MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRICULTURA E BIODIVERSIDADE

PROSPECÇÃO, CONSERVAÇÃO, CARACTERIZAÇÃO GENÉTICA, QUÍMICA, MORFOAGRONÔMICA E SAZONALIDADE EM GERMOPLASMA DE *Croton blanchetianus* Baill.

ROSEMEIRE SANTOS COSTA

2026

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Orientador
Prof. Dr. Arie Fitzgerald Blank

SÃO CRISTÓVÃO
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APROVADA em 24 de fevereiro de 2026.

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SÃO CRISTÓVÃO
SERGIPE – BRASIL

A Deus, pela sabedoria e pela força.
À minha família, por todo amor e paciência.
A todos que acreditam que o conhecimento é uma forma de transformar o mundo.
Dedico

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

BAG	Banco Ativo de Germoplasma
ISSR	Inter Simple Sequence Repeat
UFS	Universidade Federal de Sergipe
APG	Angiosperm Phylogeny Group
MEV	Ácido mevalônico
MEP	Metileritritol fosfato
IPP	Isopentenil difosfato
DOXP	1-deoxixilulose-5-P
DXR	1-desoxi-xilulose-5-P redutoisomerase
DMAPP	Difosfato de dimetilalil
AFLP	Amplified Fragment Length Polymorphism
SSR	Simple Sequence Repeats
RAPD	Random Amplified Polymorphic
N	Number of individuals
Na	Observed number of alleles
Ne	Effective number of alleles
I	Shannon Diversity Index
He	Expected heterozygosity
P%	Percentage of polymorphism
Φ_{PT}	Genetic differentiation among the populations
G_{ST}	coefficient of genetic differentiation among populations
AMOVA	Analysis of Molecular Variance
UPGMA	Unweighted Pair Group Method with Arithmetic Means
MCMC	Markov Chain Monte Carlo
PCoA	Principal Coordinate Analysis
k	Number of genetic groups
T	Annealing temperature
TNB	Total number of bands
NPB	Number of polymorphic bands
PIC	polymorphic information content
AQ	Aquidabã
GC	Graccho Cardoso
IT	Itabi
LG	Lagarto
TB	Tobias Barreto
PV	Poço Verde
CTAB	Cetyl Trimethyl Ammonium Bromide
PVP	Polyvinylpyrrolidone
DF	Degrees of freedom
SS	Sum of squares
MS	Mean of squares
Nm	Gene flow among populations
GC-MS	Gas chromatography-mass spectrometry
EOC	Essential oil concentration
EO	Essential oil
RI	Retention indices
ANOVA	Analysis of variance
PCA	Principal Component Analysis
RRIo	Relative retention index observed

RRIl	Relative retention index from the literature
TI	Total relative percentage of compounds with concentration ≥ 2%
SB	Sum of bases
CEC	Ation exchange capacity
EOY	Essential oil yield
NIST	National Institute of Standards & Technology
EI	Electron ionization
PH	Plant height
CD	Canopy diameter
LL	Leaf length
LW	Leaf width
LL/LW	Leaf length-to-width ratio
LA	Leaf area
DM	Dry matter
CV	Coefficient of variation
Rad	Adaxial red
Gad	Adaxial green
Bad	Adaxial blue
Rab	Abaxial red
Gab	Abaxial green
Bab	Abaxial blue
PC1	Principal Component 1
PC2	Principal Component 2
RI	Retention Index
RI_lit	Retention Index reported in the literature
ATP	Adenosine Triphosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
INMET	Instituto Nacional de Meteorologia
TOE	Teor de óleo essencial
ROE	Rendimento do óleo essencial
MS	Massa seca
DPPH	Radical 2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
ABTS	2,2’-azino-bis(3-etilbenzotiazolina-6-sulfônico)

RESUMO

COSTA, Rosemeire Santos. **Prospecção, conservação, caracterização genética, química, morfoagronômica e sazonalidade em germoplasma de *Croton blanchetianus* Baill.** São Cristóvão: UFS, 2026. 144p. (Tese – Doutorado em Agricultura e Biodiversidade).*

Croton blanchetianus Baill. é uma espécie medicinal e aromática nativa da Caatinga e amplamente distribuída no Nordeste brasileiro. Destaca-se pelo elevado potencial bioativo e apícola, listada no portfólio do Ministério do Meio Ambiente, considerada prioritária para a região por sua importância econômica potencial. Apesar disso, é frequente a redução de sua área de ocorrência natural, principalmente em função de intervenções antrópicas. Diante das ameaças iminentes à variabilidade genética da espécie, a Universidade Federal de Sergipe (UFS) estudou populações naturais e, posteriormente, estabeleceu uma coleção de *C. blanchetianus* em seu Banco Ativo de Germoplasma (BAG) de Plantas Medicinais e Aromáticas da UFS. Assim, é possível traçar estratégias de conservação e uso correto dessa espécie. A presente pesquisa objetivou avaliar a diversidade genética e química de populações naturais de *C. blanchetianus* de municípios do estado de Sergipe, estabelecer uma coleção e caracterizar morfoagronômica, química e sazonal, bem como realizar a atividade antioxidante dos acessos conservados no BAG. No primeiro artigo, avaliou-se a diversidade e a estrutura genética de seis populações naturais de *C. blanchetianus* de diferentes municípios do estado, utilizando marcadores ISSR. Observou-se baixa variabilidade genética entre populações (6%), não sendo possível diferenciar os genótipos de acordo com seu local de origem. No segundo artigo, avaliou-se a diversidade química do óleo essencial de 70 genótipos de *C. blanchetianus* coletados em seis populações naturais de Sergipe. Os compostos majoritários foram α-pineno (1,60-13,37%), limoneno (0,30-17,54%), β-felandreno (4,38-16,04%), 1,8-cineol (0,16-13,56%), (*E*)-cariofileno (0,31-13,14%), germacreno D (0,33-10,60%), biciclogermacreno (5,06-27,47%) e espatulenol (4,34-29,83%). Os resultados evidenciam a ampla diversidade química. No terceiro artigo, realizou-se a caracterização e a análise da diversidade, através de variáveis morfoagronômicas e químicas, de 26 acessos de *C. blanchetianus*. Evidenciou-se alta variabilidade entre os acessos para todos os descritores morfoagronômicos analisados, com destaque para altura (87,00-236,50 cm), diâmetro de copa (48,75-131,00 cm), área foliar (74,50-176,37 cm^2), massa seca (83,17-333,00 g/planta) e teor de óleo essencial (0,60-1,60%). Quimicamente, os acessos apresentaram predominância de α-pineno (3,90-12,02%), limoneno (0,88-10,72%), β-felandreno (1,30-17,51%), 1,8-cineol (0,36-11,57%), (*E*)-cariofileno (1,82-12,48%), germacreno D (0,38-10,12%), biciclogermacreno (8,37-29,13%) e espatulenol (5,57-28,04%). No quarto artigo, analisou-se o perfil químico do óleo essencial de folhas e frutos de cinco acessos de *C. blanchetianus*, revelando diferenças quantitativas e qualitativas entre os órgãos. As folhas apresentaram predominância de sesquiterpenos (37,34-62,03%), enquanto os frutos, monoterpenos (23,97-61,03%). A presença exclusiva de acetato de mirtenila no óleo essencial dos frutos indica seu potencial como marcador químico. No quinto artigo, avaliou-se a influência sazonal (três épocas de colheita) sobre variáveis agronômicas, químicas e atividade antioxidante do óleo essencial de 26 acessos de *C. blanchetianus*. Os acessos CBL-403 e CBL-503 destacaram-se quanto ao maior teor (1,93%), enquanto CBL-503 (17,41 g/planta) e CBL-507 (17,38 g/planta) exibiram maiores rendimentos. Observou-se predominância de sesquiterpenos nas colheitas 1 e 2 (59,02% e 62,01%, respectivamente), aumento de monoterpenos na colheita 3 (40,01%) e um elevado potencial antioxidante do acesso CBL-301 (DPPH: 66,24%; ABTS: 95,51%). Os resultados indicam que *C. blanchetianus* apresenta variabilidade genética, morfoagronômica e química a ser explorada, e seus óleos essenciais possuem plasticidade sazonal e atividade antioxidante.

Palavras-chave: Óleo essencial, variabilidade genética, diversidade química, variação sazonal.

* Comitê Orientador: Arie Fitzgerald Blank – UFS (Orientador), Itamara Bomfim Gois – UFS (Co-orientadora).

ABSTRACT

COSTA, Rosemeire Santos. **Prospection, conservation, genetic, chemical and morphoagronomic characterization, and seasonality in germplasm of *Croton blanchetianus* Baill.** São Cristóvão: UFS, 2026. 144p. (Thesis - Doctor of Science in Agriculture and Biodiversity).*

Croton blanchetianus Baill. is a medicinal and aromatic species native to the Caatinga biome, widely distributed in the Brazilian Northeast. It is notable for its high bioactive and apicultural potential, and it is listed by the Ministry of Environment as a priority species for the region due to its potential economic importance. Nevertheless, its natural occurrence area is frequently reduced, primarily due to anthropogenic interventions. In view of the imminent threats to the species' genetic variability, the Federal University of Sergipe (UFS) studied natural populations and subsequently established a collection of *C. blanchetianus* in its Active Germplasm Bank (AGB) for Medicinal and Aromatic Plants at UFS. This initiative allows the development of strategies for conservation and sustainable use of the species. The present research aims to evaluate the genetic and chemical diversity of natural populations of *C. blanchetianus* from municipalities in the state of Sergipe, as well as to establish a collection, characterize the accessions morpho-agronomically, chemically, and seasonally, and assess the antioxidant activity of the accessions conserved in the AGB. In the first article, the genetic diversity and structure of six natural populations of *C. blanchetianus* from different municipalities in the state were evaluated, using ISSR markers. Low genetic variability among populations (6%) was observed, and it was not possible to differentiate genotypes according to their geographic origin. In the second article, the chemical diversity of the essential oil from 70 genotypes of *C. blanchetianus,* collected from six natural populations in Sergipe, was evaluated. The major compounds found were α-pinene (1.60–13.37%), limonene (0.30–17.54%), β-phellandrene (4.38–16.04%), 1,8-cineole (0.16–13.56%), (*E*)-caryophyllene (0.31–13.14%), germacrene D (0.33–10.60%), bicyclogermacrene (5.06–27.47%), and spathulenol (4.34–29.83%), highlighting the broad chemical diversity of the species. In the third article, the morpho-agronomic and chemical diversity of 26 *C. blanchetianus* accessions was characterized and analyzed. High variability among accessions was observed for all evaluated morpho-agronomic descriptors, particularly plant height (87.00–236.50 cm), crown diameter (48.75–131.00 cm), leaf area (74.50–176.37 cm²), dry mass (83.17–333.00 g/plant), and essential oil content (0.60–1.60%). Chemically, the accessions were mostly comprised of α-pinene (3.90–12.02%), limonene (0.88–10.72%), β-phellandrene (1.30–17.51%), 1,8-cineole (0.36–11.57%), (*E*)-caryophyllene (1.82–12.48%), germacrene D (0.38–10.12%), bicyclogermacrene (8.37–29.13%), and spathulenol (5.57–28.04%). In the fourth article, the chemical profile of the essential oil from leaves and fruits of five accessions of *C. blanchetianus* was analyzed, revealing quantitative and qualitative differences between plant organs. Leaves had a predominance of sesquiterpenes (37.34–62.03%), whereas fruits had higher levels of monoterpenes (23.97–61.03%). The exclusive presence of myrtenyl acetate in fruit essential oil indicates its potential as a chemical marker. In the fifth article, the seasonal influence (three harvest periods) on agronomic variables, chemical composition, and antioxidant activity of essential oil from 26 accessions of *C. blanchetianus* was evaluated. Accessions CBL-403 and CBL-503 showed the highest oil content (1.93%), while CBL-503 (17.41 g/plant) and CBL-507 (17.38 g/plant) exhibited the highest essential oil yields. Sesquiterpenes predominated in harvests 1 and 2 (59.02% and 62.01%, respectively), while monoterpenes increased in harvest 3 (40.01%). A high antioxidant potential was observed in accession CBL-301 (DPPH: 66.24%; ABTS: 92.51%). These results indicate that *C. blanchetianus* exhibits exploitable genetic, morpho-agronomic, and chemical variability, and that its essential oils show seasonal plasticity and antioxidant activity.

Keywords: Essential oil, genetic variability, chemical diversity, seasonal variation.

* Guidance Committee: Arie Fitzgerald Blank (Advisor), Itamara Bomfim Gois (Adjunct advisor).

1. INTRODUÇÃO GERAL

Croton L. é o segundo maior gênero da família Euphorbiaceae, com cerca de 1200 espécies, e apresenta distribuição ecologicamente proeminente em áreas de vegetação secundária nos trópicos e subtrópicos em todo o mundo (Webster, 1993). No Brasil, *Croton* é o mais representativo da família, sendo composto por 311 espécies, das quais 240 são endêmicas e podem ser encontradas em todos os estados e domínios fitogeográficos do país (BFG, 2025). No estado de Sergipe, são encontradas 22 espécies, que variam de subarbustos a árvores e estão presentes principalmente em áreas de caatinga, incluindo o *Croton blanchetianus* Baill. (BFG, 2025).

Croton blanchetianus é uma planta medicinal e aromática endêmica da Caatinga, popularmente conhecida no Nordeste do Brasil como marmeleiro e/ou marmeleiro-da-caatinga. Caracteriza-se morfologicamente por ser um arbusto monóico (1,5-8 m), com lâmina foliar membranácea, oval a lanceolada, inflorescência em tirso com flores unissexuais, flor pistilada com pétalas ausentes, 5 sépalas e estiletes unidos em coluna, flor estaminada com 5 pétalas brancas, 5 sépalas e estames com antera longa; fruto em cápsula verde a amarelada esferoidal; e semente marrom a preta, elipsoide lisa, com carúncula reniforme (Rossine *et al*., 2023; BFG, 2025). Essa planta aromática apresenta uso medicinal difundido e está listada nos portfólios do Ministério do Meio Ambiente por ser considerada prioritária para a região Nordeste, devido à sua importância econômica atual e potencial (Coradin *et al*., 2018).

Na medicina popular, as folhas e cascas de *C. blanchetianus* são utilizadas para o tratamento de distúrbios gastrointestinais, reumatismo, cefaleia e bronquite (Chaves e Reinhard, 2003). Além disso, diferentes estudos têm sido publicados sobre as atividades terapêuticas de *C. blanchetianus*, as quais estão intimamente associadas aos seus metabólitos secundários, como flavonoides, alcaloides e terpenos (Aquino *et al*., 2017; Oliveira *et al*., 2022a). Dentre as atividades estudadas, destacam-se as gastroprotetora, analgésica, anti-inflamatória, antioxidante, neuroprotetora, antimicrobiana e antinociceptiva (Firmino *et al*., 2019; Freitas *et al*., 2020; Fernandes *et al*., 2021; Dantas *et al*., 2021; Oliveira *et al*., 2022a; Oliveira *et al*., 2022b; Nascimento *et al*., 2024). Outros trabalhos relatam ainda atividades biológicas de seus óleos essenciais, como antibacteriana, acaricida, antimicrobiana, antifúngica, inseticida e larvicida (Melo *et al*., 2013; Angélico *et al*., 2014; Rodrigues *et al*., 2019; Silva *et al*., 2020; Camara *et al*., 2021; Vasconcelos *et al*., 2022a e 2022b; Venancio *et al*., 2025; Lopes *et al*., 2025).

Apesar da importância desta espécie, é frequente a sua redução em áreas de sua ocorrência natural, principalmente em função de intervenções antrópicas, como o desmatamento de áreas para expansão imobiliária e agrícola, o que a expõe ao risco de perda de genótipos potencialmente úteis ao homem. Diante da ameaça iminente à espécie, o estudo da variabilidade genética e química de populações naturais, aliado à conservação em Bancos Ativos de Germoplasma (BAGs), é fundamental para acessar, preservar e manejar parte de seu reservatório genético, além de subsidiar o avanço das pesquisas científicas. Apesar de *C. blanchetianus* apresentar ampla distribuição no estado de Sergipe, ainda não existem informações disponíveis sobre sua diversidade genética. A caracterização dos acessos conservados em BAGs pode ser realizada por meio de descritores fenotípicos que avaliam aspectos morfológicos, agronômicos e químicos da espécie. A avaliação integrada desses descritores constitui uma etapa fundamental para a avaliação da diversidade entre acessos, possibilitando a identificação e a seleção de acessos agronomicamente desejáveis para cultivo e inclusão em programas de melhoramento genético, além de eliminar duplicidades de acessos e diminuir custos de manutenção (Blank, 2013; Nascimento *et al*., 2020; Oliveira *et al*., 2020b). Além disso, a caracterização química de óleos essenciais em espécies aromáticas fornece informações relevantes acerca do potencial bioativo, subsidiando a seleção de acessos superiores para múltiplas aplicações (Oliveira *et al*., 2019; Rodrigues *et al*., 2023). No entanto, a caracterização química de óleos essenciais é influenciada por fatores ambientais sazonais, tais

como precipitação, temperatura, umidade e intensidade luminosa, que podem afetar de forma significativa a biossíntese desses metabólitos, resultando em flutuações no perfil químico ao longo do ciclo anual e, consequentemente, sua atividade biológica (Sá Filho *et al*., 2022; Jerônimo *et al*., 2024).

Diante do exposto, a caracterização representa uma etapa indispensável na gestão de coleções de germoplasma. O conhecimento da variabilidade genética, química e morfoagronômica de espécies aromáticas permite a valorização de recursos genéticos locais e é ponto de partida para o desenvolvimento de novas aplicações biotecnológicas e, apesar de *C. blanchetianus* apresentar ampla distribuição no estado de Sergipe, ainda não existem informações disponíveis sobre sua diversidade genética. Nesse contexto, o presente estudo teve como objetivo realizar a caracterização genética e química de populações naturais de *C. blanchetianus* coletadas em diferentes municípios do estado de Sergipe, Brasil. Além disso, foi implementada uma coleção *ex situ* e, posteriormente, sua caracterização química, morfoagronômica e sazonal, visando identificar a variabilidade existente na espécie, para subsidiar estratégias de conservação, programas de melhoramento genético e aproveitamento sustentável da espécie.

2. REVISÃO DE LITERATURA

2.1 Aspectos botânicos, descrição morfológica e usos de *Croton blanchetianus* Baill.

A família Euphorbiaceae Juss., composta por cerca de 300 gêneros e aproximadamente 6000 espécies, apresenta distribuição cosmopolita, sendo mais comum nos trópicos (Judd *et al*., 2009). A família, segundo APG IV, pertence à ordem Malpighiales, juntamente com Achariaceae, Chrysobalanaceae, Clusiaceae, Erythroxylaceae, Hypericaceae, Linaceae, Malpighiaceae, Ochnaceae, Passifloraceae, Phyllanthaceae, Picrodendraceae, Podostemaceae, Rafflesiaceae, Rhizophoraceae, Salicaceae e Violaceae; e é considerada polifilética com base em evidências morfológicas e moleculares (Hennig, 1966; Judd *et al*., 2009).

O gênero *Croton* L., pertencente à família Euphorbiaceae, está inserido juntamente com *Ophellantha* Standl., *Sandwithia* Lanj., *Sagotia* Baill., *Brasiliocroton* Berry e Cordeiro e *Astraea* Klotzch. na tribo Crotoneae, diferindo destes por apresentar dobramento dos filetes no botão floral, inflorescências tirsóides e pétalas reduzidas ou ausentes nas flores pistiladas (Berry *et al*., 2005; Wurdack *et al*., 2005). O gênero compreende cerca de 1200 espécies (aproximadamente 16% do total de espécies da família) e tem distribuição pantropical, embora a maioria dos seus representantes ocorra nas Américas (Webster, 1993; Silva *et al*., 2010). No Brasil, *Croton* é o mais diverso da família Euphorbiaceae, com 311 espécies registradas, das quais 240 são endêmicas. Pode ser facilmente encontrado em todas as regiões e frequentemente está associado a ambientes antropizados em áreas de caatinga (Silva *et al*., 2010; BFG, 2025).

O Nordeste brasileiro é a segunda região com maior representatividade do gênero *Croton*, com 113 espécies, ficando atrás apenas do Sudeste, com 152 espécies (BFG, 2025). Estudos taxonômicos também evidenciam alta diversidade do gênero *Croton* na região Nordeste, tais como: Cordeiro (1995) com 10 espécies (*C. argyrophylloides*, *C. blanchetianus*, *C. campestris*, *C. grewioides*, *C. heliotropiifolius*, *C. jacobinensis*, *C. nepetifolius*, *C. rudolphianus*, *C. sonderianus* e *C. urticifolius*); Silva *et al*. (2009) com 15 espécies (*Croton adamantinus*, *C. argyrophyllus*, *C. blanchetianus*, *C. campestris*, *C. corchoropsis*, *C. grewioides*, *C. heliotropiifolius*, *C. hirtus*, *C. jacobinensis*, *C. nepetifolius*, *C. nummularius*, *C. rudolphianus*, *C. sonderianus*, *C. urticifolius* e *C. virgultosus*); Silva *et al*. (2010) com 35 espécies (*C. adamantinus*, *C. agrestis*, *C. argyrophylloides*, *C. blanchetianus*, *C. campestris*, *C. corchoropsis*, *C. grewioides*, *C. glandulosus*, *C. heliotropiifolius*, *C. hirtus*, *C. jacobinensis*, *C. lundianus*, *C. nepetifolius*, *C. nummularius*, *C. polyandrus*, *C. pulegioides*, *C. rudolphianus*, *C. sonderianus*, *C. urticifolius*, *C. virgultosus*, *C. argenteus*, *C. atrorufus*, *C. betaceus*, *C. echioides*, *C. fulvus*, *C. gracilipes*, *C. laceratoglandulosus*, *C. seminudus*, *C. subvillosus*, *C. tenuifolius*, *C. triqueter*, *C. sellowii*, *C. pedicellatus*, *C. echinocarpus* e *C. micans*); Lucena e Alves (2010) com 7 espécies (*C. acradenius*, *C. laceratoglandulosus*, *C. lachnocladus*, *C. parodianus*, *C. pulegioides*, *C. sapiifolius* e *C. tenuifolius*); e Crepaldi *et al*. (2016) com 6 espécies (*C. adamantinus*, *C. heliotropiifolius*, *C. jacobinensis*, *C. japirensis*, *C. tricolor* e *C. echioides*). As espécies são amplamente distribuídas nos mais diferentes domínios fitogeográficos, destacando-se a Caatinga, com 69 espécies catalogadas (BFG, 2025). No estado de Sergipe, são encontradas 22 espécies, que variam de subarbustos a árvores e estão presentes principalmente em áreas de caatinga, incluindo o *Croton blanchetianus* Baill. (BFG, 2025).

Croton blanchetianus é uma espécie endêmica da Caatinga, popularmente conhecida como marmeleiro, marmeleiro-branco e marmeleiro-da-caatinga, com ocorrência em todos os estados da região Nordeste, exceto no Maranhão e, no Sudeste, em Minas Gerais (BFG, 2025). A espécie é encontrada em áreas de vegetação de caatinga, formando densas populações sobre solo arenoso ou argiloso, entre 200 e 800 m de altitude, e está associada a ambientes antropizados (Carneiro-Torres, 2009; Pereira *et al*., 2001).

Caracteriza-se morfologicamente por ser um arbusto monóico (1,5-8 m), apresenta ramificação monopodial cilíndrica verde, amarelada a acinzentada, com tricomas estrelado-lepidotos; lâmina foliar inteira, cartácea, oval a lanceolada, ligeiramente bifacial, face adaxial

verde escuro, face abaxial opaca, verde claro a cinza; inflorescência em tirso com flores unissexuais, flor pistilada com pétalas ausentes, 5 sépalas e estiletes unidos em coluna, flor estaminada com 5 pétalas brancas, 5 sépalas e estames com antera longa; fruto em cápsula verde a amarelado esferoidal; e semente marrom a preta, elipsoide lisa, com carúncula reniforme (Figuras 1 e 2) (Rossine *et al*., 2023; BFG, 2025).

As flores do marmeleiro possuem mecanismo de abertura floral assíncrona. Inicialmente ocorre a abertura das flores pistiladas e, após a antese dessas flores, abrem-se as flores estaminadas (Santos, 2016), o que favorece a polinização cruzada e reduz a ocorrência de endogamia. A dispersão das sementes é do tipo autocórica, ocorrendo por meio da deiscência explosiva dos frutos secos, que arremessam as sementes a certa distância da progenitora (Webster, 1994; Passos e Ferreira, 1996). As sementes apresentam uma carúncula ou elaiossomo, estrutura rica em compostos lipídicos, cuja composição atrai formigas e facilita a dispersão secundária (Webster, 1994; Passos e Ferreira, 1996).

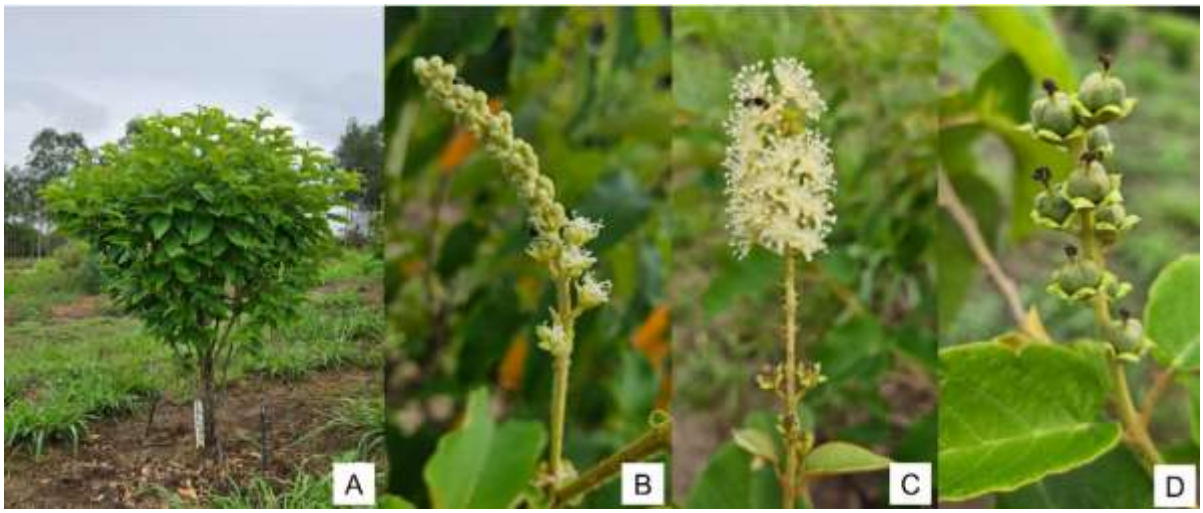

Figura 1. *Croton blanchetianus* (Euphorbiaceae): A – hábito de crescimento; B – inflorescência (com flores pistiladas na base e botões florais no ápice); C – inflorescência (com frutos jovens na base, seguidos por flores estaminadas); D – inflorescência com frutos.

O marmeleiro é uma espécie aromática que apresenta usos difundidos como medicinal, apícola, madeireiro e forrageiro e está listada no portfólio do Ministério do Meio Ambiente, sendo considerada prioritária para a região Nordeste por sua importância econômica atual e potencial (Santos *et al*., 2005; Santana, 2009; Ramos e Albuquerque, 2012; Souza *et al*., 2014; Nunes *et al*., 2016; Coradin *et al*., 2018). A espécie ainda é considerada de extrema importância para a manutenção do balanço ecológico da caatinga, por apresentar características morfológicas e anatômicas adaptadas às condições semiáridas, onde a precipitação não ultrapassa a evapotranspiração (Barros e Soares, 2013). Além disso, tem sido demonstrada a notável capacidade de adaptação da espécie às mudanças climáticas (época seca e chuvosa), a qual pode ser considerada um modelo em estudos que exploram as relações hídricas em espécies lenhosas e em estudos de reflorestamento (Mendes *et al*., 2017).

Na medicina popular, as folhas e cascas de *C. blanchetianus* são amplamente utilizadas no tratamento de distúrbios gastrointestinais, doenças hepáticas, reumatismo, cefaleia, bronquite, diabetes e no processo de cicatrização, sendo empregadas na forma de chás, xaropes, infusões, macerações e sucos (Chaves e Reinhard, 2003; Souza *et al*., 2014; Saraiva *et al*., 2015; Bitu *et al*., 2015; Macedo *et al*., 2018). Diversas atividades terapêuticas já foram cientificamente comprovadas para *C. blanchetianus*, as quais estão diretamente associadas à ampla diversidade de metabólitos secundários presentes na espécie, incluindo compostos fenólicos, alcaloides e terpenos (Aquino *et al*., 2017; Oliveira *et al*., 2022a). Dentre as atividades, destacam-se as gastroprotetora, analgésica, anti-inflamatória, antioxidante, neuroprotetora, antimicrobiana e antinociceptiva (Firmino *et al*., 2019; Freitas *et al*., 2020;

Fernandes *et al*., 2021; Dantas *et al*., 2021; Oliveira *et al*., 2022a; Oliveira *et al*., 2022b; Nascimento *et al*., 2024).

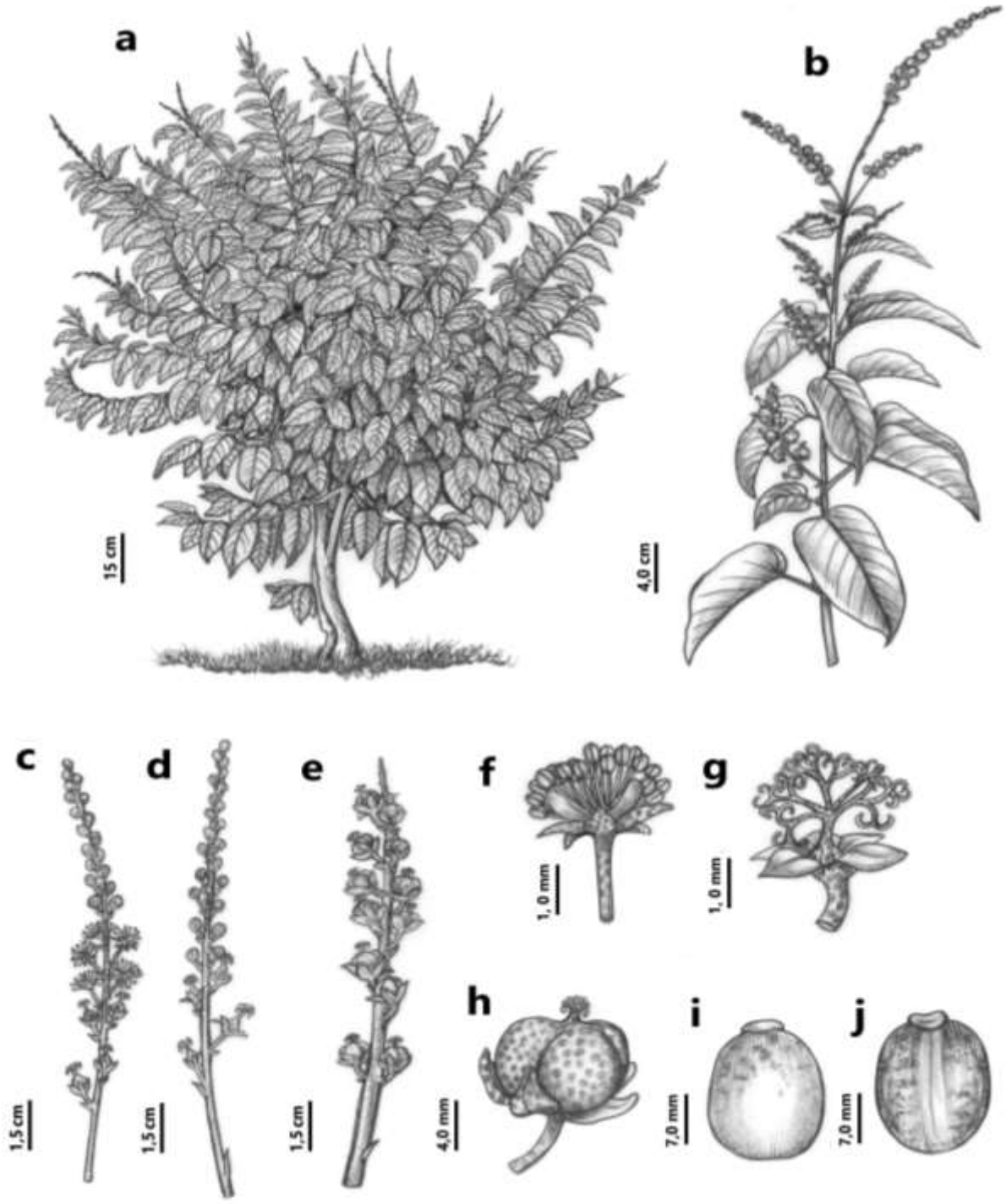

Figura 2. *Croton blanchetianus* (Euphorbiaceae): a - hábito de crescimento; b – ramo fértil; c – inflorescência (com frutos jovens na base, seguido por flores estaminadas e botões florais no ápice); d – inflorescência (com flores pistiladas na base e botões florais no ápice); e – inflorescência com frutos; f – flor estaminada; g – flor pistilada; h – fruto; i-j – semente.

2.2 Conservação e caracterização de recursos genéticos de plantas aromáticas

A conservação de recursos genéticos consiste em conservar a diversidade e a variabilidade das informações genéticas contidas nos genomas de indivíduos representativos das espécies, assegurando a manutenção de genótipos de interesse atual ou potencial (Costa *et*

al., 2012). Essa conservação é, em geral, realizada por meio de bancos de germoplasma, seja pela preservação das espécies em seus ambientes naturais (conservação *in situ*), seja pela manutenção do germoplasma fora desses ambientes, em condições artificiais (conservação *ex situ*) (Borém *et al*., 2021). O Banco Ativo de Germoplasma (BAG) consiste em uma coleção organizada de acessos do patrimônio genético de uma espécie, mantida de forma ativa e utilizada rotineiramente para fins de pesquisa, caracterização, avaliação e utilização de recursos genéticos (Costa *et al*., 2012; Borém *et al*., 2021).

A prospecção e a coleta de germoplasma constituem etapas iniciais e fundamentais para a formação e a ampliação dos BAGs, pois possibilitam a identificação, a amostragem e a incorporação da variabilidade genética existente em populações naturais e cultivadas, assegurando uma representação adequada da diversidade genética a ser conservada e utilizada em programas de pesquisa e melhoramento genético (Lleras *et al*., 1992; Costa *et al*., 2012).

Uma vez incorporados aos BAGs, os acessos passam a ser submetidos à etapa de caracterização, uma das principais atividades desenvolvidas nessas coleções, com o objetivo de conhecer e estabelecer estratégias de conservação e de uso da variabilidade genética disponível (Figura 3). A caracterização é realizada por meio de descritores que permitem avaliar os aspectos morfológicos, agronômicos e químicos das espécies (Costa *et al*., 2012; Nascimento *et al*., 2020). Os descritores são características mensuráveis ou subjetivas de um acesso, organizadas em listas específicas para cada espécie, que permitem a avaliação padronizada dos acessos por meio dos mesmos parâmetros, com o objetivo de distingui-lo dos demais. As informações obtidas podem ser de natureza quantitativa, qualitativa ou semiquantitativa (Costa *et al*., 2012).

Figura 3. Banco Ativo de Germoplasma de *Croton blanchetianus* da Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brasil, composto por 30 acessos destinados a estudos de caracterização e melhoramento genético.

A caracterização morfológica e agronômica em BAG constitui uma ferramenta fundamental para a avaliação da diversidade fenotípica entre os acessos, permitindo a identificação e a seleção de materiais agronomicamente superiores para cultivo e para inclusão em programas de melhoramento genético, além de eliminar duplicidades de acessos e diminuir custos de manutenção (Blank, 2013; Nascimento *et al*., 2020; Oliveira *et al*., 2020b). Descritores morfológicos, como a morfologia e a anatomia de órgãos vegetativos e reprodutivos

(folhas, flores, frutos, sementes e estruturas subterrâneas), e agronômicos, incluindo ciclo de maturação, época e duração da produção, produtividade por planta, teor de óleo e rendimento, quando elaborados de forma adequada para cada espécie, devem fornecer informações relevantes para a classificação e a seleção de acessos.

Estudos têm demonstrado que a caracterização morfoagronômica em BAG de plantas aromáticas tem sido uma ferramenta útil para a identificação e a seleção de acessos superiores. Em estudo conduzido por Camêlo *et al*. (2011), foram caracterizados morfoagronomicamente 48 acessos da coleção de *Lippia alba* do Banco Ativo de Germoplasma de Plantas Medicinais e Aromáticas da Universidade Federal de Sergipe, com o objetivo de identificar materiais promissores para o estado de Sergipe. Os resultados evidenciaram diferenças morfológicas entre os acessos quanto às variáveis comprimento de ramos, diâmetro da copa, coloração do caule, folhas e pétalas, hábito de crescimento, comprimento e largura foliar e relação comprimento/largura da folha. Em relação às características agronômicas, o acesso LA-37 destacou-se com maior massa seca (136,40 g/planta), o acesso LA-68, com maior teor de óleo essencial (2,80%), e o acesso LA-60, com o maior rendimento de óleo essencial (1,81 mL/planta).

Em estudo conduzido por Oliveira *et al*. (2020b), foram caracterizados morfoagronomicamente 27 acessos da coleção de *Varronia curassavica* do BAG de Plantas Medicinais e Aromáticas da Universidade Federal de Sergipe. Os acessos apresentaram ampla variabilidade fenotípica, evidenciada por diferenças morfológicas nas seguintes variáveis: coloração das folhas (diferentes tonalidades de verde), comprimento e largura foliar, relação comprimento/largura, área foliar, largura de copa, altura de planta e diâmetro do caule. Quanto ao caráter agronômico teor de óleo essencial, o acesso VCUR-105 destacou-se por apresentar o maior valor (3,20%), indicando seu potencial para utilização em programas de melhoramento genético voltados ao aumento da produção de óleo essencial.

Na caracterização da coleção composta por 22 acessos de *Lantana camara*, coletados em 15 municípios do estado de Sergipe, Nascimento *et al*. (2020) constataram ampla variabilidade fenotípica para todas as características morfológicas avaliadas. Em relação às variáveis agronômicas, a massa seca apresentou variação de 49,86 a 649,04 g/planta, enquanto o teor médio de óleo essencial oscilou entre 0,13 e 0,26% e o rendimento entre 0,10 e 1,55 mL/planta. Os acessos mais divergentes fenotipicamente foram LAC-001 (São Cristóvão) e LAC-038 (Moita Bonita), ao passo que LAC-004 (Itaporanga D'Ajuda) e LAC-019 (Siriri) apresentaram a menor divergência.

A caracterização química é de fundamental importância para o conhecimento da composição e da variabilidade dos metabólitos secundários presentes nas plantas aromáticas, especialmente dos óleos essenciais. Estudos de caracterização química de óleos essenciais em espécies aromáticas fornecem informações relevantes acerca do potencial bioativo dos acessos, subsidiando a seleção para múltiplas aplicações biotecnológicas (Oliveira *et al*., 2019; Almeida-Pereira *et al*., 2019; Rodrigues *et al*., 2023). Além disso, é possível identificar e quantificar os compostos majoritários e minoritários, estabelecer quimiotipos, compreender a influência de fatores genéticos e ambientais na biossíntese dos metabólitos, selecionar acessos superiores e orientar programas de conservação e melhoramento genético.

Em estudo realizado com seis acessos da coleção de *Varronia curassavica* do Banco Ativo de Germoplasma de Plantas Medicinais e Aromáticas da Universidade Federal de Sergipe, o óleo essencial foi caracterizado e avaliados seus efeitos letais e subletais sobre a formiga *Dorymyrmex thoracicus* (Oliveira *et al*., 2019). Foram identificados 48 compostos no óleo essencial; os principais compostos foram: (*E*)-cariofileno (6,14-22,30%), α-zimbireno (0,90-30,20%), tumerona (4,75-24,65%), 5-metil-9-metileno-2-isopropilciclodec-4-en-1-ona (4,75-27,77%) e tricicleno (1,06-22,30%). Os resultados indicam que os óleos essenciais de *V. curassavica* são uma fonte promissora para o controle da formiga urbana *D. thoracicus*, com concentrações letais que variaram de 0,69 a 2,48 μL/L.

Almeida-Pereira *et al*. (2019) avaliaram a diversidade química dos óleos essenciais de 37 plantas de *Croton tetradenius* coletadas em municípios do estado de Sergipe, bem como sua atividade antibacteriana. Foram identificados 25 compostos, sendo observada baixa variação química entre as amostras. Os constituintes majoritários foram: α-pineno, α-terpineno, p-cimeno, 1,8-cineol, *trans*-pinocarveol, cânfora, pinocarvona, *cis*-ascaridol e *trans*-ascaridol. Entretanto, os óleos essenciais não apresentaram atividade antibacteriana satisfatória frente a *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* e *Escherichia coli*.

Em estudo conduzido por Rodrigues *et al*. (2023), foram caracterizados quimicamente 29 acessos da coleção de *Croton grewioides* do Banco Ativo de Germoplasma de Plantas Medicinais e Aromáticas da Universidade Federal de Sergipe, provenientes dos estados de Sergipe e Bahia. Foram identificados 19 compostos nos óleos essenciais, sendo os majoritários: metil eugenol (90,32% em CGR-302, 87,09% em CGR-307, 82,76% em CGR-304, 84,56% em CGR-308, 82,19% em CGR-323 e 82,07% em CGR-210), metil chavicol (88,13% em CGR-324) e eugenol (80,38% em CGR-108 e 80,37% em CGR-107). Os óleos essenciais de *C. grewioides* apresentaram potencial antibacteriano frente a *Xanthomonas campestris* pv. *campestris*, com destaque para o acesso CGR-108, rico em eugenol (80,38%).

De modo geral, a conservação e a caracterização de recursos genéticos em Bancos Ativos de Germoplasma constituem estratégias essenciais para a manutenção da variabilidade genética e para o uso racional de plantas aromáticas. A integração de descritores morfológicos, agronômicos e químicos permite uma avaliação abrangente da diversidade fenotípica e química dos acessos, favorecendo a identificação de materiais superiores, a eliminação de duplicidades e a definição de estratégias de conservação e melhoramento genético. Os estudos apresentados evidenciam ampla variabilidade entre acessos de diferentes espécies, tanto em características morfoagronômicas quanto na composição de óleos essenciais, bem como no potencial bioativo desses metabólitos. Assim, a caracterização integrada em BAGs não apenas subsidia programas de melhoramento e conservação, mas também amplia as possibilidades de aplicação biotecnológica, farmacêutica e agroindustrial das espécies aromáticas, contribuindo para a valorização e o uso sustentável da biodiversidade.

2.3 Variabilidade genética de populações naturais de plantas aromáticas por meio de marcadores moleculares

Os germoplasmas nativos são considerados um valioso recurso genético para a nação, e sua conservação e uso sustentável são importantes para garantir o uso pelas atuais e futuras gerações. No entanto, fatores ambientais e antrópicos, como desmatamento e fragmentação de habitats, influenciam os padrões de diversidade das espécies vegetais (Tapia-Armijos *et al*., 2015; Silva-Júnior *et al*., 2020). Por outro lado, o interesse pelas propriedades bioativas, tanto pelas comunidades tradicionais quanto pelas indústrias de prospecção de novos produtos, leva à superexploração de espécies nativas, o que tem causado redução da base genética e aumento do risco de extinção destas antes mesmo de serem estudadas (Souza, 2015).

A estrutura genética de populações refere-se à forma como a diversidade genética está organizada e distribuída entre e dentro das populações, incluindo aspectos como a frequência alélica, os níveis de heterozigosidade, a associação entre loci (desequilíbrio de ligação) e a distribuição espacial dos genótipos (Hartl e Clark, 2007; Frankham *et al*., 2008). Em termos evolutivos, a evolução ocorre quando há alterações nas frequências alélicas ao longo do tempo, as quais podem ser impulsionadas por processos como mutação, migração (fluxo gênico), seleção natural e deriva genética (Frankham *et al*., 2008). Além desses processos, fatores ecológicos, como sistema reprodutivo, polinização, dispersão de sementes, tamanho efetivo populacional e distância entre populações, também influenciam a dinâmica e a estruturação genética (Allendorf *et al*., 2013). Esses fatores moldam a composição genética das populações ao longo das gerações, resultando em padrões de adaptação às condições ambientais e na diversidade biológica observada na natureza. Assim, a compreensão da estrutura genética

populacional é fundamental para estudos em genética de populações, conservação da biodiversidade e para o entendimento dos processos evolutivos em ambientes naturais.

O entendimento da variação genética, dentro e entre populações nativas, é importante para estabelecer práticas eficazes e eficientes de conservação de espécies aromáticas. Os marcadores moleculares são ferramentas relevantes na análise genética de populações naturais, permitindo aos pesquisadores investigar a diversidade genética e a estrutura populacional, a fim de traçar estratégias de conservação. Os marcadores moleculares consistem em sequências de nucleotídeos que podem ser exploradas a partir dos polimorfismos existentes entre indivíduos, refletindo variações nas sequências de DNA (Hasan *et al*., 2021). Esses polimorfismos resultam de eventos como deleções, inserções, substituições, duplicações e rearranjos cromossômicos, que constituem a base da variabilidade genética nas populações (Hasan *et al*., 2021). Os principais marcadores moleculares utilizados são RFLP (*Restriction Fragment Length Polymorphism*), AFLP (*Amplified Fragment Length Polymorphism*), SSR (*Simple Sequence Repeats*), ISSR (*Inter Simple Sequence Repeat*), RAPD (*Random Amplified Polymorphic Sequences*), CAPS (*Cleavable Amplified Polymorphic Sequences*), SSCP (*Single-strand Conformation Polymorphisms*) e SNPs (*Single Nucleotide Polymorphisms*) (Khlestkina, 2014). Os marcadores moleculares ISSR são geralmente empregados para estudar a diversidade genética em populações naturais e bancos de germoplasma, pois podem identificar a diversidade genética sem a necessidade de informações prévias sobre o genoma (González *et al*., 2002; Tohidi *et al*., 2019). Além disso, os marcadores ISSR possuem alto nível de polimorfismo, potencial de automação, alta reprodutibilidade e baixo custo, quando comparados com outros marcadores, além de necessitarem de baixas quantidades de DNA para iniciar a amplificação em PCR (reação em cadeia da polimerase) (Borba *et al*., 2005; Rustaiee *et al*., 2013).

A variabilidade genética de populações naturais foi acessada por meio de marcadores moleculares ISSR para as espécies: *Copernicia prunifera* (Vieira *et al*., 2015), *Capparis spinosa* (Liu *et al*., 2015), *Panax stipuleanatus* (Trieu *et al*., 2016), *Bertholletia excelsa* (Ramalho *et al*., 2016), *Dalbergia nigra* (Silva-Júnior *et al*., 2020), *Pityrocarpa moniliformis* (Felix *et al*., 2020), *Papaver bracteatum* (Hadipour *et al*., 2020), *Dorstenia elata* (Martins *et al*., 2021), *Lippia origanoides* (Feijó *et al*., 2022) e *Croton blanchetianus* (Costa *et al.*, 2025). Os estudos demonstraram que os marcadores moleculares ISSR são eficientes na determinação dos níveis de polimorfismos entre os indivíduos e na detecção da diversidade genética entre populações.

Dentro do gênero *Croton*, estudos sobre variabilidade genética utilizando marcadores moleculares têm apresentado resultados positivos na elucidação dos padrões de diversidade e estrutura genética de populações naturais (Tabela 1). Ângelo *et al*. (2005), ao avaliarem a variabilidade genética entre plantas de *Croton cajucara* (sacaca) por meio de marcadores moleculares do tipo RAPD, demonstraram que esses marcadores foram eficazes na detecção da diversidade genética entre os acessos da espécie. Em populações naturais de *Croton antisyphiliticus* coletadas nos estados de Minas Gerais, São Paulo e Goiás, a análise da variabilidade genética por meio de marcadores moleculares AFLP indicou que a espécie apresenta estrutura do tipo modelo de ilhas, evidenciando sua vulnerabilidade genética (Oliveira *et al*., 2016). Rocha *et al*. (2016) analisaram a diversidade genética de 41 genótipos de *Croton heliotropiifolius* provenientes de um fragmento florestal em Itapetinga, Bahia, e inferiram que a estrutura genética observada pode ser resultado da dinâmica de dispersão de sementes e/ou de pólen.

Em um estudo com 40 genótipos de *Croton tetradenius* provenientes do estado de Sergipe, Almeida-Pereira *et al*. (2017) observaram variância genética significativa entre e dentro de populações naturais por meio de marcadores ISSR. Por sua vez, Brito *et al*. (2021), ao avaliarem 38 genótipos da mesma espécie na mesma região utilizando marcadores SNP, relataram baixa diversidade genética e reduzida estruturação genética entre as populações analisadas.

Em estudo realizado com populações nativas de *Croton linearifolius*, utilizando marcadores ISSR, foi possível diferenciar os 61 genótipos de acordo com as populações e/ou o local de origem (Silva *et al*., 2020). Costa *et al*. (2020a) identificaram alta variabilidade genética entre três populações de *Croton urucurana* distribuídas em áreas de Cerrado e florestas tropicais no Brasil, por meio de ISSR. Oliveira *et al*. (2022c), ao estudarem populações de *Croton grewioides* nos estados de Sergipe e Bahia por meio de marcadores SNP, conseguiram acessar a diversidade e a estrutura genética dos genótipos em função do local de origem.

Tabela 1. Diversidade e estrutura genética do gênero *Croton* a partir de marcadores moleculares.

Espécies	Marcadores	Nº primers/ SNPs	Resultado	Referência
Croton cajuçara	RAPD	10 primers	Moderada diversidade genética de *Croton cajuçara*	Angelo *et al*. (2005)
Croton antisyphiliticus	AFLP	08 primers	Alta diversidade genética de *C. antisyphiliticus*	Oliveira (2016)
Croton heliotropiifolius	RAPD e ISSR	RAPD: 18 primers; ISSR: 15 primers	Moderada a alta diversidade genética de *C. heliotropiifolius*	Rocha *et al*. (2016)
Croton tetradenius	ISSR	13 primers	Moderada diversidade genética de *C. tetradenius*	Almeida-Pereira *et al*. (2017)
Croton linearifolius	ISSR	08 primers	Moderada a alta diversidade genética de *C. linearifolius*	Silva *et al*. (2020)
Croton urucurana	ISSR	12 primers	Moderada a alta diversidade genética de *C. urucurana*	Costa *et al*. (2020a)
Croton tetradenius	SNP	1387 SNPs	Baixa diversidade genética de *C. tetradenius*	Brito *et al*. (2021)
Croton grewioides	SNP	6942 SNPs	Moderada diversidade genética de *C. grewioides*	Oliveira *et al*. (2022c)
Croton blanchetianus	IRRS	15 primers	Moderada a baixa diversidade genética de *C. blanchetianus*	Costa *et al*. (2025)

Costa *et al*. (2025), ao avaliarem a diversidade e a estrutura genética de seis populações nativas de *C. blanchetianus* provenientes de diferentes municípios do estado de Sergipe, utilizando marcadores moleculares ISSR, concluíram que o processo de fragmentação das áreas em que a espécie ocorre contribui para a reduzida variabilidade genética observada no presente estudo. Essa redução é atribuída ao declínio no número de indivíduos nas áreas e, consequentemente, à ocorrência de cruzamentos entre indivíduos aparentados. Além disso, as populações apresentam baixa diferenciação genética, uma vez que não é possível diferenciar os genótipos de acordo com seu local de origem.

Em síntese, os germoplasmas nativos representam um recurso genético de grande importância, porém vêm sendo ameaçados por fatores antrópicos e ambientais que reduzem a variabilidade genética e aumentam a vulnerabilidade das populações naturais. A aplicação de marcadores moleculares, especialmente os ISSR, tem se mostrado eficiente na caracterização da diversidade e da estrutura genética, permitindo compreender os efeitos da fragmentação no fluxo gênico e nos processos evolutivos. Esses estudos são fundamentais para subsidiar estratégias de conservação e uso sustentável das espécies, incluindo aquelas do gênero *Croton*, garantindo a manutenção de sua diversidade genética ao longo das gerações.

2.4 Diversidade química dos óleos essenciais de plantas aromáticas

Os óleos voláteis são misturas de substâncias químicas complexas de baixo peso molecular, que apresentam, principalmente, em sua composição, terpenoides e fenilpropanoides, oriundos do metabolismo secundário das plantas (Simões *et al*., 2017). Apresentam-se como líquidos oleosos de aroma intenso em temperatura ambiente, obtidos de matérias-primas vegetais, como flores, frutos, sementes, brotos, folhas, galhos, cascas, madeira e raízes (Tohidi *et al*., 2019). Entretanto, sua principal característica é a volatilidade, o que os difere dos óleos fixos; além disso, não são muito estáveis, principalmente na presença de ar, luz, calor, umidade e metais (Simões *et al*., 2017).

Nas plantas, os óleos essenciais contribuem substancialmente para sua sobrevivência, pois estão envolvidos na proteção contra ataques de patógenos e herbívoros, na atração de polinizadores, além das ações de proteção contra estresses abióticos, como mudanças na temperatura e na umidade do ar, proteção contra raios solares, sinalização entre órgãos vegetais distintos e entre indivíduos da mesma espécie e no efeito alelopático (Unsicker *et al*., 2009; Glinwood *et al*., 2011; Simões *et al*., 2017). Os óleos essenciais estão presentes nas plantas em estruturas morfológicas especializadas, responsáveis pela sua secreção e estocagem. Entre as estruturas especializadas, destacam-se as células oleíferas, estruturas de depósito intracelulares (bolsas lisígenas e esquizógenas), tricomas e escamas glandulares (Simões *et al*., 2017). No gênero *Croton*, estudos comprovam que os óleos essenciais estão presentes em glândulas secretoras, como os idioblastos, dispersas nas células do mesofilo e tricomas glandulares (Mendonça *et al*., 2008; Barros e Soares, 2013; Rosa *et al*., 2021).

