coli* pode causar patologias extra intestinais, diversas infecções intra-abdominais, pulmonares, da pele e tecidos moles, meningite neonatal e patologias intestinais (DENAMUR, 2021); *A. baumannii* causa uma série de infecções hospitalares e na comunidade, incluindo pele e tecidos moles, infecções do trato urinário, meningite, bacteremia e pneumonia (DEXTER et al., 2015); enquanto que *E. aerogenes* causa um amplo espectro de infecções envolvendo o trato urinário, trato respiratório baixo, pele, tecidos moles, feridas e sistema nervoso central (DAVIN-REGLI; PAGES, 2015).

Devido aos frequentes problemas associados ao uso indiscriminado de antibióticos, como resistência microbiana e desequilíbrio da biota humana, a pesquisa relacionada às espécies vegetais com propriedades antimicrobianas tem crescido consideravelmente (YUNES; CALIXTO, 2001). O potencial antimicrobiano das plantas está relacionado aos tipos de metabólitos secundários presentes nas espécies, podendo ser determinado por pesquisas *in vitro* utilizando técnicas de difusão em ágar e diluições

(AGRIPINO et al., 2004; LANGFIELD et al., 2004) e por autobiografia, processo no qual os compostos bioativos são determinados pela inibição do crescimento dos microrganismos e determinam a Concentração Inibitória Mínima (CIM) das amostras (SASIDHARAN et al., 2011). A importância de se identificar novos compostos com potencial antimicrobiano provenientes de plantas se fundamenta na redução de possíveis efeitos colaterais provocados por substâncias sintéticas, ampliação de recursos e diminuição dos custos no desenvolvimento de medicamentos (ALVES et al., 2001).

Adicionalmente, o organismo humano produz substâncias para combater os radicais livres, chamadas antioxidantes. Eles também podem ser obtidos por meio da ingestão de produtos de origem sintética ou natural. Apresentam substituintes doadores de elétrons ou de hidrogênio em sua estrutura, capacidade quelante de metais ou de ressonância do radical formado. Entre os antioxidantes naturais pode-se citar os tocoferóis, vitamina C, carotenóides e compostos fenólicos (SUCUPIRA, 2012).

Muitas doenças, como o câncer, doenças cardíacas, alzheimer, aterosclerose e até o envelhecimento precoce, ditas doenças não transmissíveis degenerativas, têm sido associadas a um desequilíbrio entre a produção de antioxidantes e de radicais livres (NEVES, 2012). Esses últimos são substâncias químicas extremamente instáveis que geram estresse oxidativo, processo prejudicial às células (WANG et al., 2017), formados naturalmente por processos biológicos no organismo humano ou por ação de fatores ambientais como tabaco, poluição do ar, solventes orgânicos, pesticidas e radiações (SOARES, 2002).

Compostos antioxidantes sintéticos são amplamente utilizados na indústria, porém existem evidências de que alguns desses compostos podem promover o desenvolvimento de células tumorais (BOTTERWECK et al., 2000). A presença de compostos bioativos, como os antioxidantes (NEVES, 2012), tem-se mostrado uma boa alternativa de proteção para o corpo humano contra radicais livres (VALKO et al., 2007), pois atuam como antioxidantes, prevenindo ou adiando o início de várias doenças (OLIVEIRA, 2015). Os compostos fenólicos e flavonoides estão diretamente relacionados com o potencial antioxidante das plantas (ANDRADE et al., 2007).

Com base nas informações etnobotânicas sobre *B. subalternans* e observando a necessidade de comprovação científica desta planta nativa do Brasil e de ocorrência ainda não catalogada no estado de Roraima, verificou-se a necessidade de se realizar análise farmacobotânica e explorar o potencial biológico do insumo proveniente das

partes aéreas da espécie. Neste contexto, o objetivo geral deste estudo foi analisar a farmacobotânica e ação biológica das partes aéreas de *B. subalternans.* Para alcançar o objetivo geral, foram propostos os seguintes objetivos específicos: I) caracterizaros aspectos farmacoanatômicos e farmacohistoquímicos da espécie; II) Investigar, de forma qualitativa, os constituintes químicos das partes aéreas da espécie identificada; III) avaliar a ação antibacteriana, antioxidante, teor de compostos fenólicos e toxicidade dos seus constituintes químicos.

A metodologia empregada nesta pesquisa fundamentou-se primeiramente na análise abrangente dos extratos etanólicos da planta *B. subalternans*. Os extratos foram obtidos de partes aéreas de espécimes de diferentes origens: coletados diretamente do ambiente natural (designados como "Ambiente") e produzidos por meio de cultivo controlado em casa-de- vegetação (referidos como "Cultivo"). As partes aéreas da planta foram coletadas nestes locais e submetidas a identificação taxonômica por especialista na tribo Corepsidae. Posteriormente, os extratos foram obtidos por meio de um processo de maceração utilizando etanol, seguido por evaporação do solvente para concentrar os compostos bioativos.

No intuito de elucidar os componentes químicos presentes nos extratos, as amostras foram submetidas a uma triagem fitoquímica abrangente, visando a identificação de diversos metabólitos secundários. Além disso, investigou-se a atividade antioxidante dos extratos por meio de ensaios baseados nos radicais ABTS e DPPH e o teor de compostos fenólicos, os quais forneceram *insights* valiosos sobre o potencial antioxidante desses extratos.

A atividade antibacteriana dos extratos foi avaliada frente a diversas bactérias indicadoras por meio do método de disco-difusão. Para compreender a toxicidade dos extratos, empregou-se náuplios de *Artemia salina* como bioindicadores. Adicionalmente, procedeu-se análises por espectrometria de massas com ionização por APCI (Atmospheric Pressure Chemical Ionization) para caracterizar o perfil químico dos extratos.

No âmbito da anatomia vegetal, explorou-se as características anatômicas das folhas de *B. subalternans*, empregando técnicas de coloração histoquímica para evidenciar elementos estruturais e compostos específicos. Todos os ensaios foram conduzidos em triplicata e os dados resultantes foram submetidos a análises estatísticas pertinentes.

Este estudo está apresentado de forma compacta, em formato de artigo científico, conforme a Resolução nº 008/2017-CEPE da Universidade Federal de Roraima (UFRR, 2017). Desta forma, após a contextualização necessária trazida por esta Introdução, a pesquisa desenvolvida é apresentada na forma de manuscrito intitulado “Pharmacobotany, chemical composition and biological activities of greater beggar's ticks (Bidens subalternans DC.)” (Farmacobotânica, composição química e atividades biológicas do Picão-preto (*Bidens subalternans* DC.), submetido à revista "Plant Biosystems", com Qualis A2 para a área das Ciências Ambientais, ISSN 1126-3504 e fator de impacto 2,0. A redação do artigo seguiu as diretrizes de publicação da revista (subitem 2.1). Finalmente, na terceira seção, são apresentadas as conclusões gerais da pesquisa, juntamente com as referências citadas na Introdução.

Os estudos realizados neste trabalho representam uma contribuição substancial para a compreensão das propriedades e das possíveis aplicações de *B. subalternans*, abrangendo diversos aspectos. Esses estudos pioneiros para a espécie podem desempenhar um papel crucial em pesquisas futuras, especialmente no que diz respeito ao desenvolvimento da bioeconomia no Estado de Roraima.

Esta pesquisa foi custeada, inclusive a bolsa de estudo da autora, pelo projeto “Fortalecimento das pesquisas em Bioprospecção no Programa de Pós-graduação em Recursos Naturais – PRONAT/UFRR”, no escopo do Programa de Desenvolvimento da Pós-graduação (PDPG) da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Proc. 510194/2020-00 CAPES).

2. Article - Pharmacobotany, chemical composition and biological activities of greater beggar's ticks (*Bidens subalternans* DC.)

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Pharmacobotany, chemical composition and biological activities of greater beggar's ticks (*Bidens subalternans* DC.)

