Faculdade de Ciências Biológicas e Ambientais - FCBA
Programa de Pós-Graduação em
Biodiversidade e Meio Ambiente - PPGBMA

Variação sazonal do óleo essencial, características microscópicas e potencial alelopatico de *Momordica charantia* L. (Cucurbitaceae)

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Dourados-MS
Fevereiro de 2026

Universidade Federal da Grande Dourados - UFGD
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POR

MEIRYELLI DE OLIVEIRA GUILHERME

DISSERTAÇÃO APRESENTADA À UNIVERSIDADE FEDERAL DA GRANDE DOURADOS (UFGD), COMO PARTE DOS REQUISITOS EXIGIDOS PARA OBTENÇÃO DO TÍTULO DE MESTRE EM BIODIVERSIDADE E MEIO AMBIENTE - ÁREA DE CONCENTRAÇÃO: "CONSERVAÇÃO DOS RECURSOS NATURAIS".

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RESUMO

O Brasil abriga a maior biodiversidade vegetal do planeta, o que reforça a importância de estudos voltados à caracterização química, ecológica e funcional de espécies vegetais com potencial biológico. Nesse contexto, *Momordica charantia* L. (Cucurbitaceae), conhecida popularmente como melão-são-caetano, destaca-se por seu uso na medicina tradicional e pela presença de metabólitos secundários com relevância ecológica e farmacológica. O presente estudo teve como objetivo avaliar a variação sazonal do óleo essencial e características microscópicas de *M. charantia*, bem como investigar o potencial alelopático do óleo essencial e de extratos foliares sobre a germinação e o desenvolvimento inicial de *Crotalaria ochroleuca* G. Don (Fabaceae). O primeiro manuscrito avaliou o teor e a composição química do óleo essencial de *M. charantia* extraído das partes aéreas (folhas e caules) por 12 meses consecutivos. O óleo essencial foi extraído por meio de hidrodestilação e posterior análise por cromatografia gasosa acoplada à espectrometria de massa (CG-EM), além de análises microscópicas e histoquímicas das folhas e do caule. Os resultados demonstraram variações significativas no rendimento com março de 2026 apresentando o maior rendimento (0,53%) seguido de dezembro de 2025 (0,50%) e na composição química do óleo essencial, influenciadas principalmente pela fase fenológica da planta, com predominância dos compostos (*Z*)-3-hexenol e linalol. O segundo manuscrito investigou os efeitos alelopáticos de extratos aquoso e metanólico proveniente das folhas, e do óleo essencial das partes aéreas de *M. charantia* sobre a germinação e o crescimento inicial de *C. ochroleuca*. Os resultados indicaram ausência de efeito alelopáticos sobre a germinação, porém observaram-se efeitos inibitórios expressivos no crescimento radicular, especialmente associados ao extrato metanólico, evidenciando potencial alelopático dependente do tipo de extrato, concentração e tempo de exposição. De forma integrada, os resultados ampliam o conhecimento sobre a variabilidade química de *M. charantia* e seu papel em interações ecológicas, contribuindo para a compreensão de seu potencial uso em estratégias sustentáveis, especialmente no manejo de plantas de interesse agrícola.

Palavras-chave: melão-são-caetano; histoquimica; (*Z*)-3-hexenol; linalol; extrato metanólico.

ABSTRACT
Brazil harbors the greatest plant biodiversity on the planet, which highlights the importance of studies focused on the chemical, ecological, and functional characterization of plant species with biological potential. In this context, *Momordica charantia* L. (Cucurbitaceae), popularly known as bitter melon, stands out due to its widespread use in traditional medicine and the presence of secondary metabolites with ecological and pharmacological relevance. The present study aimed to evaluate the seasonal variation of the essential oil and the microscopic characteristics of *M. charantia*, as well as to investigate the allelopathic potential of its essential oil and leaf extracts on the germination and initial growth of *Crotalaria ochroleuca* G. Don (Fabaceae).The first manuscript assessed the yield and chemical composition of the essential oil extracted from the aerial parts (leaves and stems) of *M. charantia* over 12 consecutive months. The essential oil was obtained by hydrodistillation and subsequently analyzed by gas chromatography coupled to mass spectrometry (GC-MS), in addition to microscopic and histochemical analyses of leaves and stems. The results revealed significant variations in essential oil yield and chemical composition, with March 2026 showing the highest yield (0.53%), followed by December 2025 (0.50%). These variations were mainly influenced by the plant's phenological stage, with predominance of the compounds (*Z*)-3-hexenol and linalool.The second manuscript investigated the allelopathic effects of aqueous and methanolic extracts and the essential oil of *M. charantia* on the germination and early growth of *C. ochroleuca*. The results indicated no significant effect on germination; however, pronounced inhibitory effects were observed on root growth, particularly associated with the methanolic extract, demonstrating allelopathic potential dependent on extract type, concentration, and exposure time. Taken together, these findings expand the knowledge on the chemical variability of *M. charantia* and its role in ecological interactions, contributing to the understanding of its potential application in sustainable strategies, especially for the management of plants of agricultural interest.

Keywords: melão-são-caetano; histochemistry; (*Z*)-3-hexenol; linalool; methanolic extract.

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LISTA DE ABREVIATURAS E SÍMBOLOS

C_{18}	Octadecil (18)
CG-EM	Cromatografia gasosa acoplada a espectrometria de massa

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1. INTRODUÇÃO

O Brasil ocupa cerca de 47% do território da América do Sul e abriga a maior biodiversidade do planeta (MMA, 2022), com aproximadamente 44 mil espécies flora conhecidas, distribuídas entre seis biomas terrestres, Floresta Amazônica, Cerrado, Pantanal, Caatinga, Pampas (campos) e a Mata Atlântica e três grandes ecossistemas marinhos (MMA, 2022). Essa riqueza biológica é favorecida pela ampla variedade de zonas climáticas e condições ecológicas distintas (MMA, 2022).

Nesse cenário de elevada diversidade vegetal, as plantas medicinais ganham destaque por seu papel fundamental nas práticas populares de saúde, na produção de fitoterápicos e na geração de conhecimento científico. Diante da vasta diversidade florística do país, torna-se cada vez mais relevante a caracterização integrada de espécies vegetais com potencial terapêutico e ecológico.

Dentro dessa biodiversidade encontra-se *Momordica charantia* L., Cucurbitaceae, descrita pela primeira vez por Carl Linnaeus (Lineu) em 1753, na obra *Species Plantarum*, considerada o marco inicial da nomenclatura botânica moderna (Royal Botanic Gardens, Kew, 2026). É uma planta herbácea trepadeira, nativa do continente africano e asiático. Foi introduzida no continente americano e possui distribuição Pantropical, abrangendo as regiões tropicais (Lutz, 2026).

No Brasil, *M. charantia* é conhecida como melão-são-caetano, distribuída em todas as regiões do país (Lutz, 2026). É amplamente utilizada na medicina popular devido às suas propriedades hipoglicemiantes e anti-inflamatórias (Magalhães et al., 2019; Liu et al., 2024). Pode ser facilmente reconhecida durante sua fase de frutificação que inicia 10–14 dias após a antese, e os frutos amadurecem 25–30 dias após o início (Stewart, 2025), dependendo das condições climáticas locais. Os frutos são carnosos, inicialmente verdes, e quando maduros, adquirem uma tonalidade alaranjada intensa ao atingirem a maturação, o pericarpo se abre espontaneamente, expondo sementes de coloração negra envolvidas em um arilo vermelho

(Lutz, 2026). A floração geralmente antecede a frutificação, ocorrendo início da atividade reprodutiva entre 30–55 dias após germinação e a floração pode durar até 6 meses (Behera et al., 2010) com coloração amarela (Lutz, 2026). As folhas são simples, pecioladas, com formato palmatilobadas margens denteadas e denticuladas (Lutz, 2026).

Do ponto de vista anatômico, as folhas apresentam tricomas tectores e glandulares. Os tricomas glandulares possuem pedicelo unisseriado e cabeça multicelular, sendo distribuídos na epiderme foliar (Sá et al., 2018).

Apenas um estudo foi realizado no Brasil sobre a composição química dos óleos essenciais das folhas frescas de *M. charantia*, coletadas em Bom Jesus do Tocantins (PA), extraídas por hidrodestilação. Os principais constituintes identificados foram linalol (47,32%), benzaldeído (8,55%) e trans-óxido de linalol (piranoide) (4,27%) (Almeida et al., 2024). Outros estudos com o óleo essencial também extraído das folhas, foram realizados na Nigéria, dois nos estados de Ibadan com folhas frescas, sendo os principais constituintes *cis*-3-hexenol (34,7%), 2-hexanol (10,1%), fitol (8,3%) linalol (5,8%) (Moronkola et al., 2009) heneicosano (21,1%), *n*-decano (5,0%), 1,3,5-cicloheptatrieno (4,7%) e *o*-xileno (4,3%) (Aiyelaagbe et al., 2010), e um no estado de Lagos com folhas secas, sendo os principais constituintes pregeijereno (27,10%), *α*-pineno (14,0%), geijereno (11,2%), germacreno D (8,6%), y-pineno (7,1%) e (E)-cariofileno (4,6%) (Owolabi et al., 2013). Nesse sentido, a composição dos óleos essenciais pode variar conforme, o local de coleta e fatores ambientais, o que pode ter ação direta nas suas propriedades biológicas e ecológicas.

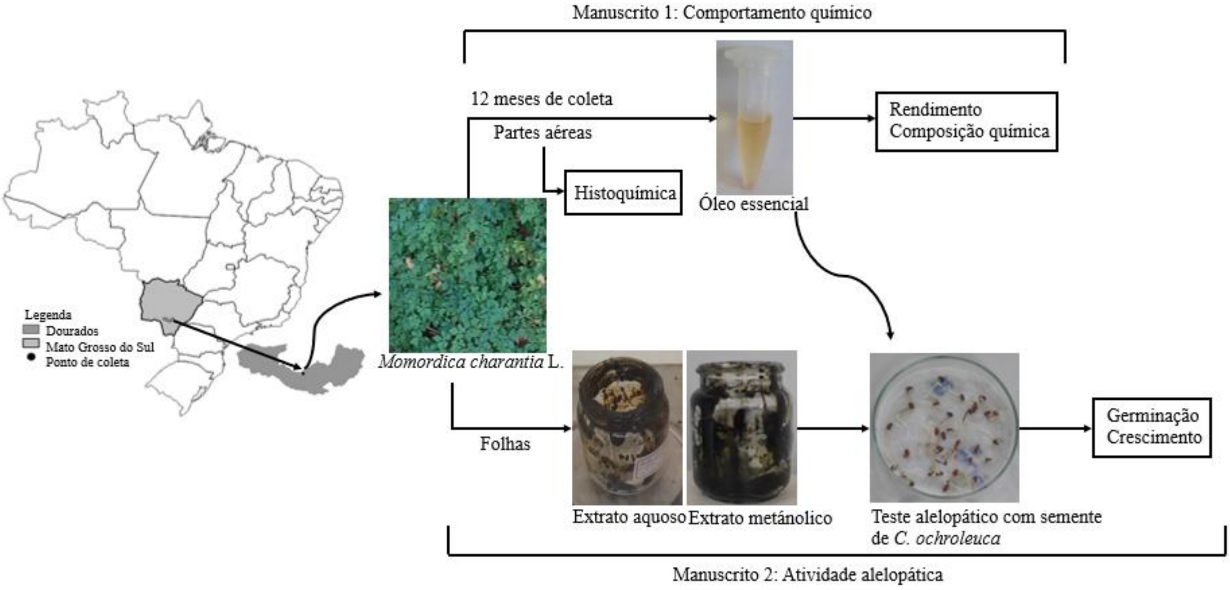
Os óleos essenciais são metabólitos secundários voláteis que desempenham funções ecológicas, como defesa contra herbívoros e patógenos, atração de polinizadores e participação em interações planta-organismo (Pichersky & Raguso, 2018; Isman, 2020). Geralmente, estão associados a estruturas secretoras especializadas, como tricomas glandulares (Lange & Turner, 2013; Huchelmann et al., 2017) distribuídos na epiderme e no mesófilo das folhas (Machado & Gregório, 2012).

A composição química dos óleos essenciais pode variar em função de fatores ambientais edáficos e sazonais (Gobbo-Neto e Lopes, 2007; Camele et al., 2019), influenciando diversas funções importantes, como na capacidade de modular respostas de defesa ao meio em que as plantas se encontram (Da Silva-Matos et al., 2022). Essas variações químicas, além de refletirem adaptações ambientais, também desempenham papel central nas interações ecológicas entre as plantas.

Nesse contexto, a alelopatia, por sua vez, é a interação bioquímica entre as plantas mediada por certos metabólitos secundários, conhecidos como aleloquímicos, que podem influenciar inibindo ou estimulando o crescimento, germinação ou desenvolvimento de outras plantas seja por liberação direta no ambiente, via compostos voláteis, lixiviados ou pela decomposição de resíduos orgânicos no solo (Ferreira & Borghetti, 2004; Valiño et al., 2023).

Espécies da família *Cucurbitaceae* como *Cucumis sativus* L. apresentam atividade alelopática, inibindo a germinação e o desenvolvimento inicial de plantas-alvo em estudos como *Amaranthus retroflexus* L., *Chenopodium album* L., *Digitaria sanguinalis* (L.) Scop., *Setaria faberi* Herrm. (Lockerman e Putnam, 1979) *Lepidium sativum* L., e *Echinochloa crus-galli* (L.) P. Beauv. (Kato-Noguchi et al., 2011). Estudos apontam o potencial alelopático de *M. charantia* sobre o desenvolvimento de outras espécies vegetais, como Singh e Sunaina (2014) observaram que os extratos aquosos das folhas e frutos inibiram significativamente o desenvolvimento de *Lycopersicon esculentum* Mill., (tomate) sendo o efeito mais intenso proveniente dos frutos. Da mesma forma, Arruda et al. (2021) relataram que extratos aquosos das folhas de *M. charantia* reduziram significativamente o crescimento radicular de *Allium cepa* L. (cebola) em todas as concentrações testadas.

Diante disso, a pesquisa foi elaborada para avaliar o teor e composição química do óleo essencial de *M. chararantia*, visando estabelecer uma padronização dos compostos ao longo de um ano, bem como avaliar seus efeitos na germinação e no crescimento inicial de plântulas de *Crotalaria Ochroleuca*, conforme ilustrado pelo Esquema 1, que mostra a descrição das inter-relações entre as atividades desenvolvidas.



Esquema 1. Inter-relações entre as atividades desenvolvidas no estudo.

2. REVISÃO DE LITERATURA

2.1. Cucurbitaceae

Cucurbitaceae compreende cerca de 95 gêneros e 965 espécies (Christenhusz e Byng, 2016), das quais 27 gêneros e 162 espécies são nativas do Brasil, amplamente distribuídas em áreas tropicais e subtropicais (Stevens, 2025). No Brasil, essa família ocorre em todas as regiões (Gomes-Klein et al., 2020).

Em sua maioria, são plantas herbáceas e trepadeiras, raramente lenhosas, com gavinhas simples ou compostas (Gomes-Klein et al., 2020). As folhas são alternadas, sem estípulas, frequentemente com base cordada e margens dentadas que possuem hidatódios (estruturas que liberam água) (Gomes-Klein et al., 2020). As flores podem ser solitárias ou dispostas em inflorescências racemosas ou cimosas, geralmente pentâmeras, epíginas e actinomorfas (Gomes-Klein et al., 2020). Os frutos variam amplamente, podendo ser cápsulas, elatérios carnosos, pixídios, pepônios, bagas, aquênios ou sâmaras, e suas sementes, geralmente exalbuminadas, podem ser comprimidas, aladas ou envoltas por um saco ariloide (Gomes-Klein et al., 2020).

Além de sua importância ecológica, a Cucurbitaceae inclui espécies de grande relevância econômica mundial, como melão (*Cucumis melo* L.), pepino (*Cucumis sativus* L.), melancia (*Citrullus lanatus* L.) e abóboras (*Cucurbita spp*.) (Schaefer & Renner, 2011; Paris, 2016).

Dentre os representantes da família Cucurbitaceae, temos o gênero *Momordica* no qual de acordo com o Royal Botanic Gardens, Kew (2026) inclui 52 espécies, e compreendem plantas anuais rasteiras ou escandentes, bem como perenes com raízes tuberosas ou arbustos trepadores de caule flexível. As folhas são simples ou compostas, pecioladas, e as gavinhas podem ser simples ou bífidas (Royal Botanic Gardens, Kew, 2026).

As flores masculinas dispõem-se em cachos ou racemos pedunculados, apresentando cinco sépalas inteiras, corola formada por cinco pétalas livres, de coloração amarela a alaranjada ou branca, e três estames, sendo dois bitecários e um unitecário, com anteras sinuosas (Royal Botanic Gardens, Kew, 2026). As flores femininas são solitárias, com ovário liso, alado, tuberculado ou espinhoso, estigma trífido e perianto semelhante ao das flores masculinas (Royal Botanic Gardens, Kew, 2026).

Os frutos variam de elipsoides a fusiformes, podendo ser deiscentes em três valvas ou indeiscentes, geralmente de coloração vermelha ou vermelho-alaranjada, contendo sementes comprimidas e esculpidas, envoltas por arilo vermelho (Royal Botanic Gardens, Kew, 2026).

Essa morfologia, caracteriza o gênero *Momordica*, no qual o nome vem do latim e significa "morder", em referência às bordas irregulares das folhas, que lembram marcas de mordidas (Grover e Yadav, 2004).