As principais vias para a formação de óleos voláteis são a via do ácido mevalônico (MEV) e a via do metileritritol fosfato (MEP), para os terpenoides, e a via do ácido chiquímico, para os fenilpropanoides (Figura 4). Os terpenoides são biossintetizados a partir de metabólitos primários, partindo-se de duas rotas principais: a via do MEV, a qual ocorre no citoplasma, e a via MEP, que ocorre no plastídio (Simões *et al*., 2017).

A via do ácido mevalônico se inicia com a condensação de piruvato e unidades acetato, na forma de acetil-CoA. Então, ocorre a condensação de três moléculas do acetil-CoA e descarboxilação subsequente para formar o ácido mevalônico. Esta molécula é então pirofosforilada, descarboxilada e desidratada para produzir o IPP (isopentenil difosfato), que é a unidade básica para a formação dos terpenos (Figura 4). Já a via do MEP ocorre inicialmente pela condensação entre piruvato e 3-fosfoglicerato para formar 1-deoxixilulose-5-P (DOXP). Em seguida, a 1-desoxi-xilulose-5-P redutoisomerase (DXR) converte DOXP em MEP (Gutbrod *et al*., 2019). Os isoprenos, precursores universais contendo cinco carbonos, após a ligação com radicais fosfato, originam as duas unidades básicas de 5 carbonos, o IPP e seu isômero, o difosfato de dimetilalil (DMAPP), a partir das quais os terpenoides são biossintetizados, via MEV e MEP, respectivamente. Os precursores (IPP e DMAPP) sofrem condensação para formar diferentes classes de terpenoides, dependendo do número de carbonos, podem ser denominados: monoterpenos (C10), sesquiterpenos (C15), diterpenos (C20), triterpenos (C30) e tetraterpenos (C40) (Simões *et al*., 2017).

Já os fenilpropanoides são formados pela via do ácido chiquímico. O ácido chiquímico é formado pela condensação de dois metabólitos da glicose (fosfoenolpiruvato e a eritrose-4-fosfato) (Figura 4) (Simões *et al*., 2017). Uma vez formado, o ácido chiquímico une-se como uma molécula de fosfoenolpiruvato para formação do ácido corísmico. O ácido corísmico sofre um rearranjo e forma o ácido prefênico, catalisado pela enzima corismato mutase. O ácido prefênico passa por uma aromatização descarboxilativa, resultando no ácido fenilpirúvico. Em seguida, por meio de uma reação de transaminação, o ácido fenilpirúvico se transforma em L-fenilalanina. Depois da biossíntese da fenilalanina, esta é convertida em ácido cinâmico, que é o precursor de diversos compostos fenólicos, incluindo o eugenol e o chavicol (Simões *et al*., 2017).

A composição dos óleos essenciais dos vegetais, bem como de outros metabólitos secundários, é determinada geneticamente, estando geralmente associada a órgãos específicos

e a determinados estágios de desenvolvimento da planta (Simões *et al*., 2017). No entanto, existe uma grande variabilidade nos compostos encontrados nos óleos essenciais, tanto entre quanto dentro das espécies. Os vegetais que são botanicamente idênticos, mas diferem em termos químicos, são denominados quimiotipos ou raças químicas (Simões *et al*., 2017). Apesar do perfil fitoquímico ser determinado pela constituição genética da espécie, as condições ambientais são capazes de causar variações significativas na composição química dos óleos essenciais.

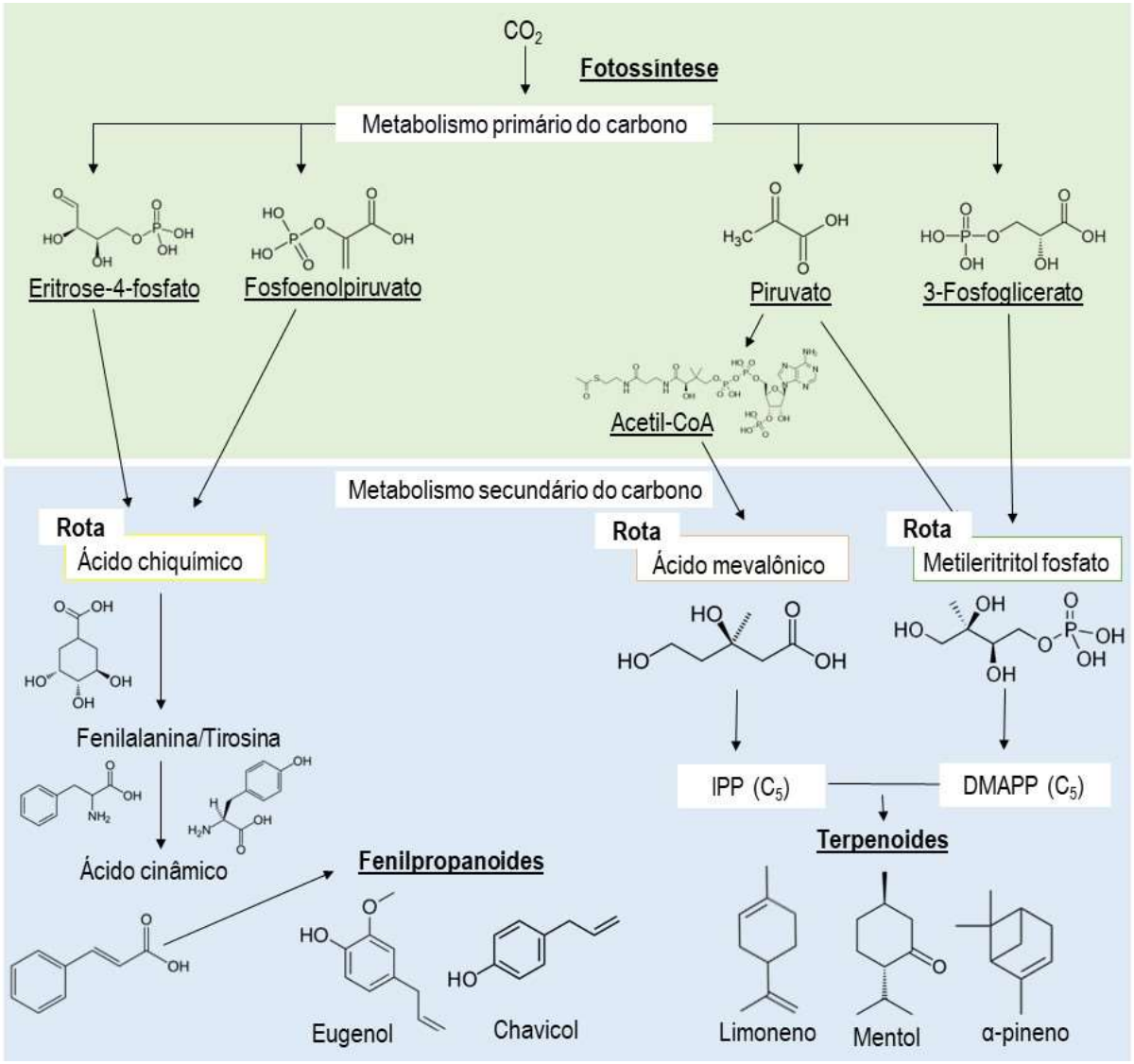

Figura 4. Esquema de síntese das vias do ácido chiquímico, ácido mevalônico e do metileritritol fosfato, de acordo com Simões *et al*. (2017).

Fatores extrínsecos, observados no ambiente em que a planta está inserida, podem influenciar a composição química dos óleos voláteis, como temperatura, umidade relativa, luminosidade, ventos, disponibilidade hídrica e nutricional, ataque de pragas e doenças, época do ano, hora da colheita das plantas e até mesmo os métodos de extração (Gobbo-Neto e Lopes, 2007; Santos *et al*., 2016a; Simões *et al*., 2017; Nizio *et al*., 2018; Ribeiro *et al*., 2018; Facanali *et al*., 2020; Sá Filho *et al*., 2022). Tais fatores podem levar a mudanças significativas na composição química, pois podem redirecionar a síntese dos metabólitos (Soares *et al*., 2019). Segundo Simões *et al*. (2017), não se pode prever ou estabelecer um único padrão, pois cada espécie pode reagir de forma diferenciada.

2.5 Composição química e atividades biológicas de óleos essenciais de plantas aromáticas

Os óleos essenciais têm sido amplamente estudados em razão de sua diversidade de compostos bioativos e da crescente demanda por produtos naturais. Constituídos por misturas complexas de substâncias voláteis, podem apresentar de 20 a 200 componentes em proporções variáveis, de acordo com a sua abundância na mistura. Até o momento, são descritos aproximadamente 3.000 óleos essenciais, dos quais cerca de 300 apresentam relevância comercial, especialmente para as indústrias farmacêutica, agrícola, alimentícia, sanitária, cosmética e de perfumaria (Bakkali *et al*., 2008). Por exemplo, o óleo essencial de *Aniba rosaeodora* é caracterizado por elevado teor de linalol (80,00-95,00%), sendo amplamente usado na indústria de perfumaria; o de *Citrus sinensis* apresenta como constituinte majoritário o limoneno (85,00-98,00%), com uso predominante como aromatizante na indústria de bebidas e alimentos; o óleo de *Eucalyptus globulus* é rico em 1,8-cineol (60,00-85,00%), sendo utilizado como aromatizante e na formulação de medicamentos; enquanto o óleo essencial de *Mentha* × *piperita*, com altos teores de mentol (30,00-60,00%) e mentona (10,00-30,00%), é amplamente empregado como aromatizante e em produtos de higiene oral (Bizzo e Rezende, 2022).

Cerca de 30% das espécies vegetais analisadas apresentam, em sua composição, óleos essenciais, distribuídos entre as principais famílias botânicas produtoras desses metabólitos, reconhecidas pela abundância, diversidade química e importância econômica (Simões *et al*., 2017). Dentre as principais destacam-se: Lamiaceae (*Ocimum*, *Rosmarinus*, *Thymus*, *Mentha*, *Lavandula*, *Origanum* e *Salvia*); Myrtaceae (*Eucalyptus*, *Melaleuca*, *Psidium*, *Eugenia* e *Syzygium*); Rutaceae (*Citrus*, *Ruta* e *Zanthoxylum*); Apiaceae (*Foeniculum*, *Coriandrum*, *Pimpinella*, *Anethum* e *Carum*); Asteraceae (*Artemisia*, *Matricaria*, *Achillea* e *Tagetes*); Lauraceae (*Cinnamomum*, *Ocotea*, *Laurus* e *Aniba*); Piperaceae (*Piper*); Euphorbiaceae (*Croton*); Zingiberaceae (*Zingiber*, *Curcuma* e *Alpinia*); Verbenaceae (*Lippia*, *Lantana* e *Aloysia*); e Poaceae (*Cymbopogon* e *Vetiveria*) (Bakkali *et al*., 2008; Simões *et al*., 2017; Bizzo e Rezende, 2022).

A diversidade química dos óleos essenciais tem sido pesquisada para as espécies: *Lippia alba* (Blank *et al*., 2015), *Lippia sidoides* (Santos *et al*., 2015), *Varronia curassavica* (Nizio *et al*., 2015), *Myrcia ovata* (Sampaio *et al*., 2016), *Croton tetradenius* (Almeida-Pereira *et al*., 2019), *Lantana camara* (Pereira *et al*., 2020), *Eplingiella fruticosa* (Pinto *et al*., 2021), *Croton grewioides* (Rodrigues *et al*., 2023) e *Ocimum basilicum* (Kirci *et al*., 2025).

Blank *et al*. (2015) evidenciaram variabilidade química ao caracterizarem os óleos essenciais de 48 acessos de *L. alba* do BAG-UFS, nos quais foram identificados 33 constituintes. Os compostos mais abundantes entre os acessos foram 1,8-cineol (0,00-9,22%), linalol (0,00-84,73%), mirceno (0,00-2,85%), limoneno (0,00-45,35%), carvona (0,00-39,58%), geranial (0,00-54,05%) e neral (0,00-32,14%), o que resultou na formação de seis grupos químicos distintos: grupo 1: linalol, 1,8-cineol e óxido de cariofileno; grupo 2: linalol, geranial, neral, 1,8-cineol e óxido de cariofileno; grupo 3: limoneno, carvona e sabineno; grupo 4: carvona, limoneno, δ-muurolene e mirceno; grupo 5: neral, geranial e óxido de cariofileno; e grupo 6: geranial, neral, o-cimeno, limoneno e óxido de cariofileno.

Em *L. sidoides*, Santos *et al*. (2015) avaliaram a diversidade química de dez acessos do BAG-UFS, bem como a influência da idade das plantas (2 e 8 anos) sobre a composição química dos óleos essenciais. O principal composto dos genótipos foi o timol (8,41-83,20%), com exceção do genótipo LSID104, que apresentou carvacrol (72,39 e 55,39%) como composto majoritário. A análise permitiu a formação de dois grupos químicos: o grupo 1, composto exclusivamente pelo genótipo LSID104, caracterizado pela predominância de carvacrol; e o grupo 2, constituído por nove genótipos (LSID002, LSID003, LSID004, LSID005, LSID006, LSID102, LSID103, LSID105 e LSID301), nos quais o timol ocorreu em maior proporção, variando de 56,65 a 83,20% em plantas com 2 anos e de 62,60 a 73,56% em plantas com 8 anos.

O estudo da composição química do óleo essencial de populações nativas de 59 plantas *de V. curassavica*, provenientes de cinco localidades do estado de Sergipe, revelou alta variabilidade química, com a identificação de 53 compostos e a formação de cinco grupos químicos, definidos com base nos constituintes majoritários. O grupo 1: representado por

turmerona (8,96-30,15%) e E-cariofileno (4,60-20,17%); grupo 2: tricicleno (20,82-35,95%) e canfeno (16,62-27,38%); grupo 3: αzingibereno (24,81-35,83%) e β-sesquifelandreno (10,72-16,19%); grupo 4: (*E*)-cariofileno (3,90-31,06%) e 7-ciclodecen-1-ona,7-metil-3-metileno-10-(1-propil); e o grupo 5: 7-ciclodecen1-ona,7-metil-3-metileno-10-(1-propil) (24,68-50,20%) (Nizio *et al*., 2015).

Sampaio *et al*. (2016), ao investigarem 37 plantas nativas de *M. ovata* coletadas no Nordeste do Brasil, observaram elevada diversidade química entre os óleos essenciais, os quais foram distribuídos em seis grupos por meio de análise de agrupamento. No grupo 1 (11 plantas): ácido nerólico (44,30-73,97%); grupo 2 (7 plantas): linalol (14,97-30,97%) e ácido nerólico (50,56-60,63%); grupo 3 (3 plantas): geraniol (73,64 a 77,07%); grupo 4 (3 plantas): neral (18,21-28,39%) e geranial (36,96-48,48%); grupo 5 (4 plantas): E-nerolidol (26,97-58,27%); e grupo 6 (9 plantas): linalol (0,44-25,35%), isopulegol (0,00-27,50%) e ácido nerólico (0,00-31,65%).

Os óleos essenciais de 105 plantas nativas de *L. camara* coletadas no Nordeste do Brasil apresentaram diversidade química, com 27 compostos identificados, formando sete grupos químicos. Os principais constituintes foram (*E*)-cariofileno (4,10-53,40%), germacreno D (2,80-48,30%), biciclogermacreno (0,00-18,90%), α-humuleno (0,70-39,00%) e α-curcumeno (24,90-34,30%), que definiram a formação dos grupos. O Grupo 1 (12 plantas) destacou-se por (*E*)-cariofileno (24,20-39,20%), α-humuleno (2,70-18,00%) e germacreno D (7,00-23,00%); o Grupo 2 (25 plantas), por (*E*)-cariofileno (22,20-42,40%) e germacreno D (13,90-35,20%); o Grupo 3 (9 plantas), por (*E*)-cariofileno (46,00-53,40%) e germacreno D (9,70-24,70%); o Grupo 4 (21 plantas), por (*E*)-cariofileno (4,10-31,40%), germacreno D (18,60-48,30%) e biciclogermacreno (0,00-18,90%); o Grupo 5 (18 plantas), por E-cariofileno (6,70-27,90%), germacreno D (3,60-24,30%) e biciclogermacreno (6,80-17,60%); o Grupo 6 (3 plantas), por (*E*)-cariofileno (13,10-18,40%), α-curcumeno (24,90-34,30%) e germacreno D (2,80-16,50%); e o Grupo 7 (16 plantas), por (*E*)-cariofileno (14,00-33,40%), α-humuleno (11,60-39,00%) e germacreno D (7,40-33,90%) (Pereira *et al*., 2020).

Kirci *et al*. (2025), ao investigarem as variações fitoquímicas entre 34 cultivares de *O. basilicum,* com o objetivo de avaliar a composição de seus óleos essenciais, identificaram 48 compostos voláteis, evidenciando acentuada variabilidade química entre os cultivares. O linalol foi o constituinte mais abundante, predominando em 28 cultivares (24,20-81,10%), e o metil chavicol apresentou abundância relativa notavelmente elevada nas cultivares ‘Cardinal’ (46,00%), ‘Mammoth’ (12,80%), ‘Napoletano’ (10,20%), ‘Nufar’ (14,90%), ‘Purple Ruffles’ (14,30%), ‘Queenette’ (53,80%), ‘Siam Queen’ (82,00%) e ‘Sweet Thai’ (70,20%). Adicionalmente, algumas cultivares exibiram altos teores de compostos específicos: em ‘Citriodorum’ foram encontrados geranial (15,70%), nerol (15,50%) e óxido de cariofileno (11,30%) em quantidades expressivas. O metil (*E*)-cinamato esteve presente a 15,00% e 24,80% nas cultivares ‘Christmas’ e ‘Cinnamon’, respectivamente. O eugenol foi detectado em ‘Aristotle’, ‘Genovese’, ‘Minette’, ‘Picolino’, ‘Pistou’ e ‘Spicy Bush’, com concentrações de 9,00%, 18,10%, 12,30%, 10,4%, 11,70% e 10,90%, respectivamente. Adicionalmente, o α-cadinol foi identificado a 15,7% em ‘Valentino’. Esses compostos podem atuar como importantes marcadores químicos característicos dos perfis de óleo essencial de suas respectivas cultivares.

No estudo da composição química do óleo essencial de seis populações de *E. fruticosa* do estado de Sergipe, foram identificados 51 compostos, sendo os majoritários: α-pineno, β-pineno, 1,8-cineol, cânfora, borneol, δ-elemeno, α-cubebeno, α-ilangeno, (*E*)-cariofileno, germacreno D, biciclogermacreno, *trans*-calameneno, espatulenol, óxido de cariofileno e viridiflorol. A análise da composição permitiu a formação de dois grupos distintos: o Grupo 1, formado pelas populações de São Cristóvão, Itaporanga d’Ajuda, Japaratuba e Malhada dos Bois, caracterizado pela predominância de cânfora (8,39-11,27%); e o Grupo 2, constituído pelas populações de Moita Bonita e Pirambu, definido pela maior presença de biciclogermacreno (7,45 e 10,98%) (Pinto *et al*., 2021).

Em estudo conduzido por Almeida-Pereira *et al*. (2019), observou-se baixa variação química nos óleos essenciais de 37 plantas de *C. tetradenius* provenientes do estado de Sergipe. A análise permitiu a formação de dois agrupamentos químicos: Grupo 1, caracterizado pelos principais compostos α-terpineno (0,90-7,74%), p-cimeno (12,22-23,38%), 1,8-cineol (8,43-19,46%), cânfora (6,82-11,45%), cis-ascaridol (4,10-7,23%) e trans-ascaridol (10,16-19,20%); e Grupo 2, definido por α-pineno (3,75-10,33%), p-cimeno (7,16-11,22%), 1,8-cineol (4,04-12,99%), trans-pinocarveol (0,00-13,61%), cânfora (11,35-17,05%), pinocarvona (0,00-8,89%) e trans-ascaridol (2,55–11,65%). Já Rodrigues *et al*. (2023), ao realizarem a caracterização química de 29 acessos da coleção de *C. grewioides* do BAG-UFS, provenientes dos estados de Sergipe e Bahia, identificaram 19 compostos nos óleos essenciais, sendo os majoritários: metil eugenol (90,32% em CGR-302, 87,09% em CGR-307, 82,76% em CGR-304, 84,56% em CGR-308, 82,19% em CGR-323 e 82,07% em CGR-210), metil chavicol (88,13% em CGR-324) e eugenol (80,38% em CGR-108 e 80,37% em CGR-107).

A diversidade química confere aos óleos essenciais ampla gama de atividades biológicas, que têm sido extensivamente investigadas. Entre as principais, destacam-se as atividades antimicrobiana, antifúngica, antioxidante, antinociceptiva, anti-inflamatória, analgésica, inseticida, acaricida, larvicida e antitumoral (Sousa *et al*., 2004; Mendes *et al.*, 2010; Lima *et al*., 2012; Silva *et al*., 2019; Oliveira *et al*., 2019; Sampaio *et al*., 2020; Penha *et al*., 2021; Oliveira *et al*., 2022c; Lopes *et al*., 2025). Tais atividades estão relacionadas à ação de um composto ou à ação sinérgica entre os diferentes constituintes, sendo frequentemente atribuídas tanto aos compostos majoritários quanto à contribuição de componentes que podem potencializar ou modular os efeitos biológicos.

Em estudo conduzido por Couto *et al*. (2021), foi realizado um ensaio de triagem com 72 amostras de óleos essenciais de *Myrcia lundiana*, *O. basilicum* e *L. alba* frente a seis bactérias patogênicas deterioradoras de alimentos (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* e *Enterobacter sakazakii*). Duas amostras de *L. alba*, três de *M. lundiana* e sete de *O. basilicum* apresentaram concentrações mínimas inibitórias (CMI) variando de 0,12 a 125 μL mL^{-1} para as bactérias avaliadas. Dentre estas, o óleo essencial de *O. basilicum* cv. Maria Bonita destacou-se por apresentar os menores valores de CMI. Adicionalmente, foi preparada uma mistura que simulou esse óleo essencial a partir de padrões comerciais de linalol, geraniol e 1,8-cineol; entretanto, essa formulação apresentou valores de CMI significativamente mais elevados em comparação ao óleo essencial natural, sugerindo a ocorrência de efeito sinérgico entre os compostos.

Na família Myrtaceae, em estudo conduzido com o óleo essencial de *M. ovata*, foi avaliada a diversidade química e o potencial antifúngico frente a *Fusarium solani*. Os óleos essenciais, caracterizados principalmente pela presença de ácido nerólico, linalol, geraniol, isômeros neral e geranial, (*E*)-nerolidol e isopulegol, promoveram inibição total (100%) do crescimento micelial de *F. solani* em todas as concentrações testadas após 96 h, evidenciando o elevado potencial antifúngico da espécie (Sampaio *et al*., 2016). Em estudo conduzido por Melo *et al*. (2021), foi avaliada a atividade formicida de óleos essenciais de dois quimiotipos de *M. lundiana* sobre operárias de *Acromyrmex balzani*. Os quimiotipos foram caracterizados principalmente por elevadas concentrações de isopulegol e citral, sendo o 1,8-cineol o segundo constituinte mais abundante. Os óleos essenciais apresentaram toxicidade significativa para *A. balzani*, promovendo rápida mortalidade e alterações no comportamento locomotor das operárias, com destaque para o isopulegol como o composto de maior toxicidade e para o quimiotipo rico em citral, que exibiu efeito repelente mais persistente.

Na família Verbenaceae, as espécies *L. alba*, *L. sidoide*, *Lippia gracilis* e *L. camara* apresentam amplas atividades biológicas. Em *L. alba*, foi avaliado o potencial acaricida do óleo essencial contra *Rhipicephalus microplus*. Foram utilizados dois quimiotipos: citral (genótipos LA-10 e LA-44), que consistiram principalmente de geranial/neral em 46,20%/33,50% e 44,20%/31,10%, respectivamente; e carvona (genótipos LA-13 e LA-57), que consistiram principalmente de carvona/limoneno em 52,90%/26,90% e 63,50%/25,80%, respectivamente.

Os genótipos exibiram elevada atividade larvicida, o que foi atribuído à presença de limoneno e carvona como componentes majoritários do óleo essencial (Peixoto *et al*., 2015). Em estudo conduzido por Melo *et al*. (2022), foi avaliada a atividade antifúngica dos óleos essenciais de genótipos de *L. gracilis* (LGRA-106 e LGRA-109) e *L. sidoides* (LSID-102 e LSID-104) frente a *Lasiodiplodia theobromae*. Os óleos essenciais ricos em timol (LGRA-106: 61,84%; LSID-102: 64,07%) e em carvacrol (LGRA-109: 54,56%; LSID-104: 69,06%) apresentaram expressiva atividade antifúngica, evidenciando o papel desses compostos como principais responsáveis pela ação inibitória. Já em *L. camara*, foi avaliada a composição química do óleo essencial de diferentes acessos em duas épocas de colheita e a atividade tripanocida frente a *Phytomonas serpens*. A composição foi dominada por *E*-cariofileno, α-humuleno, α-curcumeno e germacreno D. Os óleos essenciais apresentaram elevada atividade tripanocida, inibindo o crescimento de *P. serpens* em baixas concentrações, com valores de IC_{50} de 18,34 ± 6,60 μg mL^{-1} (LAC-018), 9,14 ± 3,87 μg mL^{-1} (LAC-027), 14,56 ± 3,40 μg mL^{-1} (LAC-037) e 14,97 ± 2,68 μg mL^{-1} (LAC-019), evidenciando o expressivo potencial biológico da espécie (Pereira *et al*., 2022).

Em Euphorbiaceae, o gênero *Croton* apresenta diferentes classes químicas de compostos com propriedades bioativas, como terpenoides e fenilpropanoides. O potencial antimicrobiano do óleo essencial de *C. grewioides* foi demonstrado por Rodrigues *et al*. (2023), que observaram elevada eficácia do óleo essencial do acesso CGR-108, rico em eugenol (80,38%), no controle do fitopatógeno *Xanthomonas campestris* pv. *campestris*. Em estudo com *Croton campinarensis*, Costa *et al*. (2022) relataram atividade antioxidante do óleo essencial, cujos principais constituintes foram germacreno D (26,95%), biciclogermacreno (17,08%), (E)-cariofileno (17,06%) e δ-elemeno (7,59%), avaliada pelos métodos ABTS e DPPH. Em estudo conduzido por Brito *et al*. (2020), foi avaliada a atividade formicida dos óleos essenciais de *Croton tetradenius* sobre *Acromyrmex balzani*. Os óleos, obtidos de cinco populações naturais do estado de Sergipe e ricos em 1,8-cineol, α-pineno, cânfora, E-pinocarveol e *p*-cimeno, apresentaram elevada toxicidade, com valores de CL_{50} variando de 1,47 a 2,40 μL L^{-1} para os óleos essenciais e de 0,45 a 11,20 μL L^{-1} para os constituintes majoritários. Dentre os compostos avaliados, a cânfora destacou-se como o mais tóxico, evidenciando sua contribuição para a atividade inseticida observada.

A análise dos estudos reunidos na Tabela 2 evidencia que os óleos essenciais de *C. blanchetianus* apresentam ampla variabilidade química, sendo predominantemente constituídos por monoterpenos e sesquiterpenos. Entre os compostos majoritários mais relatados, destacam-se biciclogermacreno (10,42-33,50%), 1,8-cineol (19,95-32,94%), (*E*)-cariofileno (9,84-27,52%), espatulenol (8,07-24,10%), α-pineno (9,14-29,43%) e germacreno D (10,89-20,60%), cujas concentrações variam consideravelmente entre os diferentes trabalhos, refletindo a influência de fatores genéticos, ambientais e metodológicos (Tabela 1) (Angélico *et al*., 2014; Ribeiro *et al*., 2018; Rodrigues *et al*., 2019; Camara *et al*., 2021; Porto *et al*., 2021; Vasconcelos *et al*., 2022a e 2022b; Cavalcante *et al*., 2022; Nunes *et al*., 2023; Nascimento *et al*., 2024; Venancio *et al*., 2025; Lopes *et al*., 2025). Os óleos essenciais foram extraídos predominantemente de folhas frescas ou secas, coletadas em diferentes estados do Nordeste brasileiro, incluindo Ceará, Paraíba, Pernambuco, Piauí e Rio Grande do Norte.

O óleo essencial de *C. blanchetianus* destaca-se por possuir diferentes propriedades biológicas e uma ampla aplicabilidade. Atividades biológicas do óleo essencial desta espécie foram reportadas por diversos autores, como atividade antibacteriana contra cepas de bactérias Gram-positivas (*Bacillus cereus, Staphylococcus aureus*) (Angélico *et al*., 2014; Venancio *et al*., 2025), e contra importantes bactérias responsáveis pela deterioração de produtos cárneos (*Listeria monocytogenes*, *Staphylococcus aureus*, *Leuconostoc mesenteroides* e *Weissella viridescens*) (Vasconcelos *et al*., 2022a e 2022b). A atividade inseticida sobre o coleóptero *Callosobruchus maculatus* em grãos armazenados de feijão-caupi foi comprovada por Silva *et al*. (2020). Rodrigues *et al*. (2019) e Camara *et al*. (2021) mostraram a atividade acaricida sobre ectoparasitos em bovinos: *Rhipicephalus* (Boophilus) *microplus* e o ácaro rajado (*Tetranychus*

urticae), respectivamente. A atividade antinociceptiva associada aos compostos α-pineno, β-felandreno e terpinoleno também foi relatada por Nascimento *et al*. (2024). Adicionalmente, os compostos α-pineno, β-felandreno, eucaliptol e β-cariofileno demonstraram atividade larvicida significativa em ensaios in vivo contra *Aedes aegypti*, reforçando o potencial de *C. blanchetianus* como fonte de compostos naturais para o controle de vetores (Lopes *et al*., 2025).

De modo geral, os estudos indicam que a diversidade química dos óleos essenciais de *C. blanchetianus* está diretamente relacionada à multiplicidade de atividades biológicas observadas, destacando essa espécie como promissora para aplicações farmacológicas, agropecuárias e no controle sustentável de pragas e vetores.

Tabela 2. Principais compostos detectados nos óleos essenciais de *C. blanchetianus.*

Compostos majoritários	Atividade relatada	Metodologia	Referência
Cedrol (28,4%), 1,8-cineol (17,4%) α-pineno (10,5%)	Antibacteriana (*Bacillus cereus* e *Staphylococcus aureus*)	Experimento *in vitro* em placa de petri	Angélico *et al*. (2014)
(*E*)-cariofileno (27,52%) Espatulenol (23,26%) ρ-cimen-8-ol (13,87%)	-	-	Ribeiro *et al*. (2018)
Cedrol (28,4%), 1,8-cineol (17,4%) α-pineno (10,5%)	Acaricida (*Rhipicephalus* (Boophilus) *microplus*)	Experimento *in vitro* em placa de petri	Rodrigues *et al*. (2019)
Espatulenol (24,10%) δ-cadineno (17,86%) β-acorenol (11,16%)	Acaricida (*Tetranychus urticae*)	Experimento *in vitro* em placa de petri	Camara *et al*. (2021)
α-pineno (19,19%) (*E*)-cariofileno (11,85%) Biciclogermacreno (10,42%) Limoneno (10,36%)	Antifúngico (Atividade não detectada)	Experimento *in vitro* em placa de petri	Porto *et al*. (2021)
1,8-cineol (16,95%) Biciclogermacreno (11,91%) (*E*)-cariofileno (9,84%) α-pineno (9,14%) Espatulenol (8,07%) Sativeno (5,87%)	Antibacteriana (*Listeria monocytogenes*, *S. aureus*, *Leuconostoc mesenteroides* e *Weissella viridescens*)	Experimento *in vitro* em placa de petri	Vasconcelos *et al*. (2022b)
1,8-cineol (32,94%) α-pineno (29,43%)	Antibacteriana (*L. mesenteroides* e *W. viridescens*)	Experimento *in vitro* em placa de petri	Vasconcelos *et al*. (2022a)
Biciclogermacreno (33,50%) Germacreno D (20,60%) E-cariofileno (17,00%) β-elemeno (12,70%)	-	-	Cavalcante *et al*. (2022)
1,8-cineol (20,97%) Espatulenol (14,73%) α-pineno (10,21%) Óxido de cariofileno (8,82%)	Antibacteriana (*S. aureus*, *Staphylococcus epidermidis* e *Escherichia coli*)	Experimento *in vitro* em placa de petri	Nunes *et al*. (2023)
α-pineno (21,23%) β-felandreno (13,92%) Terpinoleno (13,01%) Germacreno D (10,89%)	Antinociceptiva	Experimento *in vitro* em placa de petri e *in vivo* em camundongos	Nascimento *et al*. (2024)
Espatulenol (16,44%) Biciclogermacreno (16,04%)	Antibacteriana (*Staphylococcus aureus*)	Experimento *in vitro* em placa de petri	Venancio *et al*. (2025)

1,8-cineol (15,04%) Espatulenol (11,75%) β-felandreno (11,00%) Biciclogermacreno (10,21%)	Larvicida (*Aedes aegypti*)	Experimento *in vivo* em larvas	Lopes *et al*. (2025)

2.6 Diversidade química de óleos essenciais entre diferentes órgãos da planta

A composição química dos óleos essenciais é influenciada pelo órgão de origem, uma vez que cada tecido vegetal apresenta características anatômicas, fisiológicas e metabólicas específicas (Barra, 2009; Simões *et al*., 2017). Os óleos essenciais podem estar presentes nas flores (laranjeira, bergamoteira), folhas (capim-limão, eucalipto, louro), cascas do caule (canelas), madeira (sândalo, pau-rosa), raízes (vetiver), rizomas (cúrcuma, gengibre), frutos (anis-estrelado, funcho, erva-doce) e sementes (noz-moscada) (Simões *et al*., 2017). Assim, óleos essenciais extraídos de diferentes partes de uma mesma espécie podem diferir significativamente quanto ao perfil químico, às propriedades físico-químicas e ao aroma. Essas variações refletem a especialização funcional dos órgãos vegetais e a regulação das vias biossintéticas envolvidas na produção dos compostos voláteis (Barra, 2009).

Diversos estudos têm relatado variabilidade no teor e na composição química dos óleos essenciais extraídos de diferentes órgãos da planta, como observado em *Hortia oreadica*, *Cinnamomum verum*, *Cupressus arizonica*, *Myrica gale*, *Syzygium cumini*, *Zingiber zerumbet*, *Zanthoxylum mantaro*, *Ferulago* (*F. angulata*, *F. carduchorum* e *F. contracta*) e *Foeniculum vulgare* subsp. *piperitum* (Santos *et al*., 2016b; Castro *et al*., 2020; Narimani *et al*., 2022; Rawat *et al*., 2023; Cherrad *et al*., 2022; Ložienė *et al*., 2023; Asker *et al*., 2025; Morocho *et al*., 2025). Nesse sentido, os teores de óleo essencial, obtidos a partir de folhas e cones de *C. arizonica*, foram de 0,85% e 1,29%, respectivamente. A análise da composição química indicou que o óleo das folhas é predominantemente constituído por cis-muurola-4(14),5-dieno (21,27%), umbelulona (19,88%), α-pineno (9,39%) e α-muuroleno (7,87%). Em contraste, o óleo dos cones apresentou elevado teor de α-pineno (51,07%), mirceno (17,92%), limoneno (9,66%), β-pineno (4,92%), meta-cimeneno (2,60%) e α-terpineol (2,38%) (Cherrad *et al*., 2022). Diferenças na composição química entre os óleos essenciais obtidos das folhas, flores e frutos de *H. oreadica* também foram relatadas, com variações expressivas nos teores de amorfa-4,7(11)-dieno e biciclogermacreno. O amorfa-4,7(11)-dieno apresentou concentrações de 29,27% nas flores, 20,26% nos frutos e entre 27,66 e 37,89% nas folhas, enquanto o biciclogermacreno variou de 23,28% nas flores, 20,64% nos frutos e entre 14,71 e 31,37% nas folhas (Santos *et al*., 2016b).

A variabilidade química dos óleos essenciais extraídos de diferentes órgãos (raízes, caules, folhas e frutos) de *F. vulgare* subsp. *piperitum* evidenciou diferenças marcantes entre os tecidos analisados. Nas raízes, os principais constituintes foram terpinoleno (33,15%), γ-terpineno (12,18%) e acetato de fenchila (11,23%). Nos caules e nas folhas, predominaram α-felandreno (36,85% e 41,59%, respectivamente) e β-felandreno (19,68% e 25,79%, respectivamente). Já nos frutos, destacaram-se terpinoleno (20,10%) e limoneno (17,84%) como os compostos majoritários (Ilardi *et al*., 2022).

Os óleos essenciais, extraídos das folhas, ramos e frutos de um espécime de *C. verum*, coletado em Belém (Pará, Brasil), também apresentaram composições químicas distintas entre os órgãos analisados. O óleo essencial das folhas foi caracterizado pela predominância de eugenol (64,2%), enquanto, nos ramos, destacaram-se o acetato de *E*-cinamila (10,5%), o *E*-cinamaldeído (8,57%) e o óxido de cariofileno (8,68%). Por sua vez, o óleo essencial dos frutos apresentou como principais constituintes α-cadinol (8,72%), epi-α-cadinol (8,60%), δ-cadinieno (6,81%) e γ-cadinieno (6,48%) (Castro *et al*., 2020).

Rawat *et al*. (2023) realizaram a análise fitoquímica dos óleos essenciais extraídos de diferentes órgãos de *Z. zerumbet*, incluindo folhas, cones, raízes e rizomas. Os teores de óleo essencial variaram entre os órgãos analisados, sendo observados maiores rendimentos nas raízes (0,52%) e nos rizomas (0,50%), seguidos pelos cones (0,05%) e pelas folhas (0,02%). O óleo essencial das folhas apresentou como principal constituinte o (E)-nerolidol (49,44%),

seguido por β-cariofileno (20,14%), sabineno (2,92%) e α-copaeno (2,30%). De forma semelhante, o óleo essencial dos cones também foi caracterizado pela predominância de (E)-nerolidol (52,67%), além de linalol (12,22%), óxido de cariofileno (11,19%), mirtanol (2,70%) e β-cariofileno (2,34%). Em contraste, a zerumbona foi o composto majoritário nos óleos essenciais das raízes e dos rizomas, representando 72,23% e 70,25% do óleo total, respectivamente.

No gênero *Ferulago*, Narimani *et al*. (2022) investigaram as características fitoquímicas dos óleos essenciais de folhas, flores e frutos de três espécies (*F*. *angulata*, *F*. *carduchorum* e *F*. *contracta*). Os teores de óleo essencial variaram entre 0,46 e 2,65% (v/m) nas diferentes partes avaliadas, sendo os maiores valores registrados nos frutos: 2,65% em *F*. *angulata*, 2,01% em *F*. *carduchorum* e 1,78% em *F*. *contracta*. A disparidade observada no teor de óleo essencial entre estruturas vegetativas e reprodutivas pode ser atribuída ao órgão vegetal de origem e à densidade das estruturas secretoras. A maior concentração de glândulas secretoras em órgãos específicos das plantas constitui um fator morfofisiológico bem estabelecido, que influencia diretamente as variações no rendimento dos óleos essenciais (Gostin e Blidar, 2024; Ložienė, 2025). Os principais compostos do óleo essencial entre as espécies foram α-pineno (15,01-22,16%), β-felandreno (2,48-14,73%), α-felandreno (0,52-13,8%) e germacreno B (0,11-13,28%) nas folhas; (*Z*)-β-ocimeno (38,46-47,21%), α-pineno (10,25-18,32%) e α-felandreno (5,07-9,44%) nas flores; (*Z*)-β-ocimeno (10,21-41,19%), α-felandreno (7,51-31,89%), α-pineno (8,96-17,71%), β-felandreno (7,24-17,44%), terpinoleno (2,90-7,77%) e δ-3-careno (1,57-7,66%) nos frutos.

O padrão inversamente proporcional entre monoterpenos e sesquiterpenos nos óleos essenciais de órgãos vegetativos e reprodutivos tem sido descrito em diferentes espécies, caracterizando-se pela maior predominância de monoterpenos nos frutos e de sesquiterpenos nas folhas, como observado em *C*. *arizonica*, *S*. *cumini* e *Z*. *mantaro* (Cherrad *et al*., 2022; Asker *et al*., 2025; Morocho *et al*., 2025). Em *C*. *arizonica*, o óleo essencial extraído dos cones é predominantemente composto por monoterpenos, com elevados teores de hidrocarbonetos monoterpênicos (88,27%) e monoterpenos oxigenados (11,52%), enquanto o óleo essencial das folhas apresenta maior predominância de sesquiterpenos, representados por hidrocarbonetos sesquiterpênicos (42,75%) e sesquiterpenos oxigenados (11,11%) (Cherrad *et al*., 2022). Padrão semelhante também foi observado em folhas e frutos de *S*. *cumini*. Nas folhas, verificou-se menor proporção de monoterpenos (30,72%) e maior predominância de sesquiterpenos (64,06%), enquanto nos frutos ocorreu o inverso, com maior concentração de monoterpenos (53,13%) e menor de sesquiterpenos (44,62%) (Ložienė *et al*., 2023). Em *Z*. *mantaro*, as folhas também apresentaram maior proporção de sesquiterpenos (59,78%), enquanto os frutos foram caracterizados por elevados teores de monoterpenos (92,30%) (Morocho *et al*., 2025). Essas diferenças na composição química podem estar associadas a fatores ecológicos e fisiológicos, refletindo distintos papéis adaptativos e metabólicos desempenhados pelos diferentes órgãos da planta.

Do ponto de vista ecológico, a variação química dos óleos essenciais observada entre folhas e frutos, pode estar relacionada a diferentes funções adaptativas, incluindo a defesa contra patógenos, insetos, herbívoros, a atração de polinizadores e dispersores de sementes (Jin *et al*., 2025). Nas folhas, os sesquiterpenos atuam predominantemente como agentes de defesa química contra herbívoros (Sharma *et al*., 2017). Em contraste, os monoterpenos presentes em flores e frutos desempenham papel fundamental na atração de polinizadores e dispersores de sementes, além de contribuírem para a defesa química durante o processo de frutificação (Rodríguez *et al*., 2013; Byers *et al*., 2014).

A variação química dos óleos essenciais pode estar relacionada a aspectos fisiológicos e metabólicos das plantas (Barra, 2009). A biossíntese de monoterpenos e sesquiterpenos depende de dois precursores isoprenoides, o difosfato de isopentenila (IPP) e o difosfato de dimetilalila (DMAPP), originados, respectivamente, das vias do fosfato de metileritritol (MEP) e do mevalonato (MVA) (Simões *et al*., 2017). Os sesquiterpenos, constituídos por três

unidades de isopreno (C_{15}) e sintetizados predominantemente pela via do MVA no citosol, apresentam maior complexidade estrutural e demandam elevado custo energético, estimado em 9 ATPs e 6 NADPH (Bergman *et al*., 2019; Liu *et al*., 2024). Em contraste, os monoterpenos, formados por duas unidades de isopreno (C_{10}) e produzidos majoritariamente pela via do MEP nos plastídios, requerem menor investimento energético, com consumo aproximado de 6 ATPs e 6 NADPH (Bergman *et al*., 2019). Evidências sugerem que as plantas podem modular a produção de terpenoides em resposta à disponibilidade energética e às exigências da fase reprodutiva, favorecendo a síntese de compostos estruturalmente mais simples, como os monoterpenos, de modo a otimizar o uso de energia para o crescimento e o desenvolvimento dos órgãos reprodutivos (Vranová *et al*., 2013).

Os estudos evidenciam de forma consistente que a composição química e o rendimento dos óleos essenciais são fortemente influenciados pelo órgão vegetal de origem, refletindo diferenças anatômicas, fisiológicas, metabólicas e ecológicas entre diferentes tecidos. A ampla variabilidade observada entre folhas, flores, frutos, raízes, caules e sementes de uma mesma espécie demonstra que a distribuição dos metabólitos voláteis não é aleatória, mas é resultado de uma regulação biossintética específica, associada à especialização funcional de cada órgão.

2.7 Influência da sazonalidade sobre os óleos essenciais de plantas aromáticas

A variação sazonal desempenha papel crucial na composição dos óleos essenciais, influenciando tanto a quantidade quanto a qualidade dos compostos voláteis produzidos pelas plantas. Diferenças qualitativas e quantitativas têm sido amplamente relatadas para famílias botânicas produtoras de óleo essencial, como em Verbenaceae (*L. gracilis*, *L. alba*, *L. camara*, *Aloysia gratíssima*); em Lamiaceae (*O. basilicum*, *Mentha* × *piperita*, *M. viridis* e *Rosmarinus officinalis*); em Myrtaceae (*Eugenia uniflora*, *Eugenia neonitida*, *Eugenia rotundifolia*, *Psidium cattleyanum* e em clones comerciais de *Eucalyptus*); e em Euphorbiaceae (*Croton blanchetianus*, *Croton nepetifolius* e *Croton zehntneri*) (Cruz *et al*., 2014; Ribeiro *et al.*, 2018; Pinto *et al*., 2019; Sadeh *et al*., 2019; Costa *et al*., 2020b; Alvarenga *et al*., 2021; Sá Filho *et al.*, 2022; Pereira *et al*., 2022; Jesus *et al*., 2024; Souza *et al*., 2025; Khaiper *et al*., 2026). Fatores ambientais, como precipitação, temperatura, umidade e intensidade luminosa, podem afetar de forma significativa a biossíntese desses metabólitos, resultando em flutuações no perfil químico ao longo do ciclo anual (Sá Filho *et al*., 2022; Jerônimo *et al*., 2024). Segundo Pereira *et al*. (2022), as variações nos constituintes químicos e a produção de determinados compostos estão diretamente relacionadas às rotas biossintéticas, que são moduladas conforme as demandas fisiológicas da planta, as quais podem ser influenciadas por fatores ambientais. A compreensão desses padrões de variação é fundamental para otimizar a obtenção e o uso dos óleos essenciais.

Diversos estudos evidenciam que a sazonalidade pode influenciar de forma significativa tanto o teor quanto o perfil químico dos óleos essenciais em plantas aromáticas. Em *L. gracilis*, Cruz *et al*. (2014) avaliaram a composição química dos óleos essenciais de folhas de sete acessos coletados nas estações chuvosa e seca, observando variação no teor de óleo de 1,25 a 1,92% na estação chuvosa e de 1,42 a 2,70% na estação seca. Apesar dessas variações quantitativas, a composição química manteve-se relativamente estável entre as estações, indicando a consistência do perfil químico de *L. gracilis* mesmo sob diferentes condições climáticas. De modo semelhante, em estudo com 27 acessos de *V. curassavica* coletados durante as estações chuvosa e seca (Oliveira *et al*., 2020a), o teor médio de óleo essencial foi superior na estação seca (1,71%) em comparação à estação chuvosa (1,60%). O perfil químico do óleo essencial também apresentou relativa estabilidade sazonal, embora tenha sido observado aumento na concentração de viridiflorol no acesso VCUR-503, enquanto os acessos VCUR-302 e VCUR-502 apresentaram redução nos teores de α-zingibereno e (*E*)-cariofileno, respectivamente, durante a estação seca.

Pereira *et al*. (2022), ao avaliarem o efeito da sazonalidade sobre o teor e a composição química do óleo essencial de 28 acessos de *L. camara*, coletados nas estações chuvosa e seca,

verificaram que os teores variaram de 0,13 a 0,29% na estação chuvosa e de 0,13 a 0,33% na estação seca. Os grupos químicos formados em ambas as estações apresentaram similaridade quanto aos principais constituintes; entretanto, observou-se maior produção de monoterpenos durante a estação chuvosa.

Estudos têm demonstrado que temperaturas mais elevadas e maior incidência de radiação solar são fatores determinantes para o aumento da atividade fotossintética, promovendo maior suprimento de energia e de precursores metabólicos envolvidos na biossíntese de terpenos (Song *et al*., 2014; Nishimura *et al*., 2015; Malik *et al*., 2023). A intensificação da produção de metabólitos secundários sob altos níveis de radiação solar pode ser explicada pelo fato de que as reações biossintéticas dependem do fornecimento de esqueletos carbônicos e de compostos energéticos oriundos dos processos fotossintéticos (Taiz *et al*., 2017).

Em estudo conduzido por Souza *et al*. (2025), foi avaliado o efeito da sazonalidade sobre a composição química do óleo essencial de folhas de *A. gratissima* coletadas em populações naturais no município de Erval Grande, Rio Grande do Sul, ao longo de quatro estações do ano: outono (março), inverno (julho), primavera (dezembro) e verão (janeiro). Os principais constituintes foram: no outono: pinocamfona (13,57%), guaiol (10,54%) e β-pineno (8,51%); no inverno e na primavera: pinocamfona (21,44% e 14,09%, respectivamente), β-pineno (12,16% e 10,32%, respectivamente) e acetato de trans-pinocarvila (9,59% e 8,75%, respectivamente); no verão: β-pineno (15,05%), pinocamfona (13,47%) e acetato de trans-pinocarvila (7,55%). Dentre esses, β-pineno, pinocamfona e acetato de trans-pinocarvila foram predominantes em três estações, enquanto o guaiol foi o principal composto exclusivamente no outono.

Sá Filho *et al*. (2022) avaliaram a influência da localização geográfica e da sazonalidade sobre a composição química dos óleos essenciais de sete acessos de *L. alba*. Foram observadas diferenças significativas nas concentrações dos principais constituintes, evidenciando que os perfis químicos dos óleos essenciais desses acessos são afetados, de forma quantitativa e qualitativa, tanto entre colheitas mensais em uma mesma região geográfica quanto entre regiões distintas. No que se refere ao efeito da sazonalidade, o acesso LA-57 apresentou ampla variação no teor de carvona, que oscilou de 69,18% (março) a 81,01% (dezembro) em São Cristóvão e de 58,87% (maio) a 84,86% (dezembro) em Uberlândia.

Em Lamiaceae, Pinto *et al*. (2019) avaliaram o teor e a composição química do óleo essencial de 24 cultivares e híbridos de manjericão (*O. basilicum*) cultivados em diferentes épocas: estação seca (outubro a dezembro) e estação chuvosa (abril a junho), no estado de Sergipe. O teor de óleo essencial variou de 0,66% a 3,21% na estação seca e de 0,80% a 4,20% na estação chuvosa. A cultivar Maria Bonita apresentou o maior teor na estação seca (3,21%) e, na estação chuvosa, os maiores valores foram observados para as cultivares Mrs. Burns e Maria Bonita (4,20% e 3,93%, respectivamente). Os principais compostos identificados entre os genótipos foram linalol, metilchavicol, neral, geranial, eugenol e metil (*E*)-cinamato. Entre os compostos analisados, o metilchavicol e o eugenol foram os mais influenciados pela época de cultivo. A cultivar Genovese apresentou maior teor de metilchavicol na estação chuvosa (27,43%) em comparação à estação seca (2,13%). Em contraste, o eugenol atingiu 29,69% na estação seca, não sendo detectado na estação chuvosa para essa cultivar.

Sadeh *et al*. (2019) avaliaram a composição do óleo essencial de 33 amostras de germoplasma de *Rosmarinus officinalis*, classificadas em quatro grupos quimiotípicos: 1,8-cineol, verbenona, cânfora e α-pineno, coletadas durante o verão (maio) e o inverno (janeiro). Os quatro quimiotipos não foram significativamente afetados pela sazonalidade, embora tenham sido observadas pequenas variações nos constituintes minoritários (aqueles que representam menos de 5% do óleo essencial), indicando elevada estabilidade sazonal do germoplasma. Essa estabilidade pode estar relacionada ao fato de o germoplasma utilizado no estudo ter sido cultivado por quase 30 anos no mesmo local, o que pode ter minimizado os efeitos de variações ambientais. Em espécies com quimiotipos bem definidos e rotas

metabólicas altamente reguladas, a expressão enzimática relacionada à síntese de terpenoides e fenilpropanoides tende a ser menos sensível às variações ambientais, resultando em perfis químicos relativamente constantes ao longo das estações (Barra, 2009).

Alvarenga *et al.* (2021) avaliaram a influência da sazonalidade sobre o teor e a composição química do óleo essencial de *Mentha* × *piperita* e *M. viridis* cultivadas na região Sudeste do Brasil, com coletas realizadas nas estações primavera (novembro), verão (fevereiro), outono (maio) e inverno (agosto). Os maiores teores de óleo essencial foram observados para *Mentha* × *piperita* na primavera (4,26%) e para *M. viridis* na primavera (3,30%) e no verão (3,70%). Em *Mentha* × *piperita*, verificou-se aumento nos teores de mentol (de 16,31 para 41,26%), neomentol (de 3,02 para 6,39%) e mentona (de 5,56 para 41,58%) entre a primavera e o verão. Por sua vez, a composição química de *M. viridis* não apresentou um padrão consistente de variação em função da sazonalidade.

Diversos estudos evidenciam tanto a estabilidade quanto a influência da sazonalidade em espécies da família Myrtaceae, destacando-se a estabilidade observada em *E. uniflora*, *E. neonitida* e *E. rotundifolia*, bem como os efeitos sazonais registrados em *P. cattleyanum* e em clones comerciais de *Eucalyptus*. Costa *et al*. (2020b) analisaram o teor e a composição química dos óleos essenciais de *E. uniflora* durante as estações seca (junho a novembro) e chuvosa (dezembro a abril) em Belém, Pará. Não foram observadas diferenças significativas no teor de óleo entre os períodos seco (1,40 ± 0,60%) e chuvoso (1,80 ± 0,80%). O curzereno, um sesquiterpeno oxigenado, foi o principal constituinte, e sua porcentagem não apresentou diferença significativa entre os dois períodos: seco (42,70% ± 6,10) e chuvoso (40,80 ± 5,90%). De modo semelhante, em estudo conduzido por Defaveri *et al*. (2011) com *E. neonitida* e *E. rotundifolia*, coletadas trimestralmente no estado do Rio de Janeiro, Brasil, a sazonalidade não exerceu influência significativa sobre o rendimento e a composição dos óleos essenciais de ambas as espécies. A estabilidade na produção de óleos essenciais ao longo das diferentes épocas do ano pode estar associada a fatores genéticos e fisiológicos das espécies, que conferem maior controle sobre as vias biossintéticas dos metabólitos secundários (Defaveri *et al*., 2011; Ribeiro *et al*., 2016).