Abstract

This study aimed to investigate the *in vitro* antibacterial and antioxidant activity of ethanolic extracts of the aerial parts of *Bidens subalternans* collected both in a natural environment and in controlled cultivation. Chemical prospecting, chemical profile analysis by APCI-MS, toxicity in relation to *Artemia salina* and a foliar pharmacobotanical study were performed. The results showed that extracts of *B. subalternans* showed antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli*. The extracts showed significant levels of total phenolics. In the DPPH assay, the IC_{50} values of the extracts ranged from 85.8 to 30.57 $mg.mL^{-1}$. The results related to the ABTS radical showed values of 1,969.0 $\mu MTrolox.g^{-1}$ and 1,855.1 $\mu MTrolox.g^{-1}$. Chemical prospection revealed the presence of classes of metabolites such as saponins, tannins, phenolic substances, phenols and flavonols, and chemical profile analysis using APCI-MS identified compounds such as chlorogenic acid and caffeic acid. In the pharmacobotanical study, the typical anatomical aspects of the leaves of *B. subalternans* were observed. These results highlight the therapeutic potential of *B. subalternans*, due to its antioxidant and antibacterial secondary metabolites, and they indicate the need for further studies to explore its therapeutic potential and as well as its safety.

Keywords: Ethanol extracts. Amazon. Histochemistry. Antioxidant activity. Antibacterial activity.

Introduction

Brazil is a country known for its diversity of biomes, which are home to varied ecosystems rich in biodynamic species, offering numerous potentials. This richness is especially evident in the Amazon rainforest, a biome that has an abundance of natural resources widely used in folk medicine (Salati et al. 1998). Over the centuries, different ethnic groups have used different plants as healing sources, thus revealing a huge potential for the production of secondary compounds with various biological activities (Amorozo 1996). In this context, the genus *Bidens* L., which belongs to the Asteraceae Bercht family. & J. Presl, stands out and it has been the subject of extensive studies.

A notable species of this genus is *B. subalternans* DC., popularly known as greater beggar’s ticks, a terrestrial herbaceous plant with an erect stem with a quadrangular section, smooth, glabrous or slightly hairy, which branches dichotomously throughout its length. It is characterized by having an axial main root and opposite and petiolate leaves, in addition to producing yellow flowers and achenes with four to five dark brown edges (Bogosavljević and Zlatković 2015; Bringel Júnior and Reis-Silva 2020).

Although the exact origin of *B. subalternans* is not documented in the literature, it is known that it is native to Brazil and can be found in several phytogeographic regions of the country. These areas include environments such as anthropogenic vegetation, open land, riparian forests and galleries (Bringel Júnior and Reis-Silva 2020). In addition, the distribution of this species extends throughout South America, as well as in countries of Oceania (Randall 2007), Asia (Kim 2012) and Europe (Bogosavljević and Zlatković 2015).

It is important to note that there are morphological similarities between *B. subalternans* and other species of the genus, such as *B. pilosa* and *B. alba*, which are grouped in the *Bidens* complex (Guatimosin et al. 2015). These morphological similarities make accurate identification of the species within this complex a challenge. Therefore, for its correct identification, it is necessary to resort to more in-depth analyses such as taxonomic studies, and molecular, pharmacobotanic and phytochemical analyses in order to clearly distinguish the species.

The information available in the literature regarding the species *B. subalternans* is limited, since most of the research involving this plant is related to the field of agronomy, which is due to its status as an invasive plant in crops (Gazziero et al. 2003; Freitas et al. 2021; Takano et al. 2020). Therefore, the available studies are mainly focused on physiological aspects, such as the management and chemical control of the species (Mendes et al. 2019; Takano et al. 2020; Freitas et al. 2021).

The ethnobotanical use of *B. subalternans* involves the treatment of conditions such as hepatitis, inflammations, induction of infertility, diabetes, malaria, and for healing wounds (Kujawska and Schmeda-Hirschmann 2022). With regard to its biological activities, Gonçalves et al. (2018) investigated the bactericidal effect of *B. subalternans* extracts against Gram-negative bacteria and obtained positive results against *Escherichia coli*, *Acinetobacter baumannii* and *Enterobacter aerogenes*. This can be justified by the presence of metabolites such as flavonoids, alkaloids, steroids and coumarins, which were found in the ethanolic extract of *B. subalternans* by Emediato et al. (2021).

Secondary compounds are subject to modifications in their production and accumulation in response to environmental variations such as seasonality, circadian rhythm, temperature variations, exposure to ultraviolet radiation, nutrient availability, altitude, presence of atmospheric pollution, mechanical stimuli and attacks by

pathogens. In addition, the stage of development of the plant can also influence the production of these compounds (Gobbo-Neto and Lopes 2007).

Considering the scarcity of information available on this species, the aim of this study was to investigate the pharmacobotanical characteristics, chemical profile, as well as the antioxidant and antibacterial potential of ethanol extracts of *B. subalternans*.

MATERIALS AND METHODS

This study analyzed two ethanolic extracts of *B. subalternans*: one from specimens collected in its natural habitat, named here as “Environment”, and another obtained by controlled cultivation of the plant in a greenhouse, named here as “Cultivation”. The collections were carried out only in the rainy season, in vegetative and reproductive stages. The focus of this approach was to investigate whether the plant maintains the qualitative chemical characteristics when grown in a greenhouse.

Plant Material of B. subalternans

The aerial parts of *B. subalternans* were collected at the Sítio Esperança (2°49’19’N.60º 46’ 15” W), located in the urban area of the municipality of Boa Vista, Roraima, Brazil. The predominant climate in Boa Vista is tropical humid type *Aw*, according to the Köppen classification (1948), and is identified primarily by precipitation, with two seasons: rainy and dry. The rainy season occurs in the months of April to September, with an average annual temperature of 27.4 °C.

For cultivation from seeds, a greenhouse was used, which had a temperature pf between 25 and 30 °C, a photoperiod of 12 hours and daily irrigation. The cultivation method followed an adaptation of the methodology proposed by Pamplona et al. (2020). After collection at both sites, the botanical material was properly sanitized with running water and subjected to a bench air-drying process, followed by drying in a circulating air oven until a constant biomass was reached.

The taxonomic confirmation was performed by Prof. Dr. Genilson Alves dos Reis Silva, a specialist in the tribe Coreopsideae from the Federal Institute of Piauí (IFPI), and the samples were duly registered and deposited in the UFRR Herbarium under identification code UFRR 9291.

Preparation of the plant extract

To obtain the extracts, the botanical material of *B. subalternans* from the environment and controlled cultivation groups was ground separately using a Wiley knife mill with a 5 mm sieve. The resulting powders were used to prepare two ethanol solutions, adding 200 g of powder to 1,000 mL of ethanol P.A. The mixtures were macerated separately for 7 days in flat-bottomed balloon flasks covered with aluminum foil, following the method described by Matos (2009). Then, the extractive solutions were subjected to the solvent evaporation process using a rotary evaporator, as described by Simões et al. (2017).

Phytochemical screening - qualitative classification of secondary metabolites

The ethanolic extracts of the two groups (Environment and Cultivation) were subjected to a phytochemical screening using the methodology described by Barbosa (2001). The presence of the following metabolites was evaluated: tannins, phenolic substances, flavones, flavonols, chalcones, isoflavones, saponins, free steroids, free pentacyclic triterpenoids, alkaloids, flavonoids, aurones and sesquiterpenolactones.

Determination of antioxidant activity by the scavenging capacity of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) radical (ABTS)

The capacity of the ethanol extracts to sequester the ABTS radical was evaluated using the decolorization method described by Re et al. (1999). The Abts radical solution ($ABTS^{\bullet+}$) was prepared by mixing 5 mL of ABTS solution (7 $mmol.L^{-1}$) with 88 µL of K_2SO_4 solution (140 $mmol.L^{-1}$) and leaving the mixture to stand in the dark at room temperature for 16 hours before use. For the assay, the Abts $^{*+}$ radical solution was diluted in buffered saline (pH 7.4) to an absorbance of 0.7 (±0.02) at 734 nm. Then a sample of 10 µL (500 $mg.mL^{-1}$) was mixed with 1 mL of the diluted solution of the $Abts^{\bullet+}$ radical, and the absorbance was measured after 6 minutes at 734 nm. The result was expressed in micromolar of Trolox equivalent per gram of extract (µMTrolox g^{-1}).

Scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity of the extracts was evaluated using the in vitro photocolorimetric method of free radical sequestration DPPH (2,2-diphenyl-1-

picrylhydrazyl) described by Brand-Williams et al. (1995). The samples were prepared by adding 50 µL of the extract diluted in methanol (100 mg/mL) to 3.9 mL of DPPH solution (60 µM), in triplicate. After a reaction time of 45 minutes, absorbances were measured at 515 nm using a UV-Vis spectrophotometer. A negative control was prepared using 100 µL of the control solution (50% methanol, 70% acetone and water) mixed with 3.9 mL of the DPPH solution. The antioxidant activity of the sample was expressed as IC_{50}, which represents the sample concentration required to inhibit the formation of DPPH radicals by 50%.

Content of total phenolic compounds (TPCs)

TPCs were determined using the Folin-Ciocalteau method, modified by Roesler et al. (2007). From this solution, 200 µL of *B. subalternans* extracts were removed, and added to 800 µL of distilled water, 1 mL of Folin-Ciocalteau reagent and 2 mL of 20% sodium carbonate. The absorbance (Abs) of the liquid fraction was determined at 760 nm in a UV-Vis spectrophotometer. The gallic acid calibration curve was used to quantify the total phenols. The results were expressed in gallic acid equivalents ($mgGAE.100g^{-1}$).

All readings were performed in triplicate and the difference in the absorbance between the samples and the negative control was calculated based on the mean of the data. The antioxidant activity (AA) percentages were determined using Equation 1.

$$Inhibition\ of\ activity\ (\%) = \frac{(1-B)}{A}\ x\ 100 \qquad (1)$$

Where:

A = Absorbance of the control solution.

B = Absorbance of the solution in the presence of the extract.

Antibacterial activity

The evaluation of antibacterial activity was carried out using the disc-diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI 2009), and using the bacteria *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC00531), *Bacillus cereus* (ATCC9634), *Listeria monocytogenes* (ATCC7644), *Escherichia coli* (Atcc25923), ATCC10536), *Klebsiella pneumoniae* (ATCC700603) and *Salmonella enteretidis* (ATCC13076). The bacteria were cultured and maintained on

tryptone soya agar (TSA) and incubated at 37 ºC for 24 hours. The density of the bacterial suspension was adjusted to 10^8 CFU. mL^{-1} using the MacFarland scale (Biomerieux, Italy). This suspension was diluted in a 0.85% sterile saline solution and, subsequently, a bacterial field was laid in a Petri dish, which was covered with sterile discs of filter paper (6 mm Ø) and an aliquot of 20 µL of the extracts of *B. subalternans* at the concentration of 500 $\mu g.mL^{-1}$. For the disk diffusion test, halos with a diameter of ≤ 3 mm in relation to the positive control (measured with a caliper) were considered as indicators of inhibitory activity (Ostrosky et al. 2008). Discs with commercial antimicrobials were used as the positive control: amoxicillin (15 µg) and vancomycin (30 µg). To obtain the minimum inhibitory concentration (MIC), aliquots of the extracts were used in concentrations ranging from 500 to 50 $\mu g.mL^{-1}$.

Toxicity to Artemia salina

The test was performed following the methodology described by Meyer et al. (1982), with modifications. Initially, a saline solution was prepared by adding 20 g of sea salt to 1 L of distilled water. This solution was aerated for 24 hours and exposed to the light of a 45 W lamp. The pH of the solution was adjusted to between 8 and 9 using a 10% sodium carbonate (Na_2CO_3) solution. After the incubation period, the nauplii were separated and transferred to test tubes containing the solutions of ethanol extracts of *B. subalternans* dissolved in DMSO (dimethyl sulfoxide) at 1%. Each test tube received 10 nauplii and three repetitions were used. Two positive control groups were used for comparison. The first group received only saline water (Control 1), while the second group received a combination of DMSO and saline water (Control 2), and the same number of nauplii. After 24 hours, the number of living and dead nauplii in each test tube was recorded, and the percentage of mortality was calculated based on these data.

Analysis of the chemical profile using APCI-MS

The ethanol extracts of *B. subalternans* were solubilized in methanol grade HPLC, generating stock solutions of 1,000 ppm. Aliquots (10 µL) of these solutions were transferred to vials containing 1 mL of MeOH. Then, 5 µL of the diluted solutions were analyzed by direct insertion into an ion trap mass spectrometer (LCQ Fleet model), equipped with an APCI source operating in positive and negative modes. The analytical

parameters used were as follows: discharge current: 5 µA; vaporizer temperature: 320 ºC; capillary temperature: 220 ºC; sheath gas: 30 psi; aux gas: 10 arb, mass range, *m/z* 100-1000. The MS/MS spectra were acquired using helium as the collision gas and energy ranging between 20-30% (Silva et al. 2012).

Characterization of the pharmacobotanical aspects

Adult leaves of *B. subalternans* were selected (Environment and Cultivation) and washed with distilled water. Then, histological sections were made of the median region of the leaf blade, using steel blades in a manual procedure. The chosen sections were placed in a Petri dish containing sodium hypochlorite solution at a low concentration (10 mL of sodium hypochlorite P.A. and 10 mL of water), in which they remained until the total discoloration of the chloroplasts occurred. After discoloration, the sections were washed with distilled water and subjected to a staining process for three minutes using different stains for histochemical characterization: acid fuchsin (to show the lignified membrane), Sudam III in isopropanol and glycerin (for the suberin membrane), methylene blue (for mucilages), lugol (for organic inclusions) and Sudam III (for fixed and essential oils), according to Oliveira et al. (2014). Both the cross sections and the paradermal sections were mounted in distilled water, between the slide and coverslip, and analyzed by means of an inverted microscope (Coleman).

Statistical analysis

Antioxidant activity and toxicity data were reported using mean ± standard deviation and evaluated using analysis of variance (ANOVA) followed by Tukey's Test ($P < 0.05$). All experiments were performed in triplicate (n=3).

RESULTS AND DISCUSSION

In this section, the results and central discussions of phytochemical analyses are presented, covering the content of phenolic compounds, as well as the evaluation of the antioxidant and antibacterial activities and toxicity. In addition to the chemical profile using the APCI/MS method and data from the histochemical analysis of *B. subalternans* leaves.

Phytochemical screening - qualitative classification of secondary metabolites

Table 1 shows the classes of secondary metabolites found in the extracts of *B. subalternans*.

The metabolites found in *B. subalternans* have several biological effects, such as antioxidant, anti-inflammatory and antitumor activity and inhibition of damage to collagen (Cunha et al. 2016). Isoflavones fight LDL cholesterol, help control diabetes, prevent cardiovascular disease and cancer (Vizzoto et al. 2010). According to Pereira and Cardoso, saponins have detergent properties and their biological effects stand out for their antioxidant action; in addition, they act against tumor cells (Pereira and Cardoso 2012).

A phytochemical study of this species carried out by Emediato et al. (2021) evidenced the presence of alkaloids, coumarins, steroids and flavonoids. The screening performed with the methanolic extract of the leaves and inflorescences of *B. segetum*, confirmed the presence of the following classes of secondary metabolites: alkaloids, triterpenoids, phenols, tannins, flavonoids, anthraquinones in the inflorescence and steroids, phenols, tannins, flavonoids, anthraquinones in the leaves (Fabri et al. 2011). Idris et al. (2022) identified 137 compounds in hydroalkolic and aqueous extracts of *B. pilosa*, such as phenolic acids, flavonoids and fatty acids, and attributed the medicinal use of this species to the abundant phenolic compounds found in this species. Therefore, similarities with the metabolites found in *B. subalternans* from the Environment and Cultivation extracts are noted, especially in terms of the frequencies of phenolic compounds, which are a characteristic of species of the genus *Bidens*.