Espécies do gênero *Momordica*, como *M. cardiospermoides*, *M. balsamina*, *M. charantia* e *M. Foetida*, apresentam elevada diversidade de metabólitos secundários, incluindo flavonoides, terpenoides e lipídios, o que evidencia a riqueza fitoquímica do grupo e sua possível relação com funções ecológicas como defesa e competição química (Ramabulana et al., 2021). Há registro de atividade alelopática associada a extratos de *Momordica dioica* Roxb. ex Willd., indicando o potencial do gênero em interações planta-planta. Extratos aquosos de folhas de *M. dioica* foram avaliados em bioensaios utilizando sementes de *Phaseolus aconitifolius* Jacq. (variedade "Abhaya"), e demonstraram atividade alelopática, evidenciada pela redução no desenvolvimento de sementes e plântulas (Ahire e Deokule, 2012), o que reforça a hipótese de que compostos liberados por espécies do gênero *Momordica* podem atuar em interações planta-planta.

2.2. *Momordica charantia* L.

Momordica inclui 52 espécies aceitas (Royal Botanic Gardens, Kew, 2026), sendo que no Brasil ocorre apenas *M. charantia* L., uma espécie não nativa (Lutz, 2026), originária dos trópicos da África e Ásia (Dinakaran et al., 2010). No entanto, ela se adaptou bem ao ambiente brasileiro, sendo encontrada em todas as regiões do país (Lutz, 2026).

No Brasil*, M. charantia* é popularmente conhecida como melão-são-caetano (Lutz, 2026), mas pode ser reconhecida com outros nomes, como erva-das-lavadeiras, erva-de-são-caetano, erva-de-são-vicente, fruto-de-negro, melãozinho, fruto-de-cobra e melão-de-são-vicente (Moreira e Bragança, 2010). Em outros países, é chamada de *bitter gourd*, *balsam pear* (Dinakaran et al., 2010) e *bitter melon* em países de língua inglesa, além de *karela* em hindi (Grover e Yadav, 2004).

A *M. charantia* é uma trepadeira de ramos delicados (Lutz, 2026), estriados (Pacheco, 1977), e muito ramificados (Moreira e Bragança, 2010) podendo chegar de 1 a 2 metros de altura (Pacheco, 1977) (Figura 1A). Suas flores possuem hipanto verde e pétalas amarelas (Lutz, 2026), (Figura 1B). As flores estaminadas são axilares, possuindo pedicelo (Lutz, 2026). O hipanto é estreito e campanulado, com sépalas ovadas a lanceoladas e ápice agudo (Lutz, 2026). As pétalas são oblongo-obovadas, arredondadas no ápice (Lutz, 2026). Há três estames com filetes glabros e anteras sigmoides conatas (Lutz, 2026). As flores pistiladas são solitárias e axilares, semelhantes às estaminadas, mas com hipanto inferior, tuberculado e piloso, estilete glabro e estigma trilobado (Lutz, 2026). O ovário é fusiforme, rostrado e muricado (Pacheco, 1977).

Os frutos são cápsulas carnosas deiscente (Moreira e Bragança, 2010), fusiformes, trivalvares, alaranjados e tuberculados (Lutz, 2026). Podem medir de 3 a 15 cm, com extremidades agudas ou obtusas (Pacheco, 1977) (Figura 1C). As sementes são oblongas, negras, cobertas por arilo vermelho (Figura 1D) (Lutz, 2026).

Suas folhas são simples (Lutz, 2026), alternadas (Moreira e Bragança, 2010) e pilosas nas nervuras, com formato suborbicular a ovado (Pacheco, 1977) (Figura 1-E). Apresentam de 3 a 7 lobos palmatilobados ou palmatipartidos, sendo o lobo central maior que os laterais (Lutz, 2026). Os lobos têm formato obovóide a elipsóide, base estreitada, ápice agudo e mucronulado, margem denteada a denticulada e lobos basais auriculados (Lutz, 2026). A face superior é intensamente verde, enquanto a inferior é mais pálida (Pacheco, 1977). As folhas são membranáceas, com diâmetro entre 4,5 e 8 cm, e o pecíolo mede de 2,5 a 3,5 cm, sendo glabrescente a esparsamente pubescente (Lutz, 2026). As brácteas são foliáceas e reniformes, e a planta possui gavinhas simples (Lutz, 2026).

A histoquímica de *M. charantia* revela a presença e a distribuição de diferentes metabólitos secundários nos tecidos vegetais, especialmente nas folhas (Sá et al., 2018). Estudos indicam que compostos fenólicos e alcaloides estão localizados principalmente nas células epidérmicas, enquanto substâncias lipofílicas estão associadas à cutícula (Sá et al., 2018). Além disso, há evidências da presença de lignina nos tecidos vasculares (xilema) e de amido em células do parênquima (Sá et al., 2018). Outro aspecto relevante é a ocorrência de cristais de oxalato de cálcio em idioblastos, desempenhando funções como defesa e regulação iônica (Sá et al., 2018), e segundo Pacheco (1977) na epiderme superior e inferior das folhas tambem são apresentadas glândulas secretoras que contêm óleo essencial.

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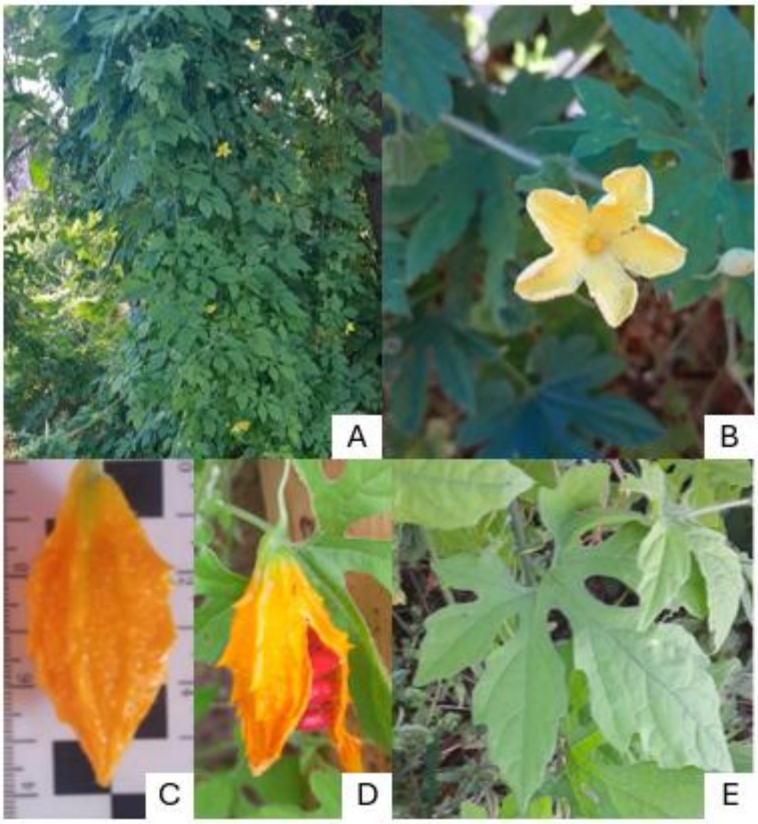

Figura 3. (A) *Momordia charantia* em seu habitat natural. (B) Flor de *M. charantia* (C) Fruto de *M. charantia* antes da sua abertura espontânea. (D) Fruto após sua abertura espontânea. (E) Folha de *M. charantia*.

2.3. Óleo essencial

Os óleos essenciais são misturas de compostos voláteis, extraídos de tecidos vegetais por métodos físicos, como hidrodestilação ou arraste a vapor (Machado, et al., 2022). Os óleos essenciais estão geralmente localizados em tricomas glandulares, cavidades ou canais secretores (Figueiredo et al., 2018) distribuídos na epiderme e no mesófilo das folhas (Machado & Gregório, 2012). Resultantes do metabolismo secundário das plantas, os óleos essenciais são constituídos por uma variedade de compostos orgânicos de baixa massa molecular, predominantemente terpenoides, cuja volatilidade à temperatura ambiente confere suas propriedades aromáticas e bioativas (Sharifi-Rad et al., 2017; Dhifi et al., 2016) -Os óleos essenciais podem ser utilizados na forma pura ou em misturas, sendo amplamente aplicados em diferentes áreas devido às suas propriedades químicas e biológicas (Sharifi-Rad et al., 2017; Dhifi et al., 2016).

A composição química dos óleos essenciais pode ser influenciada por fatores ambientais e fisiológicos, como condições de estresse e estágio de desenvolvimento, afetando tanto a quantidade quanto o perfil qualitativo e quantitativo dos compostos voláteis produzidos (Yang et al., 2018; Gershenzon, 2020; Gobbo-Neto e Lopes, 2007). Tais condições influenciam a quantidade e a composição dos compostos voláteis produzidos (Yang et al., 2018; Gershenzon, 2020).

Ecologicamente, os óleos essenciais desempenham funções adaptativas, atuando na defesa contra insetos e herbívoros, além de participarem na atração de polinizadores (Murray, 2020; Pichersky & Raguso, 2018). Além disso, os óleos essenciais podem apresentar propriedades alelopáticas, atuando na supressão da germinação e do crescimento de espécies vegetais vizinhas como estratégia competitiva (Hussain et al., 2019; Farooq et al., 2020).

Diante da relevância ecológica e funcional desses metabólitos, torna-se fundamental compreender os locais de síntese e armazenamento nos tecidos vegetais. Estudos de anatomia comparada em Cucurbitaceae indicam que gêneros como *Momordica* apresentam padrões recorrentes de estruturas secretoras, incluindo tricomas glandulares e idioblastos, compartilhados entre diferentes espécies do gênero, o que fundamenta o uso de abordagens histoquímicas para o mapeamento de compostos bioativos (Metcalfe & Chalk, 1979). Nesse sentido, a identificação de estruturas secretoras por meio de estudos histoquímicos é de grande importância, uma vez que essas estruturas estão relacionadas à produção e ao armazenamento de metabólitos secundários, influenciando sua obtenção e caracterização (Evert, 2013; Figueiredo et al., 2018).

2.4. Óleo essencial de *M. charantia* L.

Alguns estudos têm investigado a composição química do óleo essencial de *M. charantia*, revelando variações significativas de acordo com a localidade da coleta, parte da planta utilizada e condições de extração.

No Brasil, apenas um estudo foi realizado com as folhas frescas de *M. charantia*, coletadas em Bom Jesus do Tocantins (PA), submetidas ao processo de hidrodestilação. O óleo essencial apresentou um rendimento de 0,45%, e os principais constituintes foram linalol (47,32%), benzaldeído (8,55%) e *trans*-óxido de linalol (piranoide) (4,27%) (Figura 2) (Almeida et al., 2024).

Outras investigações foram realizadas no Ibadan, Nigéria, com folhas frescas submetidas também a extração por hidrodestilação, obtendo um rendimento de 0,30%. Os compostos predominantes foram *cis*-3-hexenol (34,7%), seguido por 2-hexanol (10,1%), fitol (8,3%) e linalol (5,8%) (Figura 2) (Moronkola et al., 2009).

A investigação, utilizando folhas secas de *M. charantia* colhidas em Lagos, também na Nigéria, demonstrou uma composição distinta, com predominância de pregeijereno (27,1%), *α*-pineno (14,0%), geijereno (11,2%), germacreno D (8,6%), *γ*-pineno (7,1%) e (*E*)-cariofileno (4,6%) (Figura 2) como constituintes principais e rendimento de 0,56% (Owolabi et al., 2013).

Estudo realizado em Ibadan avaliou separadamente os óleos essenciais extraídos de folhas e caules frescos da planta. No óleo essencial proveniente das folhas (0,23%), os compostos mais abundantes foram heneicosano (21,1%), *n*-decano (5,0%), 1,3,5-cicloheptatrieno (4,7%) e *o*-xileno (4,3%) (Figura 2). Já no óleo essencial extraído dos caules (0,55%), os principais constituintes incluíram heneicosano (18,7%), 8-hexil-pentadecano (16,4%), além de um composto de massa molar C_{18} não identificado (9,3%) (Aiyelaagbe et al., 2010).

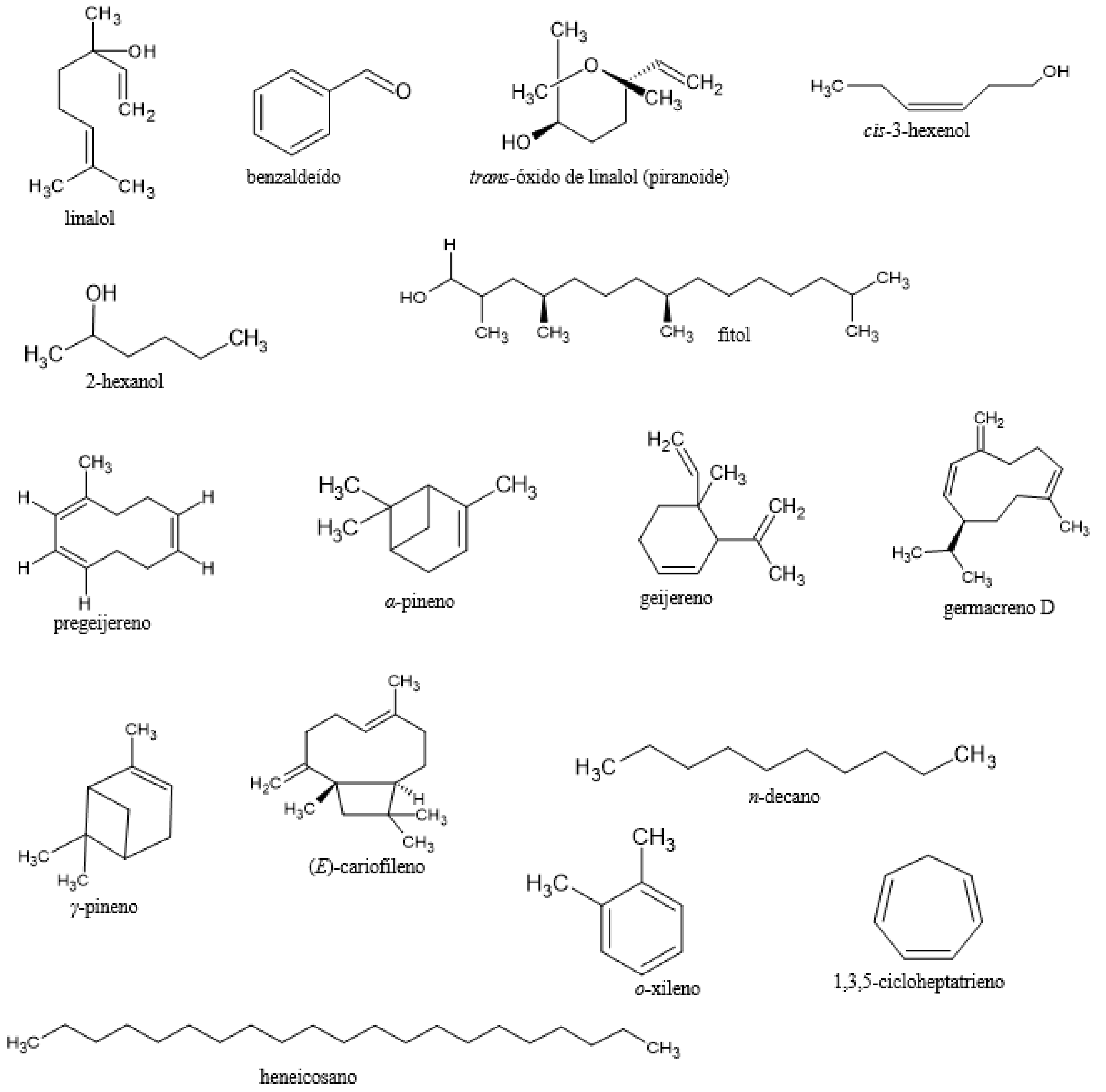

Figura 4. Estruturas químicas dos compostos majoritários presentes no óleo essencial de *M. charantia* dos estudos reportados da literatura.

Assim, embora existam relatos da presença e da variabilidade dos constituintes químicos do óleo essencial de *M. charantia* em diferentes localidades geográficas, ainda há escassez de informações sobre como esses constituintes se comportam ao longo do ciclo anual da espécie. Nesse contexto, torna-se relevante investigar a composição química do óleo essencial durante as diferentes estações do ano, buscando compreender possíveis variações sazonais que possam influenciar suas propriedades biológicas e aplicações potenciais.

Do ponto de vista anatômico, a literatura descreve que folhas e pecíolos apresentam tricomas tectores pluricelulares e tricomas glandulares, além da ocorrência de idioblastos no parênquima foliar e do pecíolo (Pacheco, 1977; Jorge et al., 1992; Sá et al., 2018). Os tricomas glandulares estão associados à presença de óleo essencial, e as folhas também possuem glândulas secretoras em número reduzido, porém bem evidentes quando observadas em secções transversais do limbo (Pacheco, 1977).