Jesus *et al*. (2024) avaliaram a composição química do óleo essencial de *P. cattleyanum* durante os períodos seco (agosto) e chuvoso (janeiro) no município de Alegre, Espírito Santo. O maior rendimento de óleo essencial foi observado em janeiro (período chuvoso), com 1,41%, em comparação a agosto (período seco), que apresentou 0,33%. No período seco, foram identificados β-bisaboleno (3,40%) e óxido de cariofileno (4,80%), os quais não foram detectados no período chuvoso. Em contraste, viridiflorol (2,90%) e guaiol (6,20%) foram encontrados exclusivamente no óleo essencial obtido no período chuvoso. De maneira análoga, Jerônimo *et al*. (2024), ao investigarem a influência da sazonalidade sobre a composição química do óleo essencial floral de *Acmella oleracea*, observaram maior rendimento de óleo na estação chuvosa (1,61%) e menor na estação seca (0,68%). Quanto à composição química, verificou-se aumento nas concentrações de (E)-cariofileno (de 13,57 para 25,74%), germacreno D (de 0,14 para 15,17%) e mirceno (de 1,08 para 11,99%) da estação chuvosa para a estação seca. Em contraste, os teores de óxido de cariofileno (0,88-31,72%) e 1-pentadeceno (5,42–16,58%) foram mais elevados durante a estação chuvosa.

As plantas utilizam a síntese de isoprenos como um mecanismo para dissipar o excesso de radiação luminosa e de temperatura, uma vez que esses compostos contribuem para a estabilidade das membranas fotossintéticas, conferindo proteção ao aparato fotossintético contra danos térmicos e foto-oxidativos (Vickers *et al*., 2009). No entanto, sob estresse hídrico e térmico, a emissão de terpenos tende a ser reduzida, resultando em alterações tanto na quantidade produzida quanto no perfil químico dos compostos emitidos (Niinemets, 2010).

Khaiper *et al*. (2026) avaliaram o efeito da sazonalidade sobre o rendimento e a composição química dos óleos essenciais das folhas de dois clones comerciais de *Eucalyptus* (C-288 e C-413). O clone C-288 apresentou rendimento de óleo variando de 0,75 a 1,75%, enquanto o clone C-413 exibiu valores entre 1,85 e 3,75%, com maiores rendimentos no verão

(abril) e menores no inverno (dezembro). A análise química evidenciou perfis distintos entre os clones. No clone C-288, os principais constituintes foram eucaliptol (17,78%), hidrato de cis-sabineno (16,38%), isolimoneno (15,15%), globulol (11,87%) e 3-careno (11,09%). Já o clone C-413 apresentou maiores proporções de isopulegol (53,53%), isolimoneno (17,01%), 3-careno (13,72%) e eucaliptol (8,60%). De modo geral, os monoterpenoides oxigenados foram mais abundantes no verão, enquanto os sesquiterpenoides oxigenados predominaram durante a estação chuvosa.

No gênero *Croton* (Euphorbiaceae), estudos têm demonstrado a influência de fatores abióticos sobre o rendimento e a composição química dos óleos essenciais. Ribeiro *et al*. (2018) avaliaram o efeito do ciclo circadiano (coletas de folhas às 8:00, 12:00 e 20:00) e da sazonalidade (períodos seco e chuvoso) na produtividade e na composição química dos óleos essenciais de três espécies do gênero (*Croton blanchetianus*, *Croton nepetifolius* e *Croton zehntneri*). Os resultados demonstraram que a composição dos óleos é influenciada tanto pelo ciclo circadiano quanto pela sazonalidade, pois certos compostos foram produzidos apenas em horários e épocas específicas de coletas. Os compostos majoritários E-cariofileno e espatulenol foram produzidos nas folhas coletadas às 8:00 e 12:00, em média 27,52% e 23,26%, respectivamente; no entanto, não foram produzidos quando as folhas foram coletadas às 20:00. Já o composto anetol (20,00%) só foi produzido quando as folhas foram coletadas às 20:00. Em relação à sazonalidade, os compostos E-cariofileno e espatulenol tiveram menores concentrações na época chuvosa e maiores na época seca (25,68 e 17,84 *versus* 29,37 e 28,69), e o ρ-cymen-8-ol (13,87%) foi sintetizado apenas na época chuvosa. Portanto, os fatores ambientais avaliados exercem influência significativa tanto sobre o rendimento quanto sobre a composição química dos óleos essenciais das espécies de *Croton*.

De forma geral, os estudos revisados demonstram que a sazonalidade exerce influência significativa sobre o teor e a composição química dos óleos essenciais, modulada por fatores ambientais, como precipitação, temperatura, umidade e luminosidade, que afetam as rotas biossintéticas dos metabólitos secundários. Entretanto, a magnitude dessas variações é dependente da espécie e do quimiotipo. Enquanto algumas plantas apresentam alterações marcantes no teor e no perfil químico ao longo do ano, outras exibem elevada estabilidade sazonal, especialmente quando possuem quimiotipos bem definidos e controle genético. Assim, a compreensão das variações sazonais permite definir épocas ideais de colheita, maximizar o rendimento e garantir a constância do perfil químico, aspectos fundamentais para o aproveitamento sustentável e para a valorização econômica das plantas aromáticas.

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4. MANUSCRIPT 1
GENETIC DIVERSITY AND GENETIC STRUCTURE OF NATURAL POPULATIONS OF *Croton blanchetianus* Baill. BASED ON INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

Artigo formatado de acordo com as normas do periódico Genetic Resources and Crop Evolution (Publicado)

ABSTRACT

Croton blanchetianus Baill. is an aromatic species endemic to the Caatinga (Brazilian biome), widely used in folk medicine, with proven therapeutic and biological activity. Due to its importance and potential applications, carrying out research on the species is of great significance to develop conservation and breeding strategies, as well as to contribute to definition of other potential biological activities. The aim of this study was to evaluate the genetic diversity and structure of six native populations of *C. blanchetianus* from different municipalities of the state of Sergipe in the Northeast region of Brazil (Aquidabã, Graccho Cardoso, Itabi, Lagarto, Tobias Barreto, and Poço Verde) using Inter Simple Sequence Repeat (ISSR) markers. A total of 264 polymorphic bands were detected, indicating a high level of polymorphism. Genetic variability was characterized based on the following estimates: observed mean number of alleles (Na = 1.768), effective mean number of alleles (Ne = 1.327), the Shannon Diversity Index (I = 0.332), expected heterozygosity (He = 0.208), and percentage of polymorphism (P% = 87.50). The greatest genetic variation was found within the populations (94%), the genetic differentiation among the populations was considered moderate (Φ_{PT} = 0.06 and G_{ST} = 0.05), and the gene flow, estimated by Nm statistic, was high (9.19). The estimates of genetic differentiation among the populations were determined using Nei's genetic distance and the pairwise fixation index (Φ_{PT}), showing differentiation of low to moderate magnitude. Multivariate analyses (principal coordinate analysis—PCoA, the dendrogram based on Jaccard's genetic distance and Bayesian population structure analysis) did not cluster the genotypes according to their geographic origin. These results contribute to conservation strategies of *C. blanchetianus* by identifying genetic variability and structure.

Key-words: Euphorbiaceae, Medicinal and aromatic plant, Caatinga biome, Genetic variability, Molecular markers.

4.1. Introduction

Native germplasms are a valuable genetic resource for a nation, and their conservation and sustainable use are important for ensuring that their properties benefit present and future generations. However, environmental and human-induced factors have led to loss of genetic variability and extinction of species with economic potential even before they have been studied (Tapia-Armijos et al. 2015; Hashemifar and Rahimmalek 2018; Silva-Júnior et al. 2020). Exclusive to Brazil, the Caatinga biome is one of the seasonally dry tropical forests of greatest biodiversity, with many endemic species (Silva et al. 2018). However, due to increasing human activity, such as deforestation for expansion of agricultural areas, development of pastures, and exploitation of energy resources, 46% of the original Caatinga vegetation has already been modified (Lapola et al. 2014; Jesus et al. 2022).

In the state of Sergipe, habitat fragmentation caused by human activity significantly affects the plant composition and structure in the Caatinga (Jesus et al. 2022). This process results in lower diversity and a reduction in the number of individuals of the species in this area, showing the importance of implementing strategies to mitigate the loss of local flora. One of

these species in Sergipe is *Croton blanchetianus*. This aromatic plant has wide medicinal use and is listed in the portfolios of the Brazilian Ministry of the Environment as a priority species for the Northeast region due to its potential economic importance (Coradin et al. 2018).

Croton blanchetianus is an aromatic species endemic to the Caatinga and is popularly known in the Northeast region as marmeleiro and/or marmeleiroda-caatinga. It is morphologically characterized as a monoecious shrub (of 1.5–8 m height), with cylindrical yellowish-green to grayish branches. Its leaves are membranous, oval to lanceolate. It has a thyrse inflorescence with unisexual flowers; pistillate flowers do not have petals and the styles are fused into a column, and staminate flowers have white petals and stamens with long anthers. The fruit is lobed and green to yellowish, and the seed is ellipsoid and brown with a reniform caruncle (Rossine et al. 2023; Caruzo et al. 2024).

In traditional medicine, the leaves and bark of *C. blanchetianus* are used to treat gastrointestinal disturbances, rheumatism, headaches, and bronchitis (Chaves and Reinhard 2003). Furthermore, various investigations have been published on the therapeutic activities of *C. blanchetianus*, which are closely associated with its secondary metabolites, such as flavonoids, alkaloids, and terpenes (Aquino et al. 2017; Oliveira et al. 2022a). Noteworthy are their gastroprotective, analgesic, anti-inflammatory, antioxidant, antimicrobial, and antinociceptive activities (Freitas et al. 2020; Dantas et al. 2021; Oliveira et al. 2022a, 2022b; Firmino et al. 2019; Nascimento et al. 2024). Other research reports the biological activities of its essential oils, with antibacterial, acaricidal, antimicrobial, antifungal, and insecticidal effects (Melo et al. 2013; Angélico et al. 2014; Rodrigues et al. 2019; Silva et al. 2020a; Camara et al. 2021; Figueiredo et al. 2022; Vasconcelos et al. 2022a, 2022b).

Given its potential for various industries, genetic research should be performed to help design strategies for its conservation. Understanding the genetic structure of plant genetic resources is essential for designing effective breeding and conservation strategies (Coskun and Gulsen 2024; Coskun et al. 2024). The genetic characterization of native germplasm has been carried out using different DNA-based molecular markers, such as Inter Simple Sequence Repeat (ISSR). These markers are commonly used to study genetic diversity in natural populations and germplasm banks as they can identify genetic diversity without the need for previous information on the genome (González et al. 2002; Tohidi et al. 2019). In addition, the ISSR markers offer advantages, such as high reproducibility, automation potential, a high level of polymorphism, the small amount of DNA required, and ease of handling (Borba et al. 2005; Rustaiee et al. 2013).

The genetic diversity of natural populations has been accessed using ISSR molecular markers in various species, such as *Copernicia prunifera* (Vieira et al. 2015), *Myrcia lundiana* (Alves et al. 2016), *Bertholletia excelsa* (Ramalho et al. 2016), *Croton heliotropiifolius* (Rocha et al. 2016), *Croton tetradenius* (Almeida-Pereira et al. 2017), *Hyptis pectinata* (Feitosa-Alcantara et al. 2017), *Myrcia ovata* (White et al. 2018), *Croton linearifolius* (Silva et al. 2020b), *Croton urucurana* (Costa et al. 2020), *Dalbergia nigra* (Silva-Júnior et al. 2020), *Pityrocarpa moniliformis* (Felix et al. 2020), *Lippia origanoides* (Feijó et al. 2022), *Harcornia speciosa* (Silva et al. 2024), and *Eplingiella* spp. (Silva et al. 2023). These investigations have shown that the ISSR molecular markers are effective in determining polymorphism levels among individuals and in detecting genetic diversity among populations.

The aim of the present study was to evaluate the genetic diversity and structure of six native populations of *C. blanchetianus* from different municipalities of the state of Sergipe, Brazil, using ISSR markers. An additional aim was to determine whether there is a correlation between the geographic and genetic distances of the populations under analysis. This will be the first study on the genetic diversity of natural populations of *C. blanchetianus*, and the data obtained will facilitate the development of a suitable approach for the conservation and selection of accessions to create a collection of *C. blanchetianus* for the Active Germplasm Bank of Medicinal and Aromatic Plants of the Universidade Federal de Sergipe.

4.2. Material and Methods

4.2.1. Plant material

Tender leaves were collected from 170 *Croton blanchetianus* genotypes across six municipalities: 30 genotypes in the municipalities of Aquidabã (AQ), Graccho Cardoso (GC), Itabi (IT), Lagarto (LG), Tobias Barreto (TB), and 20 genotypes in the municipality of Poço Verde (PV), in the state of Sergipe in the Northeast region of Brazil (Fig. 1). In this collection, a minimum distance of 50 m between plants was observed, and the geographic distances between the populations ranged from 18 to 150 km. The collected leaves were placed in Falcon tubes containing a leaf preservation solution (3 g of CTAB, 35 g of NaCl, and 100 mL of H_2O) to prevent oxidation of the samples, and they were stored in a refrigerator at 4 ºC up to the time of DNA extraction. Fertile branches (flowering and/or with fruit) were collected to prepare herbarium specimens, which were deposited in the ASE Herbarium of the Universidade Federal de Sergipe for taxonomists to confirm species identification.

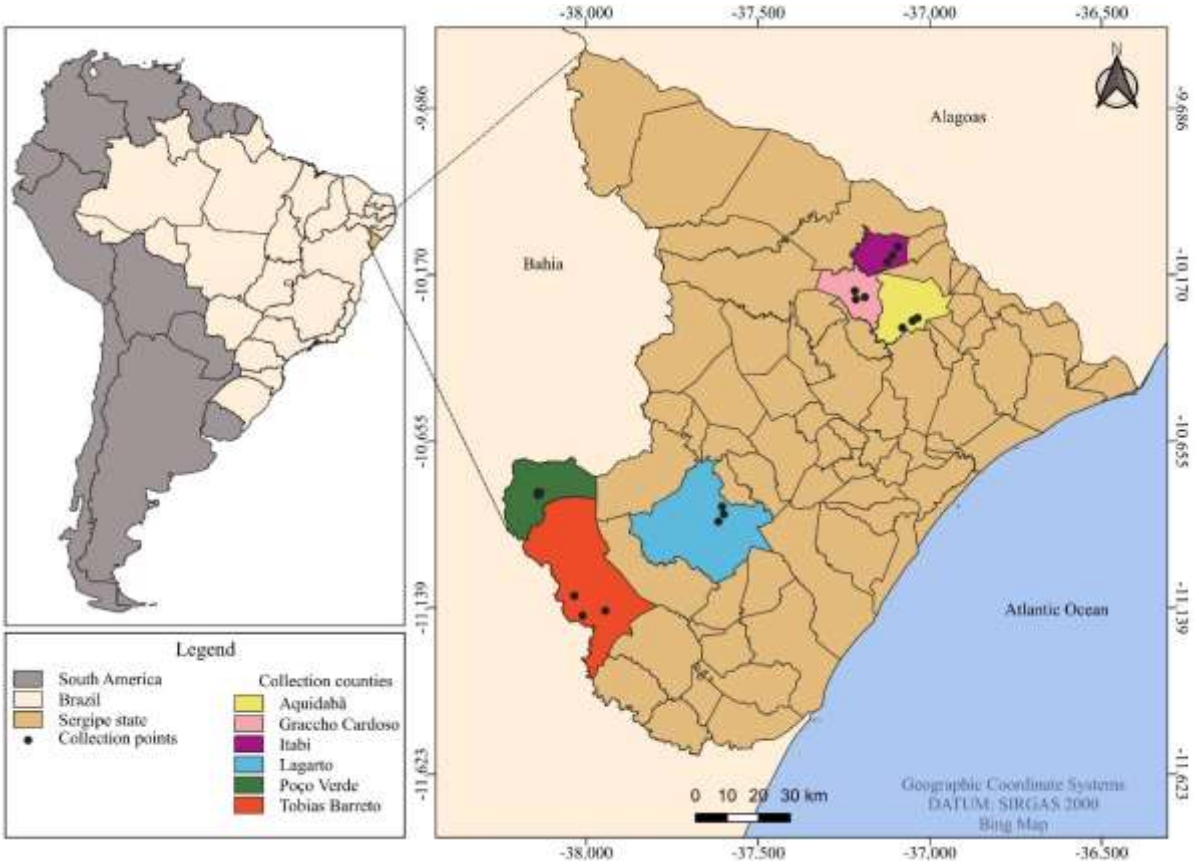

Fig. 1 Geographic location of the six natural populations of *Croton blanchetianus* Baill. collected in Sergipe, Brazil

4.2.2. DNA extraction and quantification

DNA was extracted using the CTAB (Cetyl Trimethyl Ammonium Bromide) 2X method described by Doyle and Doyle (1990), with modifications. In a mortar containing 800 µL of the CTAB 2X extraction buffer (2% CTAB; 100 mM Tris, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl; and 1% PVP), 5 µL of β-mercaptoethanol, and 2% PVP (polyvinylpyrrolidone), 200 mg of tender *C. blanchetianus* leaves were ground using a mortar and pestle. The material was placed in an Eppendorf tube and incubated at 65 °C for 60 min. After that period, 800 µL of a chloroform:isoamyl alcohol (24:1) mixture was added, and the tubes were slowly shaken until obtaining an emulsion, which was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was transferred to a tube containing 750 µL of isopropyl

alcohol, which was incubated for at least one hour at − 20 °C. The samples were then centrifuged at 12,000 rpm for 10 min at 4 °C, the supernatant was discarded, and the precipitate was washed three times with 70% alcohol. Subsequently, the precipitate (DNA) was kept at room temperature for drying and solubilized in TE solution (10 mM Tris, 1 mM EDTA, pH 8.0) containing 10 ng/µl of RNAse.

The extracted DNA was quantified through spectrophotometry using a Nanodrop 2000c (Thermo Scientific, USA), and its quality was determined in 1% agarose gel with 0.5X TBE (0.045 M tris–borate and 0.001 M EDTA). The gel underwent horizontal electrophoresis, was stained with ethidium bromide (0.5 µg mL^{-1}), and was visualized under ultraviolet light in a Gel Doc L-pix photodocumentation system (Loccus Biotecnologia, Brazil).

4.2.3. PCR—ISSR analysis

A total of 30 ISSR primers were tested. Amplification reactions were conducted in a Veriti 96 Fast thermal cycler (Applied Biosystems, USA) in a 20 µL reaction volume containing 2 µL of 10X PCR buffer, 0.4 µL of 2.5 mM dNTP, 1.0 µL of 50 mM magnesium chloride, 0.4 µL of 1U Taq DNA polymerase enzyme, 0.8 µL of 10 µM oligonucleotide primer, 2 µL of DNA (15 ng), and ultrapure sterile water to complete the volume. The PCR reaction consisted of initial denaturation at 95 °C for 5 min., followed by 40 amplification cycles with denaturation at 94 °C for 40 s, specific temperature for primer annealing for 1 min., and extension of the DNA strand at 72 °C for 1 min. After the 40 cycles, a final extension was performed at 72 °C for 7 min. The capillary electrophoresis kit used was Agilent DNF-935-K0500; and for electrophoretic separation, the capillary electrophoresis Fragment Analyzer (Advanced Analytical, Germany) was used. A total of 20 primers were amplified and used to perform statistical analyses.

4.2.4. Statistical analyses

The presence (1) and absence (0) of bands were used to construct a binary matrix, which was used to estimate the genetic parameters described below. The polymorphic information content (PIC) value for each primer was estimated using the expression $PIC=2f_i(1-f_i)$ where f_i is the frequency of the amplified allele (band presence) and $1-f_i$ is the frequency of the null allele (Roldán-Ruiz et al. 2000). The genetic diversity of the natural populations of *Croton blanchetianus* were characterized based on estimates of the observed number of alleles (Na); effective number of alleles (Ne); the Shannon diversity index (I); the expected heterozygosity (He), calculated according to the proportions expected under Hardy–Weinberg Equilibrium (Nei 1978); and the percentage of polymorphism (P%). The analyses were carried out using the Genalex 6.5 software (Peakall and Smouse 2012).

Analysis of Molecular Variance (AMOVA) was performed to estimate the genetic variance among and within populations, and the significance level was determined with 9,999 permutations. The genetic differentiation among the populations (Φ_{PT}), which corresponds to the proportion ofgenetic variation among populations compared to the total genetic variation, was estimated using the expression $\Phi_{PT}=\frac{\text{Variance among populations}}{\text{Total variance}}$, and its significance was tested through 10,000 bootstraps. Additionally, Nei's genetic distance was estimated among the populations. Analyses were carried out using the Genalex 6.5 software (Peakall and Smouse 2012). The coefficient of genetic differentiation among populations ($G_{ST}=1-H_S/H_T$), and the gene flow ($Nm=0.5(1-G_{ST})/G_{ST}$) were estimated from POPGENE v.1.32 (Yeh et al. 1997).

The Mantel test (Mantel 1967) was performed to assess the significance of correlation between the Φ_{PT} statistical and Nei's genetic distance matrices and the geographic distance matrix between the populations (km). Statistical analysis was performed using the vegan package (Oksanen et al. 2022) in the R environment (R Core Team 2022), estimating statistical significance (*p*-value) with 10,000 permutations.

Genetic distance between the genotypes was evaluated using the genetic distance of Jaccard (1908) and visualized by constructing a dendrogram using the Unweighted Pair Group

Method with Arithmetic Means (UPGMA) algorithm. The number of groups formed was determined by the method of Mojena (1977). Analysis was made with the "MultivariateAnalysis" package (Azevedo 2021) for R (R Core Team 2022), and 9999 bootstraps were performed to verify the reliability of the clusters. The MEGA 2.1.7 software was used to format the dendrogram obtained. Principal coordinate analysis (PCoA) at the genotype level was carried out using the Genalex 6.5 software (Peakall and Smouse 2012).

Bayesian analysis was carried out to estimate the genetic structure of the populations using the Structure v.2.3.4 software (Pritchard et al. 2000). Genetic cluster values (k) ranging from 1 to 10 were tested; and for each k, 10 independent replications were performed. Each replication consisted of a burn-in period of 10,000 iterations, followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations, assuming the admixture ancestry model and the alleles frequency correlated. The number of genetic groups (k) was identified by the ΔK method (Evanno et al. 2005), implemented on the StructureSelector software (Li and Liu 2018). Theaccessions with membership values lower than 0.8 were considered of mixed ancestry.

4.3. Results

4.3.1. Polymorphism of the ISSR markers

Of the 30 ISSR primers tested, 10 did not amplify for the genotypes tested. The 20 ISSR primers amplified in *C. blanchetianus* revealed 100% polymorphic bands. A total of 264 bands were detected, ranging from 7 (UBC 878) to 20 (UBC 842), with a mean of 13.20 bands per primer, indicating a high level of polymorphism. The PIC values ranged from 0.16 (UBC 864) to 0.37 (UBC 890 and 809), with a mean of 0.27 (Table 1).

Table 1 Inter Simple Sequence Repeat (ISSR) primers used to evaluate the genetic variability of *Croton blanchetianus* Baill.

Primer	Sequence (5'-3')	T (°C)	TNB	NPB	P (%)	PIC
UBC 811	GAG AGA GAG AGA GAG AC	46.8	13	13	100	0.29
UBC 812	GAG AGA GAG AGA GAG AA	54.8	13	13	100	0.31
UBC 816	CAC ACA CAC ACA CAC AT	54.8	12	12	100	0.26
UBC 842	GAG AGA GAG AGA GAG AYG	58.8	20	20	100	0.35
UBC 848	CAC ACA CAC ACA CAC ARG	58.8	14	14	100	0.34
UBC 864	ATG ATG ATG ATG ATG ATG	50.8	14	14	100	0.16
UBC 888	BDB CAC ACA CAC ACA CA	56.4	15	15	100	0.24
UBC 890	VHV GTG TGT GTG TGT G	56.4	18	18	100	0.37
UBC 007	CTC TCT CTC TCT CTC TRG	45.0	14	14	100	0.22
UBC 901	GTG TGT GTG TGT YR	41.0	14	14	100	0.26
UBC 809	AGA GAG AGA GAG AGA GG	52.0	8	8	100	0.37
UBC 841	GAG AGA GAG AGA GAG ATC	57.6	10	10	100	0.36
UBC 878	GGA TGG ATG GAT GGA	55.6	7	7	100	0.27
UBC 860	TGT GTG TGT GTG TGT GRA	52.0	11	11	100	0.21
UBC 813	CTC TCT CTC TCT CTC TT	50.0	9	9	100	0.20
UBC 815	CTC TCT CTC TCT CTC TG	57.2	11	11	100	0.23
GOOFY	GTG TGT GTG TGT GTY G	47.0	17	17	100	0.26
JOHN	AGA GAG AGA GAG AGY C	43.0	13	13	100	0.27
OMAR	GAG GAG GAG GAG RC	43.0	15	15	100	0.25
TERRY	GTG GTG GTG GTG RC	47.0	16	16	100	0.27
Mean	-	-	13.2	13.2	100	0.27

T (°C) annealing temperature; TNB total number of bands; NPB number of polymorphic bands; P (%)percentage of polymorphism; PIC polymorphic information content; Y—Stands for "pyrimidine" (C or T); R— Stands for "purine" (A or G); B—Stands for "not A" (C, G or T);

D—Stands for "not C" (A, G or T); V— Stands for "not T" (A, C or G); H—Stands for "not G" (A, C or T).

4.3.2. Genetic variability of six populations of *Croton blanchetianus*

The observed number of alleles (Na) amplified for the 170 native genotypes of *C. blanchetianus* ranged from 1.674 (AQ) to 1.860 (IT), with a mean of 1.768 (Table 2). The effective number of alleles was lower than the observed number of alleles, ranging from 1.299 (AQ) to 1.393 (IT), with a mean of 1.327. The genotypes collected in IT (0.379) and LG (0.344) had the highest estimates for the Shannon diversity index (I), which showed an overall mean of 0.332 for the populations. Similarly, the expected heterozygosity (He) was higher in IT (0.242) and LG (0.216) and lower in the AQ population (0.191), with an overall mean of 0.208. The percentage of polymorphic loci was higher than 80% across the six populations of *C. blanchetianus.*

Table 2 Estimates of genetic variability parameters for natural populations of *Croton blanchetianus* Baill.

Population	N	Na	Ne	I	He	P (%)
PV	20	1.739	1.315	0.325	0.202	85.61
TB	30	1.742	1.301	0.317	0.196	86.36
LG	30	1.799	1.345	0.344	0.216	89.02
IT	30	1.860	1.393	0.379	0.242	92.42
GC	30	1.792	1.309	0.321	0.199	88.64
AQ	30	1.674	1.299	0.306	0.191	82.95
Mean	28.333	1.768	1.327	0.332	0.208	87.50

N number of individuals; Na observed number of alleles; Ne effective number of alleles; I Shannon diversity index; He expected heterozygosity; P (%) percentage of polymorphism.

4.3.3. Analysis of molecular variance (AMOVA) and population differentiation

Analysis of molecular variance was carried out to determine the extent of genetic variation within and among the populations. This analysis showed that 94% of the total genetic variance occurs within the populations, whereas 6% of the variation occurs among the populations (Table 3). The overall genetic differentiation among the populations, measured by the pairwise fixation index Φ_{PT}), and by G_{ST} (0.06 and 0.05, respectively), was moderate (Wright 1978 *apud* Silva et al. 2023). The gene flow, estimated by Nm, was high (9.19).

Table 3 Analysis of molecular variance (AMOVA) and genetic differentiation among natural populations of *Croton blanchetianus* Baill.

Source of variation	df	SS	MS	Variance	Variance (%)	Φ_{PT}	p-value	G_{ST}	Nm
Among Populations	5	482.97	96.59	2.17	6.00**	0.06**	0.0001	0.05	9.19
Within Populations	164	5800.28	35.37	35.37	94.00**				
Total	169	6283.25		37.54	100.00				

DF degrees of freedom; SS sum of squares; MS mean of squares; ΦPT pairwise fixation index; GST Nei's coefficient of genetic diversity; Nm—gene flow among populations **Significant at 1% probability

Genetic divergence among the six populations was determined by calculating Nei's genetic distance and the pairwise fixation index (Table 4). Nei's genetic distance between populations ranged from 0.007 (AQ and GC) to 0.031 (IT and PV); while the Φ_{PT}, ranged from 0.010 between AQ and GC to 0.102 between IT and PV (Table 4). The Mantel correlation between the geographic distance and Nei's genetic distance was 0.54; and between the geographic distance and Φ_{PT}, it was 0.66, both with significant effects ($p < 0.01$).

Table 4 Nei's genetic distance (above the diagonal) and Φ_{PT}, (below the diagonal) among natural populations of *Croton blanchetianus* Baill.

	PV	TB	LG	IT	GC	AQ
PV		0.012	0.022	0.031	0.019	0.022
TB	0.032		0.011	0.023	0.016	0.016
LG	0.080	0.032		0.014	0.015	0.014
IT	0.102	0.083	0.035		0.018	0.018
GC	0.074	0.059	0.052	0.051		0.007
AQ	0.094	0.068	0.053	0.061	0.010	

PV – Poço Verde; TB – Tobias Barreto; LG – Lagarto; IT – Itabi; GC – Graccho Cardoso; AQ – Aquidabã.

4.3.4. Cluster analysis, principal coordinate analysis (PCoA), and population structure

The 170 *C. blanchetianus* genotypes were divided into 23 groups using the Mojena method and the Jaccard similarity matrix. The groups ranged from a single genotype (G-1, G-2, G-3, G-4, G-9, G-11, G-14, G-16, G-17, G-18) to 112 genotypes (G-5) (Fig. 2). Groups 5 and 7 contained the largest number of genotypes, with 112 and 17 genotypes, respectively, accounting for around 80% of the individuals. Group 7 did not have genotypes from PV and IT populations. The other groups were represented by ≤ 5 genotypes.

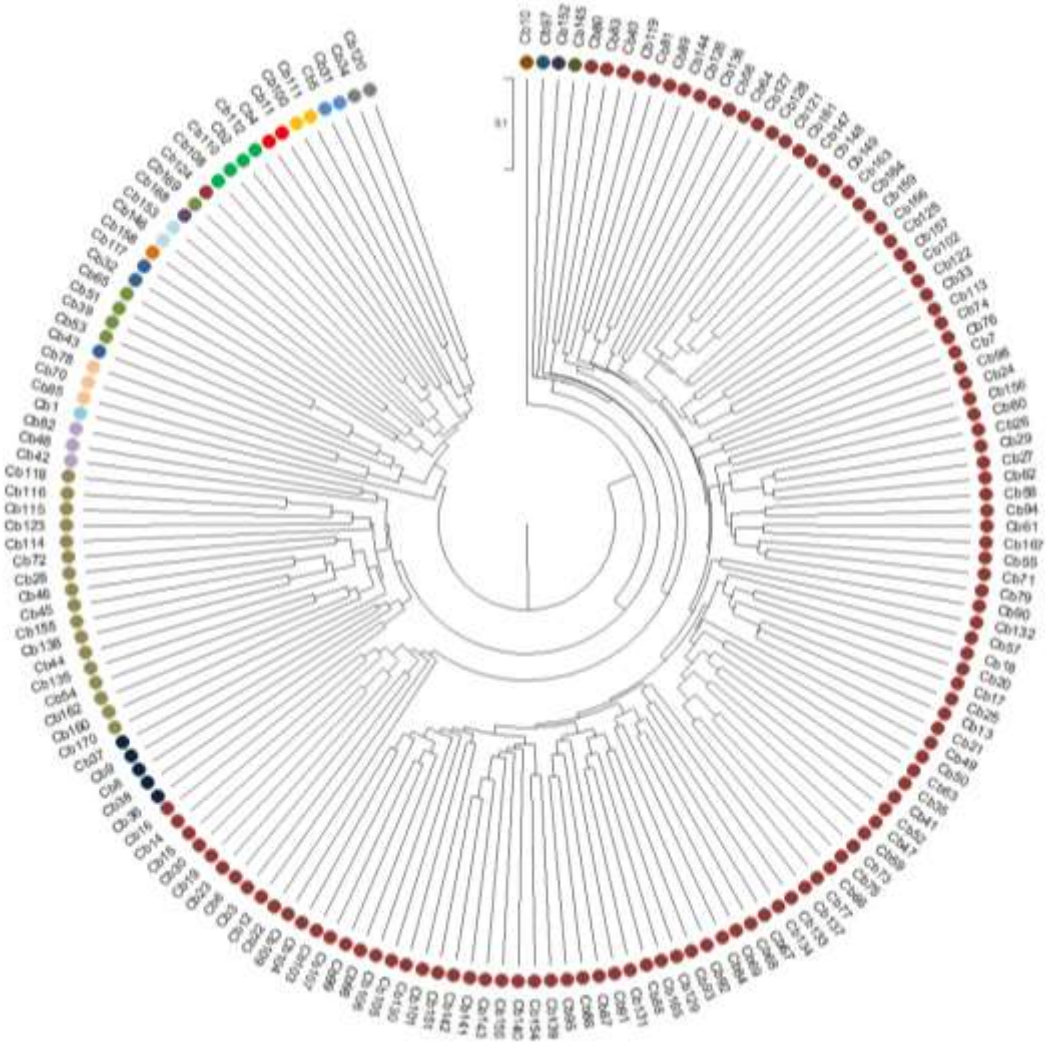

Fig. 2 Dendrogram obtained using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method based on Jaccard genetic distance among 170 genotypes of *Croton blanchetianus* Baill. PV—Cb1—Cb20; TB— Cb21—Cb50; LG—Cb21—Cb80; IT—Cb81—Cb110; GC— Cb111—Cb140; AQ—Cb141—Cb170.

The genetic relationship of the 170 native *C. blanchetianus* genotypes was subsequently investigated using Principal Coordinate Analysis (PCoA) (Fig. 3). The principal coordinates explained 27.8% of the total genetic variance, with 22.27% explained by coordinate 1 and 5.53% by coordinate 2. The genotype distribution patterns on the two-dimensional plot showed no clearly defined clustering according to the geographic origin of the populations. These results are consistent with the findings from cluster analysis (Fig. 2).

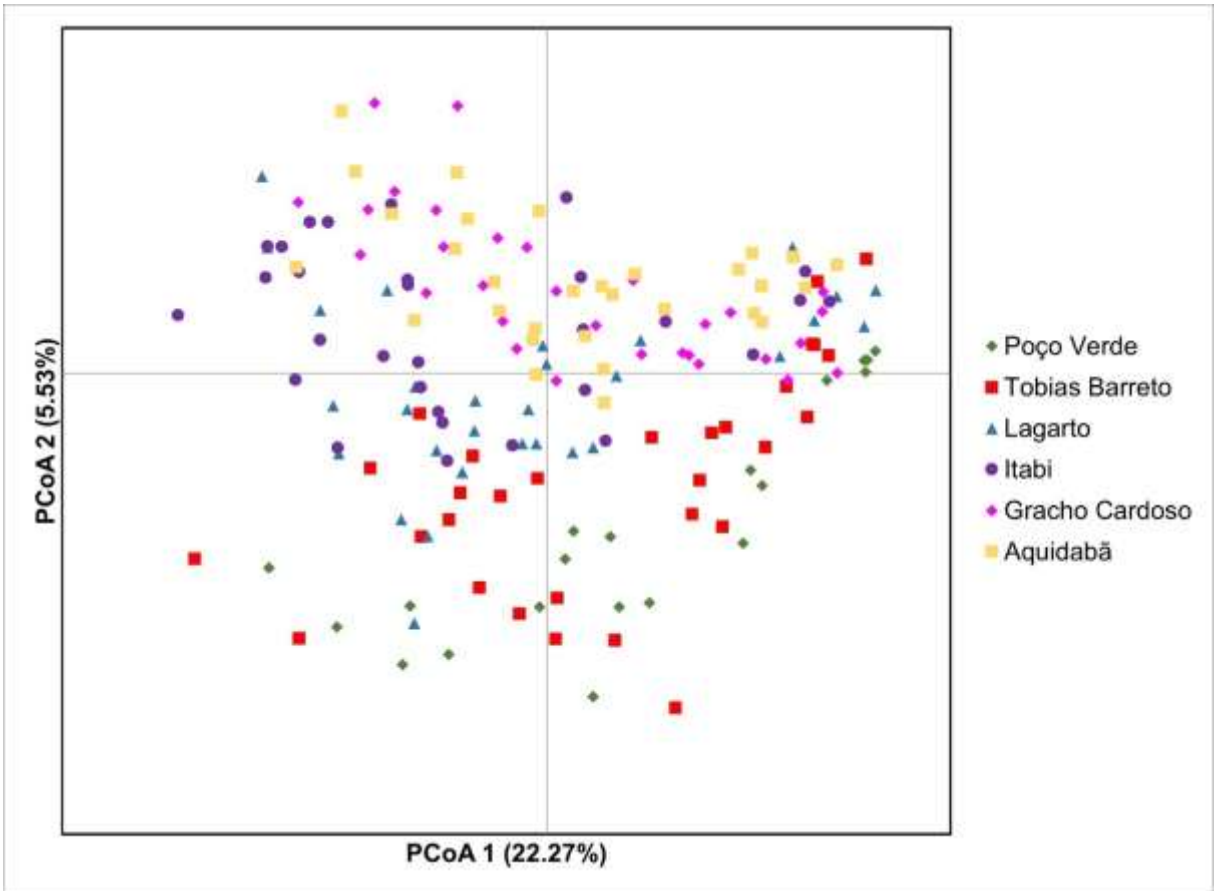

Fig. 3 Principal Coordinate Analysis (PCoA) for 170 genotypes from natural populations of *Croton blanchetianus* Baill.

The *C. blanchetianus* genotypes were analyzed regarding population structure using Bayesian analysis. The number of clusters was K = 2 (Fig. 4A), represented by red and green colors (Fig. 4B). Sixtyone individuals were found to have mixed ancestry. Consistent with the findings from cluster and principal coordinate analyses, Bayesian analysis was not able to differentiate the genotypes based on their populations of origin, suggesting low differentiation among the genotypes from the six natural populations studied.

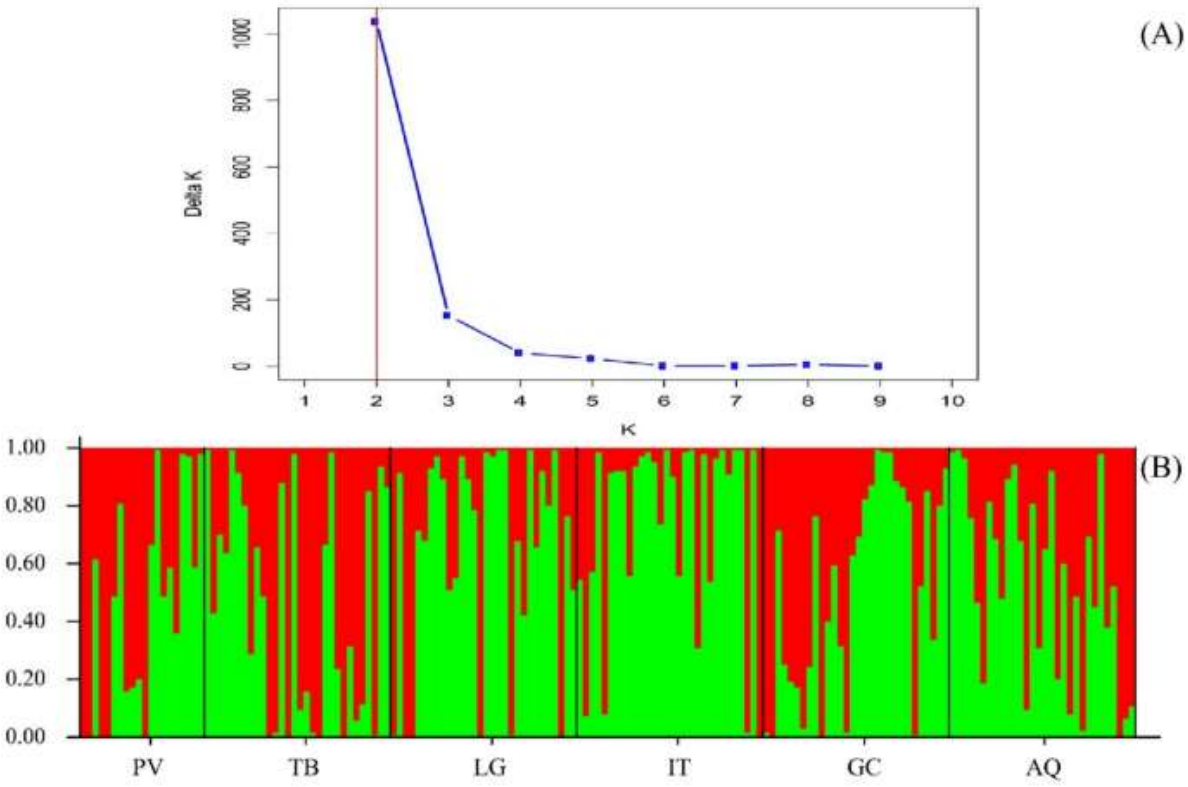

Fig. 4 STRUCTURE analysis of *Croton blanchetianus* Baill. based on ISSR data for 170 genotypes from six natural populations. A The variation of delta K vs. K values for the estimation of the number of cluster populations. B The STRUCTURE output of the analysis for K=2 clusters.

4.4. Discussion

The 20 ISSR primers amplified in *C. blanchetianus* showed a high percentage of polymorphism (100%). Genotype prospecting in different municipalities of the state of Sergipe, Brazil, allowed the sampling of a greater diversity of the species, which provided high values of polymorphism. Similar results have been reported for other species of *Croton*, as in *C. heliotropiifolius*, which showed 94.66% polymorphic bands with the use of 15 ISSR primers (Rocha et al. 2016). Similarly, in *C. tetradenius*, *C. urucurana*, and *C. linearifolius*, 93.25%, 86.83%, and 74.01% polymorphic bands were detected, respectively, using ISSR primers (Almeida-Pereira et al. 2017; Costa et al. 2020; Silva et al. 2020b). The lower polymorphism levels of the markers in these investigations may be related to the size of the sample and to the diversity of the genotypes investigated (Lacape et al. 2007).

The Polymorphic Information Content (PIC) represents the effectiveness of a molecular marker in detecting polymorphism among individuals of a population, and it is one of the marker quality indicators in genetic research (Serrote et al. 2020). According to the classification proposed by Serrote et al. (2020), the PIC values for dominant markers are classified as highly informative (0.40–0.50), informative (0.30–0.40), moderately informative (0.10–0.25), and poorly informative (0–0.10). The mean PIC value in the present study was 0.27, considered moderately informative (six primers) to informative (14 primers), a value near that observed for *C. urucurana* (the mean PIC value was 0.29) (Costa et al. 2020). Thus, considering the number of primers and the PIC values, the ISSR markers can be effectively used to access the genetic diversity present among and within the natural populations of *C. blanchetianus*.

The mean observed number of alleles (1.768) was higher than that reported by Costa et al. (2020) in a study on three natural populations of *C. urucurana* (1.418) using ISSR markers. The effective number of alleles was lower than the observed number of alleles, with a mean of

1.327, suggesting that many alleles are rare ($p<0.05$) or are of low frequency ($0.05> p <0.25$) (Viegas et al. 2011).

The Shannon diversity index (I) and the expected heterozygosity (He) were estimated to better understand the genetic diversity of the germplasm under study (Table 2). Considering these estimates, the populations with the highest values, in decreasing order, were IT, LG, PV, GC, TB, and AQ. The values of these genetic estimates are directly related to the sample size; however, it is observed that the PV population, despite having a smaller number of individuals (20), presented estimates for the parameters I and He that were higher than those of the GC, TB, and AQ populations. The exchange of alleles with neighboring populations may explain the higher values observed in the PV population, associated with the absence of rare alleles. The estimated values for these statistics are considered low (I = 0.332; He = 0.208), which may be directly related to the occupation and use process of the areas where the samples were collected and to the species' reproductive mechanism (Santos et al. 2017). Reduction in the number of individuals present in these areas likely resulted in crosses between related individuals, which affects the genetic diversity pattern of preferentially allogamous species such as *C. blanchetianus*.

Marmeleiro is a monoecious species with an asynchronous flower opening mechanism. First, pistillate flowers open, followed by the anthesis of staminate flowers (Santos 2016). This favors cross pollination and reduces inbreeding. However, biparental inbreeding has been identified as leading to the high values of the inbreeding coefficient observed in natural populations (Costa et al. 2017). The values found in this study are similar to those reported for *C. urucurana* (I = 0.334; He = 0.221) but lower than those obtained for *C. tetradenius* in Caatinga vegetation (I = 0.450; He = 0.300) (Almeida-Pereira et al. 2017; Costa et al. 2020).

The low heterozygosity values observed may also be related to the manner of seed dispersal used by the species of the *Croton* genus (autochory and zoochory). Autochorous dispersal is characterized by explosive dehiscence of the dry fruit, projecting the seeds far from the parent plant, to a maximum distance of 3.2–3.4 m (Webster 1994; Passos and Ferreira 1996). Zoochorous dispersal, in turn, is a secondary dispersal that occurs through the attraction of ants to the caruncles or elaiosomes (structures rich in lipid compounds present in the seeds), which can travel maximum distances from 1 to 2.5 m (Webster 1994; Passos and Ferreira 1996). This can facilitate crosses between individuals that share common ancestry.

The percentages of polymorphic loci were higher than 80% for the six populations of *C. blanchetianus* (Table 2), confirming the genetic variability of the genotypes evaluated. The highest values were observed for populations, in decreasing order, IT, LG, GC, TB, PV, and AQ. The PV population presented a higher value only than the AQ population, different from what was observed for the I and He parameters. This result may be directly associated with the smaller number of individuals sampled in the PV population (20). These estimates were higher than those reported for three populations of *C. urucurana* (65.93%) (Costa et al. 2020). This may be related to the smaller number of individuals evaluated (30 genotypes) by the authors.

Genetic variation within and among populations is affected by various factors, such as genetic isolation, genetic drift, pollination, reproductive systems, and geographic dispersion (Kamali et al. 2023). In *C. blanchetianus*, the variation within populations (94%) was greater than the variation among populations (6%) (Table 3), a result expected for allogamous species. *C. blanchetianus* has a large group of insects that visit its staminate and pistillate flowers, belonging to the orders Coleoptera (Cerambycidae, Curculionoidae, and Elateridae), Diptera (Drosophilidae and Tachinidae), Hymenoptera (Apidae, Formicidae, and Vespidae), and Lepidoptera (Dutra 2018). However, *C. blanchetianus* relies on a functional set of main pollinators, including bees (Apidae: *Apis mellifera* and *Trigona spinipes*) and two occasional groups, such as beetles (Elateridae *sp*.1, Curculionoidae *sp*.1, Curculionoidae *sp*.2, and Cerambycidae *sp*.1) and ants (Formicidae: *Camponotus crassus* and *Camponotus substitutus*) (Neves 2008; Dutra 2018). Due to its allogamous nature, the effectiveness of thepollinators plays a crucial role in pollen flow between the flowers of different individuals, directly affecting

the reproductive success of the species and preventing the genetic isolation of the different populations.

In cross-pollinated plants, most genetic variability is commonly dispersed within populations, with only a small variation occurring among populations (Murthy et al. 1993). For *C. blanchetianus*, deforestation, resulting from expanding land ownership and conversion of land to agricultural use, has led to fragmentation of the areas in which the species occurs, and this has culminated in the distancing of remaining populations. This fragmentation may explain the current distribution of the species, along with ecological conditions. The hypothesis that the populations studied once constituted a single population may explain the low genetic variation observed among them. This dispersion pattern has been observed in other *Croton* species, such as *C. antisyphiliticus* (Oliveira et al. 2016), *C. tetradenius* (Almeida-Pereira et al. 2017; Brito et al. 2021), and *C. grewioides* (Oliveira et al. 2022c).

The overall genetic differentiation measured (Φ_{PT} = 0.06 and G_{ST} = 0.05) is classified as moderate, implying that there was high gene flow among the populations (Terefe et al. 2022). The results obtained are in agreement with those reported for *C. grewioides*, which also showed moderate genetic differentiation (Oliveira et al. 2022c). The pairwise fixation index (Φ_{PT}) and the Nei's coefficient of genetic diversity (G_{ST}) are measurements that evaluate the genetic differentiation among populations based on allele frequencies, and they can be categorized as low (0–0.05), moderate (0.05–0.15), high (0.15–0.25), and very high (>0.25) population differentiation (Silva et al. 2023 *apud* Wright 1978).

Nei's genetic distance and the pairwise fixation index (Φ_{PT}) had lower values between the AQ and GC populations (0.007 and 0.010, respectively) and higher values between the IT and PV populations (0.031 and 0.102, respectively) (Table 4). It can be said that geographic distance also contributes to genetic differentiation among populations, as confirmed by the significance observed in the Mantel test (0.54 e 0.66, respectively). The distance between the collection sites of the genotypes from AQ and GC is approximately 18 km. This may explain the smaller genetic distance between these populations compared to the IT and PV populations, which are 140 km distant from each other, resulting in greater differentiation between the populations. Therefore, differentiation among the populations studied may have been affected by geographic distance, which results in genetic isolation, as has been shown by some investigations (Chang et al. 2020; Oliveira et al. 2022c). In addition, forest fragmentation and unsustainable exploitation of plant resources may lead to population decline, affecting pollination effectiveness and seed dispersal (Brito et al. 2021). The gene flow (Nm = 9.119) was high, but this is probably the result of the recent fragmentation process and of sharing a recent common ancestor.

Confirming the low genetic diversity observed among the studied populations, the dendrogram clustering (based on Jaccard distance), the principal coordinate analysis (PCoA), and Bayesian statistics did not show a clustering pattern of the genotypes based on their sites of origin. These results indicate low population differentiation, consistent with the result for overall population differentiation obtained through AMOVA (Φ_{PT} = 0.06 and G_{ST} =0.05). Our findings are in agreement with what was observed for *C. tetradenius*, for which high allele sharing among the populations was found, due to gene flow (Brito et al. 2021). We hypothesize that these populations probably formed a single population in the past, and after the fragmentation process, they have not yet differentiated through the emergence of new alleles. An exclusive allele was observed in the PV, TB, and LG populations, but it was not sufficient to confer high values of genetic differentiation between them.

4.5. Conclusion

The results of the present study provide information on the genetic variability among and within native populations of *Croton blanchetianus* from the Caatinga biome of the state of Sergipe, Brazil. The process of fragmentation of the areas in which the species occurs

corroborates the reduced genetic variability observed in the present study due to decline in the number of individuals in the areas and, consequently, the occurrence of crosses between related individuals. Additionally, the populations exhibit low genetic differentiation, since it is not possible to differentiate the genotypes according to their site of origin. These results are essential for selecting genotypes to compose the species collection in the active germplasm bank, as well as for using them in future breeding programs.

4.6. References

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5. MANUSCRIPT 2
CHEMICAL DIVERSITY OF THE ESSENTIAL OIL OF *Croton blanchetianus* Baill.: AN ENDEMIC PLANT FROM THE CAATINGA WITH BIOACTIVE POTENTIAL

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ABSTRACT

The aim of this study was to evaluate the chemical diversity of the essential oil of *Croton blanchetianus* collected from six natural populations in the state of Sergipe, Brazil. Essential oils from 70 genotypes were extracted by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The major compounds identified were α-pinene (1.60–13.37%), limonene (0.30–17.54%), β-phellandrene (4.38–16.04%), 1,8-cineole (0.16–13.56%), (E)-caryophyllene (0.31–13.14%), germacrene D (0.33–10.60%), bicyclogermacrene (5.06–27.47%), and spathulenol (4.34–29.83%). Cluster analysis showed the formation of two distinct chemical clusters, with the presence or absence of limonene and β-phellandrene determining this difference. These results suggest chemotypes within the species. The clusters were not influenced by the geographic origin of the genotypes, indicating strong influence of genetic factors. Results indicate chemical variability in the essential oil of *C. blanchetianus* genotypes and confirm its biotechnological potential, given its importance as a source of bioactive compounds.

Key-words: Euphorbiaceae, *Croton blanchetianus*, essential oils, terpenes, chemical variability, Caatinga biome.

5.1. Introduction

Croton blanchetianus Baill. is an aromatic species endemic to the Caatinga biome (xeric shrubland and thorn forest), whose presence has been confirmed in all states of the Northeast region of Brazil, except Maranhão (1). The species is found in *caatinga* vegetation areas, forming dense populations on sandy or clayey soils in association with anthropized environments and along the edges of forest fragments (1, 2). Morphologically, it is described as a monoecious shrub (1.5-8 m height), with monopodial cylindrical branching, green to yellowish or grayish, with stellate-lepidote trichomes; chartaceous leaf blades, oval to lanceolate, slightly bifacial, with dark green adaxial surfaces and dull, light-green to gray abaxial surfaces; inflorescences in thyrses with unisexual flowers, pistillate flowers without petals and with styles joined in a column, staminate flowers with white petals and stamens with long anthers; fruit consisting of green to yellowish spherical capsules; and smooth brown to black ellipsoid seeds with reniform caruncles (1, 3).

C. blanchetianus is a species with widespread medicinal, beekeeping, and timber uses, and it is listed in the portfolio of the Brazilian Ministry of the Environment as a priority species for the Northeast region of Brazil because of its potential economic importance (4). Furthermore, the species is regarded as extremely important for maintaining ecological balance in the Caatinga, due to its morphological and anatomical traits adapted to semi-arid conditions. Despite its importance, areas in which the species naturally occurs are frequently reduced through expanding human activities, such as increasing real-estate development and deforestation of areas for agriculture. This exposes the species to risks of genetic erosion and loss of genotypes that may be useful to humans.