Antioxidant activity (ABTS, DPPH and phenolic compounds)

The extracts of *B. subalternans* showed significant results against the radicals used. In addition, the Environment extract showed values close to those of the antioxidants ascorbic acid and BHT (butylated hydroxytoluene), as shown in Table 2.

From the data obtained, it is noted that the antioxidant potential and content of phenolic compounds of the same species vary according to the collection site. For the tests with the DPPH and ABTS radicals, it is observed that the extract of the leaves collected in the natural habitat (Environment) presents values that are close to/superior to synthetic antioxidants (ascorbic acid and BHT). In addition, the Environment extract has a higher content of phenolic compounds than the extract grown in the greenhouse.

The difference between the values obtained can be attributed to the characteristics of the soils where the botanical material was collected. The Cultivation extract was derived from parts of the plant grown in a greenhouse environment, with fertilized soil, while the Environment extract was obtained from plants growing in croplands.

These differences can be contextualized via the characteristics of the soils in Roraima. According to Miranda and Absy (2000), the soils of the croplands exhibit reduced levels of calcium and magnesium, which are characteristics that are indicative of low soil fertility. Studies such as that of Jacobson et al. (2005) indicate that soils of low chemical fertility may result in higher levels of total phenols and tannins.

Despite the significant discrepancy between the results obtained and the values described in the literature for the species *B. pilosa*, such as the studies by Deba et al. (2007) and Wu et al. (2012), which reported considerably lower total phenolic contents in different parts of the plant collected in Western Asia, it is important to highlight that several factors may contribute to these divergences. Luminosity is one of these influential factors. As mentioned by Dudt and Shure (1994), an increase in luminous intensity can result in higher levels of phenolic compounds in various plants. This difference in exposure to sunlight may partly explain the variations in phenolic contents between plant material collected in the natural environment in relation to material from plants grown in greenhouse conditions.

The study by Borella et al. (2019) investigated the response of *B. pilosa* to different levels of organic fertilization and shade and revealed that the best responses were obtained in the conditions of absence of fertilization and without light restriction. These findings are in line with the results of the present research, in which plant material grown under controlled and fertilized conditions exhibited significant differences in relation to plant material collected in their natural habitat. Full exposure to sunlight and the absence of fertilization may have contributed to an increased production of phenolic compounds in plants in the natural environment. Therefore, the results of the research corroborate the idea that the difference in phenolic contents can be attributed to factors such as luminosity, fertilization and environmental exposure, thus demonstrating the complexity of the interactions between plants and their environment.

According to Lima et al. (2000), the antioxidant activity presented by several plants is correlated to their content of total phenolic compounds. In this research, the ethanolic extracts of *B. subalternans* showed promising values for antioxidant activities. In the DPPH assay, the IC_{50} of the two extracts ranged from 85.8 to 30.57 $mg.mL^{-1}$, with inhibition percentages of 69.1% and 12.5%, respectively. The antioxidant potential of plants is influenced by environmental conditions (Melo et al. 2006). Therefore, the difference in the IC_{50} values of the two extracts can be explained by the divergence in the collection sites, thus altering the biotic and abiotic factors, which influence the production of agents that act as antioxidants.

The results obtained in the ABTS radical assay highlight the efficacy of the ethanolic extracts of *B. subalternans* as potential sources of antioxidant activity. In the Environment extract, a value of 1,969.0 µMTrolox g^{-1} was observed, accompanied by a remarkable inhibition rate of 99.2%; while the Cultivation extract exhibited a value of 1,855.1 µMTrolox g^{-1} and an inhibition percentage of 93.57%. It is relevant to note that these values are close to the antioxidant activity of ascorbic acid, which recorded a value of 1,978.4 ± 7.12. In addition, both extracts exceeded the antioxidant capacity of BHT, a synthetic antioxidant widely used in industry, which recorded a value of 1,368.7 ± 4.98.

Freitas et al. (2006) observed a positive correlation between the average content of total polyphenols and the average values of antioxidant activity equivalent to Trolox, which indicates a directly proportional relationship, substantiating the results obtained in the ABTS assay. Thus, the data acquired in this research confirm the antioxidant action of ethanolic extracts of the species *B. subalternas*. In addition, the result provides s possible low-cost, safe and sustainable alternative to the use of BHT in food and other products, as synthetic antioxidants are unstable at high temperatures and can be toxic when stored for prolonged periods (Mansour et al. 2022).

The proximity of the values for the antioxidant activity of *B. subalternans* extracts in relation to ascorbic acid, as well as their superiority over BHT, highlights the substantial antioxidant potential of these extracts. Antioxidant activity is a desirable characteristic for the prevention of oxidative damage in the human body, which is related to aging and the development of chronic diseases. The fact that the Environment and Cultivation extracts showed such significant antioxidant activity suggests that *B.*

subalternans may be a promising source of natural antioxidant compounds, which can be used in food, pharmaceutical and cosmetic applications.

Taken together, these results reinforce the potential of *B. subalternans* as a natural source of valuable antioxidant compounds and highlight the importance of further studies to better understand the chemical composition of these extracts, as well as their impact on biological activities and their practical applications.

Antibacterial activity

The antibacterial activity was performed in triplicate. The inhibition halos and the minimum inhibitory concentration (MIC) were obtained, as shown in Table 3.

The Environment extract showed significant activity against the microorganisms tested. The antibacterial nature of *B. subalternans* extracts is directly attributed to the presence of secondary metabolites, which include saponins, tannins, alkaloids, phenols, flavonoids and sesquiterpenes (Pisoschi et al. 2018).

The halos in this research ranged from 2 to 10 mm. Singh et al. (2017) demonstrated significant antibacterial activity for the extract of *B. pilosa*, with a variation in halos from 9.1 to 18.2 mm, in which *E. coli* presented the largest halo (18.2 mm) and *S. aureus* a halo of 15.66 mm. Deba et al. (2008) found an inhibition halo of 11.8 mm for *Bacillus cereus*. The inhibition halos found in the literature were higher, which can be attributed to several environmental factors that can influence the production of antimicrobial compounds, in addition to the fact that they are in fact different species.

When confronted with synthetic antibiotics, the ethanolic extract of *B. subalternans* (Environment) demonstrated encouraging results, exhibiting a larger diameter halo when compared to vancomycin with respect to *B. cereus*. It is important to note that vancomycin is a frequently selected antibiotic for empirical therapy against *B. cereus* infections (Ikeda et al. 2015). In addition, minimum inhibitory concentrations (MIC) of 400 to 500 µg mL^{-1} for the Environment extract and 500 to 300 µg mL^{-1} for the Cultivation extract were observed. A *B. pilosa* methanolic extract showed significant activity against selected bacterial pathogens with a MIC ranging from 80 to 870 µg mL^{-1}. The extract showed maximum activity against *E. coli* with a MIC of 80 µg mL^{-1}, followed by *S. aureus* with 110 µg mL^{-1} (Singh et al. 2017). Angelini et al. (2021) found a MIC of < 31 µg $mL^{-1}$for *E. coli* and *B. cereus* and 39.031 µg.$mL^{-1}$for *S. aureus*. The

discrepancies in the MICs reported by these researchers can be attributed to variations in the chemical composition of the extracts, in the bacterial strains used, in the cultivation conditions, in the culture medium used and in the concentration of the substances tested, among other factors (Nascimento et al. 2007).

Toxicity against Artemia salina

For the toxicity test against *A. salina*, live, dead, or paralyzed nauplii were counted and the lethal concentration 50% (LC_{50}) was determined. It is important to highlight that in control groups 1 and 2 no dead nauplii were observed, indicating the absence of toxicity under these conditions. Both the extract from the natural environment and the extract obtained from the controlled cultivation did not present dead nauplii, suggesting the absence of significant toxic effects on the organisms tested.