2.5. Alelopatia

Alelopatia é um fenômeno ecológico de interações químicas entre plantas mediadas por metabólitos especializados (aleloquímicos) produzidos e liberados por determinadas espécies, capazes de afetar o estabelecimento, crescimento e desempenho de outras plantas, geralmente com efeitos negativos, embora também possam ocorrer efeitos neutros ou positivos (Kong et al., 2024). O crescimento vegetal é definido pelo aumento irreversível no tamanho (massa, volume ou número de células), enquanto o desenvolvimento abrange os processos de diferenciação e transição entre as fases do ciclo de vida, como a germinação e o florescimento (Taiz et al., 2017). Compostos alelopáticos podem ser liberados diretamente no ambiente ou atuar de maneira indireta, sendo frequentemente transformados por microrganismos da rizosfera, o que altera as propriedades químicas do solo e a disponibilidade de nutrientes para as espécies vizinhas (Asaduzzaman & Asao, 2014; Scavo and Mauromicale, 2019). Além do potencial inibitório, os aleloquímicos podem exercer efeitos estimulantes (hormese) sobre o crescimento vegetal, oferecendo vantagens competitivas que fundamentam seu uso como defensivos naturais e estratégias de manejo sustentável na agricultura moderna (Belz & Duke, 2014)

Estudos demonstram que espécies da família *Cucurbitaceae*, como *Cucumis sativus* apresenta atividade alelopática, inibindo a germinação e o desenvolvimento inicial de plantas-alvo (Lockerman e Putnam, 1979; Kato-Noguchi et al., 2011). Em estudo conduzido por Singh e Sunaina (2014), foi avaliado o efeito alelopático de *M. charantia* sobre o desenvolvimento de

Lycopersicon esculentum Mill.(tomate) durante 21 dias, os resultados demonstraram que os extratos aquosos (25, 50, 75 e 100%) promoveram uma inibição das atividades metabólicas (biofísicas e bioquímicas) de *L. esculentum*, resultando em redução do crescimento e desenvolvimento das plantas. Embora ambos os órgãos da planta (folhas e frutos) apresentem potencial alelopático, os autores observaram que o extrato aquoso proveniente dos frutos exibiu efeito inibitório mais acentuado quando comparado ao das folhas.

Arruda et al. (2021) reportou que o extrato aquoso obtido das folhas secas da *M. charantia* em diferentes concentrações (0,5, 1, 2 e 4g/L), avaliado frente as sementes de *Allium cepa* L. (cebola), apresentam efeito alelopático em todas as concentrações, com diminuição significativa nos comprimentos médios das raízes em relação ao controle. A avaliação das sementes foi feita em três dias, que foi o que período que as sementes começaram a germinar.

A eficiência da extração de compostos alelopáticos e, consequentemente, a intensidade dos efeitos alelopáticos observados, variam conforme o sistema de solventes utilizado e o método de extração, uma vez que diferentes solventes têm afinidades químicas distintas pelos aleloquímicos de interesse e influenciam a composição final dos extratos (Krumsri *et al.*, 2024). Enquanto o extrato aquoso solubiliza compostos de alta polaridade, como ácidos fenólicos que afetam a embebição das sementes (Solodka & Solodka, 2025), o extrato metanólico isola uma gama maior de metabólitos, incluindo flavonoides e alcaloides, que interferem diretamente na divisão celular e no alongamento radicular (Chatepa *et al.* 2024). Já os óleos essenciais, de caráter apolar, concentram terpenos voláteis que podem comprometer a integridade das membranas celulares e os processos respiratórios das espécies receptoras (Kostina-Bednarz, et al., 2023).

Plantas com efeito alelopático podem ser utilizadas como estratégia agroecológica, podendo ser aplicadas em hortos e cultivos sustentáveis (Schulz & Tabaglio, 2024), na agricultura sustentável como ferramenta para manejo de plantas daninhas e redução de insumos químicos (Ain, et al., 2023) e em agroecossistemas como parte de práticas integradas de manejo e produção ecológica (Schulz & Tabaglio, 2025). A *Crotalaria ochroleuca* G. Don, é uma leguminosa da família Fabaceae, não endêmica do Brasil (Flores et al., 2026), amplamente utilizada como adubo verde devido à sua elevada capacidade de fixação biológica de nitrogênio (Berriel, 2024), promovendo o aumento da fertilidade do solo, incremento da matéria orgânica e redução na necessidade de fertilizantes químicos (Arone, et al., 2024). Devido ao seu rápido crescimento e elevada produção de biomassa, é eficiente no controle da erosão do solo, protegendo a superfície contra o impacto da chuva e do vento (Koo, et al., 2023). Além desses benefícios, plantas de cobertura como a *C. ochroleuca* podem produzir compostos alelopáticos e competir por recursos com outras espécies (Silva et al., 2012; Fernando & Shrestha, 2023), e a incorporação de grandes volumes de biomassa pode dificultar a germinação de plantas sensíveis, seja por barreiras físicas ou por alterações temporárias na disponibilidade de nutrientes durante o processo de decomposição (Shekoofa, et al., 2020; Cherr et al., 2006).

Apesar de apresentar vantagens agronômicas, como adubação verde e ciclagem de nutrientes, a utilização de *C. ochroleuca* em sistemas diversificados, como hortas e hortas de plantas medicinais, pode ser prejudicial. A incorporação de grandes volumes de sua biomassa pode dificultar a germinação e o estabelecimento de espécies sensíveis, não apenas por barreiras físicas e modificações temporárias no solo, mas também pela liberação de compostos alelopáticos capazes de inibir o desenvolvimento de culturas de interesse. Dessa forma substituir herbicidas sintéticos por compostos derivados de plantas permite um manejo de plantas invasoras mais alinhado com os sistemas de produção de ervas medicinais e com a preservação ambiental.

3. OBJETIVOS

3.1. Objetivo geral

Caracterizar a composição química do óleo essencial proveniente das partes aéreas (folhas e caules) da *M. charantia* em relação a variação sazonal, bem como avaliar a atividade alopática dos extratos metanólico e aquoso obtidos das folhas e do óleo essencial.

3.2. Objetivos específicos

- Avaliar a composição quimica por cromatográfica gasosa acoplada a espectrometria de massa (CG-EM) e o rendimento do óleo essencial extraídos das partes aéreas (folhas e caules) da *M. charantia* em relação a sazonalidade;
- Investigar, por meio de técnicas de microscopia e análises histoquímicas, características que contribuam para a identificação e o controle de qualidade da espécie.
- Avaliar o potencial alelopático do óleo essencial, extrato metanólico e extrato aquoso de *M. charantia* sobre a germinação e o desenvolvimento inicial de *C. ochroleuca*, correlacionando os efeitos biológicos com a composição química dos extratos e possíveis variações sazonais.

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Seasonal variation in essential oil chemical composition and structural characterization of *Momordica charantia* L. **(Cucurbitaceae)**

Abreviation

C Labmu – Multiuser Laboratory Complex; CG-MS – Gas chromatography–mass spectrometry; CH_2Cl_2 – dichloromethane; CO_2 – carbon dioxide; EDS – Energy-dispersive X-ray spectroscopy; EO – Essential oil; EO-APR – Essential oil (April); EO-AUG – Essential oil (August); EO-DEC – Essential oil (December); EO-FEB – Essential oil (February); EO-JAN – Essential oil (January); EO-JUL – Essential oil (July); EO-JUN – Essential oil (June); EO-MAR – Essential oil (March); EO-MAY – Essential oil (May); EO-NOV – Essential oil (November0; EO-OCT – Essential oil (October0; EO-SEP – Essential oil (September); FAA 70 – formalin–acetic acid–70% ethanol solution; HCl – hydrochloric acid; Na_2SO_4 – anhydrous sodium sulfate; Rf – retention fator; SEM – Scanning electron microscopy; TLC – Thin-layer chromatography.

Abstract

Momordica charantia L. (Cucurbitaceae), commonly known as "melão-de-são-caetano," is widely distributed throughout Brazil. Essential oils have been identified in its leaves, and previous studies have demonstrated seasonal variations in their chemical composition. This study investigated the monthly variation in the chemical composition of essential oils from *M. charantia* leaves and stems over a one-year period (June 2024 to May 2025), as well as analyses of their micromorphology and histochemistry. Leaves and stems were collected monthly in Dourados, Mato Grosso do Sul, Brazil. Essential oils were extracted from the leaves and stems of *M.*

charantia by hydrodistillation and subsequently analyzed using gas chromatography–mass spectrometry (GC–MS). The micromorphological and histochemical features of *M. charantia* leaves and stems were examined using prepared sections, specific reagents, light microscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS). The essential oils ranged in color from colorless to dark orange, with yields varying between 0.01% and 0.53%. GC–MS analysis identified thirteen compounds, predominantly alcohols (1.26%–64.06%) and monoterpenes (5.36%–68.76%), with (Z)-3-hexenol (20.14%–64.06%) and linalool (5.77%–78.76%) as the major constituents. Histochemical analyses revealed the presence of terpenoids, lipophilic compounds, phenolics, mucilage, and crystals in the leaves and stems, supporting anatomical, phytochemical correlations and providing reliable markers for plant authentication and standardization. These findings indicate that seasonal variation can influence the chemical composition of *M. charantia* essential oil.

Keywords: linalool, “melão-de-são-caetano”, micromorphology, (*Z*)-3-hexenol, histochemical tests.

1. Introduction

Momordica charantia L. (Cucurbitaceae) is a well-known herbaceous vine widely recognized by various vernacular names globally, including the popular Brazilian name “melão-de-são-caetano” (Lutz, 2025), along with “bitter melon” or “bitter gourd” in others regions (Dinakaran et al., 2010; Grover & Yadav, 2004). The species has a pantropical distribution, occurring throughout tropical regions worldwide (Royal Botanic Gardens, Kew, 2025; Lutz, 2025), and is present in all regions of Brazil (Lutz, 2025). Morphologically, *M. charantia* is characterized as a vigorous, highly branched climbing vine with delicate (Gomes-Klein et al., 2025), striated stems (Pacheco, 1977), typically reaching a height of 1-2 meters (Pacheco, 1977).

The species is widely used in popular medicine in Africa, Central America, and South

America due to its hypoglycemic and anti-inflammatory properties (Polito et al., 2016). Pharmacological studies of *M. charantia* have been well documented, especially its antidiabetic properties (Grover & Yadav, 2004; Oyelere et al., 2022; Ansari et al., 2024; Xiao et al., 2024) and anti-inflammatory (Srividya et al., 2023; Armis et al., 2019; Lee et al., 2020). Phytochemical studies showed the presence of essential oil (EO) in *M. charantia*, specially extracted from seeds and leaves (Ishikawa et al., 1985; Braca et al., 2008). Studies conducted on the leaves have demonstrated chemical diversity in the essential oil composition. In Brazil, at Bom Jesus do Tocantins (Pará), the oils were predominantly oxygenated monoterpenes, with linalool accounting for 47.32% (Almeida et al., 2024). In Nigeria, at Ibadan, aliphatic alcohols (cis-3-hexenol, 34.7%) and alkanes (heneicosane, 21.1%) were dominant (Aiyelaagbe et al., 2010; Moronkola et al., 2009), whereas in Lagos, the essential oils contained mono- and sesquiterpenes, including pregeijerene (27.10%) (Owolabi et al., 2013). This divergent composition may be attributed to several factors, such as environmental conditions and the plant's developmental stage. Therefore, understanding how these factors collectively influence the chemical composition of *M. charantia* EO is highly relevant for its phytochemical prospecting.

This study aimed to investigate the monthly variation in the chemical composition of *M. charantia* EO over the course of one year, as well as to analyze the micromorphology and histochemistry of its leaves and stems. Although the chemical composition of *M. charantia* EO is already known, no studies to date have provided a detailed assessment of how seasonal and phenological factors may be associated with the production, chemical composition, and relative abundance of its constituents. Additionally, histochemical tests, combined with microscopy, can contribute to distinguishing morphologically similar species.

2. Materials and methods

2.1. Plant material

Fresh aerial parts (leaves and stem) of *M. charantia* were collected in the city of Dourados, Mato Grosso do Sul, Brazil (22°12′36.111″S, 54°50′13.889″W) (**Fig. 1**) from June 2024 to May 2025, between 7:00 and 7:30 a.m., once at the end of each month. A voucher specimen (14829) was deposited in the Herbarium of the Federal University of Grande Dourados (UFGD) and registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (A2CFB4D).

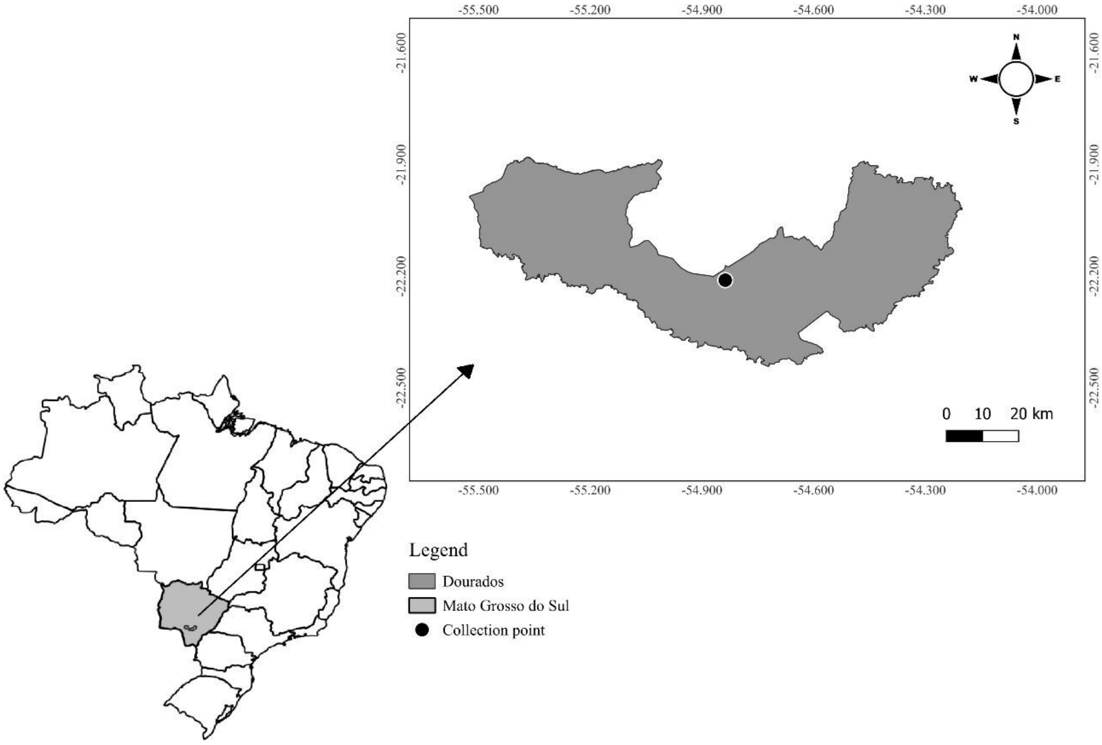


Fig. 1. Location of the collection site of aerial parts of *M. charantia* in the municipality of Dourados, Mato Grosso do Sul State, Brazil (22°12′36.111″S; 54°50′13.889″W). The map highlights the municipality within the state and indicates the precise sampling point.

2.2 Microscopy analyzes

Fresh, healthy, fully expanded leaves and stems collected from the sixth node downward were fixed in FAA 70 for 3 days (Johansen, 1940), rinsed in distilled water, and transferred to 70% ethanol (v/v) for storage (Berlyn et al., 1976). Transverse and longitudinal free hand sections were prepared using razor blades. Sections were rehydrated as needed and stained with toluidine blue O (O'Brien et al., 1964) and with the Astra blue plus basic fuchsin combination (Kraus et al., 1998). After staining, sections were mounted in 50% glycerin (Berlyn et al., 1976) on glass slides, covered with a coverslip, and sealed with clear nail polish. For epidermal surface analysis, small leaf fragments were washed and cleared in 50% sodium hypochlorite until translucent. Samples were then rinsed in distilled water and neutralized in 5% acetic acid. After a further rinse in distilled water, the material was stained with safranin (Fuchs, 1963) and mounted as described above.

2.3 Histochemical analyses

Fresh leaves and stems collected from the sixth node downward were used for histochemical assays. To investigate the chemical composition of cell walls and cellular contents, the following reagents were applied: phloroglucinol plus hydrochloric acid for lignin detection (Sass, 1951); ruthenium red solution (0.002%) for pectins (Johansen, 1940), ferric chloride solution and potassium dichromate for phenolic compounds (Gabe, 1968; Johansen, 1940); Sudan III and Oil red solution for lipophilic components (Foster, 1949; Jayabalan, Sahah, 1986); NADI reagent for terpenoids (David, Carde, 1964); Toluidine Blue O as a metachromatic stain for mucilage; and iodine solution (Lugol's reagent) for starch detection (Berlyn et al., 1976).

2.4 Scanning electron microscopy (SEM)

FAA fixed samples were dehydrated through a graded ethanol series (70%, 80%, 90%, and 100% v/v) and critical point dried in a Balzers CPD 030 apparatus (BalTec AG, Balzers, Liechtenstein) using liquid CO_2 as the transitional fluid. Dried specimens were sputter coated with gold in a Quorum SC7620 coater. Micrographs were acquired with a Mira 3 field emission scanning electron microscope (Tescan, Brno Kohoutovice, Czech Republic).

2.5 Energy dispersive X ray spectroscopy (EDS)

Elemental microanalysis was performed during SEM observation by energy dispersive X ray spectroscopy (EDS) to determine the chemical composition of the crystals. Measurements were conducted at random on crystals and on adjacent crystal free cells used as internal controls, using an EDS detector coupled to the same variable pressure SEM operated at 15 kV. SEM and EDS analyses were carried out at the Multiuser Laboratory Complex (C Labmu), State University of Ponta Grossa.

2.6. Extraction and yield of the essential oil

The aerial parts (leaves and stem, 300 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. The product obtained from hydrodistillation, referred to as hydrosol (water + EO), was partitioned using liquid-liquid extraction in a 1:1 ratio (hydrosol: ethyl acetate). After filtration, the organic phase containing the EO was dried over anhydrous sodium sulfate (Na_2SO_4) and subsequently concentrated using a rotary evaporator to obtain the pure EO (Almeida et al., 2024).