The essential oil of *C. blanchetianus* contains promising bioactive compounds that stand out for their diverse biological activities and broad applicability. Prominent compounds include

α-pinene, bicyclogermacrene, spathulenol, 1,8-cineole, (*E*)-caryophyllene, cedrol, limonene, germacrene D, and *δ*-cadinene, which have proven biological activity, including antibacterial, acaricidal, antifungal, and insecticidal properties (5-10). The chemical composition of essential oils is determined by the genetic makeup of the species, but it is also influenced by various environmental factors, such as temperature, relative humidity, rainfall, light intensity, winds, soil nutrients, seasonality, and even the time at which plants are harvested (11, 12). These factors can lead to significant changes in the production of these constituents because they modulate the expression and functionality of the different enzymes responsible for the wide diversity of compounds in essential oils, such as terpene synthases (13, 14). However, it is not possible to foresee or establish a single pattern, as each species reacts differently (11).

Given the potential economic importance and diverse applications of the species, it is essential to know the chemical diversity of naturally occurring genotypes in the state of Sergipe, Brazil, to develop strategies for use, conservation, and breeding. Thus, the aim of the present study was to evaluate the chemical diversity of six natural populations of *C. blanchetianus* from different municipalities/counties of Sergipe. Although the species is widely distributed across the state, this is the first study to explore its chemical diversity. The data obtained will facilitate selection of accessions to establish the collection of this species in the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe. This initiative will contribute to the conservation and sustainable use of the genetic resources of the species.

5.2. Material and Methods

5.2.1. Plant material

Leaves from 70 genotypes of *C. blanchetianus* were collected from six municipalities (Aquidabã, Graccho Cardoso, Itabi, Lagarto, Tobias Barreto, and Poço Verde) of the state of Sergipe in the Northeast region of Brazil between June and August 2022 (Table 1). In each municipality, from 8 to 14 genotypes were collected, maintaining a distance of at least 50 m between individuals and from 18 to 150 Km between populations (Table 1). The municipalities of Aquidabã, Graccho Cardoso, and Itabi are in the *Médio Sertão* region of Sergipe. These municipalities have a climate ranging from sub-humid to semi-arid, with a mean annual temperature of 24.7 °C and mean annual rainfall in the last five years of 1267.08 mm (15, 16). The municipalities of Lagarto, Tobias Barreto, and Poço Verde are in the *Centro-Sul* region of Sergipe and have a climate ranging from tropical rainy to semi-arid, with a mean annual temperature of 27 °C and mean annual rainfall in the last five years of 833.68 mm (16, 17). Fertile branches in the flowering or fruiting phase were collected for preparing the specimens, which were deposited in the ASE Herbarium of the Federal University of Sergipe for species identity to be confirmed by taxonomists (Table 1). Plant samples were made available according to the National System for Management of Genetic Heritage and Associated Traditional Knowledge (*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* - SisGen), registered under no. A8CCB3B.

5.2.2. Isolation and analysis of the chemical composition of the essential oils

The collected leaves were dried in a forced-air circulation laboratory oven at 40 ± 1 °C for five days. The essential oils were extracted by hydrodistillation for 120 minutes after the water began to boil using a modified Clevenger apparatus connected to a 3-L round-bottom flask and a heating mantle as a heat source. Isolation was carried out in triplicate using samples of 75-g of dried leaves and 2.0 L of water. The extracted oils were collected and stored in amber vials at -20 °C until analysis of chemical composition. The essential oil content (EOC) of each sample was calculated using the following equation:

$$\text{EOC}\ (\%) = \left(\frac{\text{Volume extracted from the sample}}{\text{Sample dry matter}}\right) \times 100$$

Chemical analyses of the essential oil (EO) samples were carried out using a gas chromatograph (model 7820A, Agilent Technologies) coupled to a mass spectrometer (model 5975 MSD, Agilent Technologies). The system was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness, Agilent).

Samples were injected using an autosampler (model G4513A, Agilent). The injector was fitted with a split-type liner (inner diameter 4.0 mm, outer diameter 6.25 mm, length 78.5 mm, volume 870 μL), and the injection was performed in split mode (split ratio 10:1). The carrier gas was helium 5.0 (purity grade 99.999%) at a constant flow rate of 1.2 mL min^{-1}. One microliter (1.0 μL) of each essential oil sample (10 mg mL^{-1} solution in ethyl acetate) was injected.

The oven temperature program was as follows: initial temperature of 60 °C (held for 1 min), increased to 170 °C at 3 °C min^{-1} (no hold), then to 220 °C at 5 °C min^{-1} (no hold), and finally to 280 °C at 20 °C min^{-1} (no hold). The transfer line temperature was maintained at 280 °C. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, with an ion source temperature of 230 °C and a quadrupole temperature of 150 °C. The mass scan range was 40–550 m/z.

Retention indices (RI) were calculated from the retention times of a homologous series of n-alkanes (C7–C30, certified reference material, Merck) analyzed under the same chromatographic conditions. Compound identification was performed using the Agilent MassHunter Qualitative Analysis software (version 10.0), with support from the NIST MS Search 2.2 library for mass spectral comparison. Additionally, spectra were verified and compared with those available in the NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/) and with data reported in the reference work (18).

5.2.3. Statistical analysis

Analysis of variance (ANOVA) was carried out on the data on essential oil concentration of *C. blanchetianus*, and mean values were clustered using the Scott-Knott test with significance of $p \leq 0.05$ using the Sisvar® software (19).

Based on the chemical composition of the essential oils, two multivariate analyses were performed: cluster analysis and principal component analysis (PCA), using the Statistica® software. For cluster analysis, a dissimilarity matrix was constructed based on Euclidean distance. This matrix was simplified by means of a dendrogram created using Ward's clustering method. Additionally, histograms representing the means and standard deviations of the main compounds in each cluster identified by cluster analysis were prepared using the GraphPad Prism® software. Correlation analysis was also carried out among the chemical constituents of the essential oils (concentrations ≥ 2%) in at least one sample among the analyzed genotypes were considered.

5.3. Results

Chemical analyses of the essential oil from the *C. blanchetianus* genotypes detected the presence of 138 compounds, of which 89 were identified. For statistical analyses, only the compounds with relative percentage ≥ 2% were considered in all the genotypes analyzed, resulting in 32 selected compounds, representing an average of 87.34% of the total concentration (Table 2). The essential oil relative percentage of *C. blanchetianus* ranged from 0.30% for the CBI-13 plant (Itabi) to 1.07% detected in plant CBL-21 (Lagarto), with an overall mean of 0.65% (Table 2). The compounds with relative percentage ≥ 2% were predominantly classified as monoterpenes (33.33%) and sesquiterpenes (66.67%). The compounds with the highest concentrations (≥5%) in at least one sample among, were α-pinene (1.60-13.37%), myrcene (0.10-7.36%), α-phellandrene (0.07-6.80%), ρ-cymene (0.16-5.97%), limonene (0.30-17.54%), β-phellandrene (4.38-16.04%), 1,8-cineole (0.17-13.56%), terpinolene (0.28 -9.36%),

β-elemene (0.15-8.95%), (*E*)-caryophyllene (0.31-13.14%), γ-muurolene (0.07-9.98%), germacrene D (0.33-10.60%), bicyclogermacrene (5.06-27.47%), spathulenol (4.34-29.83%), and 14-hydroxy-9-epi-(*E*)-caryophyllene (0.19-5.21%) (Table 2).

The genotypes CBA-01, CBA-02, and CBA-07, originating from Aquidabã, exhibited the highest relative percentage of limonene (17.31%, 17.54%, and 16.35%, respectively), whereas the accessions CBI-02, CBI-12, and CBL-03, collected in the municipalities of Itabi and Lagarto, stood out for their higher relative percentage s of β-phellandrene (15.58%, 14.83%, and 16.04%, respectively). The compounds bicyclogermacrene and spathulenol showed elevated relative percentage in all analyzed genotypes. The genotypes CBL-12, CBI-03, and CBG-23, collected in Lagarto, Itabi, and Graccho Cardoso, exhibited the highest relative percentage of bicyclogermacrene (27.47%, 24.43%, and 24.31%, respectively), while genotypes CBP-03, CBT-22, and CBP-12, collected in Poço Verde and Tobias Barreto, presented the highest relative percentage of spathulenol (29.83%, 28.57%, and 27.74%, respectively). The genotypes CBL-22, CBL-02, CBG-22, and CBG-21, originating from the municipalities of Lagarto and Graccho Cardoso, exhibited the highest relative percentage of *α*-pinene (13.37%, 11.54%, 11.01%, and 10.81%, respectively). Regarding the compounds 1,8-cineole and (*E*)-caryophyllene, genotypes CBA-06 and CBI-23 stood out (13.56% and 12.33% of 1,8-cineole, respectively), as well as CBP-04 and CBI-23 (13.56% and 12.33% of (*E*)-caryophyllene, respectively). The highest relative percentage of myrcene, α-phellandrene, ρ-cymene, terpinolene, β-elemene, γ-muurolene, and 14-hydroxy-9-epi-(*E*)-caryophyllene were observed in genotypes CBT-01 (5.70%), CBP-11 (6.92%), CBP-12 (5.97%), CBG-11 (9.36%), CBT-03 (8.95%), CBA-01 (9.98%), and CBG-12 (5.21%), respectively, collected in Tobias Barreto, Poço Verde, Graccho Cardoso, and Aquidabã.

The major compounds determined the formation of two distinct clusters based on chemical composition (Figure 1). Cluster I, with 49 genotypes (CBA-01, CBA-02, CBG-04, CBG-02, CBG-03, CBI-01, CBI-24, CBL-14, CBL-21, CBL-24, CBA-04, CBA-05, CBG-11, CBG-05, CBT-03, CBA-06, CBI-23, CBA-11, CBG-13, CBA-12, CBT-21, CBL-02, CBT-11, CBP-14, CBP-11, CBA-08, CBI-13, CBT-01, CBT-14, CBG-12, CBL-12, CBG-22, CBP-13, CBA-07, CBA-09, CBG-01, CBI-14, CBI-22, CBG-14, CBI-21, CBP-02, CBT-24, CBT-13, CBL-13, CBP-01, CBP-04, CBT-22, CBP-03, and CBP-12), was mainly characterized by the presence of *α*-pinene (1.60-11.54%), limonene (0.00-17.54%), 1,8-cineole (0.00-13.56%), (*E*)-caryophyllene (2.38-12.30%), germacrene D (0.00-10.60%), bicyclogermacrene (5.06-27.47%), and spathulenol (4.34-29.83%) as the main compounds, and the absence of β-phellandrene (0.00%). Cluster II, in turn, with 21 genotypes (CBA-03, CBI-03, CBG-24, CBG-06, CBG-23, CBL-01, CBA-10, CBI-11, CBT-04, CBI-04, CBL-11, CBI-12, CBT-23, CBT-12, CBG-21, CBI-02, CBT-02, CBL-23, CBL-03, CBL-22 e CBL-04), was mainly characterized by the presence of α-pinene (3.03-13.37%), β-phellandrene (4.38-16.04%), (*E*)-caryophyllene (1.44-11.97%), germacrene D (1.33-10.17%), bicyclogermacrene (6.71-25.31%), and spathulenol (5.77-20.62%) as the major compounds, and the absence of limonene (0.00%) (Table 2 and Figure 2).

The compounds *α*-pinene, *γ*-terpinene, α-terpineol, (*E*)-caryophyllene, aromadendrene, bicyclogermacrene, γ-cadinene, spathulenol, caryophyllene oxide, guaiol, and muurola-4,10(14)-dien-1-β-ol were detected in the essential oil of all the sampled genotypes (Table 2). The chemical compound limonene was identified in the genotypes of Cluster 1, except for genotypes CBA-06 and CBI-23, whereas β-phellandrene was identified only in the genotypes of Cluster II (Figure 1, Figure 2 and Table 2).

According to principal component analysis, the first and second components accounted for 25.28% of the total cumulative variance (Figure 3). The first principal component explained 14.24% of the total variance and was negatively correlated with sabinene (r = -0.85). The second principal component, in turn, accounted for 11.04% of the total variance and was positively correlated with spathulenol (r = 0.70) and negatively correlated with germacrene D (r = -0.70).

Correlation analysis among the compounds showed strong positive correlations between (*E*)-caryophyllene and *α*-humulene ($r = 0.96$) and between 1,8-cineole and α-terpineol ($r = 0.70$) (Figure 4). Moderate correlations were observed between the compounds sabinene and α-terpineol ($r = 0.69$), sabinene and 1,8-cineole ($r = 0.68$), spathulenol and *ρ*-cymene ($r = 0.65$), spathulenol and muurola-4,10(14)-dien-1-β-ol ($r = 0.53$), caryophyllene oxide and *α*-humulene ($r = 0.58$), caryophyllene oxide and (*E*)-caryophyllene ($r = 0.56$), γ-cadinene and δ-cadinene ($r = 0.65$), and C29 and *β*-elemene ($r = 0.54$). Moderate negative correlations were found for the compounds ρ-cymene and bicyclogermacrene ($r = -0.58$), and limonene and β-phellandrene ($r = -0.53$) (Figure 4).

5.4. Discussion

The essential oils of the *C. blanchetianus* genotypes collected from native populations in the state of Sergipe showed high chemical variability. Although the genotypes were collected from different regions of Sergipe (*Médio Sertão* and *Centro-Sul*), characterized by different edaphic and climatic conditions, geographic origin did not influence the distribution of the genotypes within each cluster in cluster analysis. The general clustering pattern did not strictly correspond to the distribution of genotypes in their different geographic origins, as observed for *Varronia curassavica*, *Eplingiella fruticosa*, and *Lantana camara* (20-22). Although biosynthesis of essential oils is affected by both genetic and environmental factors, the results of this study suggest that genetic factors may have a greater influence on the chemical composition of the genotypes analyzed, as the genotypes collected from the same location / region were grouped into different clusters.

Genetic variation within and among populations is influenced by several factors, such as genetic isolation, genetic drift, pollination, reproductive systems, and geographic dispersion (23). *C. blanchetianus* is a monoecious species, with an asynchronous flower-opening mechanism, where the pistillate flowers open first, followed by the staminate, favoring cross pollination and reducing inbreeding (24). However, this does not impede mating between related individuals. The staminate and pistillate flowers of the species are visited by a large array of insects, but it is mainly pollinated by bees (Apidae: *Apis mellifera* and *Trigona spinipes*) (25). As it is an allogamous species, the efficiency of pollinators is essential for pollen transport between flowers of different individuals, and this directly influences the reproductive success of the species, preventing genetic isolation of the different populations.

The lack of clustering based on geographic origin may be related to the existence of gene flow among the populations and/or fragmentation of a single initial population. As *C. blanchetianus* often occurs in deforested areas, resulting from real-estate expansion and from conversion of land to agricultural use, it exhibits remnant populations separated by large distances, as a result of the habitat fragmentation process. This fragmentation may explain the current distribution of the species and suggests that the populations were part of a single population in the past. Furthermore, although samples were from distinct regions in the state of Sergipe, caatinga vegetation predominated in the collection areas, as both are in the semi-arid region of the state.

The *C. blanchetianus* genotypes also showed high variability regarding essential oil concentration. Essential oil production is a quantitative trait, controlled by multiple genes and influenced by environmental factors. Thus, environmental influences, together with intrinsic genetic variation, may explain the differences in the essential oil concentration among the collected genotypes. Previous studies on the chemical composition of the *C. blanchetianus* essential oils extracted by hydrodistillation reported concentrations ranging from 0.70% to 0.72% in fresh leaves and of 0.96% in dry leaves (6, 8, 26). Ribeiro et al. (12) studied the influence of the circadian cycle (leaves collected at 8:00 a.m., 12:00 noon, and 8:00 p.m.) and of seasonality (dry and rainy seasons) on *C. blanchetianus* essential oil concentration and found variation from 0.25-0.40% and 0.33-0.49%, respectively.

Studies on the chemical composition of *C. blanchetianus* essential oil have shown the predominance of monoterpenes and sesquiterpenes, such as bicyclogermacrene (10.42–33.0%), 1,8-cineole (19.95–32.94%), (*E*)-caryophyllene (11.85–27.52%), spathulenol (8.07–24.10%), germacrene D (20.60%), α-pinene (9.14–19.19%), β-phellandrene (13.92%), and limonene (10.36%) (7, 8, 10, 12, 27-29). The present study found the same predominant major compounds as previous studies, such as *α*-pinene (1.60-13.37%), limonene (0.00-17.54%), β-phellandrene (0.00-16.04%), 1,8-cineole (0.00-13.56%), (*E*)-caryophyllene (0.31-13.14%), germacrene D (0.00-10.60%), bicyclogermacrene (5.06-27.47%), and spathulenol (4.34-29.83%). However, Rodrigues et al. (6) identified cedrol (28.4%) as a major compound in the essential oil of *C. blanchetianus* leaves collected in Patos, Paraíba, which differs from the compounds found in the present study. In this respect, the intraspecific variation found in the chemical composition of essential oils may be attributed to environmental factors such as temperature, rainfall, light intensity, and relative humidity; to geographic variations; to seasonality; and even to the time the plants are harvested (12). Additionally, genetic factors may also contribute to these differences (20, 21).

The compounds identified in this study are also consistent with studies in the literature on other species of *Croton*, which highlight terpenoids as the main constituents of the essential oils in this genus. For example, the essential oils of the leaves of *Croton tetradenius* contain *α*-pinene, *α*-terpinene, *p*-cymene, and 1,8-cineole in their composition (30). Moreover, the leaf essential oils of *Croton heliotropiifolius* include *β*-caryophyllene, bicyclogermacrene, germacrene D, limonene, and 1,8-cineole (31). In *Croton conduplicatus*, the predominant compounds were 1,8-cineole, bicyclogermacrene, (*E*)-caryophyllene, and spathulenol (32). The essential oil of *Croton argyratus*, in turn, contained *β*-caryophyllene, spathulenol, caryophyllene oxide, and germacrene D (33).

The large number of species within the genus *Croton* accounts for the high chemical diversity observed among its members, as can be seen, for example, in *Croton grewioides*, which has eugenol, methyl eugenol, and methyl chavicol as the main constituents of its essential oil (34). In *Croton pulegiodorus*, the major compounds identified were ascaridole (22.75%) and camphor (18.11%), whereas in *Croton delpyi* the predominant compounds were *β*-caryophyllene (54.34%), *α*-humulene (18.19%), *β*-caryophyllene epoxide (3.99%), valencene (3.64%), and linalool (3.22%) (35, 36).

The characterization of essential oils from *C. blanchetianus* genotypes collected from six municipalities in Sergipe showed high variability of the compounds, which formed two chemical clusters. Although the major compounds of α-pinene, (*E*)-caryophyllene, bicyclogermacrene, and spathulenol appeared in all groups, the presence or absence of limonene and β-phellandrene contributed to the formation and differentiation of the two groups. Limonene and β-phellandrene are synthesized from the *α*-terpinyl cation, where proton loss catalyzed by limonene synthase leads to the production of limonene. In contrast, β-phellandrene is produced through hydride shifts mediated by β-phellandrene synthase, followed by proton loss (14, 37, 38). The terpene synthase enzymes are numerous and are able to catalyze multiple products from a single substrate, which may explain the preferential production of one compound over another (13).

Understanding the correlations between chemical compounds is a valuable tool in selecting specific genotypes, which can be useful for conservation and breeding programs. In this study, the strong positive correlations between *α*-terpineol / 1,8-cineole and between *α*-humulene / (*E*)-caryophyllene may be related to the metabolic pathways for the production of these compounds, which share the same precursors (geranyl diphosphate and farnesyl diphosphate, respectively) (14). The monoterpenes *α*-terpineol and 1,8-cineole are formed from the *α*-terpinyl cation. Hydration of the *α*-terpinyl cation by an H_2O molecule generates α-terpineol, which, through additional internal cyclization of the alcohol oxygen, produces 1,8-cineole (13). The sesquiterpenes *α*-humulene and (*E*)-caryophyllene are formed from the nerolidyl diphosphate cation, which undergoes cyclization and deprotonation, leading to the

formation of α-humulene. This compound, in turn, undergoes further cyclization and deprotonation, forming (*E*)-caryophyllene (14). Negative correlations can also be useful in selecting specific genotypes, as they help identify those with a high content of one compound at the expense of another. These correlations may be associated with distinct metabolic pathways through which the compounds are synthesized. For example, the compounds ρ-cymene and bicyclogermacrene are negatively correlated. The monoterpene ρ-cymene has geranyl diphosphate as its main precursor, whereas the sesquiterpene bicyclogermacrene is derived from farnesyl diphosphate (14).

The essential oil of *C. blanchetianus* stands out for its high diversity of compounds, which enhances its biological potential. Several studies have reported bioactive properties for its essential oil, including antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Leuconostoc mesenteroides*, and *Weissella viridescens* (39, 8, 40); insecticidal activity against the coleopteran *Callosobruchus maculatus* in stored grains; and acaricidal activity against ectoparasites in cattle - *Rhipicephalus (Boophilus) microplus* and the two-spotted spider mite (*Tetranychus urticae*) (41, 6, 42). The species has also shown larvicidal activity against *Aedes aegypti* (43). In addition to the activities already studied, the essential oil of this species exhibits herbicidal potential due to the presence of compounds such as α-pinene, limonene, 1,8-cineole, and spathulenol (44–46), indicating that new biological activities may still be explored.

The broad range of genotypes evaluated in the present study is particularly relevant, as it demonstrates that individuals of the same species may exhibit different chemotypes, as observed in *Croton grewioides*, which shows chemical variations characterized by the predominance of eugenol, methyl eugenol, and methyl chavicol (34). Therefore, this study stands out as a pioneering initiative, both by its broad geographic scope and by the considerable number of genotypes analyzed. Its importance goes beyond the regional context, as it not only opens new routes for conservation strategies and sustainable use of the species as a source of bioactive compounds, but also highlights its as yet unexplored biological potential, establishing a solid foundation for future research and applications of this genetic resource.

5.5. Conclusion

Based on this study of the chemical diversity of essential oils of *Croton blanchetianus* in Sergipe, Brazil, it can be concluded that the species has high chemical variability among the natural populations studied. Results suggest that genetic factors may have greater influence on the chemical composition of the essential oils of the genotypes analyzed. The compounds found in higher concentrations were α-pinene, limonene, β-phellandrene, 1,8-cineole, (*E*)-caryophyllene, germacrene D, bicyclogermacrene, and spathulenol, which revealed the formation of two distinct clusters based on the chemical composition of the essential oils. The presence of specific compounds in the clusters, such as β-phellandrene and limonene, suggests the potential for identifying chemotypes within the species. The results highlight the importance of *C. blanchetianus* as a promising source of bioactive compounds with therapeutic properties and biotechnological applications, and they clarify the chemical diversity of the species, which is essential for developing strategies directed toward conservation and breeding.

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Table 1. Identification and origin of *Croton blanchetianus* plants collected from six natural populations in the state of Sergipe, Brazil.

Plant	Municipality of origin	Geographic coordinates	Voucher no.
CBA-01	Aquidabã	S 10º17'45.2" W 37º02'10.7"	42844
CBA-02	Aquidabã	S 10º17'45.1" W 37º02'09.2"	42845
CBA-03	Aquidabã	S 10º18'14.0" W 37º02'57.8"	42846
CBA-04	Aquidabã	S 10º18'14.9" W 37º02'58.8"	42847
CBA-05	Aquidabã	S 10º18'16.2" W 37º03'00.4"	42848
CBA-06	Aquidabã	S 10º19'26.6" W 37º04'41.1"	42849
CBA-07	Aquidabã	S 10º19'27.0" W 37º04'39.9"	42850
CBA-08	Aquidabã	S 10º19'27.7" W 37º04'44.3"	42851
CBA-09	Aquidabã	S 10º19'25.5" W 37º04'43.3"	42852
CBA-10	Aquidabã	S 10º19'22.9" W 37º04'42.5"	42853
CBA-11	Aquidabã	S 10º19'22.4" W 37º04'41.3"	42854
CBA-12	Aquidabã	S 10º19'22.4" W 37º04'40.4"	42855
CBG-01	Graccho Cardoso	S 10º14'02.9" W 37º11'21.5"	42856
CBG-02	Graccho Cardoso	S 10º14'04.5" W 37º11'23.5"	42857
CBG-03	Graccho Cardoso	S 10º14'05.8" W 37º11'23.7"	42858
CBG-04	Graccho Cardoso	S 10º14'06.8" W 37º11'25.6"	42859
CBG-05	Graccho Cardoso	S 10º14'06.4" W 37º11'26.9"	42860
CBG-06	Graccho Cardoso	S 10º14'03.7" W 37º11'25.8"	42861
CBG-11	Graccho Cardoso	S 10º14'29.8" W 37º12'53.6"	42862
CBG-12	Graccho Cardoso	S10º14'30.6" W 37º12'52.8"	42863
CBG-13	Graccho Cardoso	S 10º14'30.3" W 37º12'54.1"	42864
CBG-14	Graccho Cardoso	S 10º14'29.5" W 37º12'50.5"	42865
CBG-21	Graccho Cardoso	S 10º13'49.5" W 37º13'16.2"	42866
CBG-22	Graccho Cardoso	S 10º13'49.6" W 37º13'16.9"	42867
CBG-23	Graccho Cardoso	S 10º13'48.6" W 37º13'16.4"	42868
CBG-24	Graccho Cardoso	S 10º13'48.0" W 37º13'16.4"	42869
CBI-01	Itabi	S 10º05'19.6" W 37º05'33.4"	42870
CBI-02	Itabi	S 10º05'19.4" W 37º05'33.3"	42871
CBI-03	Itabi	S 10º05'19.3" W 37º05'36.0"	42872
CBI-04	Itabi	S 10º05'18.5" W 37º05'35.1"	42873
CBI-11	Itabi	S 10º06'50.9" W 37º06'32.1"	42874
CBI-12	Itabi	S 10º06'49.9" W 37º06'33.3"	42875
CBI-13	Itabi	S 10º06'51.2" W 37º06'29.7"	42876
CBI-14	Itabi	S 10º06'49.9" W 37º06'28.6"	42877
CBI-21	Itabi	S 10º07'54.1" W 37º07'17.9"	42878
CBI-22	Itabi	S 10º07'59.1" W 37º07'26.8"	42879
CBI-23	Itabi	S 10º07'49.8" W 37º07'16.1"	42880
CBI-24	Itabi	S 10º07'47.4" W 37º07'18.1"	42881
CBL-01	Lagarto	S 10º53'10.5" W 37º36'55.9"	42882
CBL-02	Lagarto	S 10º53'11.3" W 37º36'56.2"	42883
CBL-03	Lagarto	S 10º53'12.0" W 37º36'55.4"	42884
CBL-04	Lagarto	S 10º53'13.7" W 37º36'55.8"	42885
CBL-11	Lagarto	S 10º51'49.5" W 37º36'00.7"	42886
CBL-12	Lagarto	S 10º51'50.2" W 37º35'59.0"	42887
CBL-13	Lagarto	S 10º51'55.7" W 37º36'00.7"	42888
CBL-14	Lagarto	S 10º52'01.5" W 37º35'59.2"	42889
CBL-21	Lagarto	S 10º50'52.5" W 37º36'19.5"	42890
CBL-22	Lagarto	S 10º50'51.0" W 37º36'17.8"	42891
CBL-23	Lagarto	S 10º50'41.3" W 37º36'17.4"	42892
CBL-24	Lagarto	S 10º50'40.8" W 37º36'16.7"	42893
CBT-01	Tobias Barreto	S 11º08'53.0" W 37º56'41.2"	42894
CBT-02	Tobias Barreto	S 11º08'52.9" W 37º56'41.6"	42895
CBT-03	Tobias Barreto	S 11º08'54.1" W 37º56'43.4"	42896
CBT-04	Tobias Barreto	S 11º08'59.4" W 37º56'50.4";	42897
CBT-11	Tobias Barreto	S 11º09'42.6" W 38º00'37.6"	42898
CBT-12	Tobias Barreto	S 11º10'06.4" W 37º58'44.0"	42899
CBT-13	Tobias Barreto	S 11º10'05.2" W 37º58'45.9"	42900
CBT-14	Tobias Barreto	S 11º10'03.6" W 37º58'52.0"	42901
CBT-21	Tobias Barreto	S 11º06'22.6" W 38º02'02.6"	42902

CBT-22	Tobias Barreto	S 11º06'20.6" W 38º02'03.6"	42903
CBT-23	Tobias Barreto	S 11º06'15.7" W 38º02'03.6"	42904
CBT-24	Tobias Barreto	S 11º06'25.4" W 38º02'01.6"	42905
CBP-01	Poço Verde	S 10º48'33.1" W 38º08'21.4"	42906
CBP-02	Poço Verde	S 10º48'30.6" W 38º08'22.4"	42907
CBP-03	Poço Verde	S 10º48'27.5" W 38º08'23.4"	42908
CBP-04	Poço Verde	S 10º48'38.7" W 38º08'24.2"	42909
CBP-11	Poço Verde	S 10º48'15.6" W 38º08'14.3"	42910
CBP-12	Poço Verde	S 10º48'14.5" W 38º08'13.0"	42911
CBP-13	Poço Verde	S 10º48'13.6" W 38º08'10.4"	42912
CBP-14	Poço Verde	S 10º48'17.3" W 38º08'12.6"	42913

Table 2. Relative percentage (%) of chemical compounds and content of the essential oils from *Croton blanchetianus* plants collected from six natural populations in the state of Sergipe, Brazil.

Plant	Compound											
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
CBA-01	6.86 ± 0.10	2.75 ± 0.12	1.36 ± 0.12	-	-	17.31 ± 0.95	-	5.02 ± 0.64	0.57 ± 0.00	-	2.15 ± 0.08	0.28 ± 0.02
CBA-02	10.36 ± 0.76	3.16 ± 0.10	1.62 ± 0.06	0.28 ± 0.01	0.16 ± 0.14	17.54 ± 0.76	-	6.26 ± 1.22	0.98 ± 0.06	0.47 ± 0.03	3.88 ± 0.12	0.42 ± 0.03
CBA-03	4.42 ± 0.04	0.93 ± 0.04	1.09 ± 0.05	4.90 ± 0.03	0.96 ± 0.04	-	10.59 ± 0.14	1.99 ± 0.17	0.70 ± 0.02	0.39 ± 0.01	0.57 ± 0.02	-
CBA-04	3.61 ± 0.25	1.02 ± 0.01	0.76 ± 0.01	1.17 ± 0.02	1.88 ± 0.04	2.88 ± 0.05	-	4.10 ± 0.13	1.06 ± 0.02	5.12 ± 0.16	1.37 ± 0.01	7.22 ± 0.06
CBA-05	5.04 ± 0.27	1.49 ± 0.01	0.63 ± 0.01	1.33 ± 0.02	1.75 ± 0.04	3.07 ± 0.08	-	5.47 ± 0.16	0.78 ± 0.00	6.02 ± 0.12	2.30 ± 0.03	5.81 ± 0.07
CBA-06	5.75 ± 0.32	2.48 ± 0.01	0.74 ± 0.02	-	0.22 ± 0.01	-	-	13.56 ± 0.30	0.47 ± 0.01	0.06 ± 0.10	1.35 ± 0.01	1.53 ± 0.03
CBA-07	9.42 ± 0.50	1.58 ± 0.02	1.02 ± 0.02	0.37 ± 0.00	0.79 ± 0.0	16.35 ± 0.22	-	6.12 ± 0.55	1.34 ± 0.04	0.70 ± 0.01	1.50 ± 0.04	0.70 ± 0.01
CBA-08	3.06 ± 0.17	0.38 ± 0.02	2.78 ± 0.09	0.48 ± 0.02	1.62 ± 0.04	3.85 ± 0.13	-	0.70 ± 0.03	0.25 ± 0.01	2.71 ± 0.10	0.54 ± 0.02	0.47 ± 0.02
CBA-09	6.36 ± 0.23	2.67 ± 0.03	1.16 ± 0.01	1.31 ± 0.01	1.83 ± 0.03	13.02 ± 0.15	-	6.04 ± 0.37	1.22 ± 0.03	6.38 ± 0.02	2.29 ± 0.04	0.33 ± 0.01
CBA-10	5.72 ± 0.11	1.32 ± 0.05	0.96 ± 0.04	0.90 ± 0.03	0.96 ± 0.06	-	8.87 ± 0.21	4.02 ± 0.13	0.75 ± 0.03	2.97 ± 0.05	1.14 ± 0.05	1.37 ± 0.04
CBA-11	9.58 ± 0.65	1.45 ± 0.04	0.95 ± 0.02	1.02 ± 0.03	2.30 ± 0.04	2.88 ± 0.04	-	4.91 ± 0.12	0.91 ± 0.04	4.56 ± 0.09	1.48 ± 0.04	0.86 ± 0.03
CBA-12	5.83 ± 0.42	2.26 ± 0.09	0.77 ± 0.02	1.00 ± 0.04	2.51 ± 0.06	4.29 ± 4.61	-	5.57 ± 4.46	0.43 ± 0.21	4.83 ± 0.18	2.23 ± 0.02	0.27 ± 0.02
CBG-01	5.85 ± 0.94	0.81 ± 0.19	1.02 ± 0.13	1.17 ± 0.09	1.91 ± 0.11	12.36 ± 1.08	-	-	0.93 ± 0.09	3.63 ± 0.32	0.16 ± 0.03	1.18 ± 0.16
CBG-02	6.06 ± 0.42	1.43 ± 0.15	1.45 ± 0.17	1.59 ± 0.09	0.85 ± 0.08	2.42 ± 0.34	-	4.83 ± 0.52	1.34 ± 0.20	5.56 ± 1.05	1.59 ± 0.16	-
CBG-03	6.90 ± 1.53	0.66 ± 0.10	2.53 ± 0.51	1.64 ± 0.31	0.72 ± 0.07	2.81 ± 0.47	-	3.97 ± 0.69	1.60 ± 0.18	6.38 ± 1.40	1.04 ± 0.00	1.33 ± 0.13
CBG-04	9.86 ± 1.13	1.12 ± 0.24	1.31 ± 0.33	0.71 ± 0.18	0.53 ± 0.08	13.80 ± 1.68	-	2.40 ± 0.31	1.19 ± 0.19	1.30 ± 0.26	0.39 ± 0.13	1.20 ± 0.15
CBG-05	8.87 ± 0.25	1.90 ± 0.05	0.69 ± 0.02	3.45 ± 0.02	2.61 ± 0.06	1.80 ± 0.05	-	6.87 ± 0.11	0.79 ± 0.02	4.33 ± 0.05	2.15 ± 0.04	6.50 ± 0.07
CBG-06	9.21 ± 0.49	0.35 ± 0.04	1.04 ± 0.08	1.73 ± 0.11	1.31 ± 0.10	-	11.64 ± 0.12	-	0.71 ± 0.05	5.71 ± 0.23	0.54 ± 0.04	1.32 ± 0.05
CBG-11	6.07 ± 0.55	2.18 ± 0.28	0.71 ± 0.10	2.17 ± 0.25	1.60 ± 0.06	2.48 ± 0.15	-	8.12 ± 0.84	1.48 ± 0.11	9.36 ± 0.68	2.30 ± 0.09	3.23 ± 0.11
CBG-12	5.30 ± 0.62	1.91 ± 0.27	0.56 ± 0.09	-	-	3.53 ± 0.38	-	6.46 ± 0.45	0.18 ± 0.16	-	2.17 ± 0.45	2.15 ± 0.20
CBG-13	8.31 ± 0.50	1.94 ± 0.28	0.64 ± 0.18	0.52 ± 0.12	1.64 ± 0.22	1.31 ± 0.06	-	8.88 ± 0.66	0.45 ± 0.07	2.75 ± 0.65	2.83 ± 0.11	0.76 ± 0.13
CBG-14	4.35 ± 0.38	1.25 ± 0.19	0.87 ± 0.13	1.60 ± 0.20	2.07 ± 0.35	9.22 ± 2.44	-	2.22 ± 0.59	1.03 ± 0.17	7.63 ± 1.85	1.01 ± 0.19	2.95 ± 0.28
CBG-21	10.81 ± 0.61	2.45 ± 0.14	1.05 ± 0.06	1.86 ± 0.09	1.59 ± 0.08	-	7.39 ± 0.21	10.79 ± 0.50	1.27 ± 0.07	8.47 ± 0.05	3.21 ± 0.11	1.22 ± 0.01
CBG-22	11.01 ± 0.29	0.39 ± 0.01	0.50 ± 0.01	0.98 ± 0.02	0.72 ± 0.03	1.75 ± 0.06	-	1.07 ± 0.05	0.59 ± 0.01	5.22 ± 0.10	0.58 ± 0.02	2.74 ± 0.04
CBG-23	7.44 ± 0.24	1.13 ± 0.11	0.73 ± 0.07	0.99 ± 0.08	1.29 ± 0.09	-	6.89 ± 0.16	4.19 ± 0.01	0.48 ± 0.04	3.78 ± 0.13	1.28 ± 0.05	1.48 ± 0.08
CBG-24	5.89 ± 0.48	0.37 ± 0.05	7.36 ± 0.46	4.57 ± 0.30	1.69 ± 0.18	-	7.16 ± 0.45	1.12 ± 0.12	0.57 ± 0.06	0.54 ± 0.06	0.55 ± 0.04	4.09 ± 0.03
CBI-01	5.42 ± 0.09	1.84 ± 0.01	1.04 ± 0.00	1.21 ± 0.01	1.19 ± 0.01	8.59 ± 0.11	-	4.41 ± 0.25	0.99 ± 0.00	4.60 ± 0.06	1.80 ± 0.01	0.35 ± 0.00
CBI-02	5.61 ± 0.29	2.05 ± 0.07	1.68 ± 0.06	2.19 ± 0.09	0.86 ± 0.02	-	15.58 ± 0.54	3.26 ± 0.14	1.30 ± 0.12	6.41 ± 0.20	3.22 ± 0.07	4.56 ± 0.20
CBI-03	6.59 ± 0.61	1.33 ± 0.11	1.22 ± 0.06	1.55 ± 0.08	0.73 ± 0.04	-	9.98 ± 0.53	2.60 ± 0.52	1.07 ± 0.17	4.51 ± 0.33	1.29 ± 0.04	2.63 ± 0.13
CBI-04	5.86 ± 0.38	0.96 ± 0.07	0.80 ± 0.04	1.32 ± 0.07	2.45 ± 0.09	-	5.81 ± 0.24	2.73 ± 0.14	0.89 ± 0.04	5.02 ± 0.16	0.85 ± 0.04	3.91 ± 0.14
CBI-11	5.96 ± 0.19	0.29 ± 0.01	0.89 ± 0.02	1.06 ± 0.03	1.37 ± 0.06	-	8.40 ± 0.24	-	0.25 ± 0.01	2.80 ± 0.06	0.20 ± 0.01	1.08 ± 0.04
CBI-12	6.29 ± 0.18	0.67 ± 0.02	1.59 ± 0.02	-	2.43 ± 0.05	-	14.83 ± 0.12	-	0.71 ± 0.02	6.07 ± 0.14	0.93 ± 0.03	1.37 ± 0.16
CBI-13	6.13 ± 0.49	0.89 ± 0.06	0.57 ± 0.05	-	1.89 ± 0.08	3.90 ± 0.29	-	3.11 ± 0.17	0.98 ± 0.06	0.32 ± 0.00	0.65 ± 0.07	0.63 ± 0.03
CBI-14	7.45 ± 0.53	1.22 ± 0.06	1.31 ± 0.06	2.07 ± 0.10	2.45 ± 0.10	13.51 ± 0.61	-	-	1.31 ± 0.06	9.08 ± 0.41	0.92 ± 0.03	3.58 ± 0.16
CBI-21	6.15 ± 0.36	1.04 ± 0.03	0.98 ± 0.03	1.05 ± 0.03	4.25 ± 0.15	10.18 ± 0.41	-	1.28 ± 0.11	0.85 ± 0.02	4.96 ± 0.23	1.53 ± 0.12	0.16 ± 0.14
CBI-22	4.27 ± 0.26	2.92 ± 0.11	1.25 ± 0.04	0.90 ± 0.03	0.49 ± 0.43	14.02 ± 1.02	-	5.64 ± 1.00	1.29 ± 0.04	3.75 ± 0.08	3.13 ± 0.10	2.27 ± 0.40
CBI-23	6.19 ± 0.13	2.56 ± 0.02	1.01 ± 0.03	0.26 ± 0.01	0.55 ± 0.02	-	-	12.33 ± 0.24	0.86 ± 0.05	0.58 ± 0.03	2.20 ± 0.11	1.04 ± 0.03
CBI-24	8.06 ± 0.75	1.14 ± 0.06	1.32 ± 0.06	0.59 ± 0.03	0.66 ± 0.02	8.48 ± 0.48	-	2.32 ± 0.25	1.06 ± 0.04	1.10 ± 0.05	1.01 ± 0.01	4.03 ± 0.23

Table 2. (Continuation)

Plant	Compound											
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
CBT-01	7.22 ± 0.54	1.06 ± 0.06	5.70 ± 0.35	0.07 ± 0.12	0.38 ± 0.02	-	-	9.65 ± 0.50	0.62 ± 0.02	0.45 ± 0.02	2.16 ± 0.05	-
CBT-02	7.65 ± 0.05	0.57 ± 0.04	1.47 ± 0.06	1.76 ± 0.10	0.84 ± 0.05	-	8.35 ± 0.24	2.21 ± 0.05	0.54 ± 0.04	0.45 ± 0.03	1.15 ± 0.04	3.01 ± 0.13
CBT-03	6.29 ± 0.50	1.28 ± 0.04	0.58 ± 0.05	0.36 ± 0.01	0.32 ± 0.03	3.49 ± 0.29	-	5.96 ± 0.37	0.46 ± 0.03	1.72 ± 0.08	0.96 ± 0.09	8.95 ± 0.34
CBT-04	3.03 ± 0.04	0.47 ± 0.02	0.10 ± 0.09	1.01 ± 0.03	0.97 ± 0.05	-	6.19 ± 0.27	2.14 ± 0.17	0.59 ± 0.02	3.54 ± 0.05	0.77 ± 0.07	3.95 ± 0.26
CBT-11	8.19 ± 0.38	0.35 ± 0.03	0.32 ± 0.03	3.27 ± 0.09	3.38 ± 0.08	1.78 ± 0.04	-	2.05 ± 0.05	0.82 ± 0.05	3.30 ± 0.06	1.15 ± 0.03	0.47 ± 0.03
CBT-12	5.38 ± 0.12	1.23 ± 0.07	0.66 ± 0.06	-	2.91 ± 0.15	-	8.51 ± 0.28	5.30 ± 0.38	0.89 ± 0.04	3.43 ± 0.07	2.25 ± 0.07	-
CBT-13	8.93 ± 1.21	0.90 ± 0.04	3.65 ± 0.12	0.72 ± 0.04	1.30 ± 0.07	9.88 ± 0.08	-	3.14 ± 0.43	1.09 ± 0.04	3.01 ± 0.07	1.90 ± 0.11	0.15 ± 0.03
CBT-14	3.77 ± 0.25	0.66 ± 0.01	0.31 ± 0.02	-	-	-	-	5.49 ± 0.15	0.37 ± 0.03	-	1.61 ± 0.04	1.65 ± 0.03
CBT-21	5.80 ± 0.80	1.09 ± 0.34	0.23 ± 0.20	2.96 ± 0.58	3.06 ± 0.57	0.30 ± 0.52	-	7.78 ± 1.60	0.88 ± 0.34	0.40 ± 0.11	3.16 ± 0.21	1.18 ± 0.09
CBT-22	8.27 ± 0.09	0.74 ± 0.02	0.63 ± 0.02	1.57 ± 0.01	5.85 ± 0.14	0.76 ± 1.31	-	7.47 ± 1.55	0.67 ± 0.02	2.82 ± 0.08	2.61 ± 0.10	-
CBT-23	6.31 ± 0.21	1.27 ± 0.03	4.14 ± 0.10	0.73 ± 0.02	1.94 ± 0.06	-	12.25 ± 0.63	5.67 ± 0.72	2.17 ± 0.07	0.77 ± 0.04	3.18 ± 0.14	0.32 ± 0.04
CBT-24	6.68 ± 0.27	1.12 ± 0.02	0.67 ± 0.06	2.37 ± 0.02	4.50 ± 0.11	8.07 ± 0.08	-	5.52 ± 0.27	1.54 ± 0.05	2.82 ± 0.05	2.69 ± 0.23	0.96 ± 0.02
CBL-01	5.08 ± 0.22	1.53 ± 0.08	0.87 ± 0.05	-	-	-	9.90 ± 0.48	4.45 ± 0.17	0.26 ± 0.02	0.43 ± 0.02	1.06 ± 0.02	1.72 ± 0.04
CBL-02	11.54 ± 0.64	1.13 ± 0.06	0.62 ± 0.03	4.67 ± 0.10	2.41 ± 0.08	4.16 ± 0.15	-	6.75 ± 0.17	1.13 ± 0.07	0.43 ± 0.03	2.17 ± 0.05	0.19 ± 0.01
CBL-03	8.22 ± 0.39	0.83 ± 0.01	0.53 ± 0.01	-	0.49 ± 0.01	-	16.04 ± 1.63	0.83 ± 1.44	1.07 ± 0.03	0.91 ± 0.03	0.41 ± 0.01	1.08 ± 0.01
CBL-04	9.77 ± 0.09	1.28 ± 0.06	0.67 ± 0.03	6.80 ± 0.03	2.55 ± 0.08	-	11.99 ± 0.53	5.71 ± 0.38	1.50 ± 0.04	0.67 ± 0.03	1.72 ± 0.03	0.22 ± 0.02
CBL-11	5.33 ± 0.29	1.60 ± 0.09	0.76 ± 0.06	0.76 ± 0.06	1.48 ± 0.10	-	4.38 ± 0.29	5.88 ± 0.35	0.59 ± 0.05	2.71 ± 0.18	1.53 ± 0.10	-
CBL-12	3.60 ± 0.10	1.79 ± 0.02	0.64 ± 0.01	0.29 ± 0.01	0.60 ± 0.01	10.12 ± 0.12	-	6.79 ± 0.42	1.47 ± 0.19	0.28 ± 0.01	1.90 ± 0.05	0.58 ± 0.02
CBL-13	1.60 ± 0.08	0.47 ± 0.03	0.53 ± 0.03	0.64 ± 0.04	1.73 ± 0.06	9.55 ± 0.26	-	1.11 ± 0.01	0.47 ± 0.01	3.26 ± 0.11	0.47 ± 0.03	0.97 ± 0.03
CBL-14	4.13 ± 0.09	0.82 ± 0.01	0.93 ± 0.01	5.21 ± 0.13	1.38 ± 0.02	11.08 ± 0.18	-	0.10 ± 0.17	0.97 ± 0.01	0.72 ± 0.01	0.25 ± 0.01	2.25 ± 0.06
CBL-21	5.23 ± 0.15	1.75 ± 0.11	0.78 ± 0.04	4.69 ± 0.11	1.66 ± 0.10	9.20 ± 0.52	-	6.00 ± 0.24	1.27 ± 0.06	0.74 ± 0.04	1.94 ± 0.05	1.60 ± 0.02
CBL-22	13.37 ± 0.61	0.43 ± 0.02	0.93 ± 0.02	1.80 ± 0.04	1.03 ± 0.03	-	11.22 ± 0.87	1.26 ± 1.10	1.01 ± 0.02	5.64 ± 0.03	0.73 ± 0.01	1.00 ± 0.05
CBL-23	5.45 ± 0.22	-	4.78 ± 0.12	1.14 ± 0.04	0.90 ± 0.03	-	5.94 ± 0.24	0.17 ± 0.29	0.56 ± 0.02	4.32 ± 0.11	0.22 ± 0.01	1.31 ± 0.02
CBL-24	5.50 ± 0.05	1.15 ± 0.01	0.65 ± 0.01	3.65 ± 0.04	2.42 ± 0.03	8.50 ± 0.08	-	5.27 ± 0.09	1.24 ± 0.00	4.01 ± 0.02	2.70 ± 0.02	0.45 ± 0.01
CBP-01	6.73 ± 0.27	0.08 ± 0.14	-	2.63 ± 0.31	3.29 ± 0.24	8.12 ± 0.46	-	-	0.54 ± 0.11	2.22 ± 0.31	0.47 ± 0.06	0.58 ± 0.02
CBP-02	4.90 ± 0.52	0.83 ± 0.00	0.26 ± 0.00	0.83 ± 0.00	2.13 ± 0.02	10.42 ± 0.53	-	3.98 ± 0.38	0.77 ± 0.01	3.85 ± 0.08	1.66 ± 0.06	0.50 ± 0.02
CBP-03	5.00 ± 0.02	-	-	0.90 ± 0.06	2.42 ± 0.05	2.52 ± 0.05	-	2.71 ± 0.05	0.59 ± 0.03	4.51 ± 0.03	1.18 ± 0.02	0.58 ± 0.01
CBP-04	6.48 ± 0.05	0.66 ± 0.01	-	0.53 ± 0.00	0.66 ± 0.01	7.65 ± 0.15	-	3.22 ± 0.06	0.50 ± 0.01	2.64 ± 0.04	0.49 ± 0.02	1.71 ± 0.06
CBP-11	5.10 ± 0.16	0.38 ± 0.03	3.33 ± 0.13	6.92 ± 0.13	4.11 ± 0.08	1.69 ± 0.12	-	2.76 ± 0.07	1.15 ± 0.05	0.64 ± 0.04	0.70 ± 0.03	2.98 ± 0.06
CBP-12	5.74 ± 0.23	0.24 ± 0.21	-	3.42 ± 0.23	5.97 ± 0.13	2.26 ± 0.17	-	3.52 ± 0.19	0.76 ± 0.07	3.46 ± 0.20	1.66 ± 0.06	0.45 ± 0.01
CBP-13	7.61 ± 0.75	0.33 ± 0.03	0.50 ± 0.05	5.83 ± 0.38	1.80 ± 0.10	2.31 ± 0.13	-	2.66 ± 0.17	1.17 ± 0.07	0.97 ± 0.06	0.81 ± 0.03	0.86 ± 0.02
CBP-14	6.60 ± 0.25	0.55 ± 0.08	0.31 ± 0.04	4.36 ± 0.21	2.62 ± 0.18	4.54 ± 0.33	-	4.82 ± 0.18	0.78 ± 0.07	0.37 ± 0.03	0.88 ± 0.03	1.28 ± 0.07
RRIo	932	971	990	1004	1023	1029	1029	1031	1056	1087	1189	1390
RRII	932	969	988	1002	1020	1024	1025	1026	1054	1086	1186	1389

Compounds: (C1) α-pinene, (C2) sabinene, (C3) myrcene, (C4) α-phellandrene, (C5) ρ-cymene, (C6) limonene, (C7) β-phellandrene, (C8) 1,8-cineole, (C9) γ-terpinene, (C10) terpinolene, (C11) α-terpineol, (C12) β-elemene. RRIo: relative retention index observed; RRIl: relative retention index from the literature.