The determination of the lethal concentration 50% (LC_{50}) was performed using the linear regression formula Y = A + BX (Silva et al. 2021). After calculating the linear regression, the following values were obtained (Table 4).

The concentration of the extracts was 31,750.0 $\mu g.mL^{-1}$ for the Environment extract and 16,150.0 for the Cultivation extract. According to Meyer et al. (1982), toxicity is considered low when the lethal concentration 50% (LC_{50}) is greater than 1,000 $\mu g.mL^{-1}$. Therefore, according to the data, it can be confirmed that the ethanol extracts of the leaves of *B. subalternans* do not present lethality against the microcrustaceans *A. salina*.

According to Khan et al. (2021), the crude extract of *B. chinensis* showed lethality of 63.3% at 1,000 µg/mL; in turn, Sanabria-Galindo et al. (1997) highlighted that the extract of *B. pilosa* did not show lethality against *A. salina*. Thus, there is a concordance in the low toxicity of the species of the genus *Bidens*, reinforcing the results obtained in this research. These findings underscore the safety of these extracts with regard to any future applications.

Analysis of the chemical profile using APCI-MS

The analysis of the Environment extract using high resolution mass spectrometry with chemical ionization at atmospheric pressure allowed the identification of several classes of chemical constituents. The possible compounds identified in the negative

ionization mode are described in Table 5. The possible compounds identified for the Cultivation extract, in negative ionization mode, are described in Table 6.

Via the APCI/MS method, it was possible to perform a preliminary analysis of the compounds present in the ethanol extracts, highlighting the predominance of elements from the group of phenolic compounds. In the Environment extract, compounds such as dihydrocoumaric acid, caffeic acid-3-glycoside, tricine hexoside, apigenin 6,8-di-C arabinoside and X"-O-rhamnosyl C-(6-deoxy-pentohexose-ulosyl) luteolin were identified. On the other hand, in the Cultivation extract, the presence of caffeic acid, 1,3-O-dicaffeoyglycerol and caffeic acid-3-glycoside was observed. This difference can be attributed to several factors, such as soil characteristics at the collection sites, fertilization practices, irrigation regime and environmental conditions, as discussed by Nascimento et al. (2007). Although the collections were carried out during the rainy season, it is relevant to highlight that plants grown under controlled environmental conditions experienced a different irrigation regime in relation to natural precipitation. In addition, it is crucial to consider the substantial role of luminosity in this scenario, since the protective structure present in the greenhouse limits direct exposure to solar radiation. It is also pertinent to emphasize the significant influence of herbivory, given that plants grown in a controlled environment have a reduced exposure to herbivorous agents compared to those grown in their natural habitat.

The comparative analysis of the extracts Environment and Cultivation evidenced that there was a variation in chemical composition between the two; however, it is notable the recurrent presence of three specific compounds (the fatty acids palmitic acid and hexacosyl palmitate), along with the phenolic compound caffeic acid 3-glycoside. This observation is supported in the literature, which has reported the recurrence of these substances in species belonging to the genus *Bidens*.

The results of this research corroborate previous studies that identified caffeic acid in hydroalcoholic extracts of species such as *B. frondosa* L., as demonstrated by Le et al. (2015). In addition, the compounds palmitic acid and caffeic acid were identified in *B. pilosa*, which was reported by Silva et al. (2011) when compiling information on the secondary metabolites of this species. This regularity in the presence of these compounds suggests a common feature within the genus *Bidens* and is possibly related to physiological or defense functions of the plants.

The presence of phenolic compounds in species of the genus *Bidens* has been widely documented in the literature. Studies conducted by several researchers with different species, such as *B. subalternans*, *B. tripartita* and *B. gardneri*, identified the occurrence of phenolic acids, flavonoids and their derivatives (Ortega et al. 2000; Silva et al. 2013; Mendel et al. 2020). These secondary metabolites, often present in plants of the genus *Bidens*, are known for having potential biological activities, including antioxidant and anti-inflammatory properties.

Caffeic acid, which is present in the extracts analyzed, arouses considerable interest in the scientific community due to its potential therapeutic properties. This compound has been the target of studies for its antimicrobial activity and has been explored as an alternative strategy in the fight against pathogens and chronic infections caused by microorganisms, such as bacteria, fungi and viruses (Khan et al. 2021). The relevance of this compound is increased when considering the results of the antibacterial test conducted with *B. subalternans* in the present study, which indicated a positive response to some bacterial strains. In addition, other research also associates caffeic acid with an anti-inflammatory potential (Borges et al. 2013; Amoah et al. 2016), further reinforcing its multifaceted role.

In addition, previous studies have pointed out that phenolic compounds found in different species of the genus *Bidens*, such as flavones, flavonols and their glycosides, are recognized as essential sources of antioxidants in the human diet (Bohm and Stuessy 2001). The antioxidant capacity of these compounds is directly related to their oxidation potential, which is an important measure for their effectiveness in neutralizing free radicals (Farinazzi et al. 2017). However, the effectiveness of these compounds in human food also depends on efficient digestion, assimilation and metabolic processing (Oliveira and Bastos 2011).

The significant abundance of phenolic compounds in *B. subalternans* may offer an explanation for its remarkable antioxidant potential, as evidenced by this research, in addition to other beneficial properties for human health. Nonetheless, it is essential to emphasize the importance of targeted investigations that look into the biological activities and mechanisms of action of these compounds in *B. subalternans* in order to authenticate their promising therapeutic properties.

Characterization of the pharmacobotanical aspects

The anatomy of the leaf mesophyll of *B. subalternans* was observed between Cultivation samples and Environment samples, though no significant differences were detected in the anatomical structure of the mesophyll between the samples of the two groups. The results obtained in the pharmacobotanical analysis of the leaves of the different specimens of *B. subalternans* were consistent with the data previously reported in the literature, both for this species and for closely related species, such as *B. pilosa*.

The paradermal sections of the leaf show the slightly sinuous shape of the epidermal cells on both faces (Figure 1), which is similar to what is reported by Medeiros and Silva (2022) for *B. subalternans*; and for *B. pilosa* (Sá et al. 2017), *B. odorata* and other species of the tribe Coreopsideae, such as *Cosmos bipinnatus, C. parviflorus* and *Heterosperma pinnatum* (Rivera et al. 2019). As well as for other species of the Asteraceae family, such as *Tridax procumbens* (Ramos et al. 2022).

The stomata present in the leaf blade of *B. subalternans* are predominantly of the anomocytic type (Figure 1), frequent in the Asteraceae family (Metcalfe and Chalk 1950); with a small amount of anisocytic stomata distributed on both faces, thus confirming the findings of Medeiros and Silva (2022). This characteristic is also observed in *B. pilosa* and was described by Sá et al. (2017). In the species described by Younis et al. (2020), a hypoestomatic distribution pattern and the presence of diacytic and paracytic stomata were observed. In addition, other types of stomata in *Bidens* are also mentioned in the literature, such as the tetracytic stomata found on the abaxial face of the leaf blade of *B. bipinnata* (Tahir et al. 2017).

The characteristic of having amphistomatic leaves is relatively common in the Asteraceae family and has great adaptive significance as it increases the flow of carbon dioxide in a short time interval (Metcalfe and Chalk 1950; Fahn and Cutler 1992). This characteristic can be advantageous to the plant when it is in an environment with high luminosity and low water availability.

In cross section, the main rib, exhibits a biconvex contour (Figure 2-A), also found by Medeiros and Silva (2022), and similar to *B. pilosa* (Sá et al. 2017). The mesophyll has a heterogeneous and asymmetric structure, and is characterized by a layer of palisade parenchyma followed by 5-6 layers of predominantly sinuous cells in the spongy parenchyma, as illustrated in Figure 2-B.

Several tector trichomes were found in the leaf indument of *B. subalternans*, as well as glandular trichomes. This is different from Medeiros and Silva (2022), who reported only the presence of tector trichomes for the species.