The EO were coded according to the collection month: EO-JUN (June/2024), EO-JUL (July/2024), EO-AUG (August/2024), EO-SEP (September/2024), EO-OCT (October/2024), EO-NOV (November/2024), EO-DEC (December/2024), EO-JAN (January/2025), EO-FEB (February/2025), EO-MAR (March/2025), EO-APR (April/2025), and EO-MAY (May/2025). The EO was stored at 4 °C until analysis, and the yields were expressed as the volume of EO/weight of plant material.

2.7. Thin-layer chromatography (TLC)

The TLC was performed to analyze the EO composition. Silica gel plates were used, and *n*-hexane/CH_2Cl_2 (40%) served as the mobile phase. The plates were visualized using a sulfuric acid/p-anisaldehyde/acetic acid reagent (1:0.5:50 mL). R_f values were determined experimentally by measuring the distance traveled by each spot relative to the solvent front under the described conditions. This approach allowed a preliminary assessment of the number and relative polarity of the components in the EO.

2.8. Analysis

Chemical characterization of all the EOs was performed using a gas chromatograph coupled with a mass spectrometer (GC-MS), diluted in 500 µL of n-hexane. The GC-MS conditions were as follows: DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness); helium (99.999% purity) was used as the carrier gas at a flow rate of 1.0 mL min^{-1}, with an injection volume of 1 µL (split ratio 1:10). The oven temperature was programmed from 50 °C to 280 °C at a rate of 3 °C min^{-1}. The injector temperature was set at 220 °C, and the transfer line and quadrupole detector were maintained at 280 °C. The mass spectra were obtained using electron impact ionization at 70 eV, scanning a mass range of 50-600 Da with a scan interval of 0.3 s. Retention indices were calculated using a C_8-C_{40} n-alkane series as external standards, and compound identification was achieved by comparing mass spectra with the NIST21 and WILEY229 libraries, as well as data reported in the literature (Adams, 2007).

2.9. Meteorological data

Precipitation and temperature data were obtained from the Brazilian Agricultural Research Corporation (EMBRAPA, https://www.embrapa.br/), which provides monthly averages of rainfall and temperature for the period from June 2024 to May 2025.

3. Results and discussion

Momordica charantia L., or “melão-de-são-caetano,” has long been used in popular medicine due to its hypoglycemic and anti-inflammatory effects (Polito et al., 2016). Therefore, investigating its bioactive constituents, particularly the terpenes that comprise its EOs, is of great relevance, since these metabolites contribute, at least in part, to its biological activities and are essential for chemical characterization and standardization. In this study, we evaluated the influence of the collection period on the yield and chemical composition of the EO of *M. charantia*. Additionally, morphoanatomical and histochemical analyses of the leaves and stems were performed as complementary pharmacognostic tools to support accurate botanical identification, ensure the quality of the plant material, and strengthen the reliability of the chemical analyses.

The leaves of *M. charantia* (**Fig. 2a**) show sinuous anticlinal epidermal cell walls on both surfaces in frontal view (**Fig. 2b, c**). Anomocytic stomata occurs on the abaxial surface (**Fig. 2c**), characterizing a hypostomatic leaf. In cross-section, the epidermis is uniseriate and covered by a thin striated cuticle. Non-glandular trichomes are uniseriate and multicellular, whereas glandular trichomes have a uniseriate pedicel and a uni- or multicellular head. Glandular trichomes react positively to the NADI reagent, indicating terpenoid secretion compatible with essential oils found in this study. Mucilaginous idioblasts occur beneath the epidermis, especially on the abaxial side. The presence of mucilage was confirmed by metachromatic staining with toluidine blue O (**Fig. 2d**), a classical indicator of pectic and mucilaginous polysaccharides (Ribeiro & Leitão, 2020; Retamales & Scharaschkin, 2014). This finding adds a useful microdiagnostic character for *M. charantia* leaves, because mucilage-bearing idioblasts are not always explicitly reported in routine anatomical descriptions, yet they can contribute to a more robust identification matrix when combined with other characters, as observed in the present study.

The mesophyll showed a typical dorsiventral organization, with a single palisade layer and five to six layers of spongy parenchyma (**Fig. 2d**), in agreement with descriptions of the species from distinct geographic origins and with the prevailing Cucurbitaceae pattern, where dorsiventral leaves, anomocytic stomata, and both non-glandular and glandular trichomes are common. Consequently, the diagnostic value of the leaf drug increases when such structural traits are interpreted in combination with additional markers, including crystals and oil-containing idioblasts, thereby strengthening authentication and quality control of powdered or fragmented materials (Guarniz et al., 2019; Kadiri & Olowokudejo, 2016; Sá et al., 2018; Sârbu et al., 2022). In this context, the histochemical evidence of terpenoids in glandular trichomes is consistent with the essential-oil profile of aerial organs and helps link chemical composition to specific anatomical sites of secretion and storage (Demarco, 2017).

In the midrib (**Fig. 2e-h**), the epidermis is uniseriate and covered by a thin cuticle, bearing discrete papillae on the adaxial surface (**Fig. 2e**). Two to four layers of angular collenchyma occur beneath the epidermis (**Fig. 2g, h**), followed by a starch sheath, in which starch grains (**Fig. 2g**) were evidenced by Lugol's reagent in the histochemical tests. The vascular system is represented by an open bicollateral bundle surrounded by ground parenchyma containing calcium oxalate druses (**Fig. 2f**).

The petiole (**Fig. 2i-m**) has a nearly plano-convex outline with two wings on the adaxial side (**Fig. 2i**) and an epidermis similar to that of the midrib (**Fig. 2k**). One to two continuous layers of angular collenchyma and five free U-shaped vascular bundles (**Fig. 2i, j**) are present, each associated with a cap of perivascular fibers (**Fig. 2j**). Fibers and xylem reacted positively with phloroglucinol/HCl, confirming lignification. In the wings, approximately five layers of angular collenchyma and one vascular bundle are present (**Fig. 2k**), and druses occur in the ground parenchyma (**Fig. 2l, m**). In addition, NADI-positive cells consistent with terpenoid-bearing idioblast-like elements were observed in the ground parenchyma adjacent to the vascular tissues (**Fig. 2f**), indicating localized internal accumulation of terpenoid-related lipophilic compounds in the midrib region. Pharmacognostic and taxonomic studies on *M. charantia* and related species highlight bicollateral bundles and calcium crystals as important diagnostic characters for the aerial organs of Cucurbitaceae (Guarniz et al., 2019; Sampiev et al., 2021; Sârbu et al., 2022).

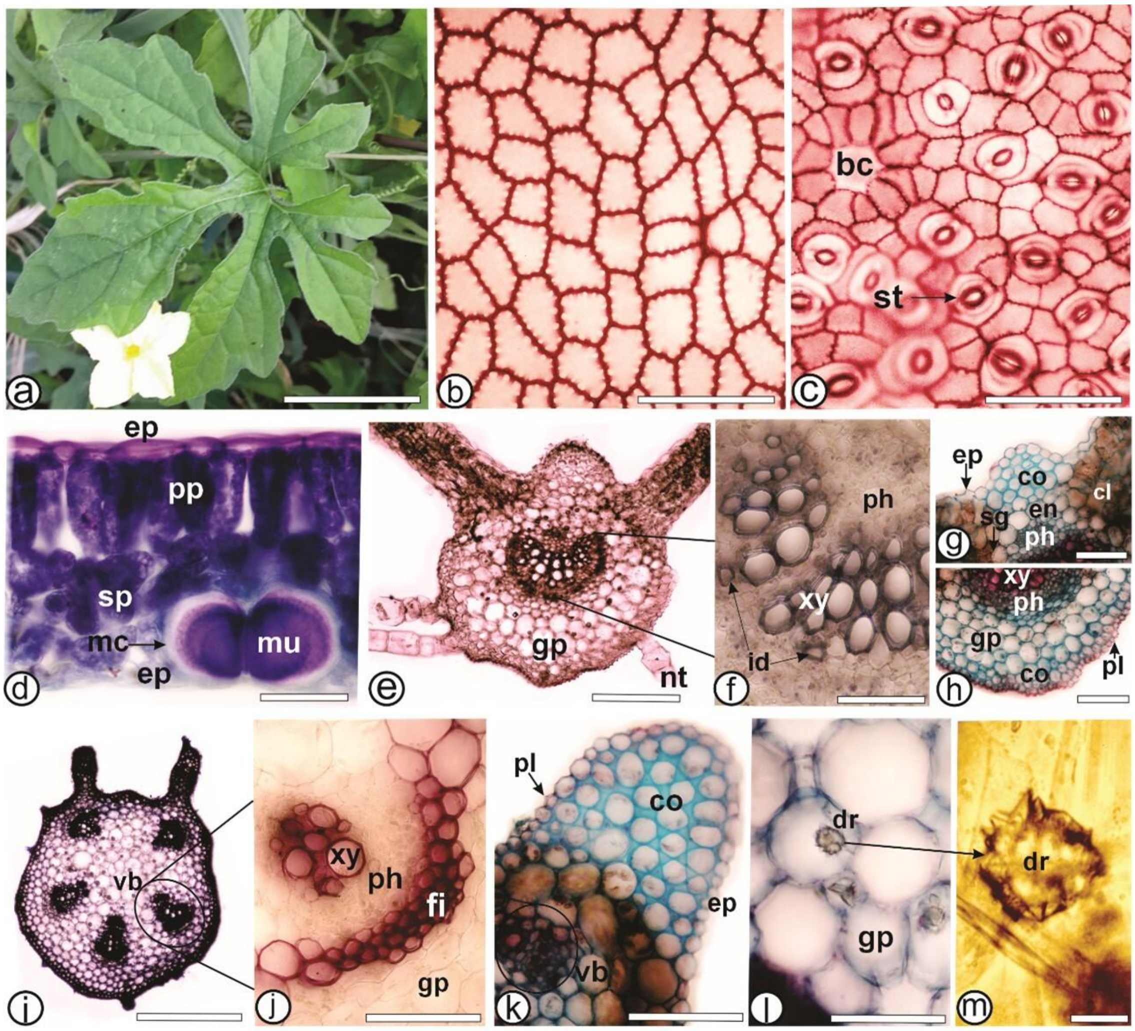


Fig. 2. *Momordica charantia* L. leaves. a. Plant habit. b, c. Frontal view of epidermis: adaxial (b) and abaxial (c). d-m. Cross-section: d. Lamina, e-h. Midrib. i-m. Petiole. [bc: base cell of trichome, ep: epidermis, cl: chlorenchyma, co: collenchyma, dr: druse, en: endodermis, fi: fiber, gp: ground parenchyma, mc: mucilaginous cell, mu: mucilage, nt: non-glandular trichome, ph: phloem, pl: papilla, pp: palisade parenchyma, sg: starch grain, sp: spongy parenchyma, st: stomata, vb: vascular bundle, xy: xylem]. Scale bars: a: 2 cm; b, c, d, f, g, h, k, l: 50 µm; e, j: 500 µm; m: 10 µm.

The stem of *M. charantia*, in cross-section, shows five conspicuous ribs (**Fig. 3a**). The epidermis is uniseriate, covered by a thin striated cuticle that reacted positively with Sudam III (**Fig. 3i**), and bears long uniseriate non-glandular trichomes (**Fig. 3a, e**) and rare glandular trichomes. Beneath the epidermis, layers of angular collenchyma are conspicuous, especially in the ribs (**Fig. 3b**), followed by a cortex of thin-walled irregular parenchyma cells (**Fig. 3i**). Perivascular fiber caps occur adjacent to the vascular bundles (**Fig. 3a-d**) and react completely with phloroglucinol/HC for lignified compounds. Internally, a uniseriate endodermis delimits the cortex. Idioblasts containing lipophilic material were observed in the cortical parenchyma and in tissues associated with the phloem (**Fig. 3j**). These elements stained positively with Sudan III, indicating intracellular accumulation of neutral lipids and other hydrophobic substances, and also showed a positive NADI reaction, which is commonly interpreted as evidence of terpenoid-related constituents (David & Carde, 1964; Demarco, 2017). Together, these histochemical signals support the occurrence of localized internal reservoirs of lipophilic, terpenoid-associated compounds in the stem, consistent with the contribution of internal tissues, in addition to epidermal secretory structures, to the pool of volatiles detected in the essential-oil fraction.

Ten vascular bundles are arranged in two concentric rings, with a well-developed cambium (**Fig. 3j**). The metaxylem of the central bundles is larger than that of the fibrovascular bundles located at the angles (**Fig. 3a, b, f, g**). The central cylinder contains abundant xylem, including large vessel elements, and sclerenchyma fibers surround the internal phloem. Between the bundles of the inner ring, parenchymatic rays converge toward a parenchymatic pith (**Fig. 3f, g**). Cells containing mucilage were observed predominantly in the cortical parenchyma and, less frequently, in the pith. These cells stained positively with ruthenium red (**Fig. 3d**), a classical histochemical reagent for acidic pectic polysaccharides and mucilaginous substances, supporting the presence of mucilage-rich cell contents. This result is consistent with the metachromatic response to toluidine blue O observed in leaf tissues and reinforces the occurrence of a polysaccharide accumulation system across different aerial organs (Ribeiro & Leitão, 2020).

Phenolic compounds were predominantly detected in the cortical tissues (**Fig. 3g**) and in the inner phloem (**Fig. 3k**), indicating that phenolic accumulation in *M. charantia* stems is not restricted to superficial tissues but also involves perivascular regions. This tissue-specific distribution is consistent with the dual role commonly attributed to phenolics in aerial organs, namely, local chemical defense and modulation of oxidative stress, particularly in tissues exposed to mechanical constraints and to the transport interface (Saini et al., 2024).

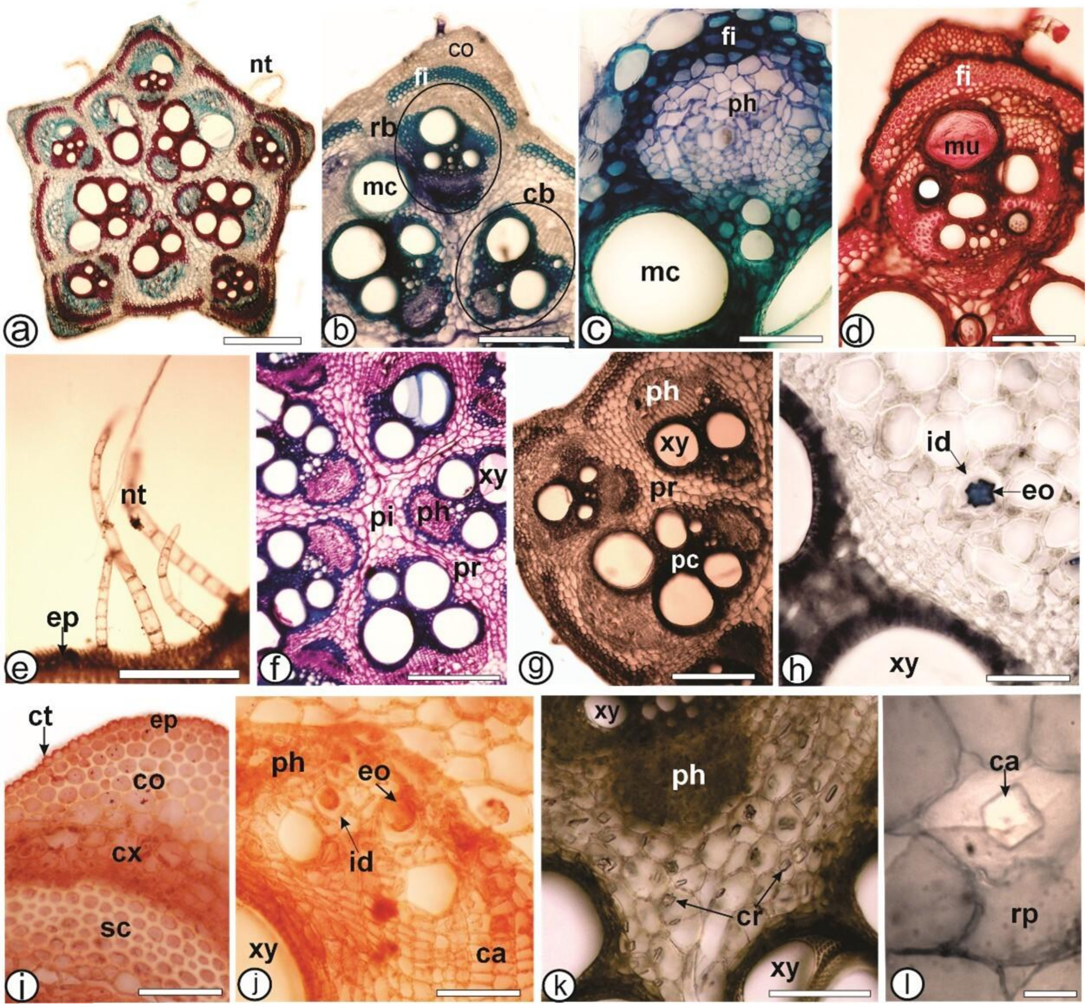


Fig. 3. *Momordica charantia* L. stems. Cross-section. a. General view. b. Detail of central and rib bundles. c. Detail of the rib. d. Ribs. e. Non-glandular trichomes. f. Center of the stem, showing pith. g. Phenolic compounds in the stem. h. Terpene content in reaction with NADI reagent. i. Rib, evidencing cuticle and idioblasts containing lipophilic material in reaction with Sudan III. j. Idioblasts containing lipophilic material in the phloem. k. Crystals in the parenchymatic ray. l. Arrow-shaped crystal. [ac: arrow-shaped crystal, ca: cambium, cb: central bundle, co: collenchyma, cr: crystal, ct: cuticle, cx: cortex, ep: epidermis, fi: fiber, id: idioblast, lc: lipophilic compounds, mc: mucilage cavity, mu: mucilage, nt: non-glandular trichome, ph: phloem, pi: pith, pc: parenchymatous cells, rp: parenchymatic ray, rb: rib bundle, xy: xylem]. Scale bars: a: 500 µm; b, f, g, j: 300 µm; c, d, e, h-k: 50 µm; l: 10 µm.