Table 2. (Continuation)

Plant	Compound											
	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
CBA-01	2.67 ± 0.04	-	0.43 ± 0.03	0.49 ± 0.01	1.20 ± 0.01	9.98 ± 0.29	-	-	23.76 ± 0.75	-	0.38 ± 0.03	0.73 ± 0.01
CBA-02	3.16 ± 0.15	-	0.44 ± 0.02	0.63 ± 0.10	0.96 ± 0.09	2.20 ± 0.09	-	-	20.57 ± 0.25	-	0.34 ± 0.05	0.42 ± 0.02
CBA-03	6.01 ± 0.04	-	0.57 ± 0.01	1.18 ± 0.01	1.20 ± 0.02	0.88 ± 0.05	6.88 ± 0.02	-	25.31 ± 0.18	-	1.71 ± 0.04	1.50 ± 0.05
CBA-04	5.69 ± 0.02	0.20 ± 0.01	0.44 ± 0.02	1.36 ± 0.07	0.77 ± 0.05	0.07 ± 0.12	5.79 ± 0.04	1.53 ± 0.02	14.89 ± 0.38	2.53 ± 0.10	0.30 ± 0.02	0.60 ± 0.02
CBA-05	2.38 ± 0.04	0.33 ± 0.00	0.62 ± 0.01	1.16 ± 0.07	-	0.42 ± 0.03	4.56 ± 0.09	1.13 ± 0.04	19.04 ± 0.12	1.94 ± 0.22	0.39 ± 0.13	0.66 ± 0.02
CBA-06	10.45 ± 0.14	0.31 ± 0.01	0.53 ± 0.01	2.06 ± 0.01	1.16 ± 0.07	0.33 ± 0.57	9.30 ± 0.55	0.36 ± 0.00	17.97 ± 0.40	-	0.75 ± 0.04	1.14 ± 0.05
CBA-07	3.98 ± 0.05	-	2.02 ± 0.03	0.98 ± 0.02	1.14 ± 0.05	0.97 ± 0.07	3.37 ± 0.09	0.93 ± 0.10	14.49 ± 0.08	-	0.61 ± 0.02	1.04 ± 0.03
CBA-08	7.63 ± 0.22	0.14 ± 0.12	0.69 ± 0.02	1.78 ± 0.15	1.58 ± 0.09	0.12 ± 0.21	7.09 ± 0.11	-	18.35 ± 0.53	-	0.46 ± 0.01	1.49 ± 0.02
CBA-09	0.31 ± 0.01	-	0.42 ± 0.01	-	1.09 ± 0.06	0.06 ± 0.10	3.60 ± 0.08	-	14.36 ± 0.13	-	0.21 ± 0.02	0.44 ± 0.02
CBA-10	5.42 ± 0.03	0.22 ± 0.01	0.52 ± 0.01	1.15 ± 0.02	0.97 ± 0.02	0.58 ± 0.50	7.04 ± 0.36	0.37 ± 0.04	14.42 ± 0.23	-	0.64 ± 0.02	1.04 ± 0.03
CBA-11	5.37 ± 0.04	0.21 ± 0.00	0.33 ± 0.00	1.26 ± 0.04	0.94 ± 0.08	0.09 ± 0.15	6.03 ± 0.14	0.19 ± 0.02	11.79 ± 0.19	-	0.49 ± 0.04	0.59 ± 0.05
CBA-12	3.22 ± 0.06	0.32 ± 0.00	0.57 ± 0.01	0.74 ± 0.02	1.12 ± 0.04	0.25 ± 0.04	4.29 ± 0.13	-	15.83 ± 0.62	-	0.42 ± 0.04	0.98 ± 0.04
CBG-01	3.47 ± 0.39	1.29 ± 0.18	0.28 ± 0.05	0.83 ± 0.11	0.82 ± 0.11	0.05 ± 0.08	8.49 ± 0.80	0.42 ± 0.06	10.05 ± 0.95	3.13 ± 0.36	0.51 ± 0.07	1.40 ± 0.09
CBG-02	3.73 ± 0.69	-	1.62 ± 0.52	0.87 ± 0.10	1.13 ± 0.26	-	9.82 ± 0.75	1.04 ± 0.07	18.20 ± 1.34	1.79 ± 0.18	0.81 ± 0.06	1.58 ± 0.13
CBG-03	4.22 ± 0.15	2.17 ± 0.10	0.20 ± 0.04	1.31 ± 0.21	0.86 ± 0.13	1.68 ± 1.49	10.60 ± 1.01	1.67 ± 0.08	15.43 ± 0.84	2.71 ± 0.10	1.19 ± 0.05	2.41 ± 0.12
CBG-04	4.31 ± 0.70	0.28 ± 0.03	0.60 ± 0.07	0.79 ± 0.07	1.25 ± 0.13	0.48 ± 0.14	3.57 ± 0.46	0.08 ± 0.14	21.14 ± 3.07	-	1.82 ± 0.26	2.23 ± 0.30
CBG-05	5.16 ± 0.03	0.33 ± 0.06	0.10 ± 0.09	0.91 ± 0.09	0.66 ± 0.09	0.13 ± 0.12	3.05 ± 0.13	1.22 ± 0.20	11.05 ± 0.33	0.21 ± 0.09	0.23 ± 0.10	0.84 ± 0.06
CBG-06	3.10 ± 0.08	1.42 ± 0.03	0.30 ± 0.05	0.63 ± 0.01	1.29 ± 0.00	0.07 ± 0.13	2.51 ± 0.09	0.42 ± 0.36	21.12 ± 1.01	-	0.28 ± 0.03	0.68 ± 0.03
CBG-11	2.80 ± 0.28	0.56 ± 0.05	0.11 ± 0.10	0.59 ± 0.08	0.91 ± 0.03	0.43 ± 0.08	2.58 ± 0.10	0.46 ± 0.01	15.47 ± 1.04	-	0.97 ± 0.02	1.57 ± 0.02
CBG-12	3.72 ± 0.58	1.31 ± 0.25	0.38 ± 0.07	0.75 ± 0.13	1.67 ± 0.23	0.39 ± 0.34	8.06 ± 0.33	0.59 ± 0.17	24.41 ± 0.78	-	0.52 ± 0.00	1.20 ± 0.12
CBG-13	5.61 ± 0.50	0.62 ± 0.10	0.21 ± 0.04	1.57 ± 0.26	1.27 ± 0.13	0.48 ± 0.06	6.83 ± 0.21	0.25 ± 0.02	13.66 ± 0.09	-	0.45 ± 0.04	1.16 ± 0.08
CBG-14	5.03 ± 0.70	1.26 ± 0.23	0.21 ± 0.05	1.34 ± 0.27	1.23 ± 0.23	0.41 ± 0.09	2.70 ± 0.56	0.73 ± 0.16	14.09 ± 2.36	-	0.28 ± 0.07	1.03 ± 0.18
CBG-21	2.00 ± 0.05	0.38 ± 0.02	0.15 ± 0.02	0.44 ± 0.02	0.72 ± 0.03	0.13 ± 0.11	3.40 ± 0.11	0.20 ± 0.03	12.37 ± 0.26	-	0.19 ± 0.01	0.53 ± 0.03
CBG-22	3.08 ± 0.02	0.74 ± 0.01	0.15 ± 0.13	0.65 ± 0.01	1.21 ± 0.01	0.73 ± 0.02	2.83 ± 0.02	0.47 ± 0.02	21.69 ± 0.37	0.25 ± 0.00	2.56 ± 0.10	2.81 ± 0.06
CBG-23	1.44 ± 0.05	0.89 ± 0.01	0.36 ± 0.01	0.40 ± 0.02	1.78 ± 0.06	0.27 ± 0.01	4.45 ± 0.07	-	24.31 ± 0.60	-	0.30 ± 0.02	0.66 ± 0.02
CBG-24	1.49 ± 0.04	0.64 ± 0.01	0.08 ± 0.14	0.30 ± 0.01	1.35 ± 0.03	0.26 ± 0.43	10.16 ± 0.40	0.80 ± 0.05	23.29 ± 0.84	-	0.64 ± 0.07	1.58 ± 0.08
CBI-01	3.39 ± 0.03	-	0.28 ± 0.05	0.72 ± 0.01	0.97 ± 0.04	0.61 ± 0.04	9.32 ± 0.30	-	15.58 ± 0.33	-	3.90 ± 0.02	1.71 ± 0.02
CBI-02	5.93 ± 0.08	0.84 ± 0.03	0.05 ± 0.09	1.43 ± 0.07	0.74 ± 0.07	0.20 ± 0.34	6.14 ± 0.36	0.80 ± 0.03	11.88 ± 0.29	3.28 ± 0.08	0.32 ± 0.04	0.87 ± 0.06
CBI-03	6.55 ± 0.26	-	0.63 ± 0.11	1.33 ± 0.09	1.58 ± 0.12	-	5.19 ± 0.29	0.47 ± 0.05	24.43 ± 0.51	1.11 ± 0.18	0.44 ± 0.05	0.80 ± 0.08
CBI-04	2.05 ± 0.22	0.25 ± 0.05	0.24 ± 0.04	0.31 ± 0.08	0.72 ± 0.09	-	10.17 ± 1.14	0.95 ± 0.12	9.57 ± 0.66	0.26 ± 0.07	0.96 ± 0.06	1.36 ± 0.06
CBI-11	6.75 ± 0.24	0.18 ± 0.01	0.40 ± 0.02	1.41 ± 0.04	0.93 ± 0.02	-	7.41 ± 0.33	0.68 ± 0.10	13.03 ± 0.46	0.12 ± 0.11	0.50 ± 0.03	1.22 ± 0.05
CBI-12	3.08 ± 0.02	0.33 ± 0.00	0.55 ± 0.03	0.70 ± 0.09	1.45 ± 0.10	0.24 ± 0.04	2.14 ± 0.15	0.26 ± 0.06	12.59 ± 0.19	0.37 ± 0.04	0.41 ± 0.03	0.65 ± 0.03
CBI-13	6.32 ± 0.18	0.54 ± 0.01	0.66 ± 0.02	1.29 ± 0.09	1.44 ± 0.16	-	9.18 ± 0.12	0.06 ± 0.11	18.20 ± 0.20	0.15 ± 0.13	0.76 ± 0.04	1.63 ± 0.04
CBI-14	2.06 ± 0.15	0.35 ± 0.03	0.34 ± 0.03	0.50 ± 0.04	0.70 ± 0.04	0.17 ± 0.29	5.58 ± 0.21	0.70 ± 0.10	9.42 ± 0.21	0.18 ± 0.04	0.36 ± 0.02	1.02 ± 0.08
CBI-21	5.98 ± 0.72	0.33 ± 0.03	0.29 ± 0.01	1.42 ± 0.13	0.84 ± 0.07	0.28 ± 0.01	3.27 ± 0.08	-	6.54 ± 0.09	-	0.34 ± 0.04	0.97 ± 0.17
CBI-22	4.40 ± 0.34	-	0.32 ± 0.02	0.96 ± 0.05	0.63 ± 0.10	-	6.00 ± 0.22	0.19 ± 0.05	10.20 ± 0.74	0.14 ± 0.13	0.29 ± 0.07	0.80 ± 0.12
CBI-23	4.41 ± 0.10	0.17 ± 0.01	0.42 ± 0.01	1.18 ± 0.11	0.87 ± 0.07	-	8.45 ± 0.12	0.19 ± 0.01	11.21 ± 0.06	0.23 ± 0.02	0.50 ± 0.01	0.99 ± 0.02
CBI-24	5.31 ± 0.18	0.17 ± 0.01	0.62 ± 0.01	1.41 ± 0.11	1.15 ± 0.18	-	7.44 ± 0.34	0.75 ± 0.17	15.22 ± 0.24	0.06 ± 0.11	0.40 ± 0.09	0.91 ± 0.00

Table 2. (Continuation)

Plant	Compound											
	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
CBT-01	5.05 ± 0.12	-	3.65 ± 0.05	1.39 ± 0.12	1.86 ± 0.17	1.07 ± 0.10	3.94 ± 0.45	-	20.55 ± 0.42	-	0.61 ± 0.04	1.34 ± 0.08
CBT-02	9.96 ± 0.23	-	1.46 ± 0.03	2.65 ± 0.30	1.10 ± 0.13	1.18 ± 0.13	3.39 ± 0.16	1.86 ± 0.16	15.40 ± 1.66	-	1.29 ± 0.04	2.04 ± 0.11
CBT-03	2.83 ± 0.11	-	0.99 ± 0.20	0.92 ± 0.20	1.12 ± 0.23	0.21 ± 0.19	4.02 ± 0.29	2.88 ± 0.22	15.59 ± 0.73	0.35 ± 0.31	0.43 ± 0.02	1.01 ± 0.02
CBT-04	8.89 ± 1.18	-	1.32 ± 0.28	2.35 ± 0.31	1.09 ± 0.11	1.26 ± 1.12	5.95 ± 1.17	2.73 ± 0.25	12.28 ± 0.31	-	0.92 ± 0.06	1.68 ± 0.07
CBT-11	6.23 ± 0.89	-	2.88 ± 0.38	1.75 ± 0.11	2.08 ± 0.08	-	3.23 ± 0.10	0.74 ± 0.02	16.02 ± 2.02	-	0.55 ± 0.06	1.13 ± 0.13
CBT-12	9.33 ± 0.20	-	2.15 ± 0.19	2.24 ± 0.10	1.44 ± 0.03	1.07 ± 0.08	2.16 ± 0.13	0.29 ± 0.05	6.71 ± 0.19	-	0.62 ± 0.01	1.32 ± 0.07
CBT-13	7.52 ± 1.11	-	1.84 ± 0.22	1.89 ± 0.14	0.91 ± 0.06	1.49 ± 0.28	4.20 ± 0.09	0.28 ± 0.01	7.46 ± 0.24	-	0.73 ± 0.05	1.72 ± 0.16
CBT-14	10.52 ± 0.16	-	3.38 ± 0.04	2.71 ± 0.05	1.99 ± 0.04	0.35 ± 0.60	4.18 ± 0.53	1.97 ± 0.06	21.88 ± 0.72	0.20 ± 0.18	0.66 ± 0.02	1.41 ± 0.03
CBT-21	3.74 ± 0.18	2.66 ± 0.28	0.15 ± 0.13	0.87 ± 0.22	1.42 ± 0.27	1.45 ± 0.50	4.66 ± 0.59	1.29 ± 0.46	15.38 ± 0.96	0.28 ± 0.04	0.87 ± 0.04	1.71 ± 0.06
CBT-22	2.80 ± 0.05	-	2.95 ± 0.07	0.90 ± 0.05	1.54 ± 0.04	0.65 ± 0.04	0.54 ± 0.06	1.00 ± 0.05	5.63 ± 0.53	1.09 ± 0.09	0.44 ± 0.13	0.54 ± 0.17
CBT-23	5.20 ± 0.23	2.57 ± 0.16	0.30 ± 0.03	1.43 ± 0.09	1.47 ± 0.12	0.91 ± 0.11	1.87 ± 0.07	0.82 ± 0.05	11.84 ± 0.15	0.37 ± 0.02	0.50 ± 0.02	1.00 ± 0.05
CBT-24	5.67 ± 0.70	0.96 ± 0.10	0.10 ± 0.09	1.54 ± 0.16	0.86 ± 0.08	1.28 ± 0.08	2.58 ± 0.23	1.61 ± 0.08	5.06 ± 0.11	0.23 ± 0.08	0.60 ± 0.02	1.26 ± 0.08
CBL-01	3.57 ± 0.05	0.21 ± 0.00	1.06 ± 0.02	0.80 ± 0.01	1.25 ± 0.03	0.20 ± 0.34	6.58 ± 0.19	0.53 ± 0.10	20.38 ± 0.23	-	0.81 ± 0.06	1.03 ± 0.07
CBL-02	2.53 ± 0.06	-	2.57 ± 0.03	0.55 ± 0.00	1.28 ± 0.02	1.37 ± 0.03	4.14 ± 0.06	0.24 ± 0.01	14.30 ± 0.39	0.16 ± 0.15	0.87 ± 0.01	1.41 ± 0.01
CBL-03	11.97 ± 0.20	-	1.52 ± 0.00	3.03 ± 0.03	1.10 ± 0.02	1.09 ± 0.07	4.21 ± 0.02	1.12 ± 0.07	13.62 ± 0.20	-	0.87 ± 0.01	1.33 ± 0.02
CBL-04	11.50 ± 0.08	-	1.73 ± 0.01	2.52 ± 0.00	0.94 ± 0.05	0.38 ± 0.04	1.33 ± 0.02	0.35 ± 0.02	12.96 ± 0.16	-	0.31 ± 0.00	0.55 ± 0.02
CBL-11	4.36 ± 0.21	-	0.27 ± 0.02	1.05 ± 0.06	0.93 ± 0.05	0.60 ± 0.03	3.58 ± 0.15	0.28 ± 0.01	10.79 ± 0.49	-	0.35 ± 0.02	0.88 ± 0.04
CBL-12	3.77 ± 0.11	-	3.04 ± 0.14	0.95 ± 0.14	1.90 ± 0.16	0.26 ± 0.45	6.99 ± 0.61	0.14 ± 0.25	27.47 ± 0.63	-	0.73 ± 0.07	1.60 ± 0.07
CBL-13	4.16 ± 0.09	-	2.93 ± 0.07	1.11 ± 0.03	1.85 ± 0.04	1.60 ± 0.06	3.89 ± 0.06	1.99 ± 0.05	15.62 ± 0.25	-	0.82 ± 0.02	1.57 ± 0.03
CBL-14	4.36 ± 0.10	-	0.57 ± 0.04	0.84 ± 0.04	1.65 ± 0.08	0.84 ± 0.06	5.31 ± 0.16	0.30 ± 0.04	22.52 ± 0.27	-	2.75 ± 0.12	1.68 ± 0.09
CBL-21	10.16 ± 0.04	-	1.20 ± 0.01	2.22 ± 0.03	0.94 ± 0.09	-	10.09 ± 0.33	0.76 ± 0.18	12.75 ± 0.48	-	0.59 ± 0.02	1.34 ± 0.02
CBL-22	8.88 ± 0.13	-	2.31 ± 0.07	2.06 ± 0.05	0.98 ± 0.03	1.02 ± 0.03	1.42 ± 0.04	1.54 ± 0.03	15.35 ± 0.21	-	0.53 ± 0.01	0.90 ± 0.02
CBL-23	8.10 ± 0.06	-	2.39 ± 0.14	1.60 ± 0.05	1.53 ± 0.05	1.50 ± 0.05	4.05 ± 0.09	1.37 ± 0.03	19.44 ± 0.36	-	1.71 ± 0.08	2.17 ± 0.04
CBL-24	12.30 ± 0.18	-	0.70 ± 0.02	2.81 ± 0.05	0.38 ± 0.01	2.36 ± 0.06	8.35 ± 0.07	0.69 ± 0.02	5.51 ± 0.09	-	1.15 ± 0.02	2.43 ± 0.06
CBP-01	5.03 ± 0.23	2.01 ± 0.07	0.32 ± 0.02	1.35 ± 0.07	1.32 ± 0.03	1.84 ± 0.09	3.16 ± 0.20	1.66 ± 0.13	9.49 ± 0.85	-	1.11 ± 0.06	2.33 ± 0.19
CBP-02	6.78 ± 0.18	0.97 ± 0.04	0.30 ± 0.03	1.66 ± 0.04	0.83 ± 0.04	1.02 ± 0.09	3.66 ± 0.05	0.87 ± 0.08	6.73 ± 0.17	-	0.53 ± 0.02	1.25 ± 0.04
CBP-03	4.58 ± 0.08	-	3.14 ± 0.10	1.20 ± 0.05	2.09 ± 0.09	1.16 ± 0.15	1.46 ± 0.10	2.17 ± 0.24	12.69 ± 0.65	-	0.31 ± 0.28	1.00 ± 0.05
CBP-04	13.14 ± 0.50	1.31 ± 0.07	0.23 ± 0.03	3.59 ± 0.15	1.26 ± 0.01	4.14 ± 0.16	2.01 ± 0.04	-	13.15 ± 0.71	0.25 ± 0.02	0.52 ± 0.03	1.02 ± 0.06
CBP-11	8.29 ± 0.06	1.37 ± 0.01	0.15 ± 0.00	1.65 ± 0.01	1.03 ± 0.01	1.46 ± 0.01	4.71 ± 0.01	3.32 ± 0.02	13.14 ± 0.16	-	0.64 ± 0.01	1.14 ± 0.01
CBP-12	5.35 ± 0.10	-	2.89 ± 0.03	1.43 ± 0.05	1.58 ± 0.02	0.67 ± 0.05	0.33 ± 0.02	1.99 ± 0.03	9.23 ± 0.33	-	0.40 ± 0.04	-
CBP-13	5.88 ± 0.04	3.28 ± 0.06	0.31 ± 0.01	1.53 ± 0.04	2.03 ± 0.04	1.30 ± 0.01	4.67 ± 0.10	1.27 ± 0.11	22.48 ± 0.43	-	0.84 ± 0.02	2.23 ± 0.05
CBP-14	4.77 ± 0.22	2.86 ± 0.17	0.43 ± 0.05	1.35 ± 0.09	1.72 ± 0.10	0.49 ± 0.05	1.38 ± 0.06	3.15 ± 0.24	16.81 ± 1.21	0.26 ± 0.04	0.33 ± 0.02	1.05 ± 0.09
RRIo	1416	1428	1435	1449	1457	1479	1479	1483	1497	1506	1511	1521
RRII	1417	1437	1439	1452	1458	1478	1480	1489	1500	1509	1513	1522

Compounds: (C13) (*E*)-caryophyllene, (C14) α-guaiene, (C15) aromadendrene, (C16) α-humulene, (C17) *allo*-aromadendrene, (C18) γ-muurolene, (C19) germacrene D, (C20) β-selinene, (C21) bicyclogermacrene, (C22) α-bulnesene, (C23) γ-cadinene, (C24) δ-cadinene. RRIo: relative retention index observed; RRIl: relative retention index from the literature.

Table 2. (Continuation)

Plant	Compound									
	C25	C26	C27	C28	C29	C30	C31	C32	TI	EOC (%)
CBA-01	0.25 ± 0.01	8.69 ± 0.17	1.54 ± 0.02	1.11 ± 0.02	1.91 ± 0.11	0.45 ± 0.07	0.53 ± 0.12	2.78 ± 0.12	93.18 ± 0.57	0.67 ± 0.06 e
CBA-02	0.30 ± 0.01	9.09 ± 0.14	2.05 ± 0.05	1.17 ± 0.06	1.63 ± 0.11	0.79 ± 0.15	0.97 ± 0.13	2.26 ± 0.15	92.12 ± 0.62	0.62 ± 0.03 f
CBA-03	0.37 ± 0.00	5.77 ± 0.03	2.96 ± 0.00	2.01 ± 0.02	2.38 ± 0.03	1.21 ± 0.01	1.47 ± 0.01	4.21 ± 0.05	92.16 ± 0.18	0.68 ± 0.08 e
CBA-04	0.27 ± 0.01	13.59 ± 0.38	2.14 ± 0.16	0.79 ± 0.09	0.64 ± 0.08	0.32 ± 0.09	1.99 ± 0.13	2.32 ± 0.11	86.43 ± 1.00	0.58 ± 0.03 g
CBA-05	0.26 ± 0.01	12.64 ± 0.14	1.66 ± 0.04	1.12 ± 0.02	0.57 ± 0.01	0.77 ± 0.04	1.58 ± 0.04	3.06 ± 0.03	87.96 ± 0.20	0.65 ± 0.05 e
CBA-06	0.73 ± 0.02	8.59 ± 0.02	2.99 ± 0.08	1.48 ± 0.04	0.63 ± 0.42	0.72 ± 0.26	0.93 ± 0.38	2.51 ± 0.05	89.09 ± 0.45	0.57 ± 0.06 g
CBA-07	0.46 ± 0.03	14.17 ± 0.25	2.35 ± 0.19	1.10 ± 0.05	1.17 ± 0.03	0.26 ± 0.06	0.80 ± 0.07	0.68 ± 0.07	90.39 ± 0.11	0.73 ± 0.06 d
CBA-08	0.52 ± 0.01	20.37 ± 0.66	2.33 ± 0.47	1.39 ± 0.13	1.69 ± 0.25	0.70 ± 0.21	1.73 ± 0.11	2.87 ± 0.10	87.79 ± 1.20	0.58 ± 0.03 g
CBA-09	0.75 ± 0.02	16.29 ± 0.26	1.07 ± 0.05	0.81 ± 0.03	1.71 ± 0.05	2.81 ± 0.02	1.12 ± 0.02	1.79 ± 0.03	89.43 ± 0.53	0.93 ± 0.06 b
CBA-10	0.41 ± 0.01	12.15 ± 0.10	2.97 ± 0.09	1.74 ± 0.03	1.29 ± 0.06	1.16 ± 0.04	1.65 ± 0.03	2.78 ± 0.03	85.52 ± 0.10	0.77 ± 0.06 d
CBA-11	1.15 ± 0.04	16.22 ± 0.11	2.07 ± 0.26	0.78 ± 0.10	2.06 ± 0.17	0.94 ± 0.08	1.31 ± 0.08	2.20 ± 0.10	84.92 ± 0.64	0.85 ± 0.05 c
CBA-12	0.42 ± 0.04	19.17 ± 0.63	1.40 ± 0.07	0.96 ± 0.25	1.19 ± 0.36	0.79 ± 0.25	1.34 ± 0.18	3.36 ± 0.06	86.34 ± 1.44	0.72 ± 0.08 e
CBG-01	0.09 ± 0.08	13.15 ± 1.14	2.21 ± 0.27	3.64 ± 0.33	0.86 ± 0.06	0.93 ± 0.03	2.16 ± 0.18	2.52 ± 0.36	85.32 ± 0.77	0.43 ± 0.06 h
CBG-02	1.28 ± 0.25	6.00 ± 0.30	1.36 ± 0.08	1.32 ± 0.09	1.15 ± 0.36	0.95 ± 0.23	2.11 ± 0.36	2.96 ± 0.56	84.85 ± 1.05	0.67 ± 0.06 e
CBG-03	1.41 ± 0.20	4.34 ± 0.81	1.34 ± 0.29	1.33 ± 0.29	0.99 ± 0.16	0.39 ± 0.07	1.82 ± 0.11	0.19 ± 0.17	85.81 ± 0.88	0.83 ± 0.06 c
CBG-04	-	8.11 ± 1.28	1.71 ± 0.24	1.26 ± 0.20	0.96 ± 0.10	1.45 ± 0.29	0.91 ± 0.27	2.29 ± 0.46	87.06 ± 1.60	0.70 ± 0.00 e
CBG-05	1.29 ± 0.05	12.15 ± 0.31	2.41 ± 0.10	0.91 ± 0.09	1.37 ± 0.05	0.68 ± 0.04	1.33 ± 0.10	2.36 ± 0.18	86.35 ± 0.50	0.67 ± 0.06 e
CBG-06	-	14.34 ± 0.11	2.17 ± 0.15	1.12 ± 0.08	1.72 ± 0.12	0.63 ± 0.04	1.27 ± 0.08	3.76 ± 0.13	90.40 ± 0.56	0.75 ± 0.05 d
CBG-11	-	11.09 ± 0.66	1.68 ± 0.19	1.44 ± 0.12	2.01 ± 0.20	1.13 ± 0.09	1.34 ± 0.13	4.33 ± 0.41	89.14 ± 1.35	0.60 ± 0.00 f
CBG-12	-	12.30 ± 0.26	2.50 ± 0.09	1.68 ± 0.18	2.45 ± 0.20	0.98 ± 0.09	2.00 ± 0.15	5.21 ± 0.41	92.38 ± 0.51	0.43 ± 0.06 h
CBG-13	0.30 ± 0.04	17.30 ± 0.67	2.61 ± 0.29	1.41 ± 0.20	1.94 ± 0.12	0.79 ± 0.02	1.57 ± 0.20	3.41 ± 0.56	91.47 ± 1.06	0.53 ± 0.06 g
CBG-14	0.05 ± 0.09	13.82 ± 1.50	2.15 ± 0.16	1.33 ± 0.09	2.00 ± 0.10	0.52 ± 0.03	1.54 ± 0.11	4.26 ± 0.48	88.15 ± 0.88	0.57 ± 0.06 g
CBG-21	0.42 ± 0.02	9.99 ± 0.31	1.23 ± 0.05	0.71 ± 0.04	1.55 ± 0.03	0.45 ± 0.09	1.12 ± 0.11	3.62 ± 0.07	89.69 ± 0.57	0.70 ± 0.00 e
CBG-22	-	10.19 ± 0.09	1.51 ± 0.18	1.38 ± 0.08	2.15 ± 0.08	1.73 ± 0.18	1.27 ± 0.25	4.52 ± 0.27	85.50 ± 0.07	0.68 ± 0.03 e
CBG-23	0.46 ± 0.02	15.59 ± 0.32	1.90 ± 0.03	1.45 ± 0.06	2.31 ± 0.06	0.60 ± 0.10	1.37 ± 0.06	4.41 ± 0.13	92.60 ± 0.44	0.87 ± 0.06 c
CBG-24	-	8.70 ± 0.33	1.57 ± 0.07	1.47 ± 0.07	1.46 ± 0.10	0.54 ± 0.07	1.33 ± 0.14	2.33 ± 0.19	91.89 ± 0.50	0.67 ± 0.06 e
CBI-01	1.03 ± 0.02	8.64 ± 0.07	1.40 ± 0.03	0.70 ± 0.04	1.39 ± 0.09	1.45 ± 0.06	1.30 ± 0.06	2.27 ± 0.07	86.10 ± 0.66	0.93 ± 0.06 b
CBI-02	-	6.35 ± 0.25	1.68 ± 0.04	0.79 ± 0.03	0.32 ± 0.03	0.26 ± 0.02	0.90 ± 0.03	1.23 ± 0.07	90.73 ± 0.17	0.62 ± 0.03 f
CBI-03	-	8.49 ± 0.44	1.62 ± 0.03	1.03 ± 0.05	0.42 ± 0.04	0.53 ± 0.05	0.97 ± 0.09	2.44 ± 0.14	91.53 ± 0.16	0.70 ± 0.00 e
CBI-04	0.28 ± 0.03	12.82 ± 0.53	1.67 ± 0.21	1.40 ± 0.04	0.45 ± 0.04	1.79 ± 0.15	2.11 ± 0.17	2.44 ± 0.36	80.41 ± 0.68	0.63 ± 0.06 f
CBI-11	0.96 ± 0.03	15.96 ± 0.39	1.83 ± 0.09	1.35 ± 0.02	0.62 ± 0.04	1.12 ± 0.15	1.84 ± 0.04	3.02 ± 0.07	81.63 ± 1.26	0.55 ± 0.05 g
CBI-12	-	18.67 ± 0.45	1.55 ± 0.01	1.07 ± 0.10	2.32 ± 0.18	1.04 ± 0.09	1.27 ± 0.06	3.61 ± 0.15	87.19 ± 0.66	0.53 ± 0.06 g
CBI-13	0.23 ± 0.02	15.75 ± 0.34	2.52 ± 0.41	1.20 ± 0.08	2.64 ± 0.22	0.94 ± 0.10	2.09 ± 0.23	3.57 ± 0.19	88.26 ± 0.79	0.30 ± 0.00 i
CBI-14	1.13 ± 0.14	9.84 ± 0.40	0.98 ± 0.09	0.27 ± 0.10	1.51 ± 0.17	0.90 ± 0.10	1.55 ± 0.15	2.44 ± 0.28	82.88 ± 0.90	0.55 ± 0.05 g
CBI-21	0.85 ± 0.10	20.10 ± 2.90	1.92 ± 0.17	0.77 ± 0.03	1.93 ± 0.04	0.79 ± 0.07	1.45 ± 0.12	2.71 ± 0.47	83.20 ± 1.15	0.47 ± 0.06 h
CBI-22	1.17 ± 0.03	11.04 ± 0.84	1.57 ± 0.54	0.64 ± 0.19	1.71 ± 0.34	0.75 ± 0.23	1.55 ± 0.19	2.00 ± 0.06	84.30 ± 0.59	0.80 ± 0.00 d
CBI-23	1.86 ± 0.22	11.92 ± 0.35	1.91 ± 0.18	1.39 ± 0.02	1.92 ± 0.02	1.03 ± 0.02	1.86 ± 0.04	1.80 ± 0.07	79.88 ± 0.20	0.53 ± 0.06 g
CBI-24	1.62 ± 0.14	8.84 ± 0.44	1.64 ± 0.07	0.80 ± 0.02	1.55 ± 0.26	0.80 ± 0.16	1.55 ± 0.25	1.50 ± 0.11	81.50 ± 0.58	0.50 ± 0.00 h

Table 2. (Continuation)

Plant	Compound									
	C25	C26	C27	C28	C29	C30	C31	C32	TI	EOC (%)
CBT-01	0.49 ± 0.02	13.40 ± 0.34	2.67 ± 0.13	2.60 ± 0.16	1.29 ± 0.11	0.37 ± 0.04	0.67 ± 0.12	1.06 ± 0.05	89.34 ± 0.31	0.67 ± 0.06 e
CBT-02	1.05 ± 0.05	9.97 ± 0.13	2.49 ± 0.05	1.64 ± 0.07	0.86 ± 0.07	0.38 ± 0.02	1.27 ± 0.10	0.98 ± 0.12	86.95 ± 0.74	0.70 ± 0.10 e
CBT-03	2.26 ± 0.16	10.00 ± 1.29	1.99 ± 0.10	1.73 ± 0.17	0.87 ± 0.02	0.38 ± 0.21	2.99 ± 0.09	0.81 ± 0.12	81.76 ± 0.35	0.93 ± 0.06 b
CBT-04	0.47 ± 0.10	13.05 ± 1.58	3.21 ± 0.63	2.53 ± 0.21	1.21 ± 0.10	-	1.75 ± 0.17	1.85 ± 0.10	85.30 ± 1.08	0.52 ± 0.03 h
CBT-11	0.38 ± 0.03	20.08 ± 0.55	2.14 ± 0.12	2.02 ± 0.09	1.75 ± 0.17	0.07 ± 0.11	0.84 ± 0.03	1.17 ± 0.02	88.14 ± 0.24	0.65 ± 0.05 e
CBT-12	0.37 ± 0.02	20.62 ± 0.53	2.40 ± 0.30	1.00 ± 0.16	1.72 ± 0.06	0.29 ± 0.03	0.85 ± 0.06	1.52 ± 0.21	86.63 ± 0.36	0.70 ± 0.00 e
CBT-13	0.77 ± 0.13	12.02 ± 0.51	2.29 ± 0.40	2.03 ± 0.13	1.11 ± 0.13	0.80 ± 0.08	0.58 ± 0.06	1.37 ± 0.23	83.68 ± 0.79	0.68 ± 0.03 e
CBT-14	0.15 ± 0.13	15.16 ± 0.92	2.46 ± 0.01	2.15 ± 0.16	2.10 ± 0.08	0.80 ± 0.07	1.74 ± 0.13	1.88 ± 0.40	89.54 ± 1.30	0.72 ± 0.03 e
CBT-21	0.91 ± 0.11	17.67 ± 1.71	1.70 ± 0.28	1.26 ± 0.02	2.65 ± 0.21	-	1.59 ± 0.14	1.40 ± 0.03	88.52 ± 1.15	0.63 ± 0.06 f
CBT-22	-	28.57 ± 0.33	1.54 ± 0.11	1.94 ± 0.02	2.84 ± 0.13	0.50 ± 0.03	1.06 ± 0.03	2.08 ± 0.22	87.99 ± 0.66	0.50 ± 0.00 h
CBT-23	0.44 ± 0.02	12.67 ± 0.15	1.95 ± 0.03	1.58 ± 0.07	1.84 ± 0.18	-	0.94 ± 0.01	0.89 ± 0.13	87.33 ± 0.43	0.75 ± 0.05 d
CBT-24	0.29 ± 0.03	14.84 ± 2.04	2.61 ± 0.27	1.16 ± 0.10	1.28 ± 0.31	-	1.98 ± 0.09	1.61 ± 0.26	82.46 ± 0.28	0.50 ± 0.00 h
CBL-01	1.11 ± 0.03	11.85 ± 0.15	2.03 ± 0.04	1.10 ± 0.03	2.32 ± 0.01	1.53 ± 0.03	1.76 ± 0.04	3.42 ± 0.03	86.81 ± 0.46	0.50 ± 0.00 h
CBL-02	1.88 ± 0.05	13.80 ± 0.21	1.51 ± 0.06	0.96 ± 0.06	2.47 ± 0.04	0.55 ± 0.01	0.36 ± 0.01	0.87 ± 0.02	87.01 ± 0.60	0.70 ± 0.00 e
CBL-03	1.06 ± 0.01	8.30 ± 0.12	2.77 ± 0.01	0.83 ± 0.05	1.04 ± 0.22	1.48 ± 0.02	0.92 ± 0.02	0.64 ± 0.06	87.32 ± 1.16	0.80 ± 0.00 d
CBL-04	-	9.42 ± 0.45	2.63 ± 0.05	0.87 ± 0.02	0.80 ± 0.04	0.44 ± 0.05	0.42 ± 0.05	0.82 ± 0.03	90.83 ± 0.21	0.67 ± 0.06 e
CBL-11	0.99 ± 0.05	14.50 ± 0.63	1.04 ± 0.21	1.37 ± 0.37	1.41 ± 0.30	0.44 ± 0.42	0.92 ± 0.80	1.88 ± 0.17	70.69 ± 1.59	0.70 ± 0.00 e
CBL-12	0.68 ± 0.06	10.30 ± 0.37	2.09 ± 0.26	1.36 ± 0.20	0.61 ± 0.53	0.22 ± 0.19	0.88 ± 0.10	0.56 ± 0.04	91.61 ± 0.58	0.57 ± 0.06 g
CBL-13	1.07 ± 0.05	20.76 ± 0.44	2.08 ± 0.17	1.37 ± 0.14	1.80 ± 0.07	0.64 ± 0.12	1.59 ± 0.08	1.15 ± 0.07	86.81 ± 0.86	0.50 ± 0.00 h
CBL-14	1.14 ± 0.04	8.57 ± 0.27	1.17 ± 0.39	1.01 ± 0.17	0.57 ± 0.04	1.40 ± 0.16	1.11 ± 0.15	2.03 ± 0.06	85.65 ± 0.23	0.60 ± 0.00 f
CBL-21	1.00 ± 0.01	7.96 ± 0.09	1.65 ± 0.39	0.87 ± 0.21	0.46 ± 0.16	0.29 ± 0.14	0.94 ± 0.17	0.49 ± 0.06	88.56 ± 0.31	1.07 ± 0.06 a
CBL-22	-	9.44 ± 0.18	2.53 ± 0.06	1.11 ± 0.05	0.79 ± 0.03	0.25 ± 0.02	0.62 ± 0.02	0.57 ± 0.05	88.73 ± 0.18	0.70 ± 0.00 e
CBL-23	0.66 ± 0.03	10.42 ± 0.07	2.45 ± 0.15	1.32 ± 0.09	0.95 ± 0.06	1.44 ± 0.07	1.07 ± 0.06	0.85 ± 0.07	87.82 ± 0.41	0.57 ± 0.06 g
CBL-24	1.46 ± 0.03	5.65 ± 0.08	2.90 ± 0.03	0.60 ± 0.05	1.46 ± 0.03	0.65 ± 0.02	0.71 ± 0.02	0.97 ± 0.01	86.62 ± 0.35	0.70 ± 0.00 e
CBP-01	0.67 ± 0.03	22.40 ± 0.2.93	3.02 ± 0.51	1.40 ± 0.45	2.39 ± 0.55	-	1.39 ± 0.21	1.48 ± 0.34	87.03 ± 2.49	0.70 ± 0.00 e
CBP-02	0.41 ± 0.01	18.08 ± 0.49	4.28 ± 0.41	0.88 ± 0.09	2.43 ± 0.29	0.84 ± 0.19	1.35 ± 0.09	1.79 ± 0.40	84.77 ± 1.17	0.87 ± 0.06 c
CBP-03	-	29.83 ± 1.66	3.54 ± 0.07	1.90 ± 0.15	1.95 ± 1.69	0.48 ± 0.10	1.72 ± 0.06	1.57 ± 0.03	91.19 ± 0.58	0.47 ± 0.06 h
CBP-04	0.88 ± 0.05	15.16 ± 0.97	3.71 ± 0.83	1.06 ± 0.11	1.89 ± 0.80	0.18 ± 0.16	1.22 ± 0.35	1.40 ± 0.34	90.66 ± 0.49	0.73 ± 0.06 d
CBP-11	0.69 ± 0.02	14.01 ± 0.08	2.85 ± 0.19	0.87 ± 0.13	1.73 ± 0.12	0.32 ± 0.02	1.98 ± 0.16	0.94 ± 0.06	90.04 ± 0.29	0.50 ± 0.00 h
CBP-12	-	27.74 ± 1.51	4.15 ± 0.59	1.89 ± 0.04	2.56 ± 0.29	0.66 ± 0.26	0.90 ± 0.26	1.56 ± 0.02	90.81 ± 0.60	0.50 ± 0.00 h
CBP-13	0.75 ± 0.01	11.02 ± 0.37	2.08 ± 0.10	1.70 ± 0.07	1.17 ± 0.23	0.28 ± 0.01	1.06 ± 0.15	0.52 ± 0.02	89.26 ± 0.21	0.73 ± 0.06 d
CBP-14	0.23 ± 0.01	18.62 ± 1.82	2.94 ± 0.36	2.13 ± 0.47	1.66 ± 0.10	0.42 ± 0.19	1.49 ± 0.14	1.30 ± 0.21	90.50 ± 1.38	0.63 ± 0.06 f
RRIo	1552	1576	1581	1599	1636	1643	1651	1667	-	-
RRII	1559	1577	1582	1600	1630	1645	1651	1668	-	-
Mean	-	-	-	-	-	-	-	-	87.34	0.65

Compounds: (C25) germacrene B, (C26) spathulenol, (C27) caryophyllene oxide, (C28) guaiol, (C29) muurola-4,10(14)-dien-1-β-ol, (C30) cubenol, (C31) pogostol, (C32) 14-hydroxy-9-epi-(*E*)- caryophyllene. EOC: essential oil concentration; TI: total relative percentage of compounds with concentration ≥ 2% in at least one sample among the analyzed genotypes were considered; RRIo: relative retention index observed; RRIl: relative retention index from the literature. Mean values followed by the same letter in the column do not differ significantly from each other according to the Scott-Knott test ($p \leq 0.05$).

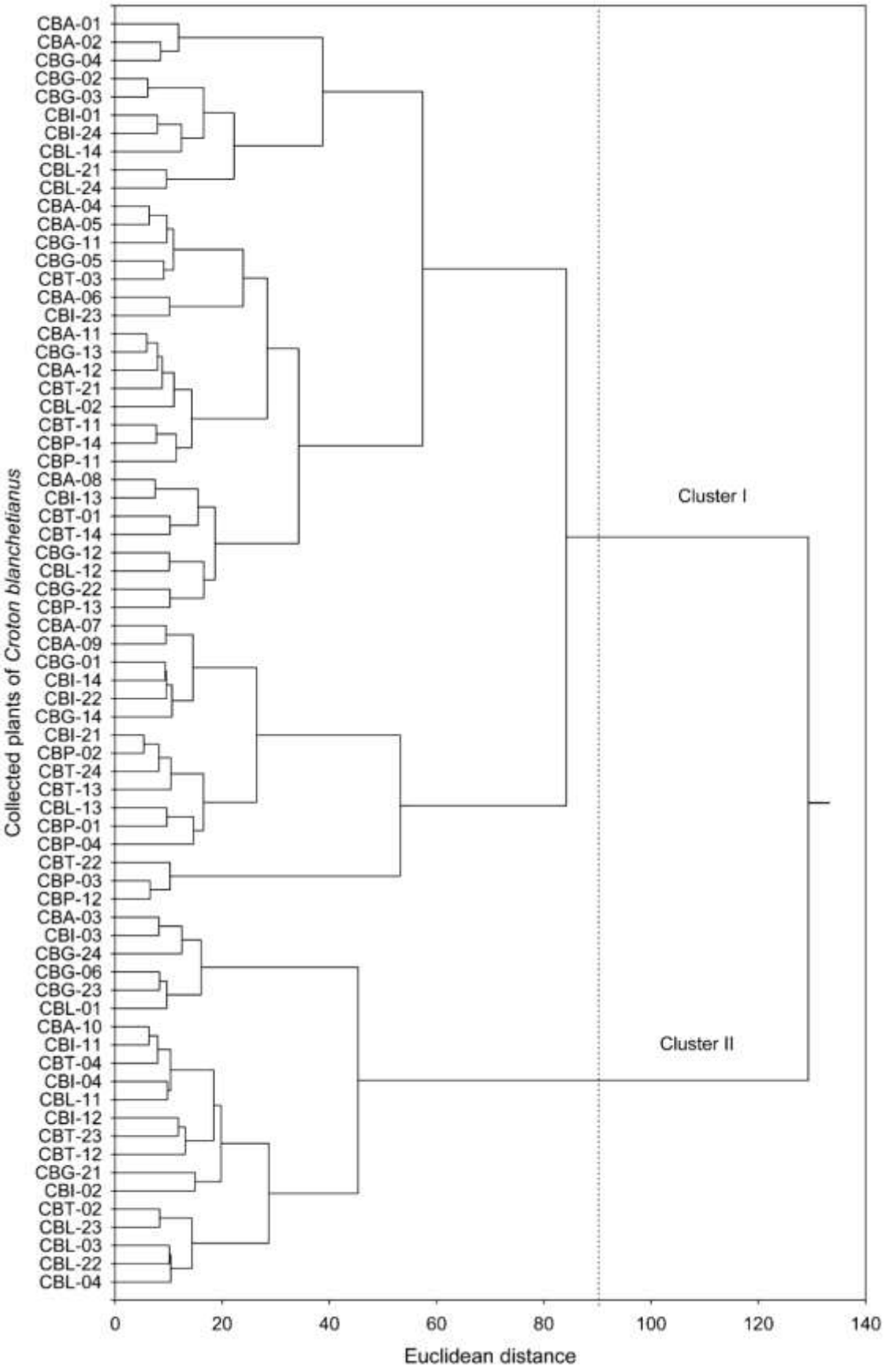

Figure 1. Two-dimensional dendrogram representing the similarity among 70 *Croton blanchetianus* genotypes based on chemical composition of their essential oils.

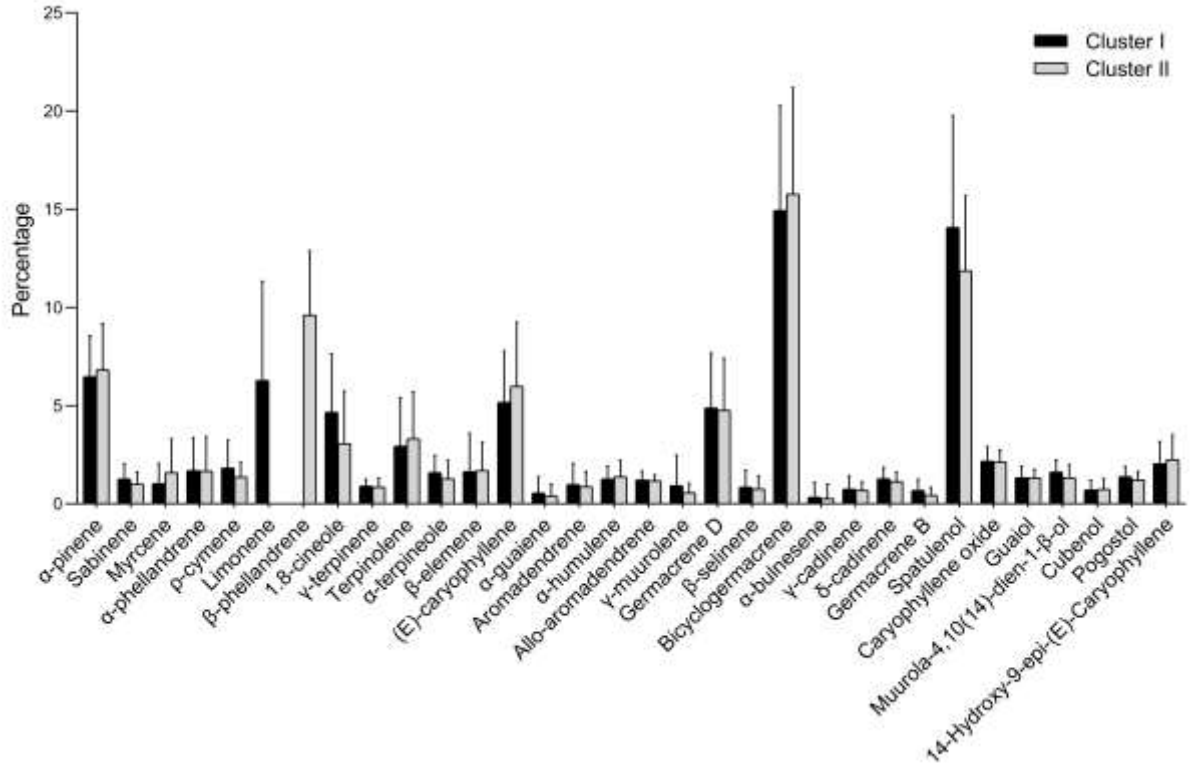

Figure 2. Mean values of the chemical constituents of the essential oils from *Croton blanchetianus* genotypes.

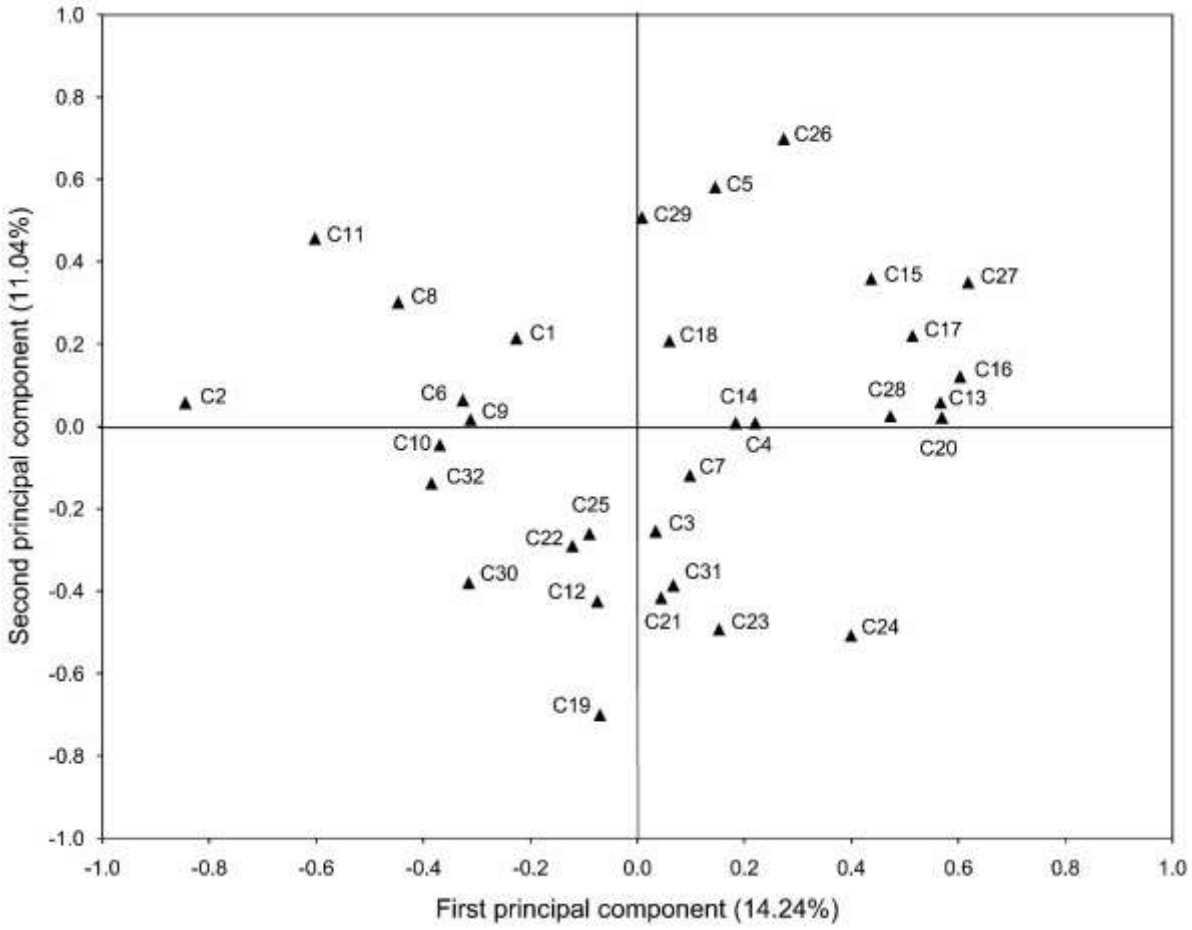

Figure 3. Distribution of the chemical compounds of the essential oils from *Croton blanchetianus* genotypes in relation to the two principal components based on principal component analysis (PCA).

Compounds: (C1) α-pinene, (C2) sabinene, (C3) myrcene, (C4) α-phellandrene, (C5) ρ-cymene, (C6) limonene, (C7) β-phellandrene, (C8) 1,8-cineole, (C9) γ-terpinene, (C10) terpinolene, (C11) α-terpineol, (C12) β-elemene, (C13) (*E*)-caryophyllene, (C14) α-guaiene, (C15) aromadendrene, (C16) α-humulene, (C17) *allo*-aromadendrene, (C18) γ-muurolene, (C19) germacrene D, (C20) β-selinene, (C21) bicyclogermacrene, (C22) α-bulnesene, (C23) γ-cadinene, (C24) δ-cadinene, (C25) germacrene B, (C26) spathulenol, (C27) caryophyllene oxide, (C28) guaiol, (C29) muurola-4,10(14)-dien-1-β-ol, (C30) cubenol, (C31) pogostol, (C32) 14-hydroxy-9-epi-(*E*)- caryophyllene.

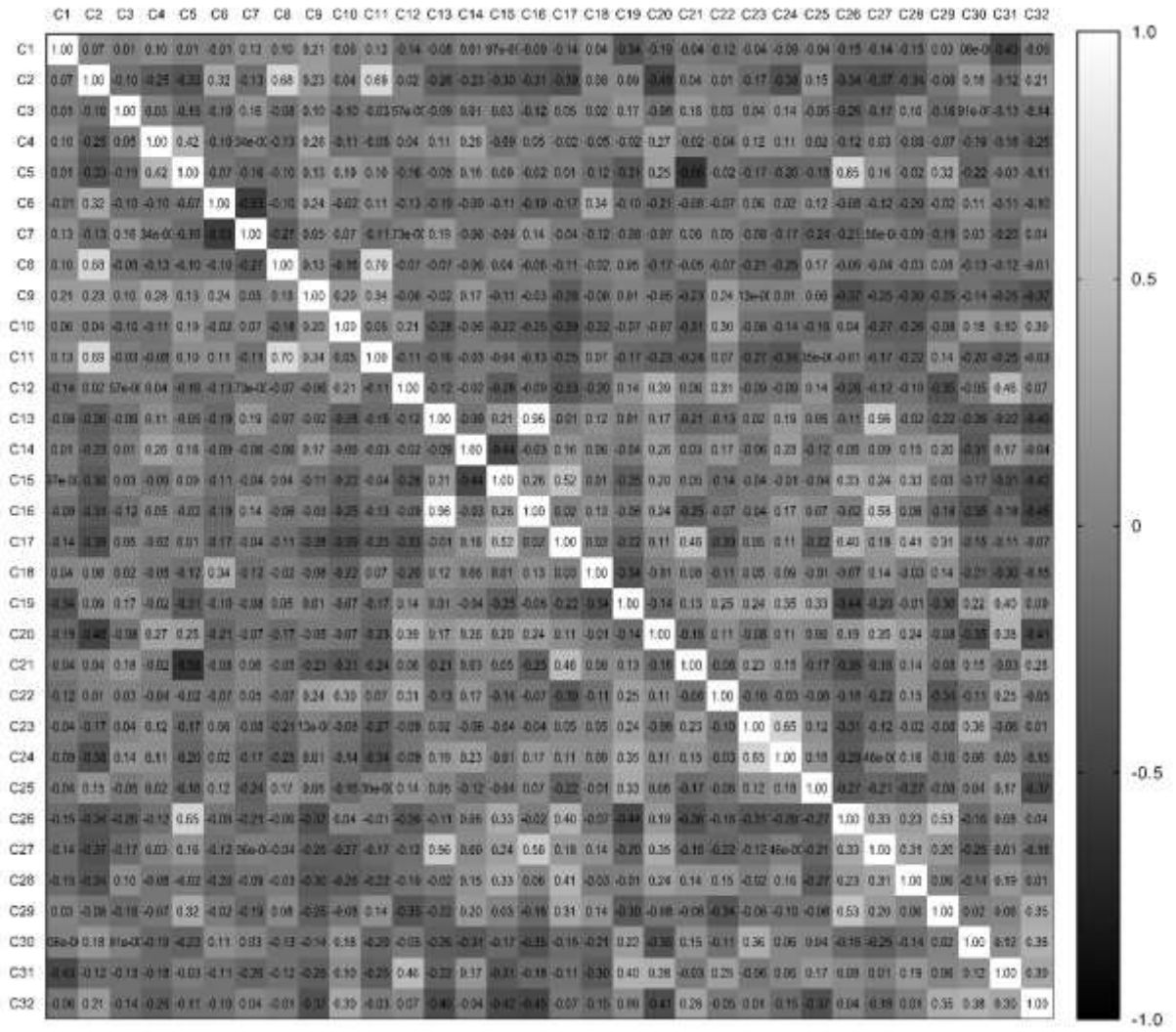

Figure 4. Correlation coefficients for the chemical constituents (C1 to C32) of the essential oils from *Croton blanchetianus* genotypes. Compounds: (C1) α-pinene, (C2) sabinene, (C3) myrcene, (C4) α-phellandrene, (C5) ρ-cymene, (C6) limonene, (C7) β-phellandrene, (C8) 1,8-cineole, (C9) γ-terpinene, (C10) terpinolene, (C11) α-terpineol, (C12) β-elemene, (C13) (*E*)-caryophyllene, (C14) α-guaiene, (C15) aromadendrene, (C16) α-humulene, (C17) *allo*-aromadendrene, (C18) γ-muurolene, (C19) germacrene D, (C20) β-selinene, (C21) bicyclogermacrene, (C22) α-bulnesene, (C23) γ-cadinene, (C24) δ-cadinene, (C25) germacrene B, (C26) spathulenol, (C27) caryophyllene oxide, (C28) guaiol, (C29) muurola-4,10(14)-dien-1-β-ol, (C30) cubenol, (C31) pogostol, (C32) 14-hydroxy-9-epi-(*E*)-caryophyllene.