This variation can be attributed both to the genetic content of the plants and to the various environmental factors to which they were exposed; it may have a probable origin in the physiological response triggered by preponderant environmental factors, among which the high temperatures, notably observed in plants collected in the natural habitat, as well as the considerable herbivorous pressure exerted. This conjunction of environmental influences suggests the presence of a marked phenotypic plasticity intrinsic to the species. In addition, in Asteraceaes, trichomes that secrete essential oils are common. These are important because they produce toxic or repellent compounds that aid in repelling herbivores (Johnson 1975; Fahn 1979; Fahn and Cutler 1992).

Tector and glandular trichomes are also found in the leaves of *B. pilosa*, as described by Sá et al. (2017) and Rehem et al. (2019). As mentioned by Liesenfeld (2018), trichomes are present in all species of the Asteraceae family and are usually distributed on both sides of the leaf blade. The success and wide distribution of the Asteraceae family in different habitats is attributed to the diversity of secretory structures, such as trichomes, as well as the ease of dispersion of its seeds by the wind and the phenotypic plasticity of its representatives (Funk et al. 2005).

During the histochemical analysis, the presence of lignin in the walls of parenchymal tissue and vascular bundle cells was evidenced by staining with acid fuchsin (Figure 3-C). Starch grains were observed in leaf parenchyma cells after lugol treatment (Figure 3-E), also found by Medeiros and Silva (2022) in *B. subalternans*; as well as in other genera of Asteraceae, such as *Acanthospermum* (Yhi-Pênê et al. 2019) and *Baccharis* (Budel et al. 2018).

The blackish coloration after the application of ferric chloride revealed the presence of phenolic compounds in regions such as the mesophyll and main leaf vein (Figure 3-F). These compounds were also found by Medeiros and Silva (2022) in *B. subalternans*. In addition, histochemical analyses performed on other species, such as *Acanthospermum* (Yhi-Pênê et al. 2019), *Baccharis* (Budel et al. 2018), *Pectis*, *Pterocaulon* and *Wedelia* (Ferraro and Scremin-Dias 2018), *Solidago* (Souza et al. 2018) and *Tagetes* (Naidoo et al. 2021) also confirmed the presence of these phenolic compounds.

By means of the Sudan III reagent, lipophilic substances were identified in the cell walls of the leaf epidermis, in addition to the presence of lipid droplets in the palisade and spongy parenchyma (Figure 3-D, H-I), as well as in the cuticle (Figure 4-G). The methylene blue test indicated the abundant presence of mucilage in the leaf mesophyll and xylem (Figure 3-B). Medeiros and Silva (2022) observed this inclusion in *B. subalternans* only in the walls of the epidermis. Lipophilic compounds have already been evidenced in several genera of the Asteraceae family, such as *Acmella* (Ramachandran and Radhakrishnan 2020), *Artemisia* (Zhang et al. 2018), *Pectis*, *Pterocaulon* and *Wedelia* (Ferraro and Scremin-Dias 2018).

The accumulation of terpenoids and flavonoids is common in trichomes in the Asteraceae family, and it confers several important functions in ecological interactions and defense against conditions of biotic and abiotic stress (Amrehn 2014). According to Werker and Fahn (1981), the species Inula viscosa (L.) Ait. secretes lipids and terpenoids via its glandular trichomes, which help in the reflection of light and in lowering the leaf temperature. In addition, this type of trichome has an important role in defending the plant against herbivory and attacks by pathogens (Werker 2000; Siebert 2004; Lusa et al. 2015).

During the analysis of the leaf blade, different types of tector trichomes were observed: uniseriate (Figure 4-A), ampoule-shaped (Figure 4-B) and multicellular with collapsed cells (Figure 4-C). In addition, glandular trichomes were also found, as illustrated in Figure 4-D.

After treatment with various stains, trichomes exhibited inclusions such as mucilages, phenolic compounds and lipid compounds. These observations were confirmed through microscopic analysis, as illustrated in Figure 5.

These findings are in agreement with the results obtained in the phytochemical prospection carried out in this study, as well as in the quantification of the content of phenolic compounds. This consistency in the results reinforces the significant presence of these compounds in the studied species and highlights their relevance in the chemical composition of the plant.

Conclusions

Although it is considered undesirable in agriculture, the species *B. subalternans* presents valuable characteristics. The increased interest in natural antioxidants, used in

the food industry and in the manufacture of cosmetic products, emphasizes the importance of researching the antioxidant properties of the species, which has demonstrated efficacy comparable to that of ascorbic acid and superior to that of butylhydroxytoluene (BHT). In this sense, it is crucial to investigate the biotechnological potential of this species, particularly its antioxidant and antimicrobial properties.

In addition, the antimicrobial activity observed in the plant extracts is relevant for addressing antimicrobial resistance and may lead to the development of therapeutic agents and natural antimicrobial products for various health areas. In the search for natural and sustainable solutions, exploring the biological capabilities of *B. subalternans* could boost innovations and health promotion.

The histochemical analyses revealed the presence of lignin, starch grains, phenolic and lipid compounds. These compounds have significant implications on plant physiology, especially in its defense against herbivores and adaptation to the environment. The consistency of the results reinforces the importance of these compounds in the chemical composition of *B. subalternans*, highlighting its pharmacological potential.

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Disclosure statement

The authors declare no potential conflict of interest.

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Appendices

Table 1. Results of the phytochemical prospection of ethanolic extracts of *Bidens subalternans*.

Secondary metabolites	**Ethanolic extract Environment**	**Ethanolic extract Cultivation**
Tannins and phenols	+	+
Flavonoids, flavones, chalcones, aurones and isoflavones	+	+
Free pentacyclic triterpenoids	+	+
Alkaloids	+	+
Phenolic substances	+	+
Saponins	+	+
Free steroids	+	+

(-) Absence; (+) Presence.

Table 2. Antioxidant activity of ethanolic extracts of *Bidens subalternans* leaves.

	Total phenolic compounds (mg.GAE.100 g^{-1}).	**DPPH radical IC_{50} (mg.mL^{-1})**	**ABTS radical (µMTrolox g^{-1})**
Ethanolic Extract Environment	1,149 ± 0.01	85.8 ± 0.3	1,969.0 ± 4.32
Ethanolic Extract Cultivation	140.6± 0.2	30.57± 2.4	1,855.1± 6.79
Gallic Acid	2,931.04 ± 8.8	-	-
Ascorbic Acid	-	100.2 ± 2.26	1,978.4 ± 7.12
BHT	-	94.5 ± 3.4	1,368.7 ± 4.98

(-) Absence.

Table 3. Inhibition of bacterial growth (mm) and minimum inhibitory concentration (MIC µg. mL^{-1}) of ethanolic extracts of *Bidens subalternans* against the listed bacteria.

BACTERIUM	**EE**	**EC**	**AMX**	**VAN**	**CIM EE ($\mu g.mL^{-1}$)**	**CIM EC ($\mu g.mL^{-1}$)**
Staphylococcus aureus (ATCC25923)	7.5	3.0	12.0	-	500	300
Enterococcus faecalis (ATCC00531)	-	2.0	8.0	-	-	500
Bacillus cereus (ATCC9634)	10	4.5	-	6.0	400	300
Listeria monocytogenes (ATCC7644)	6.0	3.0	12.0	-	400	400
Escherichia coli (ATCC10536)	3.5	3.5	6.0	-	500	500
Klebsiella pneumoniae (ATCC700603)	-	-	6.0	-	-	-
Salmonella enteritides (ATCC13076)	-	-	7.0	-	-	-

(EE): Environment extract; (EC): Cultivation extract; (AMX): Amoxicillin; (VAN): Vancomycin;
(-): Absence of activity.

Table 4. 50% lethal concentration (LC_{50}) of the ethanolic extracts of *Bidens subalternans* against *Artemia salina*.