Different calcium oxalate crystal morphotypes were recorded in the stem, occurring predominantly in the ray parenchyma (**Fig. 3k, 4a–f**), including styloid (**Fig. 4a**), druse (**Fig. 4b**), cuneiform (**Fig. 4c, d**), prismatic (**Fig. 4e**), bipyramidal (**Fig. 4f**), and arrow-shaped crystals (**Fig. 3l**). This pattern is in line with anatomical descriptions of *M. charantia* stems, which typically report prominent angular collenchyma at the ribs and bicollateral vascular bundles, and emphasize the frequent occurrence of calcium oxalate crystals in supporting and parenchymatic tissues (Sârbu et al., 2022; Sá et al., 2018). In particular, prismatic crystals in stem ray parenchyma have been previously documented for the species, supporting that crystal-bearing rays constitute a recurrent microanatomical feature in *M. charantia* (Poyraz & Derdovski, 2016).

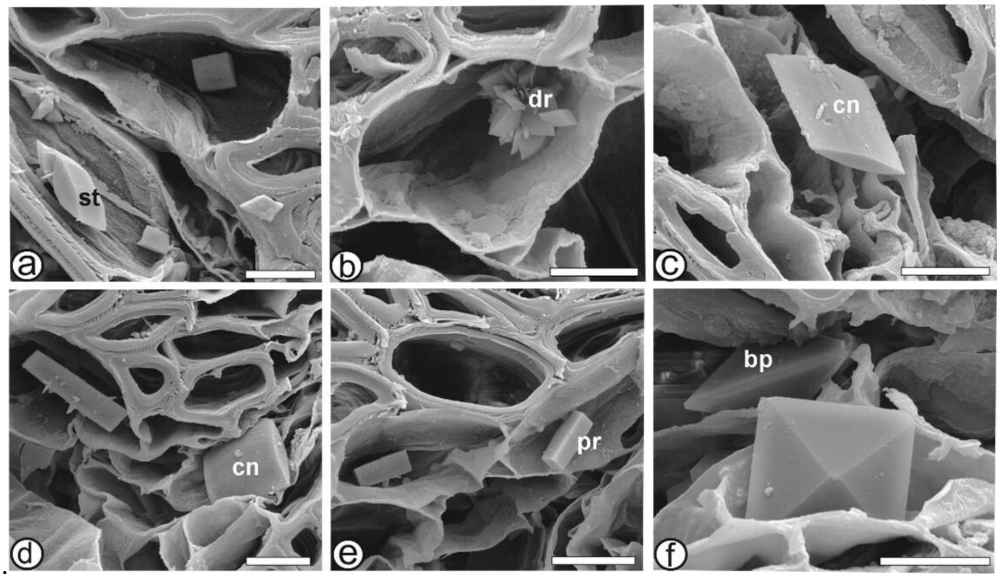


Fig. 4. Crystals in *Momordica charantia* L. stems – Scanning electron microscopy. a. Styloid. b. Druse. c, d. Cuneiform. d. Prismatic. e. Bipyramidal. [bp: bipyramidal, cn: cuneiform type, pr: prismatic, st: styloid. Scale bars: a-d, f: 10 µm; e: 20 µm.

In pharmacobotanical terms, crystal morphology and tissue distribution can be species- and tissue-informative and have long been regarded as useful microdiagnostic traits when interpreted in combination with other anatomical characters, especially for authentication of powdered or fragmented herbal materials (Raeski et al., 2023a, b). Beyond their diagnostic value, calcium oxalate crystals are often discussed in relation to calcium regulation and, secondarily, to mechanical and defensive functions, although functional inference should remain cautious in descriptive anatomical studies. Consistent with the chemical repertoire reported for *M. charantia* aerial organs, including cucurbitane-type triterpenoids and diverse phenolics (Potawale et al., 2008; Liu et al., 2009; Guarniz et al., 2019; Oliveira et al., 2025), the concurrent histochemical detection of terpenoid-related constituents (NADI), lipophilic material (Sudan III), phenolics, mucilage, and crystals in leaves and stems strengthens the correlation between anatomy, histochemistry, and phytochemical profiling. Collectively, these features provide a robust set of markers for authentication and quality control of the plant drug derived from the aerial parts of *M. charantia* (Demarco, 2017; Liu et al., 2009; Kadiri & Olowokudejo, 2016).

SEM and EDS analyses performed directly on representative crystal morphotypes revealed spectra dominated by Ca and O, with C also consistently detected (**Fig. 5a–d**). This elemental signature is compatible with calcium oxalate biomineralization, which is the predominant crystalline form reported for higher plants and is consistent with the morphology and tissue distribution observed here in ray parenchyma and perivascular regions.

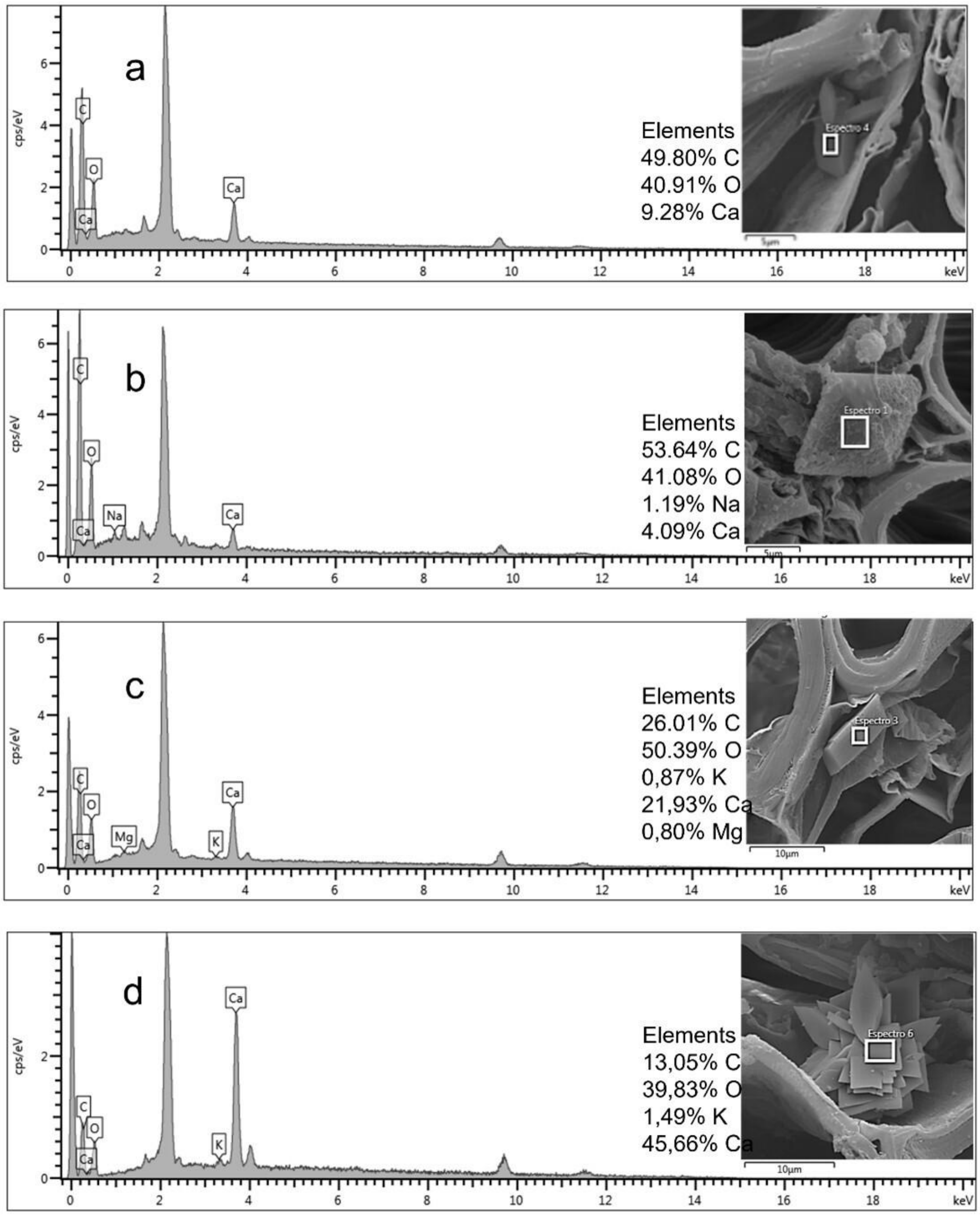


Fig. 5. SEM images and corresponding EDS spectra of stem crystals from *Momordica charantia* L.: styloid (a), bipyramidal (b), prismatic (c), and druse (d). EDS profiles are dominated by Ca and O, with C consistently detected, supporting calcium-based biominerals consistent with calcium oxalate crystals. Minor signals of K, Na, and Mg may occur and are attributed to contributions from the surrounding plant matrix and/or surface-associated material. The prominent unlabeled peak at ~0 keV corresponds to instrumental noise, and the peak near ~2.1 keV corresponds to Au from sputter-coating. Quantitative percentages are semi-quantitative EDS outputs and should not be interpreted as stoichiometric ratios.

Based on this integrated anatomical and histochemical characterization, the essential oil obtained from the aerial parts of *M. charantia* was subsequently evaluated regarding its physical characteristics and chemical variability throughout the collection periods. The EO of *M. charantia* presented its coloration ranging from dark orange to yellowish. The OE-JUN, OE-JUL, OE-AUG, EO-SEP (dark orange), OE-OCT (yellowish), OE-NOV (dark orange), OE-DEC (yellowhish), OE-JAN and OE-FEB (colorless), OE-MAR and OE-APR (light orange) and OE-MAY (yellowish) (**Fig. 6**).

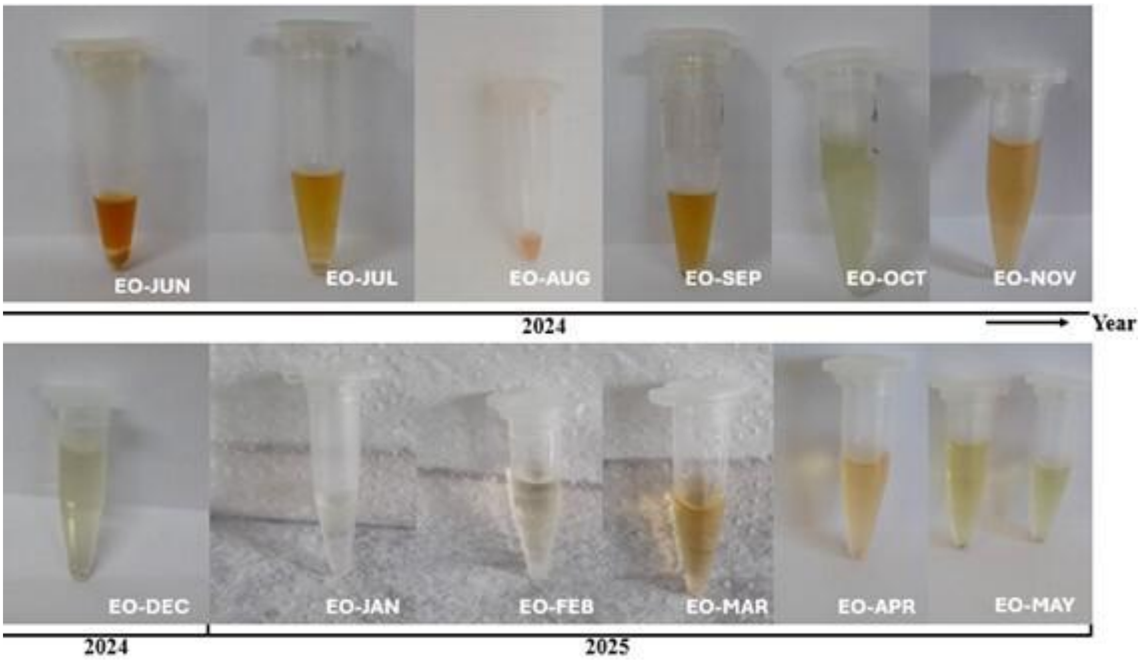


Fig. 6. Essential oils extracted from the aerial parts of *M. charantia* over one year period of sampling (June 2024 to May 2025).

Regarding the yield of *M. charantia* in the different months of collection, there was variation of 0.01% to 0.53%. The highest yields were obtained in OE-DEC (0.50%) and OE-MAY (0.53%), while the lowest occurred in OE-AUG (0.01%) (**Fig. 7**).

During the sampling period, four distinct reproductive phenophases were observed: flowering (June, August, October, November, February (2024), and April (2025), simultaneous flowering and fruiting (December (2024) and May (2025)), fruiting (July (2024) and January (2025)), and reproductive senescence (September and March (2024)).

Although the literature describes that flowering in *M. charantia* may begin a few weeks after sowing and extending for several months in tropical regions, this pattern is largely associated with crops grown under controlled conditions or with well-defined sowing dates (Stewart, 2022; Behera et al., 2010). In the present study, observations were conducted on spontaneous individuals occurring in a natural environment, which resulted in discontinuous phenological records across months, with flowering and fruiting occurring separately and non-continuously. This pattern reflects phenological asynchrony within the population, characterized by the coexistence of individuals at different ontogenetic stages (Layek et al., 2025; Morellato et al., 2016).

To understand the observed variation in EO yield during the sampling period, both biological and environmental factors were considered. The absence of flowering or fruiting records in certain consecutive months does not necessarily indicate an interruption of the species' reproductive cycle but rather reflects the punctual nature of the observations and the phenological asynchrony of the natural population (Morellato et al., 2016), which may affect the allocation of metabolic resources among individuals and, consequently, influence EO production. In this context, the highest EO yields were recorded in months when flowering and fruiting overlapped, whereas the lowest yield coincided with a uniphase reproductive stage, suggesting, at least in part, that the phenological stage is associated with variations in EO production. Additionally, notable differences in precipitation were observed during the study period, particularly in December 2024 (217 mm) and May 2025 (40 mm) (**Fig. 7**), indicating that climatic factors may also exert some influence on EO production. However, despite these differences in rainfall, both months showed high EO yields (**Fig. 7**), reinforcing the hypothesis that, under the conditions evaluated, phenological stage plays a more consistent role in determining EO yield than precipitation alone.

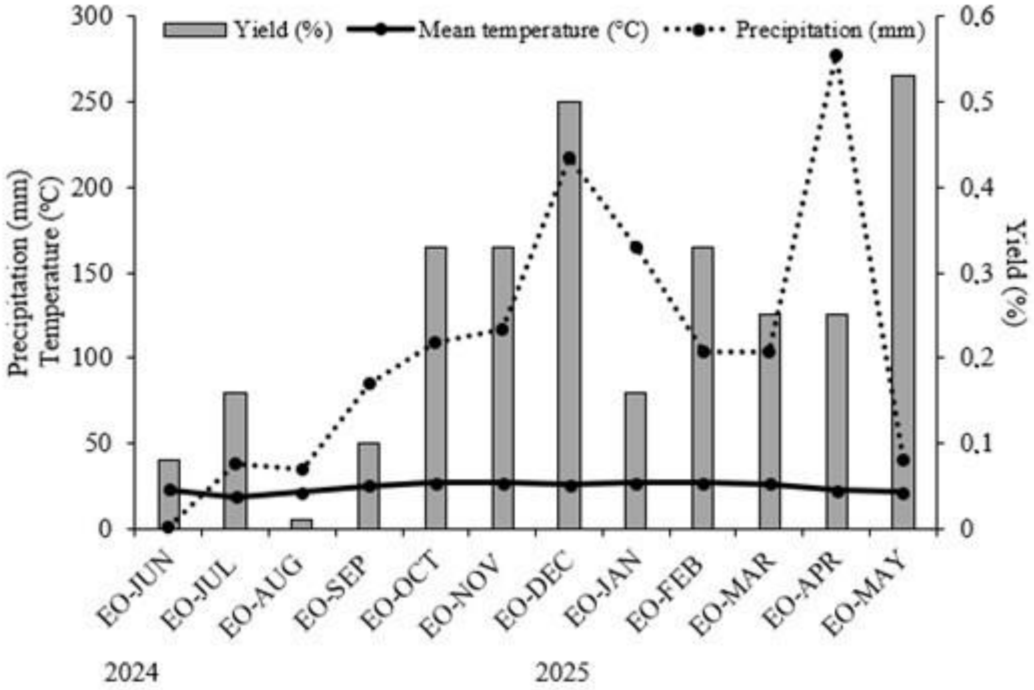


Fig. 7. Precipitation, temperature, and EO yield of *M. charantia* from June 2024 to May 2025.

TLC analysis of the essential oil revealed multiple spots, indicating the presence of a diverse mixture of compounds with varying polarities. A few spots were predominant, generally displaying higher Rf values (0.50–0.72), while less intense spots showed lower Rf values (0.20–0.45), reflecting relative differences in polarity among the constituents (data not shown). Although individual compounds could not be directly identified by TLC, this preliminary analysis provided an overview of the oil's complexity.