6. MANUSCRIPT 3
CHARACTERIZATION AND DIVERSITY ASSESSMENT OF *Croton blanchetianus* Baill. GERMPLASMS USING MORPHOLOGICAL, AGRONOMICAL AND CHEMICAL MARKERS

Artigo formatado de acordo com as normas do periódico Brazilian Journal of Botany

ABSTRACT

Croton blanchetianus Baill. is a medicinal and aromatic species native to and widely distributed across the Caatinga biome in the Brazilian Northeast region. This species exhibits ecological and phenological traits that confer high apicultural potential. The essential oil of this species contains bioactive compounds with antioxidant, anti-inflammatory, antibacterial, acaricidal, antifungal, and insecticide activities. The aim of this study was to characterize 26 accessions of *C. blanchetianus* from the active germplasm bank of the Federal University of Sergipe using morphoagronomic and chemical variables. A randomized block experimental design was used, with three replicates. High variability among the accessions was observed for all the morphoagronomic descriptors evaluated, particularly for plant height, canopy diameter, leaf area, dry matter, and essential oil content. RGB-based leaf color analysis revealed distinct groups for adaxial and abaxial surface color, with different shades of green, allowing separation into dark-green leaf and light-green leaf accessions. Chemically, the essential oils showed predominance of monoterpenes and sesquiterpenes, such as α-pinene (3.90-12.02%), limonene (0.88-10.72%), β-phellandrene (1.30-17.51%), 1,8-cineole (0.36-11.57%), (*E*)-caryophyllene (1.82-12.48%), germacrene D (0.38-10.12%), bicyclogermacrene (8.37-29.13%), and spathulenol (5.57-28.04%), resulting in the formation of three distinct clusters based on chemical similarity. Multivariate analyses indicated that the variability observed is largely genetic, highlighting the potential of these accessions for conservation purposes, breeding programs, and biotechnological applications.

Key-words: marmeleiro, active germplasm bank, essential oil, genetic variability.

6.1. Introduction

Croton blanchetianus Baill., a member of the family Euphorbiaceae, is a medicinal and aromatic species native to and widely distributed across the Caatinga biome in the Brazilian Northeast region (Caruzo et al. 2025). This species exhibits ecological and phenological traits that confer high apicultural potential, ensuring a continuous supply of nectar and pollen throughout the year, and it is listed in the portfolio of the Brazilian Ministry of the Environment as a priority species for the Northeast region due to its potential and economic importance (Borges et al. 2014; Martins et al. 2017; Coradin et al. 2018). In addition to its apicultural potential, studies have shown that *C. blanchetianus* contains bioactive compounds with antioxidant, anti-inflammatory, antibacterial, acaricidal, antifungal, and insecticide activities, reinforcing its potential for applications in pharmaceutical and biotechnological sectors (Angélico et al. 2014; Rodrigues et al. 2019; Camara et al. 2021; Dantas et al. 2021; Oliveira et al. 2022; Vasconcelos et al. 2022; Nunes et al. 2023; Venancio et al. 2024).

Despite the importance of this species, the areas in which it naturally occurs have been reduced, mainly as a result of human activity, such as deforestation of areas for building and agricultural expansion. This exposes the species to loss of genotypes that may be useful to humans. Given the imminent threat to the genetic variability of the species, the Universidade Federal de Sergipe (UFS) established a *C. blanchetianus* collection in the active germplasm bank of medicinal and aromatic plants. The conservation of species in Active Germplasm Banks is essential for preserving part of their gene pool, in addition to contributing to scientific research. One of the

activities carried out in the Active Germplasm Bank is characterization of the accessions held within them to discover and develop strategies for conservation and use of available genetic variability. These studies can be conducted using phenotypic descriptors, which allow understanding of the morphological, agronomic, and chemical aspects of a species (Nascimento et al. 2020).

Morphoagronomic characterization is a fundamental tool for evaluation of phenotypic diversity among accessions, enabling identification and selection of agronomically desirable accessions for growing and inclusion in breeding programs, as well as eliminating duplicate accessions and reducing maintenance costs (Blank 2013; Nascimento et al. 2020; Oliveira et al. 2020). In addition, chemical characterization of essential oils in aromatic species provides relevant information regarding the bioactive potential of the accessions, assisting selection of superior accessions for multiple applications in agriculture. In *Varronia curassavica* Jacq. and *Croton grewioides* Baill. accessions, for example, characterization of essential oils was essential for detecting insecticidal potential against *Dorymyrmex thoracius* and antimicrobial potential against *Xanthomonas campestris* pv. *Campestres*, respectively (Oliveira et al. 2019; Rodrigues et al. 2023).

In this context, characterization represents an indispensable step in management of germplasm collections. An understanding of morphoagronomic and chemical variability among accessions of aromatic species places value on local genetic resources and is the starting point for development of new technologies. Accordingly, the objective of the present study was to provide morphoagronomic and chemical characterization of *C. blanchetianus* accessions, aiming at identifying variability among accessions to assist conservation strategies, breeding programs, and sustainable utilization of the species.

6.2. Material and Methods

6.2.1. Plant material, location, and experimental design

The *Croton blanchetianus* collection, composed of 26 accessions originating from six municipalities (or counties) of the state of Sergipe, Brazil (Aquidabã, Graccho Cardoso, Itabi, Lagarto, Tobias Barreto, and Poço Verde) (Table 1), is maintained in the Active Germplasm Bank of Medicinal and Aromatic Plants at the Universidade Federal de Sergipe on the "Campus Rural" Experimental Farm in the municipality of São Cristóvão, Sergipe (10° 55' 27" S, 37° 12' 01" W; 46 m altitude). This collection is registered in the Brazilian system for management of genetic heritage and associated traditional knowledge (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – SisGen) under no. A8CCB3B.

Soil physicochemical analysis was carried out using samples collected from the 0-20 cm layer, with the following results: pH in water = 4.92; Ca^{2+} = 0.59 cmolc dm^{-3}; Mg^{2+} = 0.36 cmolc dm^{-3}; Al^{3+} = 0.38 cmolc dm^{-3}; organic matter = 16.5 g dm^{-3}; Na = 3.90 mg dm^{-3}; K = 14.40 mg dm^{-3}; P = 1.40 mg dm^{-3}; sum of bases (SB) = 1.77 cmolc dm^{-3}; cation exchange capacity (CEC) = 3.80 cmolc dm^{-3}; base saturation = 46.6%; sand = 820.40 g kg^{-1}; silt = 89.10 g kg^{-1}; clay = 90.5 g kg^{-1}. Based on these parameters, the soil in the area was classified as an Argissolo Vermelho Amarelo, with a sandy-loam texture. Liming was carried out 30 days before planting, based on the results of soil analysis.

The experimental area was first prepared by plowing and disking. Then 20-cm^3 plant holes were opened, into which 2 liters of cattle manure were incorporated at 15 days before transplanting seedlings. At 15 days after planting, top-dressed fertilizer was applied, consisting of 10 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5, and 20 kg ha^{-1} K_2O. The experimental design used for establishing the collection was randomized blocks, with three replicates and two plants per plot, at a spacing of 3.0 × 2.0 m (three meters between rows and two meters between plants). The crop management practices, including weeding and mowing, were carried out as needed throughout the period of conducting the experiment.

Table 1. Place of origin of the 26 accessions in the *Croton blanchetianus* Baill. collection of the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Accession	Origin (Sergipe, Brazil)	Geographic coordinates	Voucher no.
CBL-101	Aquidabã	S 10º19'27.7" W 37º04'44.3"	42851
CBL-102	Aquidabã	S 10º19'22.4" W 37º04'40.4"	42855
CBL-103	Aquidabã	S 10º17'45.1" W 37º02'09.2"	42845
CBL-201	Graccho Cardoso	S 10º14'06.8" W 37º11'25.6"	42859
CBL-202	Graccho Cardoso	S 10º14'06.4" W 37º11'26.9"	42860
CBL-203	Graccho Cardoso	S 10º14'03.7" W 37º11'25.8"	42861
CBL-204	Graccho Cardoso	S 10º14'29.8" W 37º12'53.6"	42862
CBL-205	Graccho Cardoso	S 10º14'30.6" W 37º12'52.8"	42863
CBL-206	Graccho Cardoso	S 10º14'30.3" W 37º12'54.1"	42864
CBL-207	Graccho Cardoso	S 10º14'29.5" W 37º12'50.5"	42865
CBL-301	Itabi	S 10º05'18.5" W 37º05'35.1"	42873
CBL-302	Itabi	S 10º06'49.9" W 37º06'33.3"	42875
CBL-303	Itabi	S 10º07'49.8" W 37º07'16.1"	42880
CBL-304	Itabi	S 10º07'47.4" W 37º07'18.1"	42881
CBL-401	Lagarto	S 10º53'12.0" W 37º36'55.4"	42884
CBL-402	Lagarto	S 10º51'50.2" W 37º35'59.0"	42887
CBL-403	Lagarto	S 10º50'52.5" W 37º36'19.5"	42890
CBL-404	Lagarto	S 10º50'51.0" W 37º36'17.8"	42891
CBL-501	Tobias Barreto	S 11º08'53.0" W 37º56'41.2"	42894
CBL-502	Tobias Barreto	S 11º08'52.9" W 37º56'41.6"	42895
CBL-503	Tobias Barreto	S 11º08'54.1" W 37º56'43.4"	42896
CBL-505	Tobias Barreto	S 11º10'06.4" W 37º58'44.0"	42899
CBL-602	Poço Verde	S 10º48'27.5" W 38º08'23.4"	42908
CBL-603	Poço Verde	S 10º48'17.3" W 38º08'12.6"	42913
CBL-604	Poço Verde	S 10º48'15.6" W 38º08'14.3"	42910
CBL-605	Poço Verde	S 10º48'14.5" W 38º08'13.0"	42911

6.2.2. Morphological characterization

Morphological characterization of the accessions was performed at 120 days after transplanting, in October 2023 (dry season). The following variables were analyzed: plant height (cm), canopy diameter (cm), leaf length and width (cm), leaf length-to-width ratio, and leaf area (cm^2). To measure leaf traits, three fully expanded leaves were randomly selected from each plant, placed in digital format using an HP MFP 1005 scanner, and analyzed using the Image J software. RGB color values were obtained for both leaf surfaces (adaxial and abaxial) and then converted into hexadecimal code, ensuring international standardization of the colors using Image J.

6.2.3. Agronomic characterization

The leaves were collected in October 2023 (dry season) and dried in a forced-air oven at 40 ± 1 °C for five days, after which dry matter was determined. Essential oils were extracted through hydrodistillation using 50 g of dry leaves immersed in 2 L of water in a modified Clevenger device for 120 minutes after reaching the boiling point, in triplicate. The essential oil content (EOC) and essential oil yield (EOY) of each sample were calculated using the following equations:

$$\text{EOC (\%)} = \left(\frac{\text{Sample volume extracted}}{\text{Sample dry matter}}\right) \times 100$$

$$\text{EOY (mL/plant)} = \left(\frac{\text{EOC (\%)} \times \text{Total dry matter per plant}}{100}\right)$$

6.2.4. Chemical characterization

Chemical analyses of the essential oil (EO) samples were performed using a gas chromatograph (Model 7820A, Agilent) coupled to a mass spectrometer (Model 5975 MSD, Agilent), equipped with a HP-5MS fused-silica capillary column (30 m length × 0.25 mm internal diameter × 0.25 μm thickness, Agilent).

Samples were injected using an autosampler (model G4513A, Agilent). The injector was fitted with a split-type liner (inner diameter 4.0 mm, outer diameter 6.25 mm, length 78.5 mm, volume 870 μL), and the injection was performed in split mode (split ratio 10:1). The carrier gas was helium 5.0 (purity grade 99.999%) at a constant flow rate of 1.2 mL min^{-1}. One microliter (1.0 μL) of each essential oil sample (10 mg mL^{-1} solution in ethyl acetate) was injected. The oven temperature program was as follows: initial temperature of 60 °C (held for 1 min), increased to 170 °C at 3 °C min^{-1} (no hold), then to 220 °C at 5 °C min^{-1} (no hold), and finally to 280 °C at 20 °C min^{-1} (no hold). The transfer line temperature was maintained at 280 °C. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, with an ion source temperature of 230 °C and a quadrupole temperature of 150 °C. The mass scan range was 40–550 m/z.

The chemical constituents of the essential oils were identified through the retention indices calculated using an n-alkane series (C8-C24) and by comparing the mass spectra of the samples with those reported in the literature (Adams 2017) and in the NIST (National Institute of Standards & Technology) data.

6.2.5. Statistical analysis

The data related to the morphoagronomic traits of the *C. blanchetianus* accessions underwent analysis of variance (ANOVA), and the mean values were clustered using the Scott-Knott test at a significance level of $p \leq 0.05$ with the Sisvar® software (Ferreira 2019). Based on the morphoagronomic data and the chemical composition of the essential oils, the multivariate cluster analysis and principal component analysis (PCA) were performed. Cluster analysis for the morphoagronomic variables was carried out using Euclidean distance and visualized through a dendrogram constructed with Ward's method using the Vegan package (Oksanen et al. 2017). For cluster analysis of the chemical variables, Mahalanobis distance was used, and the dendrogram was constructed through UPGMA (Unweighted Pair Group Method with Arithmetic Means) using the biotools package (Silva et al. 2017). The factoextra package (Kassambara and Mundt 2020) was used to format the dendrograms obtained. The R environment (R Core Team 2025) was used to carry out these analyses. Principal component analysis (PCA) was performed using the Statistica® software. In addition, graphs representing the mean values and the standard deviations of the main components of each cluster identified in cluster analysis were created using the GraphPad Prism® software.

6.3. Results

6.3.1. Morphoagronomic characterization

The *C. blanchetianus* accessions showed significant differences for all the quantitative variables evaluated (Table 2). Considering the plant height (PH) variable, the accessions CBL-101, CBL-103, and CBL-203 stood out, with mean values of 236.50, 220.00, and 228.00 cm, respectively. In contrast, the accession CBL-401 had the lowest height, at 87.00 cm. For the canopy diameter (CD) variable, the mean values ranged from 48.75 to 131.00 cm, with an overall mean of 88.29 cm. Regarding leaf length (LL), the mean values ranged from 12.00 cm (CBL-503) to 19.50 cm (CBL-602), with an overall mean of 15.90 cm; and for the leaf width (LW) variable, the means values ranged from 7.70 (CBL-403) to 11.90 (CBL-404), with an overall mean of 9.50 cm. The accessions exhibited a mean leaf area of 121.56 cm^2. For the leaf length-to-width ratio (LL/LW), the mean values ranged from 1.47 to 2.19, with an overall mean of 1.68, observed in accessions CBL-301 and CBL-201, respectively.

The essential oil content (EOC) of *C. blanchetianus* ranged from 0.60% in accessions CBL-205 and CBL-301 to 1.60% in accession CBL-403, with an overall mean of 0.97% (Table 2). The

accessions CBL-603 and CBL-503 had the highest values for dry matter (DM), with mean values of 279.67 g and 333.00 g, respectively (Table 2). The accession CBL-503 exhibited high mean values for the dry matter (333.00 g) and essential oil content (1.40%) variables, showing potential for use in breeding programs aimed at increasing essential oil production.

Table 2. Morphoagronomic traits of *Croton blanchetianus* accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Accession	PH	CD	LL	LW	LL/LW	LA	DM	EOC
CBL-101	236.50 a	84.75 a	16.85 b	9.99 b	1.68 c	130.47 a	150.60 c	0.90 c
CBL-102	141.00 c	70.50 b	13.75 c	8.65 c	1.60 c	79.82 b	140.75 c	0.90 c
CBL-103	220.00 a	75.00 b	15.00 c	8.60 c	1.74 c	95.60 b	114.10 c	1.20 b
CBL-201	160.50 c	48.75 b	19.50 a	8.90 c	2.19 a	133.60 a	113.30 c	0.80 d
CBL-202	172.50 b	100.75 a	17.90 a	11.50 a	1.56 d	170.20 a	168.00 c	1.20 b
CBL 203	228.00 a	93.33 a	15.77 b	9.93 b	1.59 d	129.33 a	178.53 c	0.97 c
CBL-204	170.00 b	49.17 b	16.20 b	9.77 b	1.67 c	124.13 a	83.17 c	0.80 d
CBL-205	129.17 c	77.25 b	15.17 c	10.13 b	1.52 d	117.17 b	213.73 b	0.60 e
CBL-206	184.33 b	95.50 a	17.63 a	9.40 b	1.88 b	128.57 a	158.17 c	0.80 d
CBL-207	147.67 c	100.92 a	15.70 b	9.07 b	1.74 c	109.23 b	212.28 b	0.80 d
CBL-301	174.50 b	91.50 a	14.15 c	9.65 b	1.47 d	106.00 b	198.75 b	0.60 e
CBL-302	165.83 b	86.50 a	16.53 b	9.57 b	1.74 c	176.37 a	174.23 c	0.93 c
CBL-303	153.00 c	98.83 a	15.80 b	10.10 b	1.57 c	124.25 a	216.50 b	1.00 c
CBL-304	178.67 b	112.67 a	15.47 b	9.50 b	1.63 c	114.33 b	190.17 b	0.73 d
CBL-401	87.00 e	63.83 b	15.93 b	8.83 c	1.81 c	145.67 a	145.15 c	1.50 a
CBL-402	168.00 b	110.00 a	16.50 b	9.95 b	1.67 c	123.40 a	225.55 b	0.70 d
CBL-403	153.00 c	91.00 a	13.25 c	7.70 c	1.73 c	79.35 b	171.20 c	1.60 a
CBL-404	195.00 b	82.50 a	17.70 a	11.90 a	1.49 d	164.50 a	182.40 c	1.00 c
CBL-501	175.67 b	88.33 a	17.65 a	9.45 b	1.87 b	126.25 a	146.03 c	1.00 c
CBL-502	134.50 c	87.58 a	15.90 b	9.37 b	1.70 c	112.57 b	136.07 c	1.13 b
CBL-503	120.00 d	131.00 a	12.00 c	8.00 c	1.50 d	74.50 b	333.00 a	1.40 a
CBL-505	138.83 c	86.33 a	13.57 c	8.07 c	1.69 c	86.30 b	123.07 c	1.27 b
CBL-602	142.25 c	62.00 b	19.13 a	10.15 b	1.88 b	138.10 a	89.03 c	0.70 d
CBL-603	156.17 c	102.83 a	15.93 b	9.73 b	1.64 c	132.38 a	279.67 a	1.13 b
CBL-604	177.50 b	122.25 a	14.03 c	8.33 c	1.69 c	94.93 b	239.30 b	0.73 d
CBL-605	169.50 b	82.42 a	16.33 b	10.77 a	1.52 d	143.50 a	138.60 c	0.73 d
Mean	164.58	88.29	15.90	9.50	1.68	121.56	173.90	0.97
CV (%)	11.65	20.77	7.49	7.71	6.57	20.34	28.00	8.33

Variables: PH: plant height (cm); CD: canopy diameter (cm); LL: leaf length (cm); LW: leaf width (cm); LL/LW: leaf length-to-width ratio; LA: leaf area (cm^2); DM: dry matter (g); EOC: essential oil content (%); CV: coefficient of variation. Mean values followed by the same letter in the column do not differ significantly from each other according to the Scott-Knott test ($p \leq 0.05$).

A significant difference was observed among the accessions regarding leaf color, for both the adaxial and abaxial surfaces (Table 3). On the adaxial surface, the accessions CBL-102, CBL-203, CBL-205, CBL-206, and CBL-401 had smaller proportions of green and red components, along with higher blue values, compared to the other accessions, resulting in dark-green tones. In contrast, the accession CBL-602 had a higher proportion of green and red components and a lower intensity of blue, producing light-green leaves. On the abaxial surface, the accessions exhibited grayish-green tones. The accession CBL-602 showed predominance of a light grayish-green color, associated with higher proportions of the red, green, and blue components, whereas the accession CBL-202 had dark grayish-green tones, corresponding to lower proportions of red and blue.

Table 3. Sum of the red, green, and blue color components and their hexadecimal codes for the abaxial and adaxial surfaces of the leaves of *Croton blanchetianus* accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Accession	Red (adaxial)	Green (adaxial)	Blue (adaxial)	Color	Code	Red (abaxial)	Green (abaxial)	Blue (abaxial)	Color	Code
CBL-101	60.68 b	88.97 b	38.87 c		#3D5927	120.78 c	134.96 b	88.76 b		#798759
CBL-102	46.67 c	67.91 d	44.21 a		#2F442C	112.18 e	123.06 d	89.70 b		#707B5A
CBL-103	51.60 c	79.93 c	36.98 c		#345025	117.28 c	130.40 c	87.25 b		#758257
CBL-201	48.28 c	74.44 d	41.88 b		#304A2A	116.92 d	128.71 c	94.18 a		#75815E
CBL-202	49.97 c	83.78 c	34.27 d		#325422	110.47 e	130.63 c	75.88 c		#6E834E
CBL 203	46.72 c	71.18 d	43.17 a		#2F472B	115.31 d	128.09 c	87.48 b		#738057
CBL-204	55.46 c	80.85 c	40.89 b		#375129	118.50 c	130.47 c	88.37 b		#768258
CBL-205	50.99 c	75.05 d	43.02 a		#334B2B	121.47 c	130.61 c	95.40 a		#79835F
CBL-206	49.82 c	74.05 d	42.78 a		#324A2B	113.36 e	123.97 d	85.25 b		#717C55
CBL-207	52.11 c	78.79 c	39.56 c		#344F28	121.21 c	130.18 c	91.21 b		#79825B
CBL-301	52.08 c	78.84 c	39.99 b		#344F28	118.08 c	129.17 c	90.79 b		#76815B
CBL-302	61.05 b	89.18 b	37.24 c		#3D5925	128.12 b	137.73 b	97.61 a		#808A62
CBL-303	55.14 c	81.66 c	41.02 b		#375229	129.76 b	139.68 b	99.85 a		#828C64
CBL-304	53.00 c	77.19 c	43.98 a		#354D2C	124.66 b	132.74 c	96.72 a		#7D8561
CBL-401	46.83 c	69.33 d	43.90 a		#2F452C	121.13 c	129.84 c	95.31 a		#79825F
CBL-402	51.39 c	78.86 c	40.40 b		#334F28	118.29 c	131.20 c	90.15 b		#76835A
CBL-403	45.68 c	70.67 d	38.17 c		#2E4726	116.73 d	123.80 d	80.41 c		#757C50
CBL-404	49.58 c	79.55 c	37.23 c		#325025	115.86 d	130.81 c	87.06 b		#748357
CBL-501	44.71 c	67.66 d	42.09 b		#2D442D	109.77 e	123.18 d	87.70 b		#6E7B58
CBL-502	50.27 c	76.92 c	40.63 b		#324D29	116.24 d	129.72 c	88.57 b		#748259
CBL-503	51.46 c	77.78 c	43.70 a		#334E2C	122.51 c	130.93 c	88.63 b		#7B8359
CBL-505	47.98 c	72.03 d	41.88 b		#30482A	116.03 d	125.37 d	84.38 b		#747D54
CBL-602	73.33 a	107.04 a	34.59 c		#496B22	138.34 a	147.05 a	100.62 a		#8A9365
CBL-603	48.48 c	73.94 d	42.25 b		#304A2A	120.27 c	130.97 c	91.70 b		#78835C
CBL-604	52.50 c	75.64 c	43.96 a		#344C2C	120.44 c	129.12 c	90.06 b		#78815A
CBL-605	52.24 c	78.08 c	42.35 b		#344E2A	119.32 c	129.44 c	91.90 b		#77815C
Mean	51.58	78.05	40.73	-	-	119.35	130.46	90.19	-	-
CV (%)	7.73	6.48	3.90	-	-	2.84	2.30	3.61	-	-

Mean values followed by the same letter in the column do not differ significantly from each other according to the Scott-Knott test ($p \leq 0.05$).

The morphoagronomic traits analyzed resulted in the formation of four distinct groups, identified through cluster analysis (Fig. 1). The separation into these groups was based on the similarity observed among the morphoagronomic traits and can be described as follows: Group I, composed of three accessions (CBL-505, CBL-404, and CBL-103), was mainly characterized by greater height and lower plant dry matter (Table 4); Group II, consisting of a single accession (CBL-401), stood out mainly through higher essential oil content, higher leaf length-to-width ratio and leaf area, and lower plant height and canopy diameter, as well as high values of blue tones on both the adaxial and abaxial leaf surfaces; Group III, composed of twenty-one accessions (CBL-402, CBL-207, CBL-301, CBL-604, CBL-304, CBL-206, CBL-605, CBL-204, CBL-205, CBL-302, CBL-101, CBL-201, CBL-501, CBL-102, CBL-502, CBL-203, CBL-603, CBL-303, CBL-404, CBL-202, and CBL-602), was characterized by greater leaf length and width and lower essential oil content and yield; and finally, Group IV, made up of one accession, CBL-503, was characterized by high mean values for canopy diameter, dry matter, and essential oil yield, along with low mean values for leaf length and width, leaf length-to-width ratio, and leaf area (Table 4).

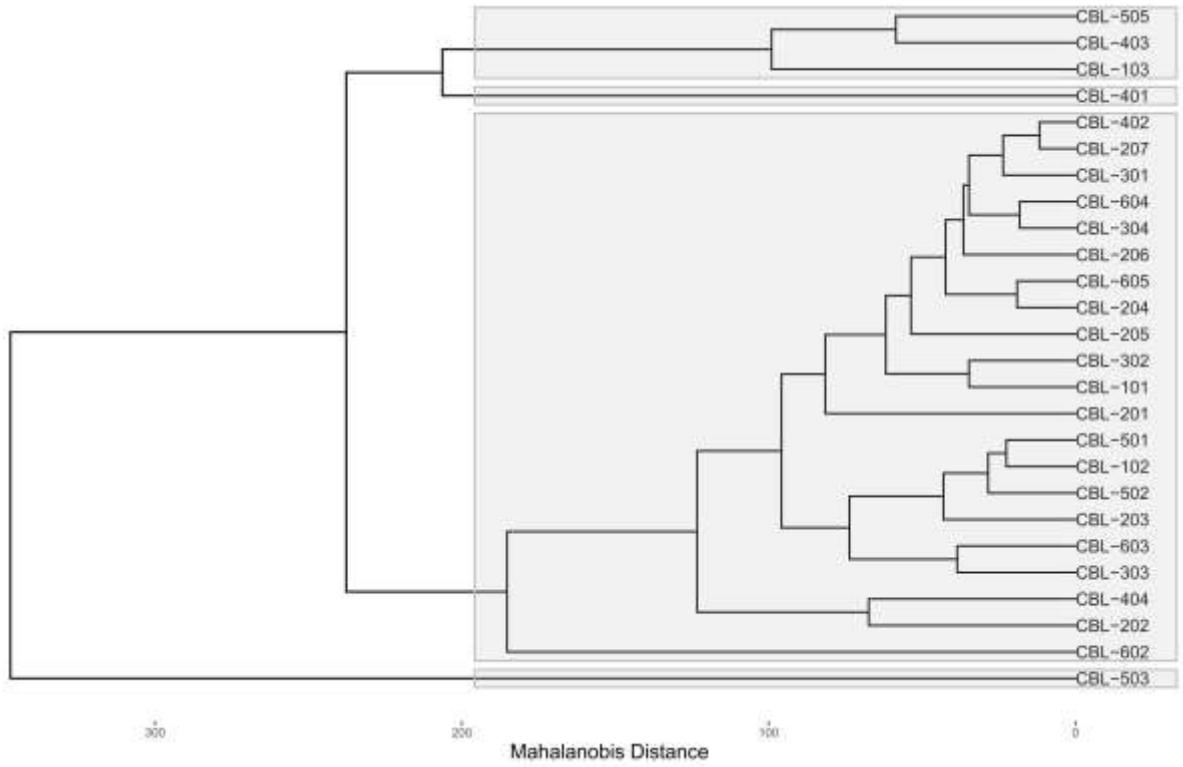

Figure 1. Two-dimensional dendrogram representing the similarities of morphoagronomic variables among the 26 accessions of *Croton blanchetianus* from the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe.

Table 4. Summaries of the two-dimensional dendrogram groups representing the clusters of the 26 accessions of *Croton blanchetianus* from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Variable	Group I	Group II	Group III	Group IV
PH (cm)	170.61 ± 43.35	87.00 ± 0.00	169.54 ± 26.86	120.00 ± 0.00
CD (cm)	84.11 ± 8.23	63.83 ± 0.00	88.02 ± 19.04	131.00 ± 0.00
DM (g/plant)	136.12 ± 30.71	145.15 ± 0.00	173.09 ± 49.22	333.00 ± 0.00
EOC (%)	1.36 ± 0.21	1.50 ± 0.00	0.87 ± 0.17	1.40 ± 0.00
EOY (mL/plant)	1.89 ± 0.74	2.18 ± 0.00	1.50 ± 0.55	4.66 ± 0.00
LL (cm)	13.94 ± 0.93	15.93 ± 0.00	16.36 ± 1.12	12.00 ± 0.00
LW (cm)	8.12 ± 0.45	8.83 ± 0.00	9.80 ± 0.84	8.00 ± 0.00
LL/LW	1.72 ± 0.03	1.81 ± 0.00	1.68 ± 0.17	1.50 ± 0.00
LA (cm^2)	87.08 ± 8.15	145.67 ± 0.00	127.58 ± 23.13	74.50 ± 0.00
Rad	48.42 ± 2.98	46.83 ± 0.00	52.59 ± 6.26	51.46 ± 0.00
Gad	74.21 ± 5.00	69.33 ± 0.00	79.03 ± 8.49	77.78 ± 0.00
Bad	39.01 ± 2.56	43.90 ± 0.00	40.68 ± 2.88	43.70 ± 0.00
Rab	116.68 ± 0.62	121.13 ± 0.00	119.49 ± 6.68	122.51 ± 0.00
Gab	126.52 ± 3.45	129.84 ± 0.00	131.02 ± 5.46	130.93 ± 0.00
Bab	84.01 ± 3.44	95.31 ± 0.00	90.90 ± 5.48	88.63 ± 0.00

Variables: PH: plant height (cm); CD: canopy diameter (cm); DM: dry matter (g); EOC: essential oil content (%); LL: leaf length (cm); LW: leaf width (cm); LL/LW: leaf length-to-width ratio; LA: leaf area (cm^2); Rad: adaxial red; Gad: adaxial green; Bad: adaxial blue; Rab: abaxial red; Gab: abaxial green; Bab: abaxial blue. Mean values ± standard deviations.

The first and second components of principal component analysis (PCA) explained 56.32% of the total accumulated variance (Fig. 2). The first principal component explained 36.11% of the total variance and was positively correlated with Rad (r = 0.92), Gad (r = 0.90), Rab (r = 0.91), and Gab (r = 0.98), indicating a strong association with red and green tones on both the adaxial and abaxial leaf surfaces. The second principal component, in turn, represented 20.21% of the total variance and

exhibited positive correlations with CD (r = 0.73), DM (r = 0.84), and EOY (r = 0.82), while it showed negative correlation with LL (r = -0.80), suggesting a positive relationship among canopy diameter, dry matter, and essential oil yield.

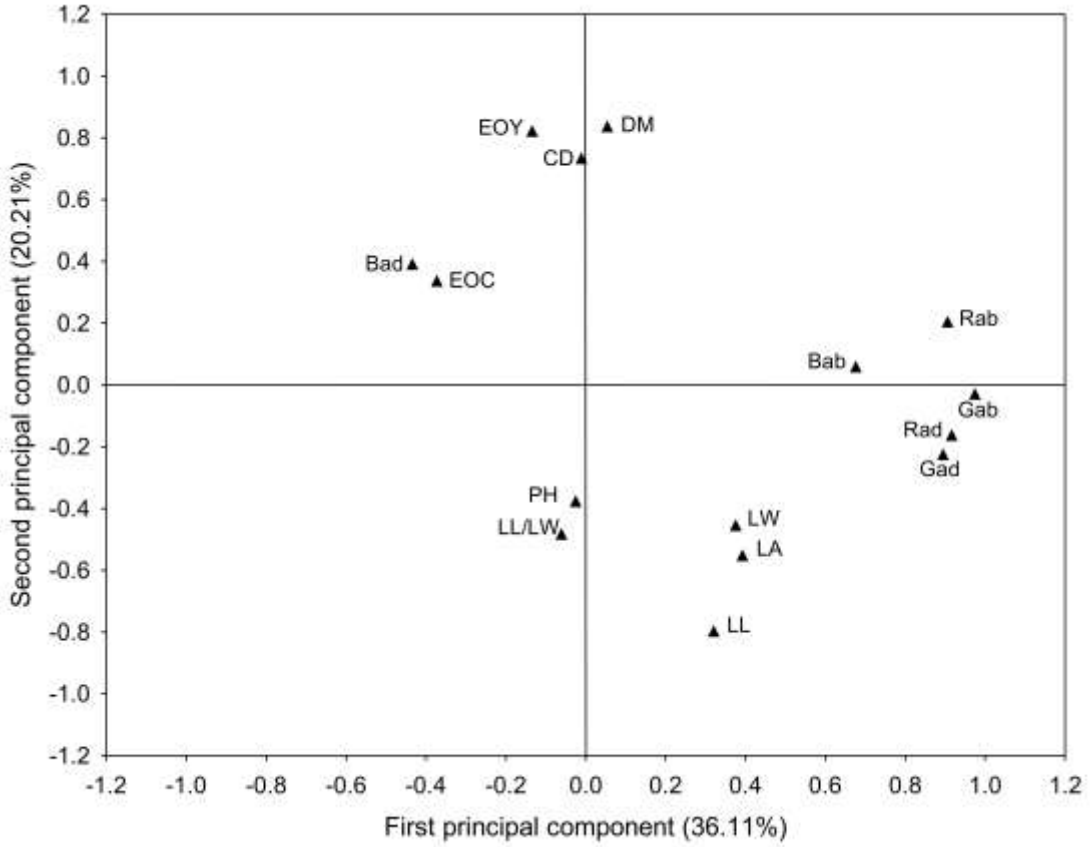

Figure 2. Distribution of the morphoagronomic variables of *Croton blanchetianus* accessions regarding the two principal components through principal component analysis (PCA). Variables: PH: plant height (cm); CD canopy diameter (cm); LL: leaf length (cm); LW: leaf width (cm); LL/LW: leaf length-to-width ratio (cm); LA: leaf area (cm^2); DM: dry matter (g); EOC: essential oil content (%); EOY: essential oil yield; Rad: adaxial red; Gad: adaxial green; Bad: adaxial blue; Rab: abaxial red; Gab: abaxial green; Bab: abaxial blue.

6.3.2. Chemical characterization

The essential oil compounds from the *C. blanchetianus* accessions that had relative contents lower than 1.5% across all the accessions were excluded from statistical analyses. As a result, 36 compounds were selected, representing, on average, 91.54% of the total essential oil composition (Table 5). The main compounds found were α-pinene (3.90-12.02%), limonene (0.88-10.72%), β-phellandrene (1.30-17.51%), 1,8-cineole (0.36-11.57%), (*E*)-caryophyllene (1.82-12.48%), germacrene D (0.38-10.12%), bicyclogermacrene (8.37-29.13%), and spathulenol (5.57-28.04%) (Table 4). Among the accessions evaluated, the highest α-pinene relative percentage were observed in accessions CBL-404 (12.02%) and CBL-502 (9.24%). Accession CBL-207 had the highest limonene relative percentage (10.72%), while accessions CBL-302 and CBL-401 had the highest concentrations of β-phellandrene (13.92% and 17.51%, respectively). The highest relative percentage of 1,8-cineole, (*E*)-caryophyllene, and germacrene D were found in accessions CBL-503 (11.57%), CBL-401 (12.48%), and CBL-403 (10.12%), respectively. All the accessions had high relative percentage of bicyclogermacrene and spathulenol, ranging from 5.57% to 29.13%, particularly CBL-501 (29.13% bicyclogermacrene) and CBL-602 (28.04% spathulenol).

Table 5. Relative percentage (%) of the chemical compounds in the essential oil of *Croton blanchetianus* Baill. accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Accession	Compound																		
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19
CBL-101	0.25	3.91	0.64	0.55	1.81	0.62	1.32	5.97	0.00	1.86	0.34	2.75	0.00	0.65	0.91	1.30	6.26	0.57	1.46
CBL-102	0.40	4.70	1.61	0.96	0.62	0.85	2.55	1.64	0.00	6.08	0.50	4.64	2.29	1.63	0.86	0.48	5.01	0.36	1.16
CBL-103	0.55	4.77	0.96	0.58	0.65	1.73	2.79	0.00	6.71	4.10	0.67	3.60	0.91	0.00	0.91	1.91	4.87	1.46	1.07
CBL-201	1.57	8.94	1.10	1.08	1.25	0.53	0.62	10.51	0.00	2.27	0.74	1.01	0.77	0.00	0.80	1.14	4.32	0.59	0.77
CBL-202	0.48	7.34	0.99	0.63	0.83	2.15	2.36	0.00	6.69	2.97	0.67	4.62	1.20	0.00	0.79	4.00	3.65	0.83	0.75
CBL 203	0.56	7.20	0.46	0.55	0.96	1.85	2.63	0.00	9.57	0.36	0.68	5.24	1.61	0.00	0.98	1.12	2.83	1.32	0.59
CBL-204	0.57	5.32	2.19	0.60	0.68	1.48	2.48	2.01	0.00	8.43	1.07	7.74	1.91	2.61	0.58	2.59	2.96	0.31	0.67
CBL-205	0.00	3.90	1.41	0.43	0.40	0.00	0.00	2.43	0.00	6.08	0.00	0.00	0.00	1.89	1.60	1.71	3.24	1.36	0.69
CBL-206	0.38	7.92	2.40	0.75	0.69	0.41	1.95	1.34	0.00	10.92	0.43	2.36	0.48	3.82	0.62	0.62	6.08	0.47	1.41
CBL-207	0.65	4.95	1.88	0.69	1.03	1.33	3.58	10.72	0.00	4.29	0.80	6.44	1.18	2.10	0.47	1.74	4.53	0.94	1.07
CBL-301	1.05	6.48	1.56	1.07	0.85	1.25	3.01	0.00	6.21	4.61	0.68	4.89	1.63	1.15	0.39	2.79	1.82	0.25	0.27
CBL-302	0.43	6.12	0.72	0.41	1.32	1.73	2.47	0.00	13.92	0.00	0.51	4.99	0.78	0.91	0.65	1.03	3.32	0.45	0.70
CBL-303	0.59	4.55	2.06	0.69	3.53	0.97	0.83	8.74	0.00	5.68	0.72	3.93	0.68	1.52	1.11	2.63	6.69	1.56	1.42
CBL-304	1.50	8.07	0.89	1.20	1.28	0.42	0.88	7.61	0.00	1.98	0.61	0.75	0.80	0.74	0.94	3.54	7.12	0.66	1.72
CBL-401	2.03	8.73	1.00	1.34	0.85	1.33	0.42	0.00	17.51	0.94	1.09	1.22	0.31	0.43	1.00	1.04	12.48	2.01	3.40
CBL-402	0.86	5.27	1.61	0.91	0.79	0.76	1.98	7.33	0.00	5.45	1.00	2.99	0.87	1.33	1.02	1.87	3.30	0.00	0.62
CBL-403	1.09	5.09	1.94	0.73	0.86	4.81	1.36	9.49	0.00	6.35	1.13	0.82	0.74	1.47	1.16	2.10	10.08	0.00	2.30
CBL-404	0.90	12.02	0.38	1.74	0.95	1.71	0.96	0.00	10.51	2.12	1.03	5.72	0.69	0.72	0.83	1.00	8.15	3.32	1.93
CBL-501	0.42	4.72	0.41	0.54	4.07	0.00	0.00	0.88	0.00	4.91	0.36	0.00	0.36	1.09	1.78	0.00	6.61	6.36	1.63
CBL-502	0.87	9.24	0.76	1.03	1.00	2.55	0.55	0.00	9.52	3.46	0.72	0.54	0.47	1.75	0.89	1.60	12.33	2.35	2.83
CBL-503	0.53	6.75	1.77	0.57	0.70	0.52	0.36	0.00	0.00	11.57	0.60	2.39	0.64	1.52	0.91	7.30	2.73	1.54	0.85
CBL-505	0.85	5.24	1.25	0.75	0.70	1.27	2.02	0.00	7.27	7.03	0.96	4.94	1.06	2.35	0.66	0.20	8.50	2.11	1.92
CBL-602	0.29	4.61	0.20	0.74	0.00	0.51	1.96	0.00	2.56	3.59	0.48	3.40	0.48	1.22	0.89	0.50	4.60	4.34	1.11
CBL-603	0.47	5.95	0.53	0.86	0.24	6.21	1.56	0.00	1.30	4.07	1.06	0.54	0.85	0.00	1.61	1.66	6.59	3.95	1.77
CBL-604	0.99	6.00	0.80	0.38	3.24	8.10	4.96	0.00	1.89	6.24	1.56	0.87	1.69	1.69	0.62	1.20	7.03	1.96	1.46
CBL-605	1.07	5.47	0.63	0.47	0.38	3.01	6.44	1.36	0.00	7.80	1.00	3.62	2.16	2.93	0.33	0.32	4.53	3.59	1.18
IRRo	924	931	970	974	989	1004	1022	1027	1029	1029	1056	1087	1100	1188	1334	1389	1416	1434	1449
IRRI	924	932	969	974	988	1002	1020	1024	1025	1026	1054	1086	1095	1186	1335	1389	1417	1439	1452

Table 5. (Continuation).

Accession	Compound																	
	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	C35	C36	TI
CBL-101	1.57	0.40	6.74	0.33	16.31	0.00	0.47	1.19	0.39	19.00	2.66	0.00	1.55	1.52	0.92	1.66	3.27	89.17
CBL-102	1.27	0.26	4.89	0.00	14.21	0.00	0.41	1.12	0.47	21.58	1.90	0.00	1.37	1.60	0.86	1.53	3.87	91.67
CBL-103	1.44	0.69	5.55	0.66	15.33	0.57	0.52	0.96	0.35	14.10	2.78	1.54	1.40	1.15	0.89	1.23	2.61	90.00
CBL-201	1.46	0.00	3.07	0.33	16.38	0.37	1.66	2.05	0.19	13.48	2.05	1.16	1.50	1.36	1.46	1.10	2.51	88.12
CBL-202	1.19	0.00	2.58	0.92	14.47	1.59	0.70	0.90	0.85	18.56	1.47	0.00	0.74	1.10	0.51	1.26	2.71	90.50
CBL 203	1.44	0.00	1.93	0.44	17.87	2.23	0.29	0.55	0.00	20.68	1.71	1.13	0.90	1.27	0.46	1.11	3.06	93.57
CBL-204	0.89	0.00	1.72	0.42	11.11	0.68	0.96	1.11	0.20	17.25	1.76	0.73	1.21	1.69	0.89	1.35	4.33	90.52
CBL-205	2.02	0.32	6.93	0.52	25.28	0.39	0.53	1.14	0.00	16.60	2.20	1.71	0.83	1.97	0.86	1.88	4.17	92.50
CBL-206	1.25	0.00	5.87	0.00	11.52	0.00	0.43	0.95	0.32	20.05	2.21	0.73	0.81	0.97	0.70	1.33	2.75	92.91
CBL-207	1.18	0.00	1.33	0.56	9.71	2.17	0.26	0.69	0.00	19.43	1.74	0.74	0.86	1.03	0.37	1.03	2.98	92.46
CBL-301	0.79	0.00	7.53	0.70	8.37	0.78	0.66	1.12	0.23	15.88	1.51	1.28	0.64	1.26	1.38	1.80	2.93	86.81
CBL-302	1.58	0.00	1.62	0.28	12.02	0.17	0.33	0.60	0.00	25.14	1.98	1.14	0.96	1.53	0.87	1.19	4.22	94.10
CBL-303	0.95	6.21	0.00	1.17	15.82	0.76	0.98	1.33	0.86	8.96	1.59	0.69	1.27	0.75	0.77	0.98	1.20	92.22
CBL-304	1.06	5.68	0.00	1.21	15.61	1.04	0.48	0.94	0.85	12.61	2.04	0.81	1.80	0.80	0.72	1.44	1.77	89.58
CBL-401	1.06	0.00	2.74	1.09	15.28	0.26	0.93	1.21	0.59	6.20	1.77	0.72	0.79	0.36	1.49	0.51	0.13	92.23
CBL-402	1.38	0.00	7.40	0.55	17.93	0.50	0.72	1.50	0.38	12.82	1.77	1.28	0.84	0.99	1.01	1.33	1.84	90.21
CBL-403	0.82	0.00	10.12	1.12	14.27	1.91	0.65	1.43	0.83	5.57	1.40	0.76	0.71	0.44	0.28	0.91	0.42	93.16
CBL-404	1.07	0.00	1.69	1.84	18.47	0.32	0.61	1.02	0.00	8.35	2.17	0.96	0.62	0.22	0.52	0.46	0.46	93.45
CBL-501	2.30	0.00	3.29	0.00	29.13	0.00	0.89	1.74	0.00	14.00	2.72	1.69	1.31	0.62	0.21	0.58	0.98	93.57
CBL-502	1.09	0.00	3.18	2.23	16.93	0.36	1.74	2.31	0.34	6.10	1.53	0.70	0.77	0.52	0.68	0.77	0.65	92.34
CBL-503	1.02	0.00	3.27	3.12	18.81	1.20	0.49	1.03	2.53	8.02	1.50	0.86	0.75	0.39	0.22	1.96	0.46	86.87
CBL-505	1.22	0.00	2.71	0.32	10.91	0.00	0.56	1.11	0.44	17.21	3.30	0.90	1.63	0.41	0.28	0.56	1.10	91.74
CBL-602	2.15	0.00	1.16	1.88	15.91	0.00	0.56	1.08	0.00	28.04	3.84	1.79	1.94	0.65	0.48	1.16	1.24	93.38
CBL-603	1.93	0.00	1.29	2.84	27.23	0.52	0.46	0.96	0.00	11.86	2.37	1.67	1.05	0.50	0.36	0.99	0.97	94.22
CBL-604	0.82	1.95	3.92	2.81	11.02	0.26	0.82	1.61	0.89	10.69	2.14	0.65	1.45	0.44	0.30	1.18	0.65	92.30
CBL-605	1.45	0.00	0.38	1.50	9.11	0.00	0.48	0.80	0.00	23.06	3.02	1.25	1.66	0.50	0.72	0.65	1.54	92.41
IRRo	1456	1474	1478	1482	1496	1501	1511	1521	1552	1578	1581	1599	1625	1639	1643	1650	1666	-
IRRI	1458	1478	1480	1489	1500	1501	1513	1522	1559	1577	1582	1604	1630	1642	1645	1651	1668	-
Mean	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91.54

Compounds: (C1) α-thujene, (C2) α-pinene, (C3) sabinene, (C4) β-pinene ,(C5) myrcene, (C6) α-phellandrene, (C7) ρ-cymene, (C8) limonene, (C9) β-phellandrene, (C10) 1.8-cineole, (C11) γ-terpinene, (C12) terpinolene, (C13) linalool, (C14) α-terpineol, (C15) δ-elemene, (C16) β-elemene, (C17) (*E*)-caryophyllene, (C18) aromadendrene, (C19) α-humulene, (C20) *allo*-aromadendrene, (C21) γ-muurolene, (C22) germacrene D, (C23) β-selinene, (C24) bicyclogermacrene, (C25) aciphyllene, (C26) γ-cadinene, (C27) δ-cadinene, (C28) germacrene B, (C29) spathulenol, (C30) caryophyllene oxide, (31) khusimone, (32) muurola-4,10(14)-dien-1-β-ol, (33) selina-3,11-dien-6-α-ol, (34) cubenol, (35) pogostol, and (C36) 14-hydroxy-9-epi-(*E*)-caryophyllene. TI: total compounds with relative percentage ≥ 1.5% in at least one sample among; IRRo: observed relative retention index; IRRl: literature relative retention index.

Cluster analysis led to identification of three distinct groups based on the chemical composition of the accessions (Fig. 3): Cluster I, with ten accessions (CBL-101, CBL-201, CBL-402, CBL-303, CBL-304, CBL-403, CBL-503, CBL-205, CBL-501, and CBL-603), characterized by higher mean concentrations of the compounds limonene, β-elemene, germacrene D, and bicyclogermacrene and by the absence of β-phellandrene (Figure 4); Cluster II, with three accessions (CBL-401, CBL-404, and CBL-502), characterized by higher mean values of the compounds α-pinene, β-phellandrene, (*E*)-caryophyllene, α-humulene, β-pinene, aromadendrene, γ-cadinene, δ-cadinene, cubenol, and bicyclogermacrene and by the absence of limonene; and Cluster III, with thirteen accessions (CBL-102, CBL-206, CBL-204, CBL-605, CBL-207, CBL-103, CBL-505, CBL-301, CBL-604, CBL-202, CBL-203, CBL-302, and CBL-602), characterized by higher mean values of the compounds ρ-cymene, terpinolene, linalool, α-terpineol, 14-hydroxy-9-epi-(*E*)-caryophyllene, and spathulenol (Fig. 3, 4).

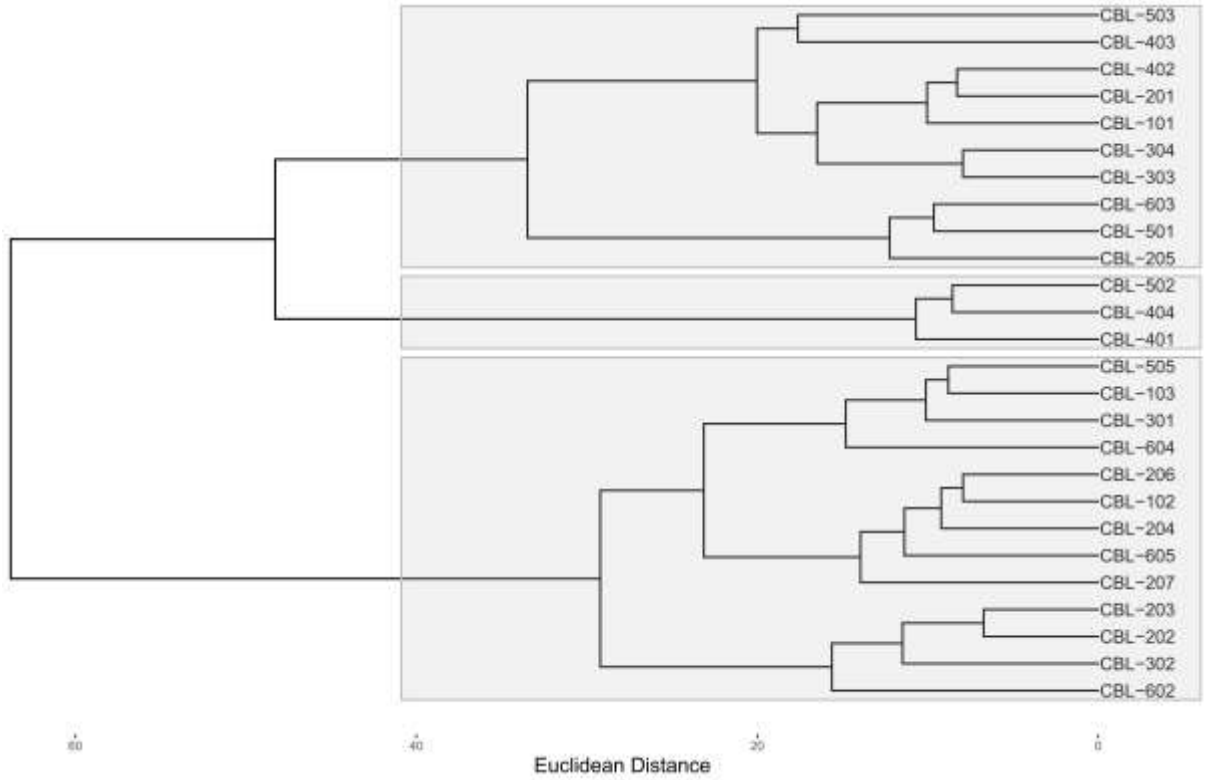

Figure 3. Two-dimensional dendrogram representing the similarity of the chemical composition of the essential oil of 26 accessions of *Croton blanchetianus* from the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe

According to principal component analysis, the first and the second component accounted for 36.07% of the total accumulated variance (Fig. 5). The first principal component explained 20.31% of the total variance and was positively correlated with (*E*)-caryophyllene (r = 0.83) and α-humulene (r = 0.82), and negatively correlated with spathulenol (r = -0.76), selina-3,11-dien-6-α-ol (r = -0.78), and 14-hydroxy-9-epi-(*E*)-caryophyllene (r = -0.87). The second principal component, in turn, accounted for 15.76% of the total variance and was negatively correlated with *allo*-aromadendrene (r = -0.96) and caryophyllene oxide (r = -0.73).