Ethanolic extract	**LC_{50} (µg.mL^{-1})**	**Linear regression**	**Standard error**
Environment	31,750.0	Y= 15,093.15,068x - 429.28493	40.0735
Cultivation	16,150.0	Y= 13,313.53,591x - 165.04793	5.66958

Table 5. Compounds identified in the ethanolic extract of *Bidens subalternans* (Environment). Base peaks are identified in bold type.[1]

Substance	m/z	MS/MS
Dihydrocoumaric acid	165	89, 101, 103, 115, 122,133, 135, **147**, 150
Undecanedioic acid	215	67, 73, 85, 89, 103,113, 116, 132,155, 161, 171, **179**, 197, 200
Palmitic acid	255	101, 137,145, 154, 163, 196, 213, 223, 227, **237**, 240
γ-linolenic acid	277	119, 141, 159, 163, 217, 231, **233**, 239, 247, 249, 257, 259, 261, 275
Caffeic acid-3-glucoside	341	101, 107, 113, 115, 119, 131, 134, 145, 149, 161, 178, **179**, 251, 263, 269, 278, 282, 312, 313, 326
Oleanic acid	455	162, 167, 293, 335, 393, **395**, 407, 408, 412, 413, 420, 422, 439, 440
Tricin hexoside	491	269, 277, **287**, 318, 329, 366, 415, 431, 449, 474, 476
Apigenin 6,8-di-C arabinoside	533	179, 243, 269, 287, 355, 397, 455, 473, **491**, 497, 515
X"-O-Rhamnosyl C-(6-desoxi-pento-hexos-ulosyl) luteolin	575	175, 219, 287, 335, 397, 401, 439, 455, 473, 497, 506, 515, 531, **533**, 537, 557, 561
Hexacosyl palmitate	620	400, 413, **446**, 447, 461, 473, 505, 519, 520, 554, 559, 588, 590, 605

[1] References: Siqueira et al. (2003); Kang (2016); Wang et al. (2017); Martini et al. (2018); Souza et al. (2020); Campelo et al. (2021).

Table 6. Compounds identified in the ethanolic extract of *Bidens subalternans* (Cultivation). Base peaks are identified in bold type [2]

Substance	m/z	MS/MS
Caffeic acid	179	57, 85, 87, 106, 113, 117, 119, 125, 129, 131, 143, 146, 149, 159, **161**
Palmitic acid	255	101, 137,145, 154, 163, 196, 213, 223, 227, **237**, 240
Mono(2-ethyl-5-hydroxyhexyl) phthalate	293	101, 113, 123, 136, 161, 179, 185, 188, 193, 197, 231, 236, 237, 249, 250, 261, 263, 265, **275**, 278, 293
Trihydroxy-octadecadienoic acid	327	97, 113, 145, 147, **165**, 171, 179, 203, 211, 221, 229, 247, 251, 263, 281, 283, 291, 309, 312
Caffeic acid-3-glucoside	341	101, 107, 113, 115, 119, 131, 134, 145, 149, 161, 178, **179**, 251, 263, 269, 278, 282, 312, 313, 326
Hexose or sucrose	377	113, 119, 125, 143, 161, 171, 197, 209, 215, 240, 255, 281, 303, 307, 320, 333, **341**, 349, 359, 362
1,3-O-Dicaffeoyglycerol	415	149, 154, 161, 179, 207, 245, 253, 279, 281, 335, 341, 931, 345, 358, 371, 373, 385, 397, **400**
Hexacosyl palmitate	620	445, **446,** 448, 461, 473, 488, 500, 505, 515, 520, 548, 562, 573, 585, 589, 605

[2] References: Siqueira et al. (2003); Kang et al. (2016); Wang et al. (2017); Yao et al. (2018); Silva et al. (2019).

Figure 1. Paradermal sections of the leaf blade of *Bidens subalternans*. A = adaxial face. White arrow indicating anomocytic stomata. B = abaxial face. White arrow indicating anisocytic stomata and red arrow indicating anomocytic stomata. Abbreviation: est = stomata

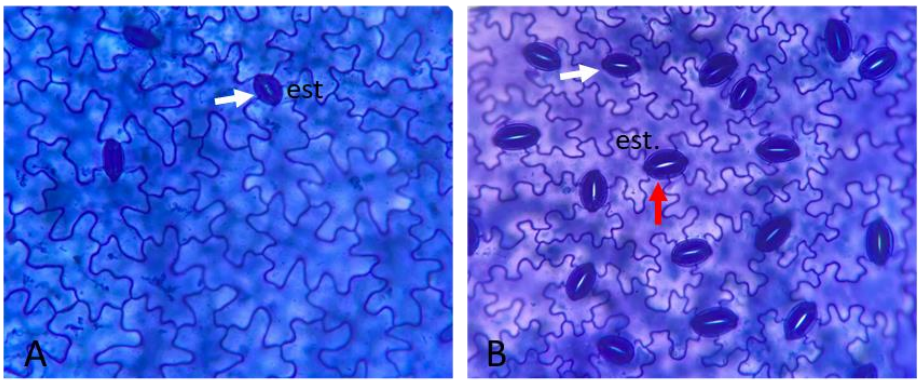


Figure 2. Cross sections of the leaf blade of *Bidens subalternans*. A. Details of the midrib showing the vascular bundle. Arrow indicating lignified walls stained in acid fuchsin. B. Mesophyll layer. Abbreviations: fv = vascular bundle; tt = trichoma tector; ad = adaxial face; ab = abaxial face; ep = epidermis; ct = cuticle; pp = palisadic parenchyma; pe = spongy parenchyma; in = inclusion; est = stomata.

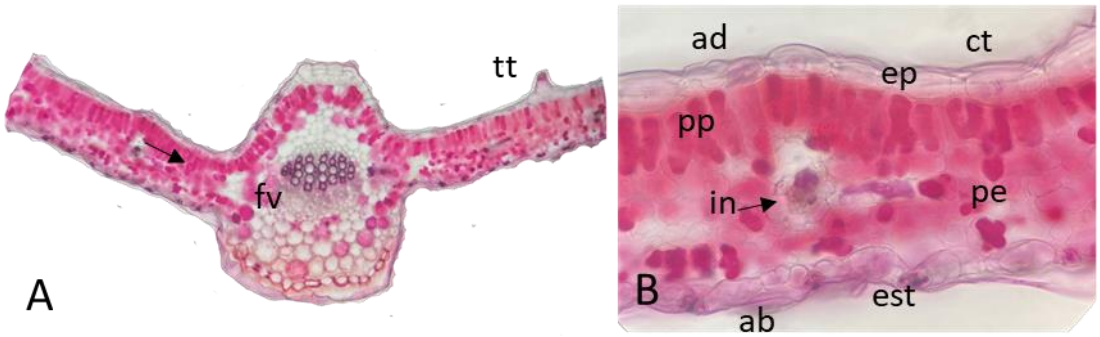


Figure 3. Cross-sections and paradermal leaf blade of *Bidens subalternans*. A. Control. B. Xylem cells showing mucilages in blue. C. Lignified walls in the vascular bundle. D. Paradermal section of the abaxial face showing phenolic compounds in the epidermis. E. Details of starch grains on the main rib. F. Phenolic compounds and lipid droplets in the main rib. G. Lipid compounds and phenolic compounds. H-I. Lipid compounds in the mesophyll and spongy parenchyma. Abbreviations: fv = vascular bundle; tt = trichoma tector; ad = adaxial face; ab = abaxial face; ep = epidermis; ct = cuticle; pp = palisadic parenchyma; pe = spongy parenchyma; in = inclusion; est = stomata.