The GC-MS analysis identified 13 compounds in the EOs samples, predominantly alcohols (1.26–64.06%) and monoterpenes (5.36–78.76%) (**Table 1**). During the 12-month sampling period, two major compounds showed variation: (Z)-3-hexenol (20.14% to 64.06%), with higher levels in EO-AUG (64.06%), EO-OCT (60.61%), and EO-DEC (59.93%), and linalool (5.37% to

78.76%), with higher levels in EO-JAN (74.23%), EO-FEB (78.67%), EO-MAR (66.98%), and EO-APR (68.76%) (Table 1). EO-JUL displayed a distinct composition, in which the major compound was (Z)-2-hexenol (43.18%), followed by (Z)-3-hexenol (27.74%) and linalool (16.78%) (**Table 1**). The combination of TLC and GC-MS thus allowed both a qualitative assessment of polarity and a detailed identification of the major constituents. The present study describes, for the first time, the seasonal variation in the chemical composition of the EO from *M. charantia* (leaves and stem).

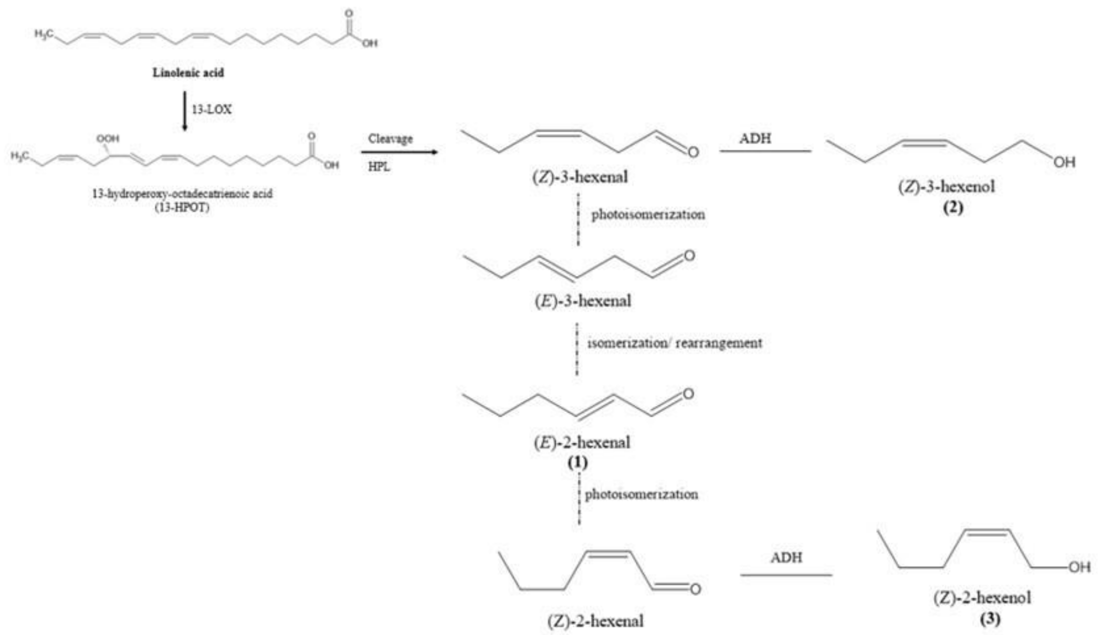
In comparison with studies conducted in Brazil and other countries, the composition of the EO exhibited considerable variability. In Brazil, samples from Bom Jesus do Tocantins (Pará) were dominated by oxygenated monoterpenes, with high levels of linalool (5.80–47.32%) and trans-linalool oxide, both furanoid (0.30–0.77%) and pyranoid (0.10–4.27%), as well as myrtenol (3.17%) and α-terpineol (1.58%) (Moronkola et al., 2009; Almeida et al., 2024). In Nigeria, samples from Ibadan were characterized by a predominance of aliphatic alcohols, such as cis-3-hexenol (34.7%) and 2-hexanol (10.1%), along with sesquiterpenes (linalool, 5.8%) and alkanes (heneicosane, 21.1%; n-decane, 5.0%; 1,3,5-cycloheptatriene, 4.7%; and o-xylene, 4.3%) (Aiyelaagbe et al., 2010; Moronkola et al., 2009). Similarly, in Lagos, the EO was dominated by mono- and sesquiterpenes, with pregeijerene (27.10%), α-pinene (14.0%), geijerene (11.2%), germacrene D (8.6%), β-pinene (7.1%), and (E)-caryophyllene (4.6%) as the major constituents (Owolabi et al., 2013). Furthermore, Owolabi et al. (2013) confirmed the occurrence of (E)-2-hexenal (0.20%), (Z)-3-hexenol (0.20%), (Z)-2-hexenol (0.10%), linalool (0.10%), α-terpineol (0.10%), myrtenol (0.20%), spathulenol (0.20%), and viridiflorol (0.30%), although at considerably lower concentrations than those observed in the present study.

The EO extract from *M. charantia* exhibits two main biosynthetic pathways: one leading to the production of green leaf volatiles (GLVs), which play a role in plant signaling and defense, and another leading to monoterpene formation (**Scheme 1A, B**). GLVs are oxylipins formed through lipid peroxidation in chloroplasts. Their biosynthesis begins with the activity of lipoxygenase (LOX), which catalyzes the conversion of α-linolenic acid, an 18-carbon polyunsaturated fatty acid (C18:3), into the hydroperoxide (9*Z*,11*E*,15*Z*)-octadecatrienoic acid (13-HPOT) (Matsui et al., 2012) (**Scheme 1A**). 13-HPOT is subsequently cleaved by hydroperoxide lyase (HPL) to release the first GLV, which can then be enzymatically reduced by alcohol dehydrogenase (ADH) to form (*Z*)-3-hexenol (**Scheme 1A**). A notable feature of GLVs is their contribution to the characteristic "green" aroma of freshly cut grass, which is consistent with the aromatic profile of the EO extracted from *M. charantia*. Linalool biosynthesis begins with the ionization of geranyl pyrophosphate (GPP) by linalool synthase, generating geranyl carbocation (**Scheme 1B**). Rotation around the C_2–C_3 bond forms the resonance-stabilized neryl cation intermediate. Subsequent nucleophilic attack by water and deprotonation yield linalool, a monoterpene alcohol that is a major component in the essential oil of *M. charantia* (**Scheme 1B**).

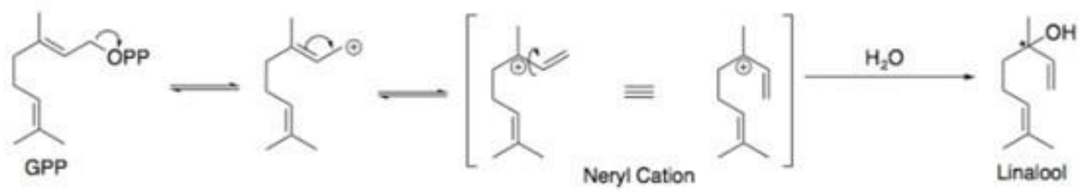
Linalool is a monoterpene that has been extensively investigated due to its broad spectrum of biological activities. Several studies have reported its anti-inflammatory properties (Goswami et al., 2025), as well as its antimicrobial activity against bacterial and fungal pathogens (He et al., 2025; Wang et al., 2026). In addition, antiviral (Astani et al., 2011) and antibiofilm activities have been demonstrated (Praseetha et al., 2023). Linalool also exhibits insecticidal and repellent effects against mosquitoes and agricultural pests (Nerio et al., 2010), in addition to allelopathic and herbicidal effects, characterized by the inhibition of seed germination and early seedling growth (Zheljazkov et al., 2021). Moreover, this compound has been associated with antioxidant properties and plays a relevant role as an ecological semiochemical in plant–insect interactions (Zhang et al., 2023). Recent evidence further indicates its involvement in the induction of plant defense responses, mediated by the activation of signaling pathways related to biotic stress (Avesani et al., 2023; Jiao et al., 2022).

According to our results, the variation in EO composition and yield observed in this study was influenced in part by phenological stage, as higher yields corresponded to months with overlapping flowering and fruiting. Despite notable variation in precipitation between months, EO yields remained high, suggesting that phenology exerts a more consistent influence on EO production than isolated climatic factors. Seasonal variation also affected the relative abundance of major compounds, reflecting environmental and developmental influences on metabolic pathways.

(A)



(B)



Scheme 1. Proposed biosynthetic pathway of for formation of the **(A)** Green Leaf Volatiles (GLVs) such as (*E*)-2-hexenal (**1**), (*Z*)-3-hexenol **(2)** and (*Z*)-2-hexenol (**3**); **(B)** monoterpene linalool (**7**) reported in *M. charantia*.

Table 1. Compounds identified by GC-MS in the EO samples extracted from *M. charantia* aerial parts from June 2024 to May 2025.

N°	Compounds	Retention index		RT	EO-JUN	EO-JUL	EO-AUG	EO-SEP	EO-OCT	EO-NOV	EO-DEC	EO-JAN	EO-FEB	EO-MAR	EO-APR	EO-MAY
		CI	LI													
(1)	(*E*)-2-hexenal	847	846	4.648	4.60	3.99	1.26	5.41	8.67	6.08	10.60	-	-	5.88	-	3.03
(2)	(*Z*)-3-hexenol	851	850	4.728	34.22	27.74	64.06	47.24	60.61	44.08	59.93	12.45	10.63	20.14	31.23	44.98
(3)	(*Z*)-2-hexenol	861	859	4.963	17.93	43.18	15.52	14.96	12.23	26.82	-	-	-	2.53	-	
(4)	*n*-hexanol	864	863	5.003	4.98	8.07	6.71	8.39	8.73	9.68	17.45	-	-	-	-	5.44
(5)	*cis*-oxide linalool (furanoid)	1070	1067	11.662	1.86	-	-	-	-	-	-	-	-	-	-	-
(6)	*trans*-oxide linalool (furanoid)	1087	1084	12.305	3.23	-	-	-	-	-	-	-	-	-	-	-
(7)	linalool	1093	1095	12.810	14.89	16.98	5.37	20.59	7.08	11.14	12.00	74.23	78.67	66.98	68.76	35.50
(8)	*trans*-oxide linalool (pyranoid)	1173	1173	15.989	1.49	-	-	-	-	-	-	-	-	-	-	-
(9)	α-terpineol	1177	1186	16.613	-	-	-	-	-	-	-	-	-	-	-	3.79

(10)	myrtenol	1193	1194	16.959	2.30	-	2.07	3.39	2.65	2.17	-	-	-	4.44	-	-
(11)	spathulenol	1574	1577	32.759	6.49	-	4.98	-	-	-	-	-	-	-	-	-
(12)	globulol	1581	1590	33.029	5.91	-	-	-	-	-	-	-	-	-	-	-
(13)	viridiflorol	1588	1592	33.029	2.04	-	-	-	-	-	-	-	-	-	-	-
Total identified					99.94	99.96	99.97	99.98	99.97	99.97	99.98	86.68	89.30	99.97	99.99	99.97

RT: Retention time; CI: Calculated retention index; LI: Literature retention index; (-) Not identified. Values are expressed above 1%.

4. Conclusion

The present study reports, for the first time, the seasonal variation in the chemical composition of EO from *M. charantia* leaves and stems, demonstrating that both composition and yield change seasonally, with phenological stage exerting a major influence. (*Z*)-3-Hexenol and linalool were the major constituents, with their predominance alternating according to the season. Micromorphological and histochemical analyses revealed the distribution of terpenoids, phenolics, and other metabolites, providing anatomical and phytochemical markers for plant authentication and standardization. These findings contribute to the understanding of seasonal dynamics in EO production and support quality control of *M. charantia*-derived products.

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Declaration of Competing Interest

The authors declare that there were no conflicts of interest.

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6. SEGUNDO MANUSCRITO

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Allelopathic effects of essential oil and extracts of *Momordica charantia* L. (Cucurbitaceae) **on the germination and initial growth of** *Crotalaria ochroleuca* G. Don

Total number of figures: 4

Palavras-chave: aleloquimicos, bioherbicida, extrato aquoso, extrato metanólico.

Keywords: Allelochemicals, bioherbicide, aqueous extract, methanolic extract.

Running Title: Allelopathy of *M. charantia* on *C. ochroleuca.*

Abstract

Allelopathy has been investigated as a sustainable alternative for weed management, particularly in cropping systems where the use of synthetic herbicides is restricted. This study evaluated the allelopathic effects of aqueous and methanolic extracts and essential oil of *M. charantia* on the germination and early development of *C. ochroleuca*. Seeds were exposed to different concentrations of the extracts and essential oil, and germination, seedling growth, and leaf fresh weight were assessed. Germination was not significantly affected at the end of the evaluation period, although early delays were observed depending on the treatment. In contrast, post-germinative parameters were more sensitive to allelopathic interference. The methanolic extract caused the greatest inhibition of root and hypocotyl growth, followed by the aqueous extract at higher concentrations. Leaf fresh weight was consistently reduced by all treatments, including the essential oil, indicating interference with physiological processes related to biomass accumulation and seedling establishment. These results demonstrate that the allelopathic potential of *M. charantia* is extract-dependent and parameter-specific, being more pronounced during the early

stages of seedling development rather than during germination.

Resumo

Efeitos alelopáticos do óleo essencial e de extratos de *Momordica charantia* L. (Cucurbitaceae) na germinação e crescimetno inicial de *Crotalaria ochroleuca* G. Don

A alelopatia tem sido investigada como uma alternativa sustentável para o manejo de plantas daninhas, especialmente em sistemas de cultivo nos quais o uso de herbicidas sintéticos é restrito. Este estudo avaliou os efeitos alelopáticos dos extratos aquoso e metanólico e do óleo essencial de *Momordica charantia* sobre a germinação e o desenvolvimento inicial de *Crotalaria ochroleuca*. As sementes foram submetidas a diferentes concentrações dos extratos e do óleo essencial, sendo avaliados a germinação, o crescimento das plântulas e a massa fresca foliar. A germinação não foi significativamente afetada ao final do período experimental, embora atrasos iniciais tenham sido observados em função dos tratamentos. Em contraste, os parâmetros pós-germinativos mostraram-se mais sensíveis à interferência alelopática. O extrato metanólico promoveu a maior inibição do crescimento radicular e do hipocótilo, seguido pelo extrato aquoso em concentrações mais elevadas. A massa fresca foliar foi consistentemente reduzida por todos os tratamentos, incluindo o óleo essencial, indicando interferência em processos fisiológicos relacionados ao acúmulo de biomassa e ao estabelecimento das plântulas. Os resultados evidenciam que o potencial alelopático de *M. charantia* é dependente do tipo de extrato e do parâmetro avaliado, sendo mais pronunciado nos estágios iniciais de desenvolvimento das plântulas do que na germinação.

1. Introduction

Allelopathy is an ecological phenomenon in which a donor plant releases secondary metabolites, known as allelochemicals, into the environment, where they influence the germination, growth, development, or competitive interactions of neighboring plants (Kong, et al., 2024). These allelochemicals are produced through specialized metabolic pathways and can be released by volatilization, leaching, root exudation, or decomposition of plant residues, playing a role in plant–plant chemical interactions and offering potential applications in sustainable agriculture and weed management (Kong, et al., 2024).

Recent studies have reported allelopathic effects of several Cucurbitaceae species, where aqueous extracts from cucurbit plants inhibited germination and early seedling growth of other species under controlled conditions, supporting the allelopathic potential of this family (Ostapchuk and Kustovska, 2024). Studies indicate the allelopathic potential of *Momordica charantia* L. on the development of other plant species. Singh and Sunaina (2014) demonstrated that aqueous leachates from leaves and fruits of *M. charantia* inhibited the early growth and physiological development of *Lycopersicon esculentum* L. seedlings, resulting in reductions in root and shoot length as well as fresh and dry biomass in a concentration-dependent manner. The effects were evaluated through the separate application of leaf and fruit leachates at concentrations of 25, 50, 75 and 100% over a 20 day experimental period, with greater reductions in growth parameters observed in treatments with fruit leachates and at the 100% concentration. Similarly, Arruda et al. (2021) reported that aqueous leaf extracts of *M. charantia* significantly reduced the primary root growth of *Allium cepa* L. at all tested concentrations. Four extracts were prepared at concentrations of 0.5 g/L, 1 g/L, 2 g/L, and 4 g/L, to which the roots were exposed for a period of three days, allowing the assessment of root growth. The results indicated allelopathic effects at all concentrations, as evidenced by a significant decrease in mean root length compared to the control.

In this context, evaluating the allelopathic effect of plant species with bioactive potential becomes essential, particularly as their secondary metabolites may represent a sustainable and low-cost alternative for the management of spontaneous plants in medicinal plant cultivation systems, especially for smallholder farmers. In the cultivation of medicinal plants, such as medicinal gardens, the use of synthetic herbicides is not recommended, as these products can compromise the phytotherapeutic quality of the cultivated species (Brasil, 2014), in addition to posing environmental and human health risks (WHO, 2020; FAO, 2018). In these areas, the presence of spontaneous plants with high germination capacity and rapid establishment can lead to competition for water, light, nutrients, and space, thereby reducing crop growth and development (Khamare et al., 2022; Dar et al., 2024).

The *Crotalaria ochroleuca* G. Don, despite its agronomic benefits when used as green manure (Arone et al., 2024), may present undesirable effects in specific cultivation areas, such as medicinal gardens, due to its rapid emergence and high biomass production, which can lead to excessive shading and increased competition for resources with species of medicinal interest (Khamare et al., 2022; Arone et al., 2024).

Therefore, evaluating the allelopathic effects of *M. charantia* leaf extracts (aqueous and methanolic) and essential oil (leaves and stems) on the germination and early growth of *C. ochroleuca* is of practical relevance.