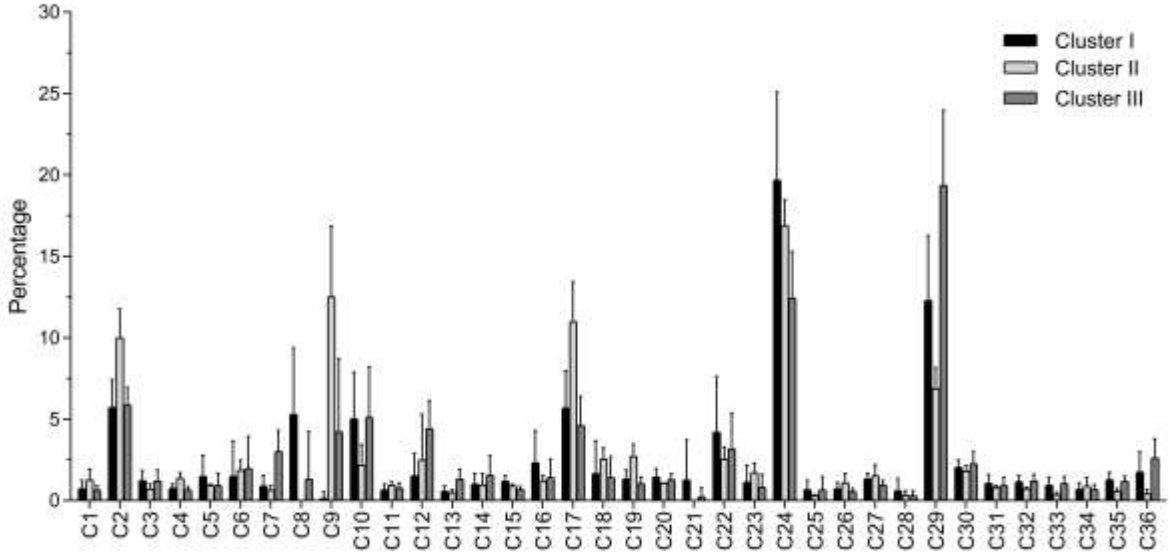

Figure 4. Mean values of the major chemical compounds of the essential oil from 26 accessions of *Croton blanchetianus* from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe. Compounds: (C1) α-thujene, (C2) α-pinene, (C3) sabinene, (C4) β-pinene, (C5) myrcene, (C6) α-phellandrene, (C7) ρ-cymene, (C8) limonene, (C9) β-phellandrene, (C10) 1,8-cineole, (C11) γ-terpinene, (C12) terpinolene, (C13) linalool, (C14) α-terpineol, (C15) δ-elemene, (C16) β-elemene, (C17) (*E*)-caryophyllene, (C18) *allo*-aromadendrene, (C19) α-humulene, (C20) *allo*-aromadendrene, (C21) γ-muurolene, (C22) germacrene D, (C23) β-selinene, (C24) bicyclogermacrene, (C25) aciphyllene, (C26) γ-cadinene, (C27) δ-cadinene, (C28) germacrene B, (C29) spathulenol, (C30) caryophyllene oxide, (31) khusimone, (32) muurola-4,10(14)-dien-1-β-ol, (33) selina-3,11-dien-6-α-ol, (34) cubenol, (35) pogostol, and (C36) 14-hydroxy-9-epi-(*E*)-caryophyllene.

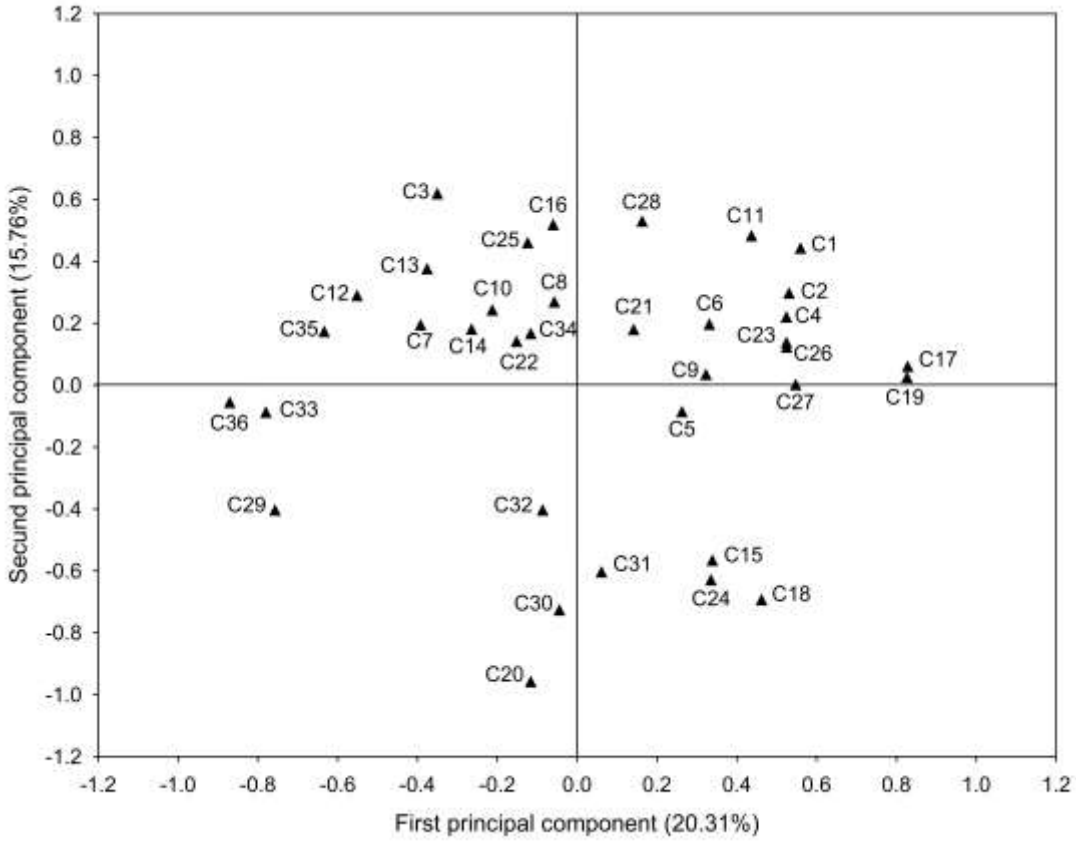

Figure 5. Distribution of the chemical compounds of the essential oil of *Croton blanchetianus* in relation to the two principal components through principal component analysis (PCA). Compounds: (C1) α-thujene, (C2) α-pinene, (C3) sabinene, (C4) β-pinene, (C5) myrcene, (C6) α-phellandrene, (C7) ρ-cymene, (C8) limonene, (C9) β-phellandrene, (C10) 1.8-cineole, (C11) γ-terpinene, (C12) terpinolene, (C13) linalool, (C14) α-terpineol, (C15) δ-elemene, (C16) β-elemene, (C17) (*E*)-caryophyllene, (C18) *allo*-aromadendrene, (C19) α-humulene, (C20) allo-aromadendrene, (C21) γ-muurolene, (C22) germacrene D, (C23) β-selinene, (C24) bicyclogermacrene, (C25) aciphyllene, (C26) γ-cadinene, (C27) δ-cadinene, (C28) germacrene B, (C29) spathulenol, (C30) caryophyllene oxide, (31) khusimone, (32) muurola-4,10(14)-dien-1-β-ol, (33) selina-3,11-dien-6-α-ol, (34) cubenol, (35) pogostol, and (C36) 14-hydroxy-9-epi-(*E*)-caryophyllene.

6.4. Discussion

6.4.1. Morphoagronomic characterization

The *C. blanchetianus* leaves are simple, entire, alternate, and cartaceous, with an ovate to lanceolate blade, a rounded to slightly cordate base, an acute to rounded apex, and entire margins, as described by Rossine et al. (2023). The length and width of the leaf blade of the accessions evaluated exhibited higher values (measuring 12-19.50 × 7.70-11.90 cm) than the taxonomic descriptors reported for the species (Rossine et al. 2023; Caruzo et al. 2025). This increase in leaf dimensions may be related to the growing conditions provided, such as fertilization, irrigation, and weed control, as the available taxonomic descriptors are based on plants collected in their natural habitats. The length-to-width ratio (LL/LW) of the leaf blade determines how leaf shape is defined. According to the classification proposed by Radford et al. (1974), the LL/LW ratios for the leaf blade are as follows: lanceolate (6:1-3:1), ovate (2:1-3:2), broadly ovate (6:5), very broadly ovate (1:1), broadly depressed ovate (5:6), and depressed ovate (2:3-1:2). The LL/LW ratio in the present study had a mean value of 1.68, corresponding to an ovate shape.

The C. *blanchetianus* leaf blade has a bifacial morphology – an adaxial surface with dark-green hues and an abaxial surface with opaque light-green to gray hues (Table 3). The leaves have ferruginous trichomes with a texture slightly rough to the touch that can cause skin irritation. Analysis of chromatic composition in the RGB system enabled differentiation of the color of the leaves sampled from the *C. blanchetianus* collection. The accessions that had higher proportions of the red component showed light-green shades, while a relative increase in the blue component led to dark-green tones. The intensity of the green component proved to be decisive for expression of overall color, such that its reduction accentuated lighter tones and its increase intensified green. The bifacial morphology of the leaf blade was also observed in a study on *V. curassavica* (Oliveira et al. 2020).

The *C. blanchetianus* accessions exhibited high variability in dry matter and essential oil content. Essential oil production is a quantitative trait regulated by multiple genes and influenced by environmental factors. Thus, the interaction between intrinsic genetic variation and environmental conditions may explain the differences observed in essential oil content among the sampled individuals. Previous studies on the chemical composition of the essential oils of *C. blanchetianus* reported concentrations ranging from 0.70% to 0.72% in fresh leaves and reaching 0.96% in dry leaves (Melo et al. 2013; Rodrigues et al. 2019; Vasconcelos et al. 2022). The accessions CBL-401, CBL-403, and CBL-503 collected in the municipalities of Lagarto/SE and Tobias Barreto/SE stood out with their mean essential oil concentrations of around 1.50%, higher than the levels reported in the literature for the species.

The results obtained from hierarchical analysis of clusters revealed high phenotypic variability among the *C. blanchetianus* accessions. Geographic origin did not affect the distribution of the accessions within each cluster, as was also observed for *V. curassavica* and *Lantana camara* L. (Nascimento et al. 2020; Oliveira et al. 2020). The accessions CBL-505 and CBL-503, both from the municipality of Tobias Barreto/SE, were most divergent. In contrast, the smallest divergence was detected between accessions CBL-402 from Lagarto/SE and CBL-207 from Graccho Cardoso/SE (Fig. 1). Similar results were reported by Costa et al. (2025) who analyzed natural populations of *C. blanchetianus* from the state of Sergipe. The authors identified high genetic variability within the populations and low variability among populations, which confirms the findings of the present study. The differences observed among the accessions for the morphoagronomic traits evaluated can be attributed to genetic variability.

Understanding the correlations among morphoagronomic variables is a valuable tool in the selection of accessions, which may be useful for conservation and breeding programs. In the present study, an inverse relationship was observed between plant height and canopy diameter, such that taller accessions had canopies of smaller diameter. In contrast, a positive correlation was found between canopy diameter and dry matter, that is, accessions with larger diameter canopies had greater dry matter values. The canopy diameter and dry matter variables reflect the accumulation of plant biomass, which is a direct indicator of essential oil yield. Thus, accessions that combine higher mean values of canopy diameter and dry matter are more promising for commercial purposes, as they exhibit traits associated with greater essential oil yield.

6.4.2. Chemical characterization

The *C. blanchetianus* accessions showed quantitative and qualitative variations in chemical composition of the essential oil (Table 5). The biosynthesis of essential oils is determined by the genetic composition of the species; however, environmental factors, such as temperature, relative humidity, rainfall, light intensity, wind regime, and soil nutrients, can lead to significant variations (Simões et al. 2017). Although the biosynthesis of these secondary metabolites is influenced by both genetic and environmental factors, the results obtained in this study indicate that genetic factors have a greater influence on the chemical composition of the plants analyzed; the accessions collected from the same location and grown under uniform environmental conditions were grouped into distinct clusters.

Studies on the chemical composition of the essential oil of *C. blanchetianus* have shown predominance of monoterpenes and sesquiterpenes, particularly compounds such as bicyclogermacrene, 1,8-cineole, (*E*)-caryophyllene, spathulenol, germacrene D, α-pinene, β-phellandrene, and limonene (Porto et al. 2021; Cavalcante et al. 2022; Vasconcelos et al. 2022; Nascimento et al. 2024). Monoterpenes and sesquiterpenes have also been described as major compounds in other species of the genus *Croton*, as in *Croton tetradenius* Baill., containing α-pinene, α-terpinene, *p*-cymene, and 1,8-cineole (Almeida-Pereira et al. 2019); *Croton heliotropiifolius* Kunth, containing β-caryophyllene, bicyclogermacrene, germacrene D, limonene, and 1,8-cineole (Alencar Filho et al. 2017); and *Croton argyratus* Blume, containing β-caryophyllene, spathulenol, caryophyllene oxide, and germacrene D (Salleh et al. 2022). The present study is consistent with previous studies in detection of predominant major compounds, including α-pinene, limonene, β-phellandrene, 1,8-cineole, (*E*)-caryophyllene, germacrene D, bicyclogermacrene, and spathulenol (Table 5).

The results obtained through cluster analysis confirmed chemical variability among the *C. blanchetianus* accessions, which were divided into three groups (Fig. 3). The accessions of Clusters I and II were differentiated by the absence or presence of β-phellandrene and limonene: the Cluster I plants did not have β-phellandrene, but had limonene. The inverse occurred in Cluster II: the plants had β-phellandrene but lacked limonene. Cluster III was composed of accessions that had ρ-cymene, terpinolene, and spathulenol as main compounds. Limonene and β-phellandrene are synthesized from a single substrate (the α-terpinyl cation); yet, they are catalyzed by different enzymes (limonene synthase and β-phellandrene, respectively), which may explain the production of one compound at the expense of the other (Bohlmann et al. 1999; Degenhardt et al. 2009; Du et al. 2014).

The accessions were not clustered according to the municipality of collection, which shows that high variability in chemical composition does not arise from origin in different municipalities. Nevertheless, high chemical variability was observed among individuals collected from the same location. These results are consistent with those reported by Costa et al. (2025), who investigated the genetic diversity of natural populations of *C. blanchetianus* in the state of Sergipe, a region that coincides with the collection areas of the accessions included in the Active Germplasm Bank. In that study, high genetic diversity among populations was not found, since the genotypes could not be differentiated according to their geographic origin. However, high genetic diversity was observed within the analyzed populations.

Principal component analysis showed correlations among the compounds present in the essential oils of *C. blanchetianus*. The compounds (*E*)-caryophyllene and α-humulene showed high positive correlation, that is, the essential oils with high concentration of (*E*)-caryophyllene also had high concentration of α-humulene. This positive correlation may be associated with the fact that both compounds originate from the same metabolic pathway, sharing farnesyl diphosphate as a common precursor (Barros et al. 2009). In addition, a high negative correlation was observed between allo-aromadendrene and caryophyllene oxide, and this can be utilized in selection of specific accessions, as it facilitates identification of accessions with high levels of one compound at the expense of the other.

Therefore, the results confirm the chemical variability among the *C. blanchetianus* accessions, attributed primarily to differences of a genetic nature. Detailed chemical characterization of these accessions is essential for identifying variations in the compounds present in the essential oils, thereby supporting the selection of accessions with distinct chemical profiles and, consequently, enhancing biotechnological utilization of this species.

6.5. Conclusion

The *C. blanchetianus* accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe show wide morphoagronomic and chemical variability. This variability is not dependent on geographic origin, indicating that genetic factors play a predominant role in differentiating the accessions. The information obtained in this study revealed accessions with higher essential oil content and specific compounds of interest, highlighting the importance of the characterization carried out to support selection of superior accessions, breeding programs, conservation strategies, and potential pharmacological, agricultural, and industrial applications of this species.

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7. MANUSCRIPT 4
CHEMICAL PROFILE OF THE ESSENTIAL OIL FROM LEAVES AND FRUIT OF *Croton blanchetianus* Baill.: A MEDICINAL AND AROMATIC PLANT ENDEMIC TO THE BRAZILIAN SEMI-ARID REGION

Artigo formatado de acordo com as normas do periódico Biochemical Systematics and Ecology (Publicado)

ABSTRACT

The aim of this study was to characterize the yield and chemical composition of the essential oils extracted from the leaves and fruit of five *Croton blanchetianus* Baill. accessions from the Active Germplasm Bank of the Federal University of Sergipe. The essential oils were extracted by the hydrodistillation method and analyzed by GC-MS. The essential oil content ranged from 1.00% to 1.33% in the leaves and from 0.61% to 1.00% in the fruit. The following constituents stood out: α-pinene (3.08-11.71%), limonene (2.87-12.11%), β-phellandrene (11.37-13.89%), 1,8-cineole (0.26-20.38%), terpinolene (0.36-9.90%), *δ*-elemene (3.24-12.83%), (*E*)-caryophyllene (2.16-8.62%), bicyclogermacrene (4.33-15.76%), and spathulenol (4.34-21.89%). A marked difference in chemical composition was observed between the vegetative and reproductive organs: the leaves had a larger proportion of sesquiterpenes, whereas the fruit exhibited predominance of monoterpenes. The compound myrtenyl acetate was detected exclusively in the essential oils extracted from the fruit and is proposed as a potential chemical marker of this organ. Principal component analysis confirmed the metabolic separation between leaves and fruit, reflecting inversely proportional patterns in the production of mono- and sesquiterpenes. The results provide the first report of the chemical composition of the essential oil from the fruit of *C. blanchetianus* and show metabolic differences between vegetative and reproductive organs, associated with possible ecological and physiological functions.

Keywords: chemical composition, marmeleiro, monoterpenes, sesquiterpenes, vegetative organs

7.1. Introduction

Essential oils are mixtures of complex, low-molecular-weight chemical substances, characterized by high volatility and an intense and pleasant aroma. The oils are obtained from plant raw materials, such as flowers, fruits, seeds, leaves, twigs, bark, wood, rhizomes, and roots (Simões et al., 2017; Tohidi et al., 2019), and are mainly composed of terpenoids and phenylpropanoids originating from the secondary metabolism of the plants (Simões et al., 2017). Terpenoids, the main constituents of essential oils, have wide structural diversity and are synthesized from isoprene (C5) units through the action of several terpenoid synthases (Siddiqui et al., 2024). According to the number of carbon atoms in the molecule, they are classified as monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and irregular terpenes (> C30) (Simões et al., 2017; Jin et al., 2025). Among these terpenoids, monoterpenes and sesquiterpenes exhibit volatile properties that determine the aromatic profiles of numerous plant species.

The ecological roles played by essential oils are related to chemical defense, attraction of pollinators and seed dispersers, and plant-plant and plant-environment communication (Jin et al., 2025). In addition to their ecological importance, essential oils have high economic potential, due to their biological and aromatic properties, and they are widely used in the pharmaceutical, cosmetics, and food industries (Sousa et al., 2023; Rodilla et al., 2024; Pezantes-Orellana et al., 2025). Regardless of their application, characterization of the chemical

composition of essential oils is indispensable, for it can vary significantly depending on location, season, time of collection and plant organ used, climate conditions, genetic factors, or even the extraction method and age of the plant (Ribeiro et al., 2018; Camara et al., 2021; Cavalcante et al., 2022; Narimani et al., 2022; Sá Filho et al., 2022; Lopes et al., 2025). These variations can lead to differences in the aroma and properties of the oils. In particular, studies on aromatic plants have shown that leaves, flowers, and fruit frequently exhibit distinct chemical profiles, as observed in species of the genus *Ferulago*, such as *F. angulata, F. carduchorum*, and *F. contracta* (Narimani et al., 2022).

The genus *Croton* (Euphorbiaceae) is recognized for its high diversity and wide distribution in tropical and subtropical regions, including the Caatinga biome, where several species show medicinal and aromatic potential (Coradin et al., 2018; Xu et al., 2018; Caruzo et al., 2025), including *Croton blanchetianus* Baill. Commonly known as marmeleiro, it is a monoecious shrub (1.5-8 m height) with entire oval to lanceolate leaf blades (12.0–19.50 × 7.70–11.90 cm); inflorescences arranged in a thyrse with unisexual flowers, pistillate flowers without petals, and staminate flowers with 5 white petals; a spheroidal capsule fruit, green to yellowish in color; and smooth, ellipsoid, brown to black seeds with a reniform caruncle. *C. blanchetianus* stands out for its use in folk medicine and for the biological activity of its essential oils (Chaves and Reinhard, 2003; Nunes et al., 2023). In folk medicine, the leaves and bark of this species are used in the treatment of gastrointestinal disorders, liver diseases, rheumatism, headaches, bronchitis, and diabetes, and also as wound-healing agents, generally in the form of teas, syrups, infusions, and macerations (Chaves and Reinhard, 2003; Souza et al., 2014; Saraiva et al., 2015; Bitu et al., 2015; Macedo et al., 2018). Among the biological activities reported for its essential oil, insecticidal, acaricidal, antifungal, antibacterial, and larvicidal activities stand out (Silva et al., 2020; Camara et al., 2021; Porto et al., 2021; Venâncio et al., 2025; Lopes et al., 2025).

Chemical studies conducted on the essential oil of *C. blanchetianus* have identified compounds belonging to the monoterpene and sesquiterpene classes. Prominent among its major components are the monoterpenes α-pinene, limonene, β-phellandrene, 1,8-cineole, and terpinolene, as well as the sesquiterpenes (*E*)-caryophyllene, bicyclogermacrene, spathulenol, germacrene D, cedrol, β-elemene, caryophyllene oxide, δ-cadinene, β-acorenol, and sylvestrene (Angélico et al., 2014; Ribeiro et al., 2018; Rodrigues et al., 2019; Camara et al., 2021; Porto et al., 2021; Vasconcelos et al., 2022a, 2022b; Cavalcante et al., 2022; Nunes et al., 2023; Nascimento et al., 2024; Venâncio et al., 2025; Lopes et al., 2025). More than 80 compounds have already been identified in *C. blanchetianus*.

Despite the wide chemical diversity observed in the species, studies have focused on the leaves, with little information available on the essential oils of the fruit, which limits understanding of possible differences in the chemical profile between vegetative and reproductive organs. Considering that variations in the chemical composition of essential oils may reflect ecological adaptations and physiological adjustments in terpenoid metabolism, the investigation of different plant organs is essential for clarifying the chemical profile of the species. Thus, the aim of the present study was to characterize the yield and chemical composition of the essential oils from the leaves and fruit of *C. blanchetianus*.

7.2. Materials and methods

7.2.1. Plant material

Leaves and fruit of the *C. blanchetianus* accessions were collected from plants maintained in the Active Germplasm Bank at the Federal University of Sergipe (SisGen Registration no. A8CCB3B) on the "Campus Rural" Experimental Farm in the municipality/county of São Cristóvão, Sergipe, Brazil (10º55'27" S, 37º12'01" W, and 46 m above sea level) (Fig. 1). The accessions originated from three municipalities in the state of Sergipe, Brazil (Aquidabã, Graccho Cardoso, and Tobias Barreto) (Table 1).

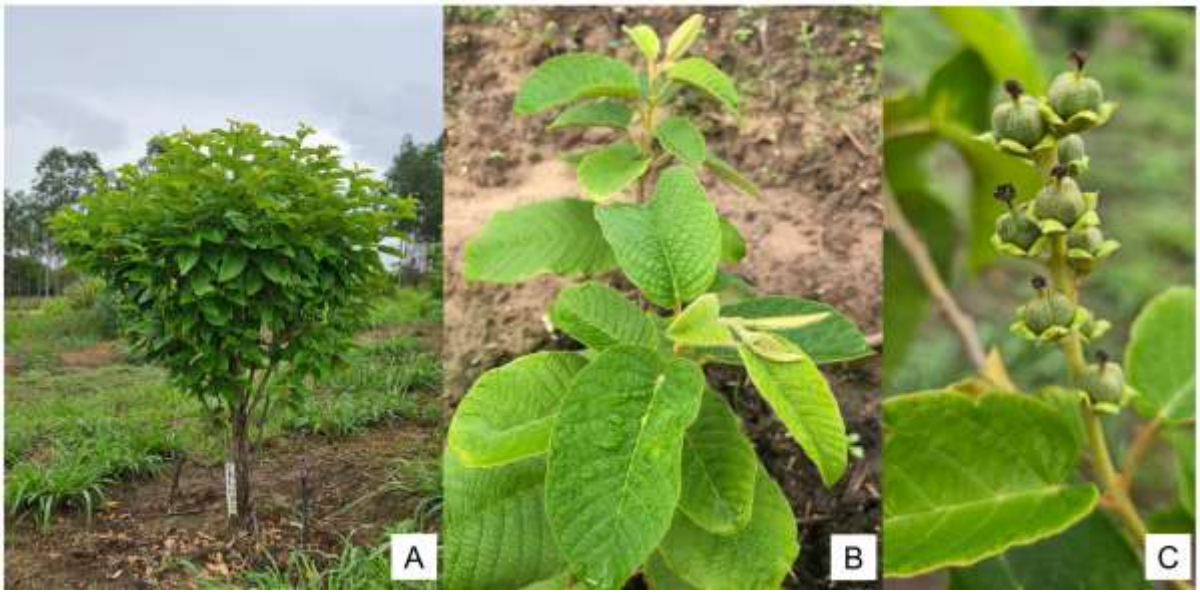

Fig. 1. *Croton blanchetianus* Baill. maintained in the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe (A) and leaves (B) and fruit (C) of *C. blanchetianus*.

Table 1. Site of origin of the five *Croton blanchetianus* Baill. accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Accessions	Origin (Sergipe, Brazil)	Geographic coordinates	Voucher no.
CBL-101	Aquidabã	10º19'27.7'' S, 37º04'44.3'' W	42851
CBL-203	Graccho Cardoso	10º14'03.7'' S, 37º11'25.8'' W	42861
CBL-207	Graccho Cardoso	10º14'29.5'' S, 37º12'50.5'' W	42865
CBL-501	Tobias Barreto	11º08'53.0'' S, 37º56'41.2'' W	42894
CBL-505	Tobias Barreto	11º10'06.4'' S, 37º58'44.0'' W	42899

7.2.2. Extraction and chemical composition of essential oils

The leaves and fruits were collected in February 2025 (dry season) and dried in a forced-air circulation laboratory oven at 40 ± 1 °C for five days. Essential oils were extracted by hydrodistillation using a modified Clevenger device for 120 minutes with 30 g of dried plant material (leaves and fruit) immersed in 2 L of water, in triplicate. The essential oils obtained were placed in amber flasks and stored at -20 ± 2 °C until chemical analyses were performed. The essential oil content (EOC) of each sample was calculated using the following formula:

$$\text{EOC (\%)} = (\text{Volume extracted from the sample} / \text{Sample dry matter}) \times 100.$$

The essential oils were analyzed using a gas chromatograph (model 7820A, Agilent Technologies), coupled to a mass spectrometer (model 5975 MSD, Agilent Technologies). The system was equipped with an HP-5MS fused-silica capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness; Agilent). The oven temperature had the following setting: initial temperature of 60 °C (maintained for 1 min), an increase to 170 °C at 3 °C min^{-1}, then to 220 °C at 5 °C min^{-1}, and finally to 280 °C at 20 °C min^{-1}. The transfer line temperature was maintained at 280 °C. The mass spectrometer operated in the electron ionization (EI) mode at 70 eV, with ion source temperature of 230 °C and quadrupole temperature of 150 °C. The mass scan range was 40-550 m/z.

The samples were injected using an autosampler (model G4513A, Agilent). The injector was equipped with split-type liner (4.0 mm internal diameter, 6.25 mm external diameter, 78.5 mm length, 870 µL volume), and injections were performed in split mode (10:1 split ratio).

Helium 5.0 (99.999% purity) was used as a carrier gas, at a constant flow rate of 1.2 mL min^{-1}. One microliter (1.0 µL) of each essential oil sample (10 mg mL^{-1} in ethyl acetate solution) was injected.

The constituents were identified based on retention indices, calculated from a homologous series of n-alkanes (C7-C30) through computerized searches using the NIST (National Institute of Standards & Technology) digital libraries of mass spectral data and through comparison of the retention indices and mass spectra with those reported in the literature (Adams, 2017).

7.2.3. Statistical analysis

Data regarding the essential oil yield of the *C. blanchetianus* accessions underwent analysis of variance (ANOVA), and mean values were compared using Tukey's test at a significance level of $p \leq 0.05$ using the Sisvar® software (Ferreira, 2019). In addition, graphs showing the proportions of monoterpenes and sesquiterpenes identified for each essential oil were constructed with the aid of the *ggplot* package (Wickham, 2016). Principal component analysis (PCA) was applied to examine the interrelationships among the chemical constituents of the essential oils of the leaves and fruit using the *factoextra* pckage (Kassambara and Mundt, 2020) and *stats* package in the R environment (R Core Team, 2022).

7.3. Results

Chemical analysis of the essential oils from the leaves and fruit of *Croton blanchetianus* accessions revealed the presence of 101 compounds, of which 83 were identified, representing an average of 97.08% of the total composition. Table 2 lists 23 compounds with relative percentage ≥ 2% in at least one sample among (representing an average of 84.80% of the total composition), along with the observed percentages of monoterpenes and sesquiterpenes. The main compounds identified in the leaves and fruit were α-pinene (3.08-11.71%), limonene (2.87-12.11%), β-phellandrene (11.37-13.89%), 1,8-cineole (0.26-20.38%), terpinolene (0.36-9.90%), δ-elemene (3.24-12.83%), (*E*)-caryophyllene (2.16-8.62%), bicyclogermacrene (4.33-15.76%), and spathulenol (4.34-21.89%) (Table 2, Fig. 2). The essential oils from the leaves and fruit exhibited the same chemical constituents, except for myrtenyl acetate (3.79-7.37%), which was present only in the fruit.

Analysis of variance showed significant interaction between the *C. blanchetianus* accessions and the parts analyzed, leaves and fruit, for the essential oil yield variable. The essential oil content of *C. blanchetianus* was higher from the leaves (1.00-1.33%) and lower from the fruit (0.61-1.00%), with an overall mean of 1.14% for leaves and 0.72% for fruit (Table 2). The accessions CBL-203, CBL-501, and CBL-505 stood out in showing the highest essential oil content from the leaves (1.17%, 1.22%, and 1.33%, respectively).

The essential oils of the accessions exhibited quantitative variation in the proportions of monoterpenes (18.11-44.89% in the leaves and 23.97-61.03% in the fruit) and sesquiterpenes (37.34-62.03% in the leaves and 23.07-49.85% in the fruit), depending on the material used for extraction, leaves or fruit of *C. blanchetianus* (Table 2). The chemical composition of the leaves had a higher proportion of sesquiterpenes and a lower proportion of monoterpenes, whereas the fruit exhibited an opposite pattern, with an increase in monoterpene relative percentage and reduction in sesquiterpene relative percentage (Fig. 3). In the fruit, increases were found in the relative percentage of monoterpenes: α-pinene, sabinene, β-phellandrene, 1,8-cineole, γ-terpinene, myrtenol, and myrtenyl acetate for all the accessions, except CBL-101. In contrast, reductions were found in the relative percentage of sesquiterpenes: δ-elemene, (*E*)-caryophyllene, aromadendrene, bicyclogermacrene, spathulenol, and 14-hydroxy-9-epi-(*E*)-caryophyllene in accessions CBL-203, CBL-207, CBL-501, and CBL-505 (Table 2). In the leaves, an opposite pattern was found – an increase in the concentrations of sesquiterpenes: δ-elemene, (*E*)-caryophyllene, aromadendrene, bicyclogermacrene, spathulenol, and 14-

hydroxy-9-epi-(*E*)-caryophyllene and reduction in monoterpenes: α-pinene, sabinene, β-phellandrene, 1,8-cineole, γ-terpinene, myrtenol, and myrtenyl acetate for all the accessions, except CBL-101.

The accession CBL-101 showed a distinct chemical profile compared to the other accessions, with higher relative percentage of the monoterpenes sabinene, limonene, and 1,8-cineole in the leaves, associated with lower relative percentage of the sesquiterpenes δ-elemene, bicyclogermacrene, caryophyllene oxide, muurola-4,10(14)-dien-1-*β*-ol, and 14-hydroxy-9-epi-(*E*)-caryophyllene.

Comparative analysis of the mean values of the compounds present in the essential oils extracted from the leaves and fruit of *C. blanchetianus* showed quantitative differences in their chemical composition (Table 2). The following monoterpenes stood out: α-pinene (6.11% in leaves, 8.94% in fruit), 1,8-cineole (5.74% in leaves, 8.40% in fruit), linalool (0.83% in leaves, 2.99% in fruit), myrtenol (0.16% in leaves, 4.37% in fruit), and myrtenyl acetate (0.00% in leaves, 4.91% in fruit), the relative percentage of which were higher in the fruit. In contrast, the main sesquiterpenes were δ-elemene (5.73% in fruit, 8.64% in leaves), (*E*)-caryophyllene (3.99% in fruit, 6.08% in leaves), bicyclogermacrene (7.70% in fruit, 10.19% in leaves), and spathulenol (6.86% in fruit, 15.21% in leaves), with higher relative percentage in the leaves compared to the fruit.

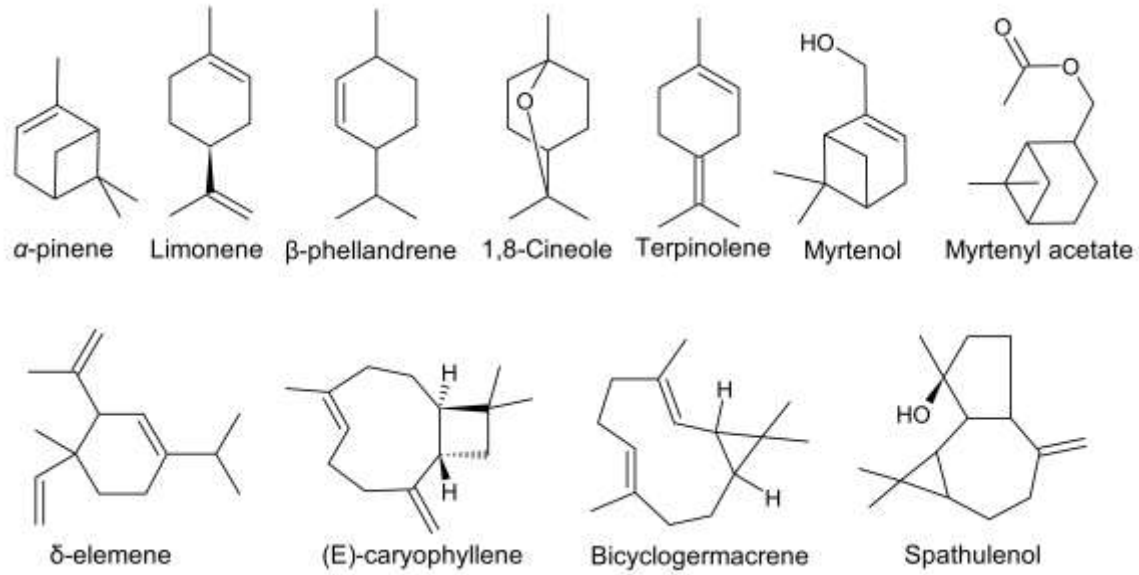

Fig. 2. Chemical structures of the main constituents of the essential oil from the leaves and fruit of *Croton blanchetianus.*

Table 2. Relative percentage (%) of the chemical compounds present in the essential oils extracted from the leaves and fruit of *Croton blanchetianus* Baill. accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe, Brazil.

No.	Compound	RI	RI_lit	Accession									
				CBL-101	CBL-203	CBL-207	CBL-501	CBL-505	CBL-101	CBL-203	CBL-207	CBL-501	CBL-505
				Leaves					Fruit				
1	α-pinene	923	932	3.08	7.84	6.02	6.75	6.85	4.33	10.53	9.28	11.71	8.87
2	Sabinene	961	969	0.48	0.54	2.58	0.89	2.02	0.26	0.90	3.17	1.95	2.90
3	Myrcene	980	988	2.50	1.17	1.14	5.00	0.95	1.39	1.08	1.06	3.47	1.38
4	α-phellandrene	995	1002	0.57	1.89	2.10	0.05	1.60	0.60	0.80	2.10	0.25	1.44
5	ρ-cymene	1014	1022	1.76	1.78	3.05	0.27	2.47	0.57	1.46	2.26	0.53	0.60
6	Limonene	1019	1024	4.20	-	12.11	-	-	2.87	-	9.49	-	-
7	β-phellandrene	1021	1025	-	11.37	-	-	-	-	13.89	-	-	-
8	1,8-Cineole	1022	1026	0.72	-	3.71	7.29	16,97	0.26	-	7.52	13.85	20.38
9	γ-terpinene	1047	1054	0.34	1.01	1.30	0.47	1.03	0.59	2.59	1.67	2.33	1.89
10	Terpinolene	1078	1086	3.36	7.21	9.90	0.36	5.68	3.78	1.80	9.82	0.60	5.08
11	Linalool	1090	1095	0.53	1.46	1.01	0.29	0.86	1.04	4.29	1.84	3.03	4.73
12	α-Terpineol	1179	1186	0.58	0.75	1.87	1.24	2.35	0.29	0.74	2.30	1.92	2.70
13	Myrtenol	1186	1194	-	0.25	0.09	0.06	0.39	3.54	3.34	2.79	5.18	7.01
14	Myrtenyl acetate	1314	1324	-	-	-	-	-	4.45	7.37	3.79	4.88	4.04
15	δ-elemene	1325	1335	8.98	10.37	6.26	12.83	4.77	9.38	5.81	3.24	6.42	3.77
16	(*E*)-caryophyllene	1401	1417	8.32	2.97	4.36	6.12	8.62	4.80	2.16	3.93	3.72	5.33
17	Aromadendrene	1426	1439	0.56	1.34	0.88	5.04	1.95	0.29	0.63	0.43	0.99	0.45
18	Germacrene D	1465	1480	5.33	1.29	1.15	2.17	2.00	4.32	3.28	1.40	3.65	3.22
19	Bicyclogermacrene	1483	1500	10.33	11.87	7.31	15.76	5.66	12.44	7.95	4.33	8.86	4.94
20	Spathulenol	1564	1577	21.89	13.74	12.84	12.78	14.78	10.74	8.56	6.28	4.36	4.34
21	Caryophyllene oxide	1568	1582	1.57	1.25	1.19	2.18	1.82	2.23	1.42	1.15	1.25	1.08
22	Muurola-4,10(14)-dien-1-β-ol	1627	1630	2.23	1.71	0.94	0.54	0.40	2.51	1.89	0.71	0.57	0.36
23	14-hydroxy-9-epi-(*E*)-caryophyllene	1654	1668	2.82	3.04	2.41	0.96	1.04	3.14	2.49	1.61	0.49	0.27
Monoterpenes (%)		-	-	18.11	35.28	44.89	22.68	41.16	23.97	48.79	57.10	49.69	61.03
Sesquiterpenes (%)		-	-	62.03	47.59	37.34	58.39	41.05	49.85	34.18	23.07	30.30	23.77
Total of compounds (≥ 2%)		-	-	80.14	82.87	82.23	81.07	82.21	73.82	82.97	80.17	79.99	84.80
Essential oil yield (%)		-	-	1.00 bA	1.17 aA	1.00 bA	1.22 aA	1.33 aA	0.67 bB	0.67 bB	0.61 bB	0.67 bB	1.00 aB

RI: calculated retention index; RI_lit: retention index reported in the literature. Mean values followed by the same lowercase letter do not differ significantly among the accessions within the organ used (leaves or fruit); and mean values followed by the same uppercase letter do not differ significantly between the organs used (fruit or leaves) within the same accession, according to Tukey's test ($p \leq 0.05$)

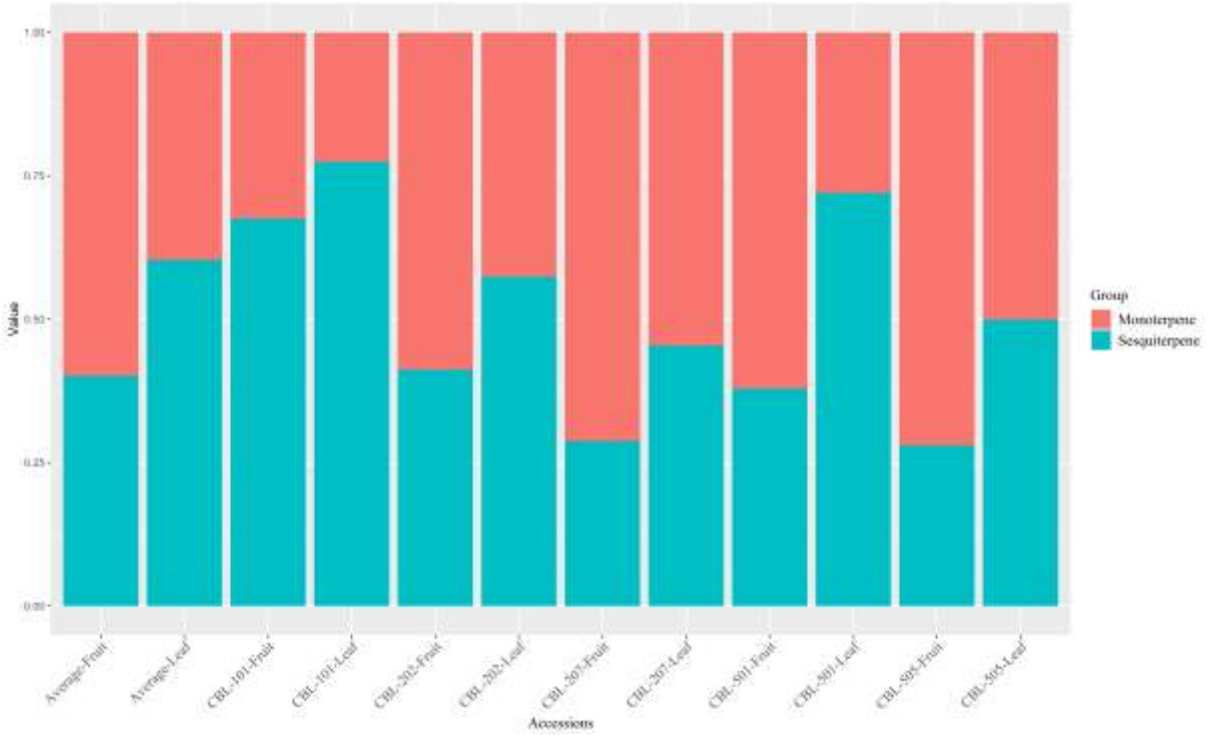

Fig. 3. Proportions of monoterpenes and sesquiterpenes in the essential oils extracted from the leaves and fruit of *Croton blanchetianus* Baill. accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe.

Principal component analysis (PCA) enabled characterization of the chemical profile of the essential oils from *C. blanchetianus* leaves and fruit. The first and second components represented 60.73% of the total accumulated variance, with PC1 accounting for 36.98% and PC2 for 23.75% (Fig. 4).

A clear separation was observed between the essential oils extracted from the leaves and from the fruit of the *C. blanchetianus* accessions, reflecting the inversely proportional pattern between the previously identified monoterpenes and sesquiterpenes. The essential oils derived from the leaves (of the CBL-101L, CBL-202L, CBL-501L, and CBL-505L accessions) clustered near the sesquiterpene compounds, such as spathulenol, 14-hydroxy-9-epi-(*E*)-caryophyllene, (*E*)-caryophyllene, *δ*-elemene, aromadendrene, caryophyllene oxide, muurola-4,10(14)-dien-1-β-ol, bicyclogermacrene, and germacrene D. In contrast, the essential oils derived from the fruit (of the CBL-202F, CBL-207F, CBL-501F, and CBL-505F accessions) were mainly associated with monoterpenes, such as sabinene, *α*-terpineol, *γ*-terpinene, *α*-pinene, linalool, myrtenol, 1,8-cineole, and myrtenyl acetate. Furthermore, corroborating the previously presented result, the essential oil derived from the fruit of the accession CBL-101 exhibited a pattern distinct from that observed in other accessions, strongly associated with the compounds *δ*-elemene, bicyclogermacrene, and caryophyllene oxide.

These results confirm the metabolic differentiation between leaves and fruit. As observed, sesquiterpenes predominated in leaves, whereas fruit was characterized by the higher concentration of monoterpenes, reinforcing the inversely proportional pattern between these two chemical classes.

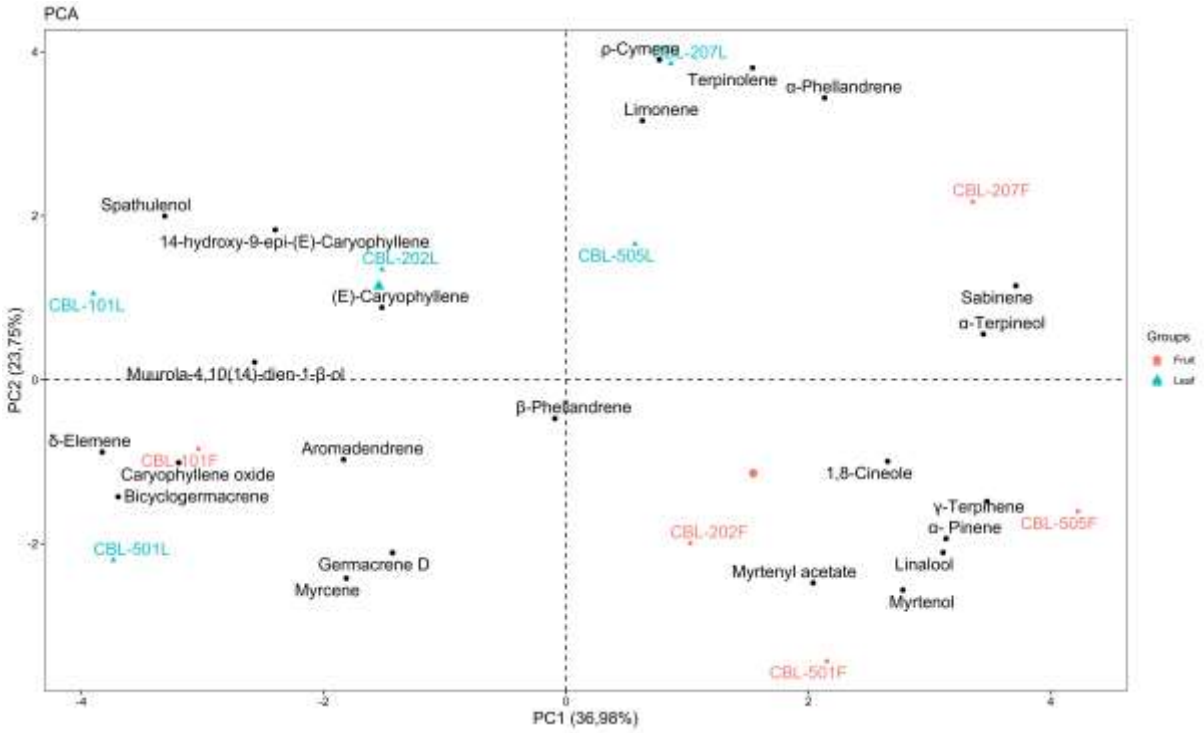

Fig. 4. Distribution of the accessions and chemical compounds of the essential oils extracted from the leaves and fruit of *Croton blanchetianus* as illustrated by principal component analysis (PCA).

7.4. Discussion

The essential oil content of *C. blanchetianus* exhibited significant variation among the plant organs analyzed, with a higher content from the leaves (1.14%) compared to the fruit (0.72%). The essential oil content extracted from the leaves observed in this study was higher than the values reported for the species in the literature, which range from 0.31 to 0.96% (Melo et al., 2013; Ribeiro et al., 2018; Rodrigues et al., 2019; Vasconcelos et al., 2022a). In contrast, the present study constitutes the first report of the yield and chemical composition of the essential oil extracted from the fruit of *C. blanchetianus*. The disparity in oil yield observed between the vegetative and reproductive structures may be attributable to the plant organ and to the density of the secretory structures. The higher concentration of secretory glands in specific plant organs is a well-established morphophysiological factor that directly influences variations in essential oil yield (Gostin and Blidar, 2024; Ložienė, 2025). In the genus *Croton*, studies have shown that essential oils are present in secretory glands, such as idioblasts dispersed in mesophyll cells and in glandular trichomes (Rosa et al., 2021).

The constituents identified in the leaves and fruit of *C. blanchetianus*, such as *α*-pinene, limonene, *β*-phellandrene, 1,8-cineole, terpinolene, *δ*-elemene, (*E*)-caryophyllene, bicyclogermacrene, and spathulenol, have already been reported in the literature for the essential oils from the leaves of this species (Malveira et al., 2022; Vasconcelos et al., 2022a; Cavalcante et al., 2022; Nunes et al., 2023; Nascimento et al., 2024). However, myrtenol and myrtenyl acetate were not previously reported as constituents of the essential oil of *C. blanchetianus*, regardless of the plant organ. Their presence in the fruit suggests that myrtenyl acetate may serve as a chemical marker to differentiate the essential oil extracted from the fruit from that obtained from the leaves of the species. This pattern of a higher percentage of myrtenol and myrtenyl acetate in fruit was observed by Pereira et al. (2009) in their analysis of the essential oils from *Myrtus communis* extracted from different organs (leaves, branches, and fruit) throughout its development.

Myrtenol ($C_{10}H_{16}O$), which was found in lower percentage in leaves (<1%) than in fruit, is a monoterpene alcohol with a menthol-like aroma widely found in the essential oils of different plant species, including *Leutea elbursensis*, *Dracocephalum kotschyi*, and *Xylopia aethiopica* (Goodner et al., 2006; Khalil-Moghaddam et al., 2022; Ndoye et al. 2024; Yadegari, 2024). Myrtenyl acetate ($C_{12}H_{20}O_2$), in turn, is a monoterpene ester characterized by a floral, fruity, and herbaceous aroma, widely identified in the essential oils of aromatic species, including *Lavandula stoechas, Myrtus communis*, and *Juniperus sabina* (Aprotosoaie et al., 2017; Ghorbanzadeh et al., 2021; Zayani et al., 2025). These monoterpenes are synthesized from the cation α-terpinyl, whose electrophilic cyclization gives rise to pinyl cations. Subsequent deprotonation at the C4 and/or C10 positions results in the formation of α-pinene and β-pinene, respectively. After that, the hydroxylation of these pinene isomers, catalyzed by enzymes of the cytochrome P450, leads to the production of myrtenol. Myrtenol is then converted into myrtenyl acetate through the action of an alcohol acetyltransferase, which uses acetyl-CoA as the acetyl group donor (Barros et al., 2009; Baharlooeian et al., 2025).

The chemical composition of the essential oils from the leaves and fruit of *C. blanchetianus* exhibited quantitative and qualitative differences. The leaves were characterized by higher relative percentage of sesquiterpenes, whereas the fruit exhibited predominance of monoterpenes. In the leaves, the sesquiterpenes δ-elemene, (*E*)-caryophyllene, bicyclogermacrene, and spathulenol predominated; while in the fruit, the monoterpenes α-pinene, 1,8-cineole, linalool, myrtenol, and myrtenyl acetate exhibited higher relative percentage. An inversely proportional pattern between monoterpenes and sesquiterpenes in essential oils from vegetative and reproductive organs has also been reported for other species, as in *Cupressus arizonica* (Cherrad et al., 2022), *Myrica gale* (Ložienė et al., 2023), *Syzygium cumini* (Asker et al., 2025), and *Zanthoxylum mantaro* (Morocho et al., 2025).

From an ecological perspective, the chemical variation in the essential oils observed when they are derived from the leaves and from the fruit may be related to different adaptive functions, including defense against pathogens, insects, and herbivores, as well as attraction of pollinators and seed dispersers (Jin et al., 2025). In leaves, the sesquiterpenes β-caryophyllene and caryophyllene oxide in *Hyptis pectinata* and (*E*)-caryophyllene and α-humulene in *Varronia curassavica* act as chemical defense agents against the ant species *Atta sexdens rubropilosa* and *Dorymyrmex thoracicus*, respectively (Feitosa-Alcântar et al., 2017; Oliveira et al., 2019). In contrast, monoterpenes present in flowers and fruit play a fundamental role in attracting pollinators and seed dispersers, as well as contributing to chemical defense during fruiting (Rodríguez et al., 2013; Byers et al., 2014).