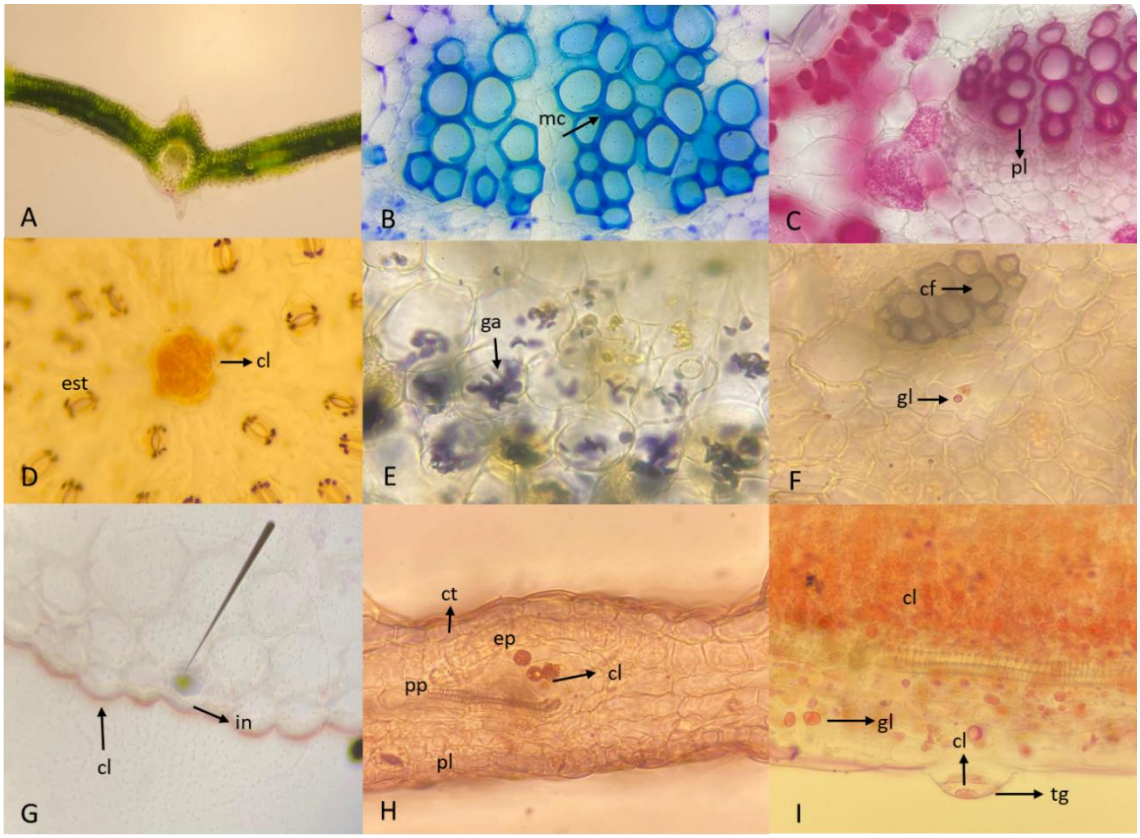


Figure 4. Trichomes of *Bidens subalternans*. A. uniseriate tector trichome. B. bulb-shaped tector trichome. C. tector trichome with collapsed cell. D. glandular trichome. Abbreviations: tt = tector trichome; tg = glandular trichome.

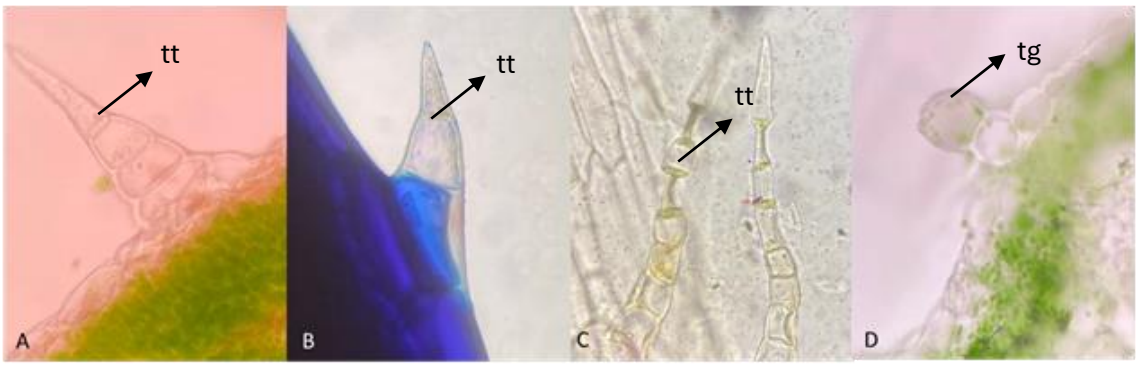
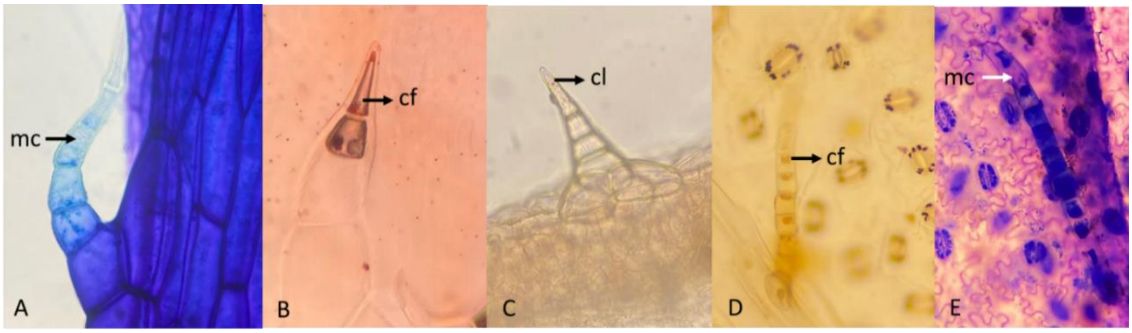


Figure 5. Trichomes found on the leaf blade of *Bidens subalternans*. A. Tector trichome showing the presence of mucilages. B. Tector trichome exhibiting inclusion of phenolic compounds. C. Tector trichome presenting lipid droplets. D. Glandular trichome with the presence of phenolic compounds. E. Glandular trichome with inclusion of mucilages. F. Glandular trichome with mucilages. Abbreviations: mc = mucilage; cf = phenolic compounds; cl=lipid droplets.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3 CONCLUSÕES

Os resultados farmacobotânicos obtidos fornecem uma caracterização abrangente da estrutura anatômica e da composição química das folhas de *B. subalternans*, contribuindo para a identificação e autenticidade da planta. Essas informações são essenciais para garantir a qualidade e segurança dos produtos fitoterápicos derivados desta planta, bem como para estudos de padronização e controle de qualidade.

A análise histoquímica das folhas revelou a presença de lignina, amido, compostos fenólicos, mucilagens e lipídios em diferentes regiões, os quais desempenham papéis importantes na defesa e adaptação da planta ao ambiente.

B. subalternans apresenta uma diversidade de metabólitos secundários com potenciais propriedades farmacológicas. A análise fitoquímica revelou a presença de saponinas, taninos, flavonoides, alcaloides, substâncias fenólicas, esteroides livres e triterpenóides pentacíclicos nos extratos, indicando uma composição química variada.

Os extratos de *B. subalternans* demonstraram atividade antioxidante significativa, sugerindo seu potencial como fonte de antioxidantes naturais. Os testes de sequestro de radicais livres (ABTS e DPPH) revelaram que os extratos exibiram atividade antioxidante com valores expressivos. Isso indica a capacidade da planta de prevenir o estresse oxidativo e combater os danos causados pelos radicais livres.

Os testes de inibição de crescimento bacteriano indicam o potencial dos extratos de *B. subalternans* no controle de infecções, enquanto que os testes de toxicidade reveleram uma baixa toxicidade.

Este estudo representa uma avaliação pioneira e preliminar das propriedades farmacológicas e toxicológicas de *B. subalternans*, sendo necessários aprofundar a compreensão dos mecanismos de ação dos compostos ativos, bem como avaliar a segurança e eficácia em modelos animais e ensaios clínicos, visto o potencial da espécie para o desenvolvimento de produtos naturais com propriedades farmacológicas, antioxidantes e antibacteriana.

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