2. Materials and Methods

2.1. Plant material

The plant material was collected in the municipality of Dourados, Mato Grosso do Sul, Brazil (22°12′36.111″ S, 54°50′13.889″ W). A voucher specimen is deposited in the Herbarium of the Federal University of Grande Dourados (UFGD) under number 14829, and access to the genetic heritage was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under code A2CFB4D.

2.2. Plant extracts

2.2.1. Preparation of the methanolic extract

For the preparation of the methanolic extract, fresh leaves of *M. charantia* (50 g) were extracted by maceration with methanol (200 mL) at room temperature (25 °C) for 4 days, followed by filtration and concentration under reduced pressure. The methanolic extract was stored in a glass flask protected from light and moisture, yielding 3.738% (w/w).

2.2.2. Preparation of aqueous extracts

For the preparation of the aqueous extract, the leaves were dried in a circulating air oven at 45 °C for 24 h. After drying, 100 g of the leaves were added to 100 mL of distilled water. The material was subjected to manual maceration with the addition of distilled water previously heated to near the boiling point, without reaching boiling, and maintained in contact until cooling to room temperature, the container was kept closed, with periodic agitation and compression of the plant material. Afterward, the resulting extract was properly stored and subjected to the lyophilization process, yielding 5.625% (w/w).

2.2.3. Extraction of essential oil

For the essential oil extraction, 300 g of aerial parts and 3 L of distilled water were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours, following the methodology of Almeida et al. (2024). The essential oil yield was 0.50% (v/w), calculated based on the ratio between the volume of oil obtained (mL) and the mass of the plant material used (g).

2.3. Allelopathic activity

The allelopathic potential of the methanolic and aqueous extracts, as well as the essential oil of *M. charantia*, was evaluated regarding their effects on the germination and initial seedling growth of *C. ochroleuca*. The seeds used in the bioassays were purchased from a commercial establishment. For the tests, different concentrations were prepared for each treatment. The methanolic extract was evaluated at concentrations of 0.5, 1.0, 1.5, and 2.5 g/L; the aqueous extract at 1.0, 2.5, 5.0, and 10.0 g/L; and the essential oil at 0.5, 1.0, 1.5, and 2.5 g/L.

The concentrations were defined based on the study by Arruda (2011) and on protocols widely adopted in allelopathy research, in which aqueous extracts are evaluated at higher concentration ranges (up to 10 g/L), whereas organic extracts and essential oils are tested at lower concentrations due to their higher biological activity (Ferreira and Áquila, 2000). The different concentration ranges tested for the essential oil and for the methanolic and aqueous extracts were established according to their chemical nature and the expected biological activity. Essential oils consist of highly concentrated mixtures of volatile and lipophilic compounds and are often capable of eliciting biological effects at low concentrations (Bakkali et al., 2008). Methanolic extracts exhibit intermediate biological activity as a result of their complex chemical composition and the wide range of secondary metabolites extracted by organic solvents (Taiz et al., 2017). In contrast, aqueous extracts generally require higher concentrations to induce measurable biological responses, as they are predominantly composed of polar compounds with lower detectable biological activity in bioassays (Ferreira and Áquila, 2000; Duke et al., 2000).

The bioassays were performed in triplicate in sterilized Petri dishes (9.0 cm diameter) lined with a sheet of filter paper. Twenty-five (25) seeds of the *C. ochroleuca* were placed in each dish. Each dish was moistened with 2 mL of the extracts/essential oil at different concentrations, or with 2 mL of a mixture of distilled water and Tween 80. A control group was prepared containing only 2 mL of distilled water and Tween 80%. Subsequently, the dishes were placed in a B.O.D. (Biochemical Oxygen Demand) germination chamber, set at a constant temperature of 25 °C and continuous white light (Formagio et al., 2012). Evaluations were conducted on the 4, 7, and 14th days of germination. The allelopathic potential of the extracts/ essential oil was assessed by counting normal seedlings at four (first count), seven, and fourteen (final count) days after sowing, with seeds showing 2 mm of root protrusion being considered germinated. The length of the primary root and hypocotyl (cm) was measured during all test stages (4, 7, and 14 days) using a graduated ruler (mm).

2.4. Statistical analysis

The growth means from the allelopathy tests were analyzed using analysis of variance (ANOVA), followed by Dunnet test for multiple comparisons ($p \leq 0.05$), using GraphPad Prism 2.0 software.

3. Results and Discussion

Weed control in medicinal plant cropping systems represents a significant challenge, particularly in environments where the use of synthetic herbicides is restricted or not recommended due to their potential impacts on the quality of plant material (Brasil, 2014) and on the environment (WHO, 2020; FAO, 2018). In this context, allelopathic compounds have been recognized as a sustainable tool for integrated weed management in agricultural systems (Schulz and Tabaglio, 2024). Therefore, the present study aimed to evaluate the allelopathic potential of the essential oil, methanolic extract, and aqueous extract on *C. ochroleuca*, a species that, despite its recognized agronomic benefits, may also present competitive effects in specific cultivation systems due to its rapid emergence and high biomass production (Arone et al., 2024; Scavo and Mauromicale, 2019; Schulz and Tabaglio, 2024). To date, there are no reports in the literature that comparatively investigate the allelopathic effects of these different plant matrices on *C. ochroleuca*, which confers a novel character to the results presented herein.

The germination percentages of *C. ochroleuca* seeds differed significantly from the control ($p \geq 0.05$) when subjected to treatments with aqueous extract, methanolic extract, and essential oil of *M. charantia* during exposure periods of 4, 7, and 14th days (Figure 1A–I). In the first germination count (4th days), reduced germination percentages (53%) were observed for the aqueous extract at 10 g/L (Figure 1A). The essential oil at concentrations of 1.5 and 2.5 g/L reduced germination to 54% and 61.45%, respectively (Figure 1C). The most pronounced inhibitory effect was observed for the methanolic extract at concentrations of 1, 1.5, and 2.5 g/L, resulting in germination percentages of 51%, 54.6%, and 56.80%, respectively, in *C. ochroleuca* seeds, compared with the untreated control (89.6%) (Figure 1B). On the 7th day of evaluation, only the essential oil at concentrations of 1.5 and 2.5 g/L reduced germination to 45% and 56%, respectively, when compared with the control (Figure 1F). On the final day (14th day) of evaluation, none of the extracts were able to inhibit germination when compared with the control (Figure 1G-I). The analysis allowed us to infer that germination was not inhibited, but rather delayed.

The evaluation of germination percentage over time provides not only information on the final seed viability, but also insights into the kinetics of the germination process, reflecting seed vigor and uniformity of response, as discussed by Sedghi (2025), who demonstrated that temporal metrics derived from germination curves capture physiologically relevant aspects under different experimental conditions. When a reduction in germination percentage is maintained throughout successive evaluations, this pattern can be interpreted as indicative of the action of substances with persistent inhibitory effects, a characteristic commonly associated with allelopathic activity, as described for allelochemicals in the literature (Ferreira and Áquila, 2000).

Compounds with allelopathic activity, such as essential oils, consist of complex mixtures of volatile secondary metabolites, including monoterpenes, sesquiterpenes, and phenolic compounds, which are capable of interfering with cell membrane integrity and essential metabolic processes, potentially resulting in the reduction or inhibition of seed germination across different plant species (Bakkali et al., 2008; Sharifi-Rad et al., 2017). Experimental studies have shown that essential oils extracted from various plant species promote a significant and dose-dependent reduction in germination percentage and early seedling growth, regardless of the functional group evaluated (Vokou et al., 2003; Synowiec et al., 2017; Schulz and Tabaglio, 2024). These effects are strongly associated with the chemical composition of the oils, as monoterpenes and phenolic compounds widely reported in the literature exhibit phytotoxic activity during the early stages of germination (Kaur et al., 2010; Synowiec et al., 2017). Furthermore, reviews indicate that, due to the high bioavailability and volatility of these compounds, essential oils tend to promote more intense inhibitory effects when compared with less concentrated plant preparations, such as aqueous extracts, reinforcing their potential as natural agents for germination control (Bakkali et al., 2008; Sharifi-Rad et al., 2017).

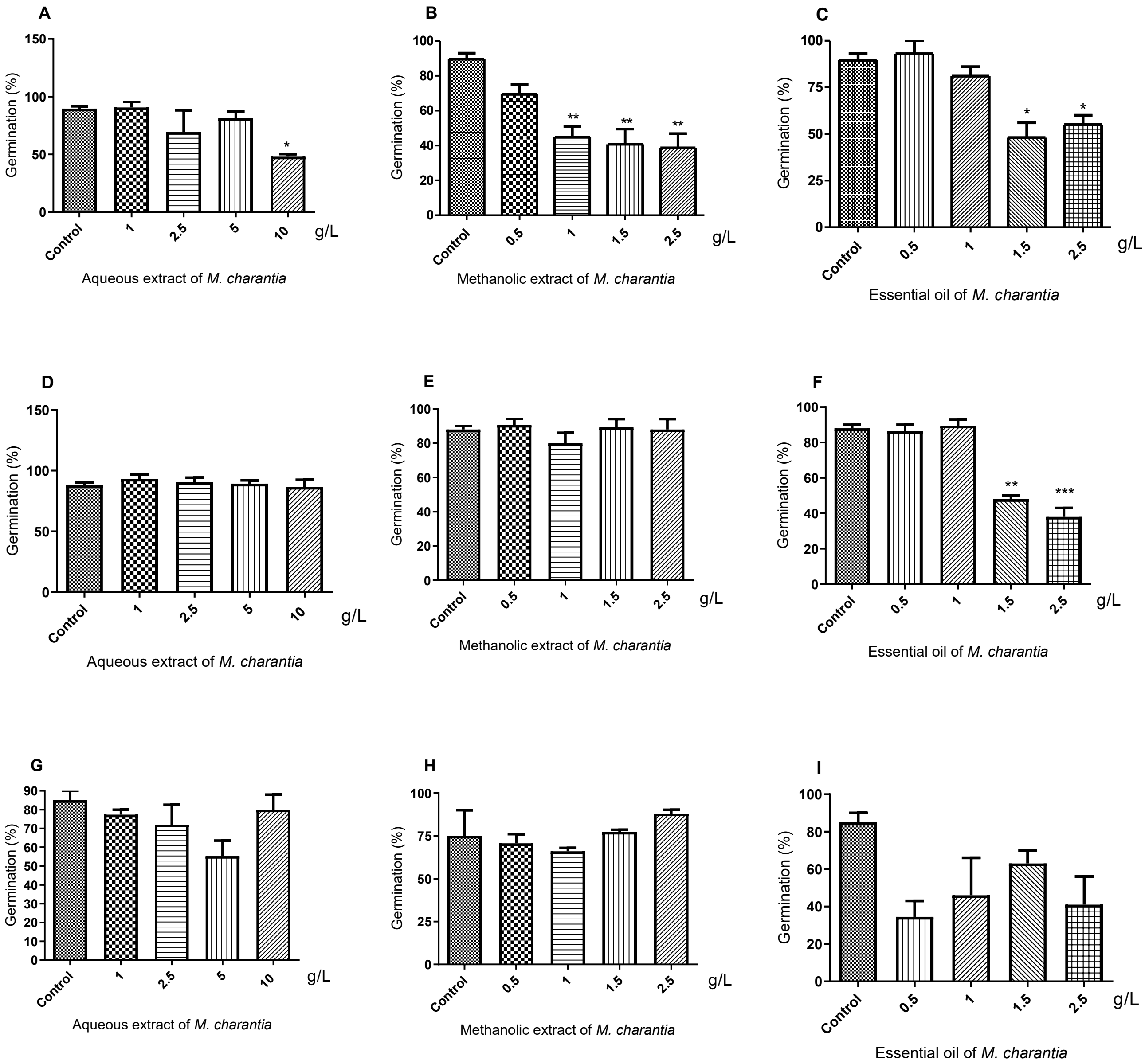

Figure 1. Germination percentage of *C. ochroleuca* seeds subjected to different concentrations of aqueous extract, methanolic extract, and essential oil of *M. charantia*. First evaluation (4th day): A–C; second evaluation (7th day): D–F; and final evaluation (14th day): G–I.

Although germination has traditionally been described as less sensitive to allelochemicals than later developmental stages, recent studies suggest that this pattern is not universal and depends on the species and experimental conditions (Ferreira and Áquila, 2000; Wang et al., 2024). This occurs because the initial germination process is largely regulated by internal physiological mechanisms, such as imbibition, enzymatic activation, and reserve mobilization, however, recent studies demonstrate that allelochemicals may affect membrane integrity, enzymatic activity, and hormonal balance, influencing germination depending on the species and compound involved (Wang et al., 2022; Li, et al., 2021).

Primary root length was significantly affected by *M. charantia* extracts, depending on the extract type, concentration, and evaluation time (4, 7, and 14th days) (Figures 2A-I). After 4 days, the aqueous extract (10 g/L) promoted a significant reduction in primary root length (0.56 cm), compared to the control (1.08 cm) (Figure 2A). For the methanolic extract, significant inhibition of root growth (0.56, 0.53, 0.46 and 0.63 cm) was observed at almost all tested concentrations (0.5, 1.0, 1.5, and 2.5 g/L), respectively, compared to the control (Figure 2B), indicating a stronger allelopathic effect already in the early stages of development. Similarly, the essential oil (10 g/L) also inhibited the primary root length of *C. ochroleuca* seedlings (0.54 cm), compared with the control (Figure 2C). On the 7th day of evaluation (Figure 2D–F), the seedlings exhibited reduced primary root lengths of less than 0.65, 0.55, and 0.38 cm in the presence of the aqueous extract (10 g/L) (Figure 2D) and the methanolic extract (0.5 and 2.5 g/L) (Figure 2E). The essential oil continued to promote no significant changes in root growth (Figure 2F). In 14th days, the development of the primary root of *C. ochroleuca* seedlings continued to be negatively affected by the aqueous (10 g/L) and methanolic extracts (0.5, 1.5, and 2.5 g/L), showing lengths of 0.64, 0.65, and 0.43 cm, respectively, compared with the control (1.52 cm) (Figure 2G, H).

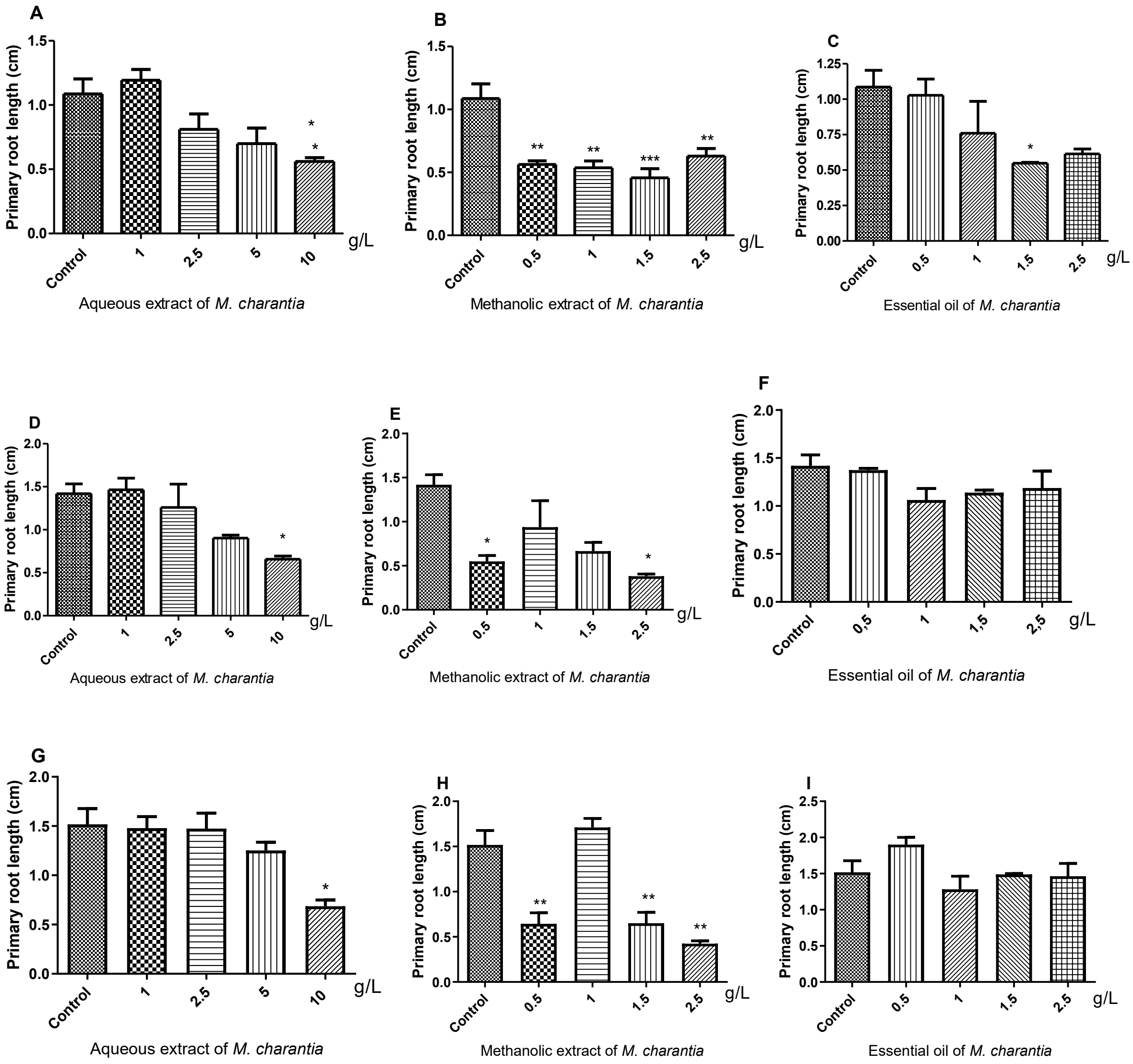

Figure 2. Primary root length of C. ochroleuca seedlings on the first evaluation day (4th day, A–C), the second evaluation day (7th day, D–F), and the last evaluation day (14th day, G–I) subjected to different concentrations of aqueous and methanolic extracts and essential oil of *M. charantia*.

Seeds and seedlings of species from the genus *Crotalaria* are frequently used as test plants in allelopathy studies due to their high sensitivity to bioactive compounds present in plant extracts (Singh & Kumar, 2015). The genus is considered an ideal model for these studies because it produces seeds of uniform size (Silva et al., 2024), which reduces experimental variation, in addition to exhibiting rapid growth and a high germination rate under controlled conditions

(Ateca, 2013), facilitating the observation of inhibitory effects.

The greater sensitivity of the primary root to allelopathic treatments is widely reported in the literature, as this organ is in direct contact with allelochemicals present in the substrate and exhibits high mitotic and metabolic activity during the early stages of development (Zhang et al., 2020; Schulz and Tabaglio, 2024). Recent studies reinforce this pattern, indicating that root growth is often more sensitive to allelochemicals than germination, although the intensity of the response may vary depending on the species and compounds involved (Wang et al., 2022).

The inhibition of root growth is considered one of the main indicators of allelopathic interference, as it reflects alterations in fundamental physiological processes such as cell division, elongation, and the uptake of water and nutrients (Farooq et al., 2011; Pan et al., 2023).

The aqueous extract (10 g/L) of *M. charantia* showed a significant inhibitory effect at 4th, 7th and 14th days of evaluation (Figure 2A, D, G). This pattern suggests that water-soluble compounds present in the aqueous extract, such as simple phenols, flavonoids, and organic acids, may be present in insufficient concentrations at lower doses to cause detectable physiological changes (Motmainna et al., 2023). Furthermore, water-soluble allelopathic compounds tend to show lower persistence in the environment and may be metabolized or diluted over time (Blum, 2011).

The methanolic extract exhibited a more intense and consistent allelopathic effect across all evaluations, with significant inhibition of root growth (Figure 2B, E, F). This result may be explained by the greater efficiency of methanol in extracting secondary compounds of medium and low polarity, such as terpenoids, alkaloids, and complex phenolics, which are frequently associated with allelopathic activity (Taiz et al., 2017). Recent allelopathic studies comparing organic (hydroalcoholic) and aqueous plant extracts report that organic solvent extracts often yield a broader diversity and higher concentrations of allelochemicals, resulting in stronger inhibitory effects on seed germination and seedling growth than aqueous extracts (Brasil Gonçalves, et al., 2024).

The persistence of the methanolic extract's effect over time suggests that the compounds present may interfere with fundamental physiological processes of root growth, including disruption of antioxidant enzyme systems, induction of oxidative stress and lipid peroxidation, and alteration of plasma membrane integrity, leading to strong inhibitory effects (Ain, et al., 2023). Furthermore, allelochemicals can induce oxidative stress, leading to the excessive production of reactive oxygen species, which compromises root growth and cellular organization (Šoln et al., 2022).

The essential oil of *M. charantia*, in turn, showed no significant inhibitory effect on primary root length in any of the evaluations (Figure 2F, I), indicating low or non-existent allelopathic activity at the tested concentrations. This result may be related to the volatility of the compounds present in essential oils, which reduces their residence time in the substrate and, consequently, their bioavailability to the seedlings (Xie et al., 2021). Additionally, the allelopathic efficacy of essential oils depends heavily on the chemical composition and concentration of the volatile compounds, which can vary between species and environmental conditions (Bakkali et al., 2008; Yang, 2024).

Overall, the results indicate that *M. charantia* possesses allelopathic potential, especially when its compounds are extracted using organic solvents, reinforcing the importance of the extraction method choice in evaluating allelopathic interactions.

The results evidence that hypocotyl length was affected differently by the various *M. charantia* extracts, varying according to the type of extract, the concentration applied, and the evaluation period, indicating distinct physiological responses between seedling organs (Figure 3A-I). The aqueous extract of *M. charantia* showed a significant inhibitory effect (0.25 cm) only at the highest concentration (10 g/L) on the 4th day of evaluation (Figure 3A), while no significant inhibitory effect was observed on the 7th and 14th days, while the methanolic extract showed the most pronounced allelopathic effect on the hypocotyl, with significant growth reductions at almost all concentrations (0.5, 1.0, 1.5 and 2.5 g/L) and evaluation times (4, 7 and 14th days) (Figure 3B).

This result indicates that the compounds extracted by methanol have a greater capacity to interfere with the physiological mechanisms responsible for cell elongation, such as auxin activity and the synthesis of cell wall components (Taiz et al., 2017). The literature indicates that organic extracts often contain a diverse range of stable and biologically active allelochemicals, including complex phenols, alkaloids, and terpenoids, which are capable of inhibiting seed germination and seedling growth, negatively affecting physiological processes in shoot and root tissues, such as phenolic acids, terpenoids, and alkaloids (Kostina-Bednarz, et al., 2023)

The inhibitory effect of the methanolic extract intensified over time, particularly on the 14th day at concentrations of 0.5, 1.0, 1.5, and 2.5 g/L, with inhibition values of 0.32, 0.83, 0.45, and 0.37 cm, respectively ($p < 0.001$) (Figure 3H) suggests a cumulative effect of allelochemicals on seedling metabolism. These compounds can induce oxidative stress, alter cell membrane permeability, and compromise enzymatic activity, resulting in reduced hypocotyl growth (Schulz and Tabaglio, 2024; Alghanem et al., 2025). Furthermore, the persistent reduction in shoot growth can affect the emergence and initial establishment of seedlings (Wang et al., 2025).

The essential oil of *M. charantia* showed no significant allelopathic effect on hypocotyl length at any of the concentrations and periods evaluated, as observed for the primary root (Figures 3C, F and I).

Taken together, the results indicate that *M. charantia* possesses allelopathic potential on hypocotyl growth, particularly when its compounds are extracted using organic solvents. The lower sensitivity of the hypocotyl compared to the primary root reinforces the importance of evaluating multiple morphological parameters for a more comprehensive understanding of allelopathic effects during initial seedling establishment (Liu et al., 2024; Fadheel et al., 2025; Wang et al., 2025;).

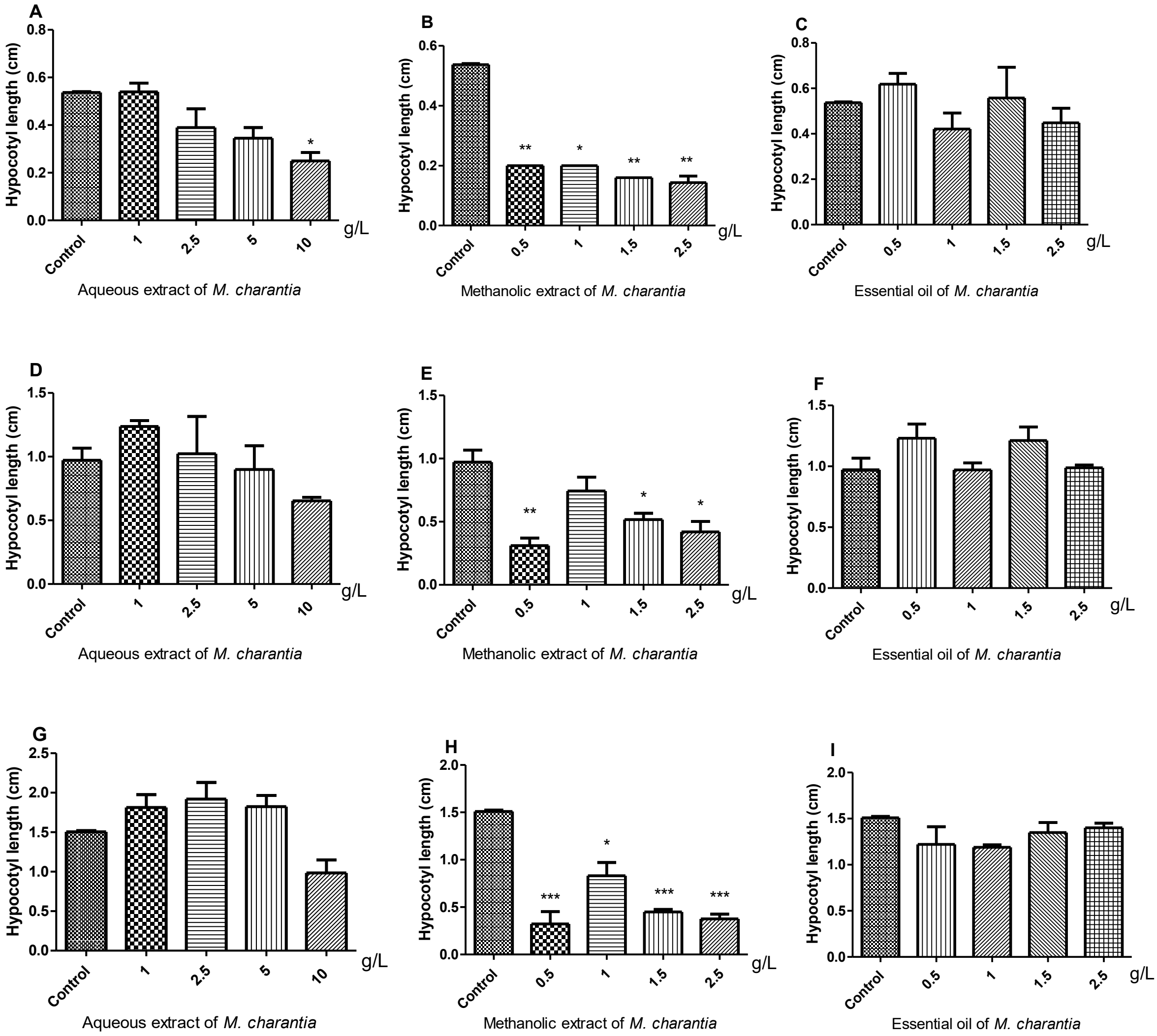

Figure 3. Hypocotyl length of C. ochroleuca seedlings on the first evaluation day (4th day, A–C), the second evaluation day (7th day, D–F), and the last evaluation day (14th day, G–I) subjected to different concentrations of aqueous and methanolic extracts and essential oil of *M. charantia.*

Leaf fresh weight was significantly affected by *M. charantia* extracts, with distinct responses depending on the extract type and applied concentration (Figure 4A-C).

The aqueous extract promoted a significant reduction in leaf fresh weight at all evaluated concentrations (1, 2.5, 5, and 10 g/L) compared to the control, evidencing a strong inhibitory effect (0.023, 0.031, 0.035 and 0,039 g). The lowest weights were observed even at the lowest concentrations, indicating high sensitivity of the leaf tissue to the water-soluble compounds present in the extract (Figure 4A). For the methanolic extract, a significant reduction in leaf fresh weight (0.082 and 0.091 g) was observed at concentrations of 1.5 and 2.5 g/L, respectively,

(Figure 4B). The essential oil of *M. charantia* also caused a significant reduction in leaf fresh weight (0.024, 0.015, 0.026 and 0.048 g) at all tested concentrations (0.5, 1, 1.5 and 2.5 g/l), repectively compared to the control (Figure 4C). The lowest weights were recorded at the intermediate and higher concentrations, suggesting that the volatile compounds present in the essential oil at least interfered negatively with the accumulation of leaf biomass (Figure 4C).

Overall, the results demonstrate that leaf fresh weight constitutes a sensitive parameter to the allelopathic action of *M. charantia*, reflecting physiological changes associated with reduced growth and plant biomass accumulation.

Leaf fresh weight is a sensitive indicator of plant growth, as it reflects cell expansion and water content, which are directly associated with metabolic activity and photosynthetic performance (Taiz et al., 2017). In the present study, all extracts of *Momordica charantia* negatively affected leaf fresh weight, although with different response patterns depending on the extraction solvent (Figure 4). The aqueous extract caused a significant reduction at all tested concentrations, indicating a strong inhibitory effect likely associated with water-soluble allelochemicals, such as phenolic acids and flavonoids. These compounds are known to interfere with membrane permeability, water balance, and enzymatic processes related to photosynthesis, ultimately limiting leaf expansion and biomass accumulation (Mushtaq and Fauconnier, 2024; Alghanem et al., 2025).

The methanolic extract showed a concentration-dependent response, with significant reductions observed only at higher concentrations (1.5 and 2.5 g L^{-1}) (Figure 4B). Methanol efficiently extracts secondary metabolites of intermediate polarity with high biological activity from fresh leaves, which may compromise photosynthetic efficiency and disrupt hormonal regulation involved in leaf development (Taiz et al., 2017; Akter et al., 2024). In contrast, aqueous extracts prepared from dried leaves may have lower concentrations of these labile compounds due to degradation during drying, potentially reducing their phytotoxic effects (Sultana et al., 2009; Dai & Mumper, 2010; Elghobashy et al. 2024) Similarly, the essential oil significantly reduced leaf fresh weight at all concentrations. Its phytotoxicity is attributed to volatile terpenoids, whose lipophilic nature facilitates interaction with cell membranes, impairing membrane integrity and cellular respiration (Wang et al. 2025; Jalilian et al., 2025).

Overall, these results demonstrate the allelopathic potential of *M. charantia* in limiting leaf development and highlight the role of solvent polarity and compound classes in determining the intensity of phytotoxic effects, reinforcing leaf fresh weight as a reliable parameter for allelopathic assessment (Mushtaq and Fauconnier, 2024).

Although germination was not affected by the essential oil, the consistent reduction in fresh weight clearly demonstrates interference with key physiological processes required for seedling establishment, confirming that allelopathic effects of *M. charantia* are predominantly expressed at post-germinative stages. Accordingly, *M. charantia* shows extract-dependent and parameter-specific allelopathic potential.

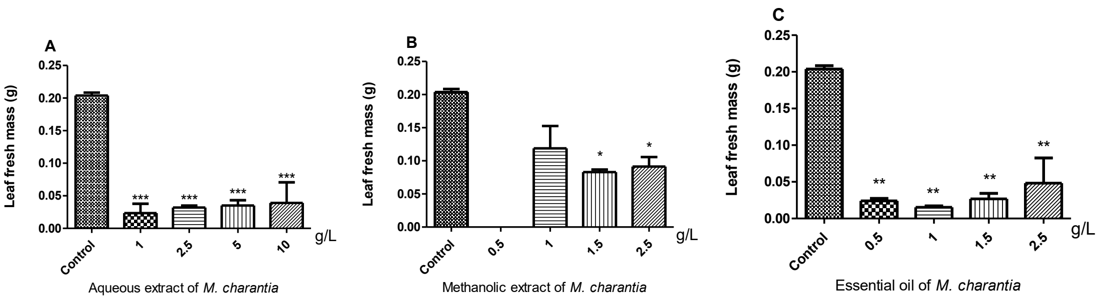

Figure 4. Fresh leaf biomass of *C. ochroleuca* seedlings subjected to different concentrations of aqueous extract (A), methanolic extract (B), and essential oil (C) of *M. charantia*.

4. Conclusion

The results indicate that *M. charantia* exhibits allelopathic potential against *C. ochroleuca*, expressed mainly at post-germinative stages. Seed germination was not significantly impaired at the end of the experimental period, whereas early growth parameters and fresh weight were more sensitive to the treatments. Importantly, the effects observed depended on both the plant part and the preparation method: methanolic extracts prepared from fresh leaves caused the greatest inhibition of seedling development, followed by aqueous extracts prepared from dried leaves at higher concentrations. Fresh weight was consistently reduced by all treatments, including the essential oil obtained from leaves and stems, indicating interference with physiological processes related to biomass accumulation and seedling establishment. Thus, the allelopathic potential of *M. charantia* is influenced not only by the type of extract and concentration but also by the plant part used and the method of preparation, highlighting the importance of these factors in assessing allelopathic activity.

Data Availability Statement

All data supporting the findings of this study are available within the article.

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7. CONCLUSÃO

Os resultados obtidos demonstram que a *Momordica charantia* L. apresenta variação significativa no rendimento e na composição química do óleo essencial ao longo do ciclo anual, com predominância dos compostos (Z)-3-hexenol e linalol, evidenciando a influência fase fenológica da espécie. As análises microscópicas e histoquímicas confirmaram a presença de estruturas secretoras associadas à produção e ao armazenamento de metabólitos voláteis, contribuindo para a caracterização anatômica da espécie e para o controle de qualidade do material vegetal.

Quanto à atividade alelopática, o óleo essencial e os extratos foliares de *M. charantia* não interferiram de forma significativa na germinação de *C. ochroleuca*, porém promoveram efeitos inibitórios relevantes sobre o crescimento inicial das plântulas, especialmente no desenvolvimento radicular. O extrato metanólico apresentou maior potencial inibitório, indicando que os efeitos alelopáticos estão relacionados ao tipo de extrato, à concentração e ao tempo de exposição.

De forma integrada, os resultados evidenciam o papel de *M. charantia* como fonte de metabólitos bioativos com importância ecológica, capazes de atuar em interações planta-planta. Este estudo contribui para o avanço do conhecimento sobre a variabilidade química da espécie e seu potencial alelopático, apontando perspectivas para o uso de compostos de origem vegetal em estratégias sustentáveis de manejo, especialmente em sistemas de cultivo nos quais o uso de herbicidas sintéticos é restrito.