The chemical variation of essential oils may also be associated with the physiological and metabolic aspects of the plants. The biosynthesis of monoterpenes and sesquiterpenes depends on two main precursors: isopentenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), derived from the methyl erythritol phosphate (MEP) pathway and/or the mevalonate (MVA) pathway (Simões et al., 2017). The sesquiterpenes, composed of three isoprene units (C15) and synthesized through the MVA pathway in the cytosol, are structurally more complex molecules and require a higher energy expenditure (9 ATP and 6 NADPH) (Bergman et al., 2019; Liu et al., 2024). Conversely, the monoterpenes, composed of two isoprene units (C10) and synthesized through the MEP pathway in plastids, require a lower energy expenditure (6 ATP and 6 NADPH) (Bergman et al., 2019). Studies indicate that plants can modulate terpene production according to energy availability and the reproductive phase, favoring simpler compounds such as monoterpenes in order to conserve energy for the growth and maturation of reproductive organs (Vranová et al., 2013).

Therefore, the results obtained indicate that there is variation in the yield and chemical composition of the essential oils extracted from the leaves and fruit of *C. blanchetianus*, which may be related to adaptive and physiological strategies intrinsic to the species. The differences observed between leaves and fruit result not only from the influence of morphophysiological factors, but also from metabolic adjustments associated with energy conservation through

which plants optimize resource allocation for fruit development, without compromising their capacity for defense and attraction of dispersers.

7.5. Conclusion

The results of this study show both quantitative and qualitative differences in the composition of the essential oils from the leaves and fruit of *Croton blanchetianus*, revealing a distinct metabolic pattern between the vegetative and reproductive organs. The leaves showed a predominance of sesquiterpenes, possibly with a chemical defense function, whereas the fruit was characterized by a higher proportion of monoterpenes, indicating a potential role in attracting dispersers and in reducing energy expenditure. The presence of myrtenyl acetate in the fruit suggests its potential as a chemical marker of the essential oil from this structure. These findings expand knowledge regarding the chemical and metabolic plasticity of *C. blanchetianus* and constitute the first report of the composition of the essential oil from the fruit of this species native to the Brazilian Caatinga.

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8. MANUSCRIPT 5
SEASONALITY AFFECTS THE CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM *Croton blanchetianus* Baill. ACCESSIONS

Artigo formatado de acordo com as normas do periódico Industrial Crops and Products

ABSTRACT

Seasonal variation can significantly influence the biosynthesis and composition of essential oils in aromatic plants. This study evaluated the content, yield, chemical composition, and antioxidant activity of the essential oils from 26 accessions of *Croton blanchetianus* from the Active Germplasm Bank of the Federal University of Sergipe, collected at three harvest times (February, June, and October 2024). The essential oils were obtained by hydrodistillation and analyzed by gas chromatography coupled to mass spectrometry (GC–MS). Significant differences were observed among accessions and harvest times, with higher values of leaf dry mass and essential oil content and yield in Harvests 1 and 2. Accessions CBL-403 and CBL-503 showed the highest essential oil contents in Harvest 1 (1.93%), whereas CBL-503 (17.41 mL plant^{-1}) and CBL-507 (17.38 mL plant^{-1}) exhibited the highest yields in Harvest 2. Analysis of chemical composition showed predominantly sesquiterpenes in Harvests 1 (59.02%) and 2 (62.01%), while an increase in monoterpenes was observed in Harvest 3 (40.01%), likely associated with seasonal climatic variations. The main constituents identified included α-pinene (0.53–18.06%), α-phellandrene (0.17–10.40%), limonene (0.83–17.34%), β-phellandrene (1.26–23.29%), 1,8-cineole (0.64–17.56%), terpinolene (0.09–10.68%), δ-elemene (0.30–10.50%), (E)-caryophyllene (2.04–12.87%), germacrene D (0.07–10.41%), bicyclogermacrene (1.78–35.74%), and spathulenol (7.63–26.68). Accession CBL-301 exhibited high antioxidant activity (DPPH: 66.24%; ABTS: 92.51%). Harvests 1 and 2 were characterized by higher biomass production and essential oil yield, with predominance of sesquiterpenes, whereas Harvest 3 showed a higher proportion of monoterpenes, evidencing seasonal variation. Antioxidant activity varied among accessions, suggesting a synergistic effect among the essential oil constituents. These results highlight the potential of *C. blanchetianus* for selection of superior accessions and definition of optimal harvest time, aiming at the production and application of bioactive compounds

Keywords: germplasm, seasonal variation, volatile compounds, aromatic species, antioxidant activity.

8.1. Introduction

Croton blanchetianus Baill. (Euphorbiaceae) is an aromatic shrub that grows spontaneously in Caatinga vegetation areas, forming dense populations on sandy, rocky, or clayey soils at altitudes ranging from 200 to 800 m. It is often associated with anthropized environments (Pereira et al., 2001; Carneiro-Torres, 2009; BFG, 2025). Endemic to the Caatinga, the species occurs in all states of the Northeast region of Brazil, except Maranhão, and in the Southeast region, in Minas Gerais, where it is popularly known as *marmeleiro*, *marmeleiro-branco*, and *marmeleiro-da-caatinga* (BFG, 2025). In the Northeast region, the fresh or dried leaves and bark of *C. blanchetianus* are traditionally used in the treatment of liver infections, stomach pain, headaches, bronchitis, and diabetes (Chaves and Reinhard, 2003). In addition, the species has ecological and phenological characteristics that confer abundant potential for beekeeping, ensuring a continuous supply of nectar and pollen throughout the year (Borges et al., 2014; Martins et al., 2017).

Previous studies have reported that the essential oils of *C. blanchetianus* have bioactive chemical compounds with pharmacological and biological activities, particularly antibacterial (Angélico et al., 2014; Vasconcelos et al., 2022a, 2022b; Nunes et al., 2023; Venancio et al., 2025), insecticidal (Silva et al., 2020), acaricidal (Rodrigues et al., 2019; Camara et al., 2021), larvicidal (Lopes et al., 2025), and antinociceptive properties (Nascimento et al., 2024). The activities of the essential oils are mainly related to their chemical compounds, mainly monoterpenes (α-pinene, limonene, β-phellandrene, 1,8-cineole, and terpinolene) and sesquiterpenes ((*E*)-caryophyllene, bicyclogermacrene, spathulenol, germacrene D, cedrol, β-elemene, caryophyllene oxide, δ-cadinene, β-acorenol, and sylvestrene). However, the chemical composition of aromatic species can vary depending on several factors, such as geographic location, climatic conditions, and harvest time (Sá Filho et al., 2022).

Seasonal variation plays a crucial role in the composition of essential oils, influencing both the quantity and quality of volatile compounds produced by plants. Qualitative and quantitative differences have been widely reported in aromatic species such as *Lippia alba*, *Varronia curassavica*, and *Lantana camara* when evaluated at different times (Oliveira et al., 2020; Sá Filho et al., 2022; Pereira et al., 2022). Environmental factors, such as precipitation, temperature, humidity, and light intensity, can significantly affect the biosynthesis of these metabolites, resulting in fluctuations in the chemical profile throughout the annual cycle (Sá Filho et al., 2022; Jerônimo et al., 2024). According to Pereira et al. (2022), variations in chemical constituents and the production of certain compounds are directly related to biosynthetic pathways modulated by the physiological demands of the plant, which in turn may be influenced by environmental factors. Understanding these variation patterns is essential for optimizing the extraction and use of essential oils, as changes in chemical composition may affect their bioactive properties (Simões et al., 2017; Guelifet et al., 2025; Khaiper et al., 2026).

Essential oils from species of the genus *Croton* have been widely investigated for their antioxidant potential, often showing moderate to high efficiency (Yagi et al., 2016; Vale et al., 2021; Oliveira et al., 2021; Costa et al., 2022a; Cruz et al., 2026). Oxygenated terpenic compounds, such as monoterpenes and sesquiterpenes, which are common in this genus, are frequently associated with the antioxidant activity of essential oils due to their ability to stabilize and neutralize reactive oxygen species (Graßmann, 2005; Miguel, 2010; Zengin and Baysal, 2014). Biological antioxidants are organic compounds that can accept electrons, neutralize reactive species (Taiz et al., 2017), and minimize the damage resulting from oxidative stress. This stress is related to the development of several diseases, such as cardiovascular disorders, Alzheimer's disease, cancer, and diabetes (Zang et al., 2015). Naturally occurring antioxidant compounds, such as essential oils, have been explored as alternatives to synthetic antioxidants, as the naturally occurring compounds pose lower risks to human health (Nascimento et al., 2020; Mutlu-Ingok et al., 2020).

The chemical variability of essential oils from *C. blanchetianus* in natural populations from Sergipe has previously been reported (Costa et al., 2026). However, it is important to evaluate how predominant the effect of climatic variation is on the composition of essential oils from bioactive plants at different times of the year. This can be carried out using accessions maintained in Active Germplasm Banks. Thus, this study aimed to analyze the leaf dry mass and the content, yield, chemical composition, and antioxidant activity of essential oils from 26 accessions of *C. blanchetianus* belonging to the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe (UFS) collected at three different harvest times.

8.2. Material and Methods

8.2.1. Plant material and climatic data

Leaf samples from 26 accessions of *C. blanchetianus* were collected from the Active Germplasm Bank (AGB) of Medicinal and Aromatic Plants of the Federal University of

Sergipe, Brazil (10°55'27" S, 37°12'01" W). The accessions from the AGB originated from six municipalities in the state of Sergipe: Aquidabã, Graccho Cardoso, Itabi, Lagarto, Tobias Barreto, and Poço Verde (Table 1). For each accession, a voucher specimen was collected; the exsiccates were deposited and registered in the "ASE" Herbarium of the Federal University of Sergipe (Table 1) and registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under number A8CCB3B.

The experiment was established in June 2023 using a randomized complete block design consisting of three replicates and two plants per plot at a spacing of 2.0 m between plants and 3.0 m between rows. The standardized crop management practice of topdressing fertilization was used for all the plots. A total of 5 L of cattle manure was applied per plant, as well as 10 kg ha^{-1} of N, 40 kg ha^{-1} of P_2O_5, and 20 kg ha^{-1} of K_2O every four months. Weeding and mowing were carried out as required. The crop was monitored over a one-year period, with harvests performed in February, June, and October 2024, for a total of three harvests.

Climatic parameters of the study area (temperature and precipitation) were obtained monthly from the database of the National Institute of Meteorology (INMET, http://www.inmet.gov.br/portal/, accessed on November 24, 2025). Meteorological data were obtained from the automatic station A-409 located in Aracaju, Sergipe, Brazil, approximately 16 km from the experimental site. In the state of Sergipe, the rainy season is from March to August, while the dry season occurs between November and February. Monthly precipitation values ranged from 8.00 mm (October 2024) to 324.80 mm (April 2024); mean temperatures ranged from 25.45 °C (July 2024) to 28.95 °C (March 2024); mean relative humidity ranged from 63.27% (September 2024) to 73.52% (April 2024); and mean solar radiation ranged from 994.28 kJ m^{-2} (June 2024) to 1714.45 kJ m^{-2} (November 2023) (Fig. 1). During the harvest months, the following values were observed for precipitation, mean temperature, relative humidity, and solar radiation, respectively: February – 109.40 mm, 28.0 °C, 71.62%, and 1480.42 kJ m^{-2}; June – 251.60 mm, 25.65 °C, 71.89%, and 994.28 kJ m^{-2}; and October – 8.00 mm, 26.50 °C, 67.33%, and 1620.63 kJ m^{-2}.

8.2.2. Extraction and chemical composition of essential oils

At all harvest times, leaf samples from all accessions were collected and dried in a forced-air circulation oven at 40 °C for 5 days, after which leaf dry mass was determined. Essential oils were extracted by hydrodistillation, in triplicate. For each extraction, 50 g of dried leaves was immersed in 2 L of water in a modified Clevenger apparatus for 120 minutes. The essential oil content (EOC) and essential oil yield (EOY) of each sample were calculated using the following equations:

(1)

$$\text{EOC (\%)} = \left(\frac{\text{Extracted volume from the sample}}{\text{Dry mass of the sample}}\right) \text{x } 100$$

(2)

$$\text{EOY } (\text{mL plant}^{-1}) = \left(\frac{\text{EOC (\%) x Total dry mass per plant}}{100}\right)$$

Annual leaf dry mass and essential oil yield per accession were determined by summing the dry masses and yields obtained at each harvest time for each plant.

The chemical composition of the essential oils was analyzed using a gas chromatograph (model 7820A, Agilent Technologies) coupled to a mass spectrometer (model 5975 MSD, Agilent Technologies). The system was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness; Agilent). The oven temperature program consisted of an initial temperature of 60 °C, held for 1 min, increased to

170 °C at a rate of 3 °C min^{-1}, then to 220 °C at 5 °C min^{-1}, and finally to 280 °C at 20 °C min^{-1}. The transfer line temperature was maintained at 280 °C. The mass spectrometer operated in electron impact (EI) ionization mode at 70 eV, with the ion source and quadrupole temperatures set at 230 °C and 150 °C, respectively. The mass scan range was 40 to 550 m/z. An automatic sampler (model G4513A, Agilent Technologies) was used for sample injection. The injector was equipped with a split-type liner (4.0 mm internal diameter, 6.25 mm external diameter, 78.5 mm length, and volume of 870 µL), and injections were performed in split mode, with a split ratio of 10:1. Helium 5.0 (99.999% purity) was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. For each analysis, 1.0 µL of each essential oil sample was injected, prepared at a concentration of 10 mg mL^{-1} in ethyl acetate solution.

The chemical constituents of the essential oils were identified based on their retention indices and mass spectra using computer-assisted searches in the NIST (National Institute of Standards & Technology) mass spectral libraries and through comparison with retention indices and mass spectra available in the literature (Adams, 2017). Retention indices (RI) were calculated based on the retention times of a homologous series of n-alkanes (C7–C30; certified reference material, Merck), analyzed under the same chromatographic conditions as the essential oil samples.

8.2.3. Antioxidant capacity analysis

Antioxidant capacity was evaluated using essential oils from 20 accessions of *C. blanchetianus* (CBL-101, CBL-201, CBL-203, CBL-205, CBL-206, CBL-207, CBL-301, CBL-302, CBL-303, CBL-304, CBL-401, CBL-402, CBL-403, CBL-502, CBL-503, CBL-505, CBL-507, CBL-603, CBL-604, and CBL-605) obtained from Harvest 1 (February 2024) and performed in triplicate. The selection of accessions for the antioxidant assays was determined according to the availability of essential oil from the accessions.

8.2.3.1. DPPH radical scavenging activity

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method as described by Boroski et al. (2015). Essential oil samples from *C. blanchetianus* accessions were individually diluted in ethanol at a concentration of 5% v/v. Aliquots of the samples (50 µL) and the DPPH solution (180 µL, 60 µM) were added to 96-well microplates. The reaction was carried out in the dark for 30 min. Ethanol was used as the negative control. Absorbance was measured at a wavelength of 517 nm using a Synergy H1 spectrophotometer (BioTek Instruments). Antioxidant activity was expressed as the percentage of DPPH radical inhibition.

8.2.3.2. Ferric reducing antioxidant power (FRAP)

The ferric-to-ferrous ion reduction assay was performed according to Jesus et al. (2024). Essential oils (EOs) from the accessions were diluted in ethanol at 5% v/v. Subsequently, in 96-well microplates, 20 µL of the essential oil solution and 180 µL of FRAP reagent (acetate buffer (0.3 mol L^{-1}, pH 3.6), TPTZ (10 mmol L^{-1}), and ferric chloride (20 mmol L^{-1})) were added. Ethanol was used as the negative control. The microplate was incubated for 30 min in the absence of light. After incubation, absorbance was measured using a Synergy H1 spectrophotometer (BioTek Instruments) at 595 nm. The calibration curve was prepared using gallic acid as the standard (12.5 to 200 mg mL^{-1}). Reducing antioxidant power was expressed as milligrams of gallic acid equivalents per milliliter (mg GAE mL^{-1}).

8.2.3.3. ABTS radical scavenging activity

The antioxidant activity based on inhibition of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical was evaluated as described by Boroski et al. (2015). Essential oils from the accessions were diluted in ethanol to a concentration of 5% v/v., The essential oil dilutions (25 µL) and the ABTS radical solution (200 µL; 7 mmol L^{-1},

diluted to an absorbance of 0.700 ± 0.02 at 734 nm) were added to microplates. Ethanol was used as the negative control. The reaction was conducted for 30 min in the dark. After incubation, absorbance was measured at 734 nm using a Synergy H1 spectrophotometer (BioTek Instruments). Antioxidant activity was expressed as the percentage of ABTS radical inhibition.

8.2.4. Statistical analysis

Data on leaf dry mass and the content, yield, and chemical composition of the essential oils of *C. blanchetianus*, obtained at different harvest times, were subjected to analysis of variance (ANOVA) using a split-plot-in-time design, and the mean values were grouped using the Scott–Knott test ($p \leq 0.05$) with the aid of Sisvar® software (Ferreira, 2019). Data related to the antioxidant activity from Harvest 1 were also subjected to analysis of variance (ANOVA), and the mean values were grouped using the Scott–Knott test ($p \leq 0.05$) through the Sisvar® software (Ferreira, 2019). For each harvest time, the chemical constituents of essential oils from the accessions with relative abundance greater than 2% in at least one sample were subjected to two multivariate analyses using Statistica software: cluster analysis and principal component analysis (PCA). For cluster analysis, a dissimilarity matrix was constructed based on Euclidean distance and visualized through a dendrogram constructed using Ward's method. The mean values and respective standard deviations of the main compounds, annual leaf dry mass, and annual essential oil yield were identified for each group and at each harvest time using GraphPad Prism® software.

8.3. Results

Analysis of variance revealed a significant interaction between *Croton blanchetianus* accessions and harvest times for the evaluated variables, indicating that both genetic factors and seasonal variations influence biomass and essential oil production (Table 2 and Table 1A). Leaf dry mass (DM) of the accessions showed wide variation across the three harvests evaluated, ranging from 208.57 to 1,448.10 g plant^{-1}, with an overall mean of 638.50 g plant^{-1}. Biomass production was higher in the second harvest (June 2024), except for accessions CBL-103 and CBL-201, which did not show significant differences across the harvests. Accessions CBL-302 (1,209.20 g plant^{-1}), CBL-503 (1,306.20 g plant^{-1}), CBL-603 (1,359.15 g plant^{-1}), and CBL-507 (1,448.10 g plant^{-1}) showed the highest DM values, not differing statistically from each other.

Essential oil content (EOC) varied among accessions and harvest times, ranging from 0.47% to 1.93%, with an overall mean of 1.05%. The highest EOC values were generally observed in Harvest 1 (February 2024), particularly for accessions CBL-403 and CBL-503, which both reached 1.93%. In Harvest 2 (June 2024), accessions CBL-101 (1.27%), CBL-503 (1.33%), CBL-401 (1.40%), and CBL-403 (1.40%) showed statistically higher EOC values than the other accessions. In Harvest 3 (October 2024), accession CBL-403 (1.60%) stood out, differing statistically from the others. Accessions CBL-201 and CBL-401 did not show significantly different values across the three harvests, with overall mean values of 1.00% and 1.38%, respectively.

Essential oil yield (EOY) exhibited expressive variation among accessions and harvest times, with values ranging from 2.43 to 17.41 mL plant^{-1} and an overall mean of 6.66 mL plant^{-1}. The pattern for EOY was similar to that observed for DM, with higher values generally associated with Harvest 2. The highest EOY values were found for accessions CBL-403, CBL-401, CBL-507, and CBL-503, at 14.69, 15.99, 17.38, and 17.41 mL plant^{-1}, respectively, indicating high yield potential in this period. Accessions CBL-103, CBL-201, CBL-205, CBL-206, CBL-402, and CBL-602 did not show significantly different EOY values among the three harvests.

C. blanchetianus accessions showed significant differences for annual leaf dry mass and annual essential oil yield (Fig. 2). Annual dry mass values ranged from 696.00 g plant^{-1} (CBL-103) to 3,062.40 g plant^{-1} (CBL-507), with an overall mean of 1,915.51 g plant^{-1}. Accessions CBL-603 and CBL-507 had the highest biomass values, at 2,934.46 and 3,062.40 g plant^{-1}, respectively. Annual essential oil yield also showed wide variation among accessions, ranging from 8.94 mL plant^{-1} (CBL-103) to 37.28 mL plant^{-1} (CBL-503), with an overall mean of 19.97 mL plant^{-1} (Fig. 2). Prominent accessions for mean yield were CBL-503 and CBL-507, with 37.28 and 34.87 mL plant^{-1}, respectively. Accessions CBL-103, CBL-201, CBL-204, CBL-205, CBL-206, CBL-304, CBL-505, CBL-602, and CBL-605 had the lowest yields, not exceeding 15 mL plant^{-1} per year.

Accession CBL-507 stood out for both annual leaf dry mass production (3,062.40 g plant^{-1}) and essential oil yield (34.87 mL plant^{-1}). In contrast. accession CBL-503 showed lower biomass accumulation (2,460.20 g plant^{-1}), yet exhibited essential oil yield equivalent to that of CBL-507, indicating high efficiency in oil production per unit of mass for CBL-503. These results highlight the potential of both accessions for inclusion in breeding programs aimed at increasing essential oil yield.

Constituents of the essential oil of *C. blanchetianus* that exhibited relative content of less than 2% in all accessions were excluded from the statistical analyses. As a result, 31 compounds were selected, representing an average of 88.15% of total essential oil composition (Table 3). Analysis of variance revealed a significant interaction between accessions and harvest times for all evaluated compounds, except β-selinene (Table 2A).

The composition of the essential oils was characterized by a predominance of monoterpenes and sesquiterpenes (Table 3). Predominant monoterpenes included α-pinene (0.53–18.06%), α-phellandrene (0.17–10.40%), limonene (0.83–17.34%), β-phellandrene (1.26–23.29%), 1,8-cineole (0.64–17.56%), and terpinolene (0.09–10.68%). The main sesquiterpenes were δ-elemene (0.30–10.50%), (*E*)-caryophyllene (2.04–12.87%), germacrene D (0.07–10.41%), bicyclogermacrene (1.78–35.74%), and spathulenol (8.04–26.68%) (Table 3). The highest α-pinene contents were observed in accessions CBL-201 (18.06%) and CBL-404 (17.97%) in Harvest 3 (October 2024). Accession CBL-604 showed the highest relative percentage of α-phellandrene across all harvests, differing statistically from the other accessions, with a peak value of 10.40% in Harvest 2 (June 2024). Accession CBL-201 stood out with the highest relative percentage of limonene throughout the study, differing statistically from the other accessions. It peaked at 17.4% in Harvest 3 (October 2024). Accessions CBL-302 and CBL-401 showed the highest relative percentages of β-phellandrene, with maximum values recorded in Harvest 3 (October 2024) at 23.29% and 23.22%, respectively. Accession CBL-204 exhibited the highest relative percentages of 1,8-cineole and terpinolene in Harvest 3 (October 2024), with values of 17.56% and 10.68%, respectively.

The compounds limonene and β-phellandrene were mutually exclusive, and showed variations among the accessions. Limonene was identified in accessions CBL-101, CBL-102, CBL-201, CBL-202, CBL-204, CBL-205, CBL-206, CBL-207, CBL-304, CBL-402, CBL-403, CBL-501, CBL-503, and CBL-605, whereas β-phellandrene was detected in accessions CBL-103, CBL-203, CBL-301, CBL-302, CBL-401, CBL-404, CBL-502, CBL-505, CBL-507, CBL-602, CBL-603, and CBL-604. This difference indicates distinct chemical profiles among the accessions, resulting in predominance of a single monoterpene without co-occurrence of these compounds in the same sample.

Among the sesquiterpenes, δ-elemene showed the highest relative percentage in accession CBL-205 (10.50%) in Harvest 3 (October 2024). The highest relative percentages of (*E*)-caryophyllene were found in accession CBL-401 in Harvest 1 (February 2024), with 12.86%, while accession CBL-502 showed high values of this compound in Harvest 1 (February 2024), with 12.12%, and Harvest 3 (October 2024), with 11.82%. The highest relative percentage of germacrene D was observed in accession CBL-403 in Harvest 2 (June 2024), with 10.41%. The highest relative percentage of bicyclogermacrene was generally

observed in Harvest 2 (June 2024), ranging from 9.72% to 35.74%, peaking in accessions CBL-205 (35.74%) and CBL-501 (35.53%), which differed statistically from the other accessions. Bicyclogermacrene levels declined by up to 81% in Harvest 3 compared to the peak levels in Harvest 2. Spathulenol concentrations were highest in accession CBL-605 (23.32%) in Harvest 1 (February 2024), in accessions CBL-602 (25.79%) and CBL-605 (25.38%) in Harvest 2 (June 2024), and in accession CBL-505 (26.68%) in Harvest 3 (October 2024). Furthermore, accession CBL-605 showed high relative percentages of spathulenol in both Harvest 2 (25.38%) and Harvest 3 (24.84%), with no significant difference observed between harvest times.

The relative percentages of compounds across the three harvests varied among the evaluated chemical classes (Table 3). Harvest 3 (October 2024) was marked by a pronounced increase in total monoterpenes (40.41%), with higher values of hydrocarbon monoterpenes (30.54%, versus 22.28% in Harvest 1 and 21.72% in Harvest 2) and oxygenated monoterpenes (9.87%, versus 6.60% in Harvest 1 and 5.71% in Harvest 2). In contrast, both Harvest 1 and 2 showed higher levels of total sesquiterpenes (59.02% and 62.01%, respectively), particularly hydrocarbon sesquiterpenes (34.99% and 40.34% in Harvest 1 and 2, respectively, versus 25.10% in Harvest 3). Oxygenated sesquiterpenes remained relatively stable, showing small variations among harvests (24.03% in Harvest 1; 21.67% in Harvest 2; and 21.59% in Harvest 3).

Cluster analysis of the chemical constituents of the essential oils from the 26 accessions of *C. blanchetianus* revealed the formation of three groups in Harvests 1 and 2 and two groups in Harvest 3 (Fig. 3A–C).

In Harvest 1 (Fig. 3A and 4A), the accessions were distributed as follows: Cluster I comprised ten accessions (CBL-101, CBL-304, CBL-402, CBL-201, CBL-403, CBL-503, CBL-205, CBL-501, CBL-507, and CBL-603) and was characterized by higher mean values of limonene, β-elemene, germacrene D, and bicyclogermacrene. Cluster II comprised six accessions (CBL-103, CBL-203, CBL-302, CBL-401, CBL-404, and CBL-502) and was characterized by higher mean values of α-pinene, β-phellandrene, and (*E*)-caryophyllene and by the absence of limonene. Cluster III comprised ten accessions (CBL-102, CBL-206, CBL-505, CBL-602, CBL-605, CBL-204, CBL-207, CBL-202, CBL-604, and CBL-301) and was characterized by higher mean values of α-phellandrene, ρ-cymene, 1,8-cineole, terpinolene, α-terpineol, and spathulenol.

In Harvest 2 (Fig. 3B and 4B), the distribution of accessions and the pattern of chemical constituents within the clusters were similar to those observed in Harvest 1, except for accession CBL-604, which was reassigned to Cluster I, due to its higher concentration of bicyclogermacrene and reduction in spathulenol.

In Harvest 3 (Fig. 3C and 4C), Cluster I comprised eighteen accessions (CBL-101, CBL-402, CBL-403, CBL-207, CBL-201, CBL-304, CBL-102, CBL-206, CBL-202, CBL-204, CBL-205, CBL-501, CBL-503, CBL-505, CBL-602, CBL-603, CBL-605, and CBL-604) and was characterized by higher mean values of ρ-cymene, limonene, 1,8-cineole, and spathulenol. Cluster II comprised eight accessions (CBL-103, CBL-507, CBL-301, CBL-404, CBL-502, CBL-203, CBL-302, and CBL-401) and stood out for higher mean values of α-pinene, β-phellandrene, terpinolene, and (*E*)-caryophyllene, as well as the absence of limonene. The compound bicyclogermacrene, which was a determinant factor for cluster formation in Harvests 1 and 2, did not influence the grouping structure in Harvest 3, likely due to a drastic reduction in its concentration in this period.

According to the principal component analysis, the first and second components of Harvests 1 (February 2024), 2 (June 2024), and 3 (October 2024) accounted for 36.78%, 36.45%, and 35.10% of the total accumulated variance, respectively (Fig. 5A–C). In Harvest 1 (Fig. 5A), the first principal component explained 21.45% of the variance and was negatively correlated with spathulenol (r = -0.86), muurola-4,10(14)-dien-1-β-ol (r = -0.86), and 14-hydroxy-9-epi-(*E*)-caryophyllene (r = -0.83). The second principal component explained 15.24% and was negatively correlated with β-elemene (r = -0.85), germacrene B (r = -0.71),

and pogostol (r = -0.77). In Harvest 2 (Fig. 5B), the first principal component accounted for 20.98% of the variance, showing negative correlations with ρ-cymene (r = -0.81), terpinolene (r = -0.73), linalool (r = -0.80), α-terpineol (r = -0.79), and spathulenol (r = -0.70). The second principal component, representing 15.47% of the variance, was positively correlated with pogostol (r = 0.76) and negatively correlated with aromadendrene (r = -0.73) and caryophyllene oxide (r = -0.77). In Harvest 3 (Fig. 5C), the first principal component explained 20.49% of the variance, showing positive correlations with sabinene (r = 0.70) and 14-hydroxy-9-epi-(*E*)-caryophyllene (r = 0.81), and negative correlations with (*E*)-caryophyllene (r = -0.75), α-humulene (r = -0.74), and caryophyllene oxide (r = -0.82). The second principal component, representing 14.61% of the variance, was positively correlated with ρ-cymene (r = 0.85) and negatively correlated with δ-elemene (r = -0.82) and bicyclogermacrene (r = -0.78).

The antioxidant activity of the essential oils of *C. blanchetianus* evaluated in Harvest 1 (February 2024) showed significant variation among accessions (Table 4), according to assays performed using the DPPH radical scavenging, ABTS, and ferric reducing antioxidant power (FRAP). In the DPPH assay, percentage inhibition values ranged from 19.01% for accession CBL-304 to 72.86% for CBL-203. Accessions CBL-203 (72.86%), CBL-503 (67.02%), and CBL-301 (66.24%) showed the highest radical scavenging capacities. For the ABTS assay, inhibition values ranged from 48.82% for accession CBL-302 to 92.51% for CBL-301. Accession CBL-301 showed the highest inhibition potential (92.51%), indicating high antioxidant capacity against this radical, followed by accessions CBL-101 (89.62%), CBL-403 (89.05%), CBL-205 (87.82%), and CBL-402 (87.73%). In contrast, the essential oils of the accessions showed low antioxidant activity in the FRAP assay, with values ranging from 0.29 for accession CBL-302 to 0.63 mg mL^{-1} for CBL-402. Overall, accession CBL-301 showed good antioxidant performance in the DPPH (66.24%) and ABTS (92.51%) assays. The essential oil of accession CBL-301 was characterized by the following major compounds in terms of relative percentage: spathulenol (15.17%), germacrene D (7.47%), α-pinene (7.29%), bicyclogermacrene (6.86%), β-phellandrene (6.61%), terpinolene (4.93%), and 1,8-cineole (4.44%).

8.4. Discussion

Seasonal and genetic variations in aromatic plants play a crucial role in determining the composition of essential oils, influencing both the yield and profile of the volatile compounds produced. Environmental factors such as precipitation, temperature, humidity, and light intensity can significantly affect the biosynthesis of these compounds, resulting in fluctuations in chemical profiles throughout the year (Sá Filho et al., 2022; Jerônimo et al., 2024). In the present study, harvest time significantly influenced the leaf dry mass and the content, yield, and chemical composition of the essential oils from *C. blanchetianus* leaves, and it assisted in revealing genetic variation among the evaluated accessions (Table 2).

Studies on essential oil yields are essential for producers, as they allow estimation of biomass requirements for oil extraction. When associated with information on optimal harvest time, these data enable exploitation of the plant's maximum productive potential. In general, the highest values for leaf dry mass and essential oil content and yield were observed in Harvests 1 and 2, particularly for accessions CBL-503 and CBL-507 (Table 2 and Fig. 2). During the periods corresponding to Harvests 1 and 2 (February and June, respectively), higher precipitation (109.40 and 251.60 mm, respectively) and relative humidity (71.62% and 71.89%, respectively) were recorded. Temperatures in these two periods ranged from 25 °C to 28 °C and solar radiation incidence from 994.28 kJ m^{-2} to 1,480.42 kJ m^{-2} (Fig. 1). In contrast, in Harvest 3, carried out in October, more intense solar radiation (1,620.63 kJ m^{-2}) was observed, associated with lower precipitation (8.00 mm) and relative humidity (67.33%), as well as a moderate mean temperature (26.50 °C) (Fig. 1). The meteorological conditions observed in Harvests 1 and 2 appear to have enhanced essential oil yields compared to Harvest 3 in October.

Favorable conditions may increase vegetative growth and photosynthetic activity, directly influencing the biosynthetic pathways involved in the production of volatile chemical constituents (Pereira et al., 2022). In contrast, the harvest carried out in October showed lower yields, possibly due to reduced precipitation combined with increased solar radiation; these factors may induce physiological stress and alter plant secondary metabolism, reducing oil accumulation (Taiz et al., 2017).

Variation in the essential oil content of *C. blanchetianus* has been reported in samples collected from different states in the Northeast region of Brazil, including values from 0.24% to 0.48% in Ceará (Ribeiro et al., 2018), 0.72% to 0.75% in Paraíba (Angélico et al., 2014; Rodrigues et al., 2019), 0.15% to 0.50% in Pernambuco (Camara et al., 2021; Venâncio et al., 2025), 0.22% in Piauí (Cavalcante et al., 2022), and 0.49% in Rio Grande do Norte (Nunes et al., 2023). Approximately 60% of the accessions evaluated in the present study showed mean essential oil contents between 1.00% and 1.93%, which are higher than those reported in the literature for this species. These findings suggest that genetic variability among accessions was responsible for the observed variations, interacting with seasonality. Such results are consistent with a previous study highlighting the interaction between genetic and environmental factors as a determinant of essential oil variability in aromatic species (Pereira et al., 2022).

Some accessions also showed stability in leaf dry mass and in essential oil content and yield across the three harvests, indicating stable production and little influence from seasonal climatic variations. The maintenance of stable levels of essential oil production under varying environmental conditions is a highly desirable trait and may be linked to intrinsic adaptive mechanisms of plants, such as greater physiological efficiency and metabolic regulation (Costa et al., 2022b). Similar patterns have also been observed in other aromatic species, such as *L. camara*, *Eugenia uniflora*, and *Myrciaria dubia* (Pereira et al., 2022; Costa et al., 2022b; Nunes et al., 2025).

The main compounds identified in the analyzed essential oils, including α-pinene, α-phellandrene, limonene, β-phellandrene, 1,8-cineole, terpinolene, δ-elemene, (*E*)-caryophyllene, germacrene D, bicyclogermacrene, and spathulenol, are consistent with the results reported in the literature for genotypes of *C. blanchetianus* from different states in the Northeast region of Brazil (Ceará, Paraíba, Pernambuco, Piauí, and Rio Grande do Norte) (Angélico et al., 2014; Ribeiro et al., 2018; Rodrigues et al., 2019; Camara et al., 2021; Porto et al., 2021; Vasconcelos et al., 2022a, 2022b; Cavalcante et al., 2022; Nunes et al., 2023; Nascimento et al., 2024; Venâncio et al., 2025; Lopes et al., 2025). However, the concentrations of these chemical constituents show high variability, resulting from geographic origin, the harvest season, and sampling time of the day (Ribeiro et al., 2018; Camara et al., 2021; Cavalcante et al., 2022; Lopes et al., 2025), as well as from genetic variability and environmental factors, including precipitation, temperature, humidity, and light intensity.

In an evaluation of the chemical diversity of the essential oil of *C. blanchetianus*, Costa et al. (2026) collected 70 genotypes from six natural populations in the state of Sergipe. They identified α-pinene (1.60–13.37%), limonene (0.00–17.54%), β-phellandrene (0.00–16.04%), 1,8-cineole (0.00–13.56%), (*E*)-caryophyllene (0.31–13.14%), germacrene D (0.00–10.60%), bicyclogermacrene (5.06–27.47%), and spathulenol (4.34–29.83%) as the main compounds. That study also revealed the formation of two distinct chemical groups (limonene and β-phellandrene) independent of the geographic origin of the genotypes, which suggests the existence of chemotypes in the species and the strong influence of genetic factors.

The analysis of the relative percentage of chemical constituents across the three harvests revealed variations among the different classes evaluated (Table 3), highlighting both the genetic variability among *C. blanchetianus* accessions and the influence of seasonal factors on essential oil composition. This suggests the existence of an interaction between genetic and environmental factors. In Harvests 1 and 2, a predominance of sesquiterpenes was observed, particularly hydrocarbon sesquiterpenes. In contrast, Harvest 3 was characterized by a marked increase in monoterpenes, both hydrocarbons and oxygenated compounds. This increase in

monoterpenes may be a response to conditions of higher solar radiation, higher temperatures, and lower precipitation.

Prior studies have shown that higher temperature and solar radiation are the main factors driving photosynthetic activity, increasing the supply of energy and metabolic precursors involved in monoterpene biosynthesis (Song et al., 2014; Nishimura et al., 2015; Malik et al., 2023). However, the production and emission of sesquiterpenes are often reduced or inhibited under conditions of water limitation, resulting in changes in the terpenoid chemical profile (Ormeno et al., 2007). This conclusion is supported by the results of the present study, in which a marked 66.19% reduction in the sesquiterpene bicyclogermacrene was observed in Harvest 3, corresponding to the period of lowest rainfall. These modifications in essential oil composition suggest an adaptive physiological response of plants to seasonal variations, reflecting redirection of terpene biosynthetic pathways in accordance with environmental conditions.

Harvest 3 showed a more distinct chemical composition, with a pronounced increase in monoterpenes compared to Harvests 1 and 2, which exhibited high similarity, confirming the results of the statistical analysis of chemical composition. This information on temporal variations is of great importance for identifying the chemical patterns of essential oils of *C. blanchetianus* produced at different times of the year. The observation of these chemical patterns allows the detection of qualitative and quantitative fluctuations in available compounds and, through identification of these differences, determination of the potential applications of the species' essential oil, since the chemical composition of the essential oil can directly influence its bioactive properties (Simões et al., 2017; Guelifet et al., 2025; Khaiper et al., 2026).

The marked variability in the antioxidant activity of *C. blanchetianus* essential oils across different accessions highlights the influence of chemical composition on the free radical scavenging capacity and reducing potential of these oils (Jesus et al., 2019). In general, the essential oils of the accessions exhibited antioxidant activity in the DPPH and ABTS assays, confirming their potential as natural antioxidants. These results may be associated with the presence of terpenes with conjugated double bonds in their structure, a characteristic that favors the stabilization of free radicals and, consequently, the antioxidant activity of these compounds (Wojtunik, 2014). In contrast, the samples showed low antioxidant activity when evaluated in the ferric reducing antioxidant power (FRAP) assay. This suggests that the chemical compounds responsible for the antioxidant activities detected by the DPPH and ABTS assays may act as hydrogen or electron donors to neutralize free radicals but have low capacity as metal-reducing agents (Mašković, 2018; Santos, 2019).

Oxygenated terpenic compounds, such as monoterpenes and sesquiterpenes, widely present in the genus *Croton*, are frequently linked to the antioxidant activity of essential oils due to their ability to stabilize and neutralize reactive oxygen species (Graßmann, 2005; Miguel, 2010; Zengin and Baysal, 2014). Studies conducted on the genus *Croton* have confirmed the antioxidant potential of its species, including *C. cajucara*, *C. zehntneri* (Donati et al., 2015), *C. zambesicus* (Yagi et al., 2016), *C. piauhiensis* (Vale et al., 2021), *C. grewioides* (Oliveira et al., 2021), *C. campinarensis* (Costa et al., 2022a), and *C. alnifolius* (Cruz et al., 2026), using methods such as DPPH, ABTS, FRAP, FIC (ferrous ion chelating potential), and BCB (β-carotene/linoleic acid oxidation inhibition),. The main constituents of the essential oil of *C. piauhiensis* (Vale et al., 2021) are β-caryophyllene (21.58%), limonene (13.47%), γ-terpinene (10.08%), and germacrene D (9.56%). These compounds also occur in the essential oil of *C. blanchetianus* and are associated with promising antioxidant activity in the DPPH assay, corroborating the results of the present study. However, the observed antioxidant potential may result from synergism among the different compounds present in the essential oil. Interactions between major and minor constituents may enhance biological activity, and it is not possible to attribute this enhanced activity to a single isolated compound (Miguel, 2010).

Studies on chemical variability and the effects of seasonality on essential oil composition in aromatic species are fundamental for understanding the production dynamics and chemical composition of these oils throughout the year. The results of this study demonstrate that the selection of genetically superior and more stable accessions, together with appropriate harvest time, constitutes a key strategy to optimize the production of *C. blanchetianus* essential oil. In addition, the data indicate the potential of certain accessions as promising sources of natural antioxidant compounds.

8.5. Conclusion

Croton blanchetianus exhibits significant variations in leaf dry mass and the content, yield, chemical composition, and antioxidant activity of its essential oils, depending on the evaluated accessions and harvest times. Seasonal conditions influenced both the quantitative and qualitative aspects of the essential oils, with changes in yield and in the distribution of terpene classes. The stability observed in some accessions indicates genetic potential for the selection of high yielding, stable germplasm. Furthermore, the observed antioxidant activity confirms the potential of the species as a source of bioactive compounds. Harvests 1 and 2 stood out for higher biomass production and essential oil yield, particularly for accessions CBL-503 and CBL-507. Sesquiterpenes were predominant in these harvest periods, whereas Harvest 3 showed a higher proportion of monoterpenes, evidencing seasonal variation in chemical composition. Antioxidant activity varied among accessions and could not be attributed to a single compound, suggesting a synergistic effect among essential oil constituents. These results highlight the potential for selection of superior accessions and for determination of the most appropriate harvest time, aiming to maximize the production and application of bioactive compounds.

8.6. References

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Table 1. Geographical origin of the 26 accessions from the *Croton blanchetianus* Baill. collection of the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe, Brazil.

Accessions	Origin	Geographic coordinates	Voucher No.
CBL-101	Aquidabã	10º19'27.7" S / 37º04'44.3" W	42851
CBL-102	Aquidabã	10º19'22.4" S / 37º04'40.4" W	42855
CBL-103	Aquidabã	10º17'45.1" S / 37º02'09.2" W	42845
CBL-201	Graccho Cardoso	10º14'06.8" S / 37º11'25.6" W	42859
CBL-202	Graccho Cardoso	10º14'06.4" S / 37º11'26.9" W	42860
CBL-203	Graccho Cardoso	10º14'03.7" S / 37º11'25.8" W	42861
CBL-204	Graccho Cardoso	10º14'29.8" S / 37º12'53.6" W	42862
CBL-205	Graccho Cardoso	10º14'30.6" S / 37º12'52.8" W	42863
CBL-206	Graccho Cardoso	10º14'30.3" S / 37º12'54.1" W	42864
CBL-207	Graccho Cardoso	10º14'29.5" S / 37º12'50.5" W	42865
CBL-301	Itabi	10º05'18.5" S / 37º05'35.1" W	42873
CBL-302	Itabi	10º06'49.9" S / 37º06'33.3" W	42875
CBL-304	Itabi	10º07'47.4" S / 37º07'18.1" W	42881
CBL-401	Lagarto	10º53'12.0" S / 37º36'55.4" W	42884
CBL-402	Lagarto	10º51'50.2" S / 37º35'59.0" W	42887
CBL-403	Lagarto	10º50'52.5" S / 37º36'19.5" W	42890
CBL-404	Lagarto	10º50'51.0" S / 37º36'17.8" W	42891
CBL-501	Tobias Barreto	11º08'53.0" S / 37º56'41.2" W	42894
CBL-502	Tobias Barreto	11º08'52.9" S / 37º56'41.6" W	42895
CBL-503	Tobias Barreto	11º08'54.1" S / 37º56'43.4" W	42896
CBL-505	Tobias Barreto	11º10'06.4" S / 37º58'44.0" W	42899
CBL-507	Tobias Barreto	11º06'15.7" S / 38º02'03.6" W	42904
CBL-602	Poço Verde	10º48'27.5" S / 38º08'23.4" W	42908
CBL-603	Poço Verde	10º48'17.3" S / 38º08'12.6" W	42913
CBL-604	Poço Verde	10º48'15.6" S / 38º08'14.3" W	42910
CBL-605	Poço Verde	10º48'14.5" S / 38º08'13.0" W	42911

Table 2. Agronomic traits of *Croton blanchetianus* accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe, Brazil, across three harvest times [1 (February 2024), 2 (June 2024), and 3 (October 2024)].

Accession	Harvest time			Harvest time			Harvest time		
	1 (Feb. 24)	**2 (Jun. 24)**	**3 (Oct. 24)**	**1 (Feb 24)**	**2 (Jun. 24)**	**3 (Oct. 24)**	**1 (Feb. 24)**	**2 (Jun. 24)**	**3 (Oct. 24)**
	---------- DM (g plant^{-1}) ----------			---------- EOC (%) ----------			---------- EOY (mL plant^{-1}) ----------		
CBL-101	565.45 aB	1007.70 bA	466.80 bB	1.07 dB	1.27 aA	0.73 gC	5.94 cB	12.69 bA	3.38 cC
CBL-102	539.50 bB	1029.80 bA	402.55 bB	1.07 dA	1.07 cA	0.73 gB	5.71 cB	11.01 bA	2.94 cC
CBL-103	220.60 bA	254.60 dA	220.80 bA	1.47 bA	1.20 bB	1.20 dB	3.24 cA	3.06 eA	2.65 cA
CBL-201	458.90 bA	669.25 cA	454.45 bA	1.13 dA	0.87 cB	0.80 fB	5.20 cA	5.71 dA	3.64 cA
CBL-202	510.00 bB	1027.40 bA	322.20 bB	1.27 cA	0.87 cB	0.87 fB	6.46 cA	8.90 cA	2.79 cB
CBL-203	475.70 bB	752.20 cA	298.77 bB	1.47 bA	1.20 bB	1.20 dB	6.86 cA	9.03 cA	3.59 cB
CBL-204	377.03 bB	588.53 cA	208.57 bB	1.00 eA	1.07 cA	0.93 eA	3.77 cB	6.34 dA	1.89 cB
CBL-205	499.97 bB	758.37 cA	354.25 bB	0.60 gB	0.47 gC	0.73 gA	3.00 cA	3.55 eA	2.56 cA
CBL-206	479.50 bB	903.10 cA	430.87 bB	0.80 fA	0.60 fB	0.80 fA	3.84 cA	5.42 dA	3.45 cA
CBL-207	719.98 aB	1102.72 bA	512.82 bB	1.20 cA	0.93 dB	1.00 eB	8.64 bA	10.26 cA	5.13 bB
CBL-301	669.65 aB	1042.90 bA	393.80 bC	0.87 eA	0.93 dA	0.73 gB	5.81 cB	9.73 cA	2.90 cC
CBL-302	596.92 aB	1209.20 aA	650.80 bB	1.27 cA	1.13 cB	1.00 eC	7.44 bB	13.55 bA	6.51 bB
CBL-304	391.50 bB	838.47 cA	485.48 bB	1.00 eA	0.80 cB	0.80 fB	3.92 cB	6.71 dA	3.88 cB
CBL-401	431.80 bB	1142.40 bA	419.93 bB	1.40 bA	1.40 aA	1.33 cA	6.05 cB	15.99 aA	5.61 bB
CBL-402	511.95 bB	875.20 cA	585.65 bB	1.07 dA	0.80 eB	0.80 fB	5.49 cA	7.00 dA	4.69 cA
CBL-403	471.35 bB	1049.35 bA	427.85 bB	1.93 aA	1.40 aC	1.60 aB	9.08 bB	14.69 aA	6.85 bB
CBL-404	329.20 bB	969.40 bA	482.00 bB	1.50 bA	1.20 bB	1.13 dB	4.94 cB	11.63 bA	5.46 bB
CBL-501	391.15 bB	865.35 cA	390.25 bB	1.17 dA	1.00 dB	1.00 eB	4.58 cB	8.65 cA	3.90 cB
CBL-502	672.87 aB	1087.15 bA	620.10 bB	1.13 dA	0.87 dB	0.80 fB	7.58 bA	9.32 cA	4.96 bB
CBL-503	694.40 aB	1306.00 aA	459.80 bC	1.93 aA	1.33 aB	1.40 bB	13.43 aB	17.41 aA	6.44 bC
CBL-505	418.55 bB	713.20 cA	280.52 bB	1.20 cA	1.07 cB	0.87 fC	5.02 cB	7.67 cA	2.43 cC
CBL-507	505.10 bC	1448.10 aA	1109.20 aB	1.27 cA	1.20 cA	1.00 eB	6.40 cC	17.38 aA	11.09 aB
CBL-602	412.78 bB	723.00 cA	411.73 bB	1.20 cA	0.93 dB	0.87 fB	4.95 cA	6.51 dA	3.68 cA
CBL-603	737.95 aB	1359.15 aA	837.35 aB	1.33 cA	0.80 eB	0.80 fB	9.68 bA	10.87 bA	6.70 bB
CBL-604	652.35 aB	1055.98 bA	585.15 bB	0.97 eA	0.80 eB	0.60 gC	6.36 cA	8.45 cA	3.51 cB
CBL-605	348.65 bB	783.35 cA	346.60 bB	1.00 eA	0.93 dB	0.80 fB	3.49 cB	7.52 cA	2.77 cB
Mean	503.18	944.69	467.63	1.20	1.01	0.94	6.03	9.58	4.36
CV 1(%)		35.39			7.30			34.07	
CV 2(%)		20.58			7.67			22.54	

Variables: DM: leaf dry mass (g plant^{-1}); EOC: essential oil content (%); EOY: essential oil yield (mL plant^{-1}). Means followed by the same lowercase letters in the column and uppercase letters in the rows do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. Relative percentage (%) of the chemical compounds of the essential oil from *Croton blanchetianus* Baill. accessions of the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe across three annual harvest times [1 (February 2024), 2 (June 2024), and 3 (October 2024)].

Accession	Compound														
	C1			C2			C3			C4			C5		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CBL-101	0.26 hC	3.33 aA	0.47 kB	4.03 gB	0.53 kC	7.09 iA	0.71 jB	0.00 lC	1.19 hA	1.52 eB	1.52 dB	2.03 eA	0.59 iA	0.55 iA	0.87 hA
CBL-102	0.37 hB	0.46 fB	0.88 iA	4.75 fC	6.16 gB	10.04 fA	1.77 dB	1.83 cB	2.97 bA	0.62 hB	0.64 hB	0.91 iA	0.92 iA	1.03 hA	1.26 gA
CBL-103	0.39 hB	0.30 gB	0.66 jA	5.53 fB	5.67 gB	8.26 gA	0.99 hB	1.03 gB	1.63 fA	0.77 gB	0.70 hB	0.92 iA	0.77 iA	0.84 hA	0.90 hA
CBL-201	1.94 aB	1.47 bC	2.94 aA	10.76 bC	11.60 bB	18.06 aA	1.36 fB	1.11 fC	1.64 fA	1.46 eB	1.32 eB	1.75 fA	0.57 iA	0.62 iA	0.67 iA
CBL-202	0.59 fB	0.45 fC	0.87 iA	8.95 cC	9.77 cB	14.02 bA	1.98 cB	1.67 dC	2.82 cA	0.75 gA	0.58 hB	0.87 iA	3.35 dA	2.62 eB	3.32 cA
CBL 203	0.53 gB	0.44 fB	1.00 hA	7.86 dC	8.79 dB	12.85 cA	0.45 kB	0.36 jB	0.68 kA	0.97 gB	0.83 gB	1.34 gA	1.40 gB	1.35 gB	2.19 eA
CBL-204	0.66 fB	0.44 fC	0.89 iA	5.55 fB	5.04 hB	7.62 hA	2.49 aB	2.08 bC	3.45 aA	0.75 gB	0.62 hB	0.97 iA	1.60 gB	1.68 gB	2.06 eA
CBL-205	0.00 iA	0.00 iA	0.00 lA	3.00 hB	3.07 jB	6.77 iA	1.30 fB	1.04 gC	1.84 eA	0.30 iB	0.00 kC	0.54 kA	0.00 lA	0.00 jA	0.00 jA
CBL-206	0.37 hB	0.38 fB	0.62 jA	7.82 dC	9.65 cB	12.45 cA	2.33 bB	2.38 aB	3.06 bA	0.69 gB	0.68 hB	0.89 iA	0.40 jA	0.49 iA	0.72 iA
CBL-207	0.58 fB	0.51 fB	0.78 iA	4.73 fB	5.10 hB	6.85 iA	1.74 dB	1.71 dB	2.42 dA	0.85 gB	0.91 gB	1.14 hA	1.16 hB	1.60 gA	1.80 fA
CBL-301	0.98 dB	0.86 dB	1.37 eA	7.29 dB	7.56 eB	11.11 eA	1.52 eB	1.44 eB	1.64 fA	0.86 gA	0.74 hA	0.92 iA	1.23 hA	1.34 gA	1.32 gA
CBL-302	0.62 fA	0.46 fB	0.69 jA	8.88 cB	8.70 dB	11.89 dA	1.09 gA	0.77 hB	1.13 hA	1.94 dA	1.22 eB	1.86 fA	2.67 eA	2.12 fB	2.58 dA
CBL-304	1.32 cB	1.18 cC	1.87 cA	8.63 cC	10.19 cB	13.88 bA	0.81 iB	0.82 hB	0.99 iA	1.20 fB	1.08 fB	1.41 gA	0.36 jA	0.40 iA	0.46 iA
CBL-401	1.61 bB	1.44 bC	2.37 bA	7.84 dC	10.13 cB	14.01 bA	0.81 iB	0.69 hB	1.07 hA	0.57 hA	0.43 iA	0.49 kA	1.09 hA	0.93 hA	1.14 gA
CBL-402	0.69 fB	0.53 fC	1.05 hA	5.08 fB	4.80 hB	6.04 jA	1.38 fB	1.20 fC	1.67 fA	0.59 hA	0.56 hA	0.71 jA	0.76 iA	0.66 iA	0.31 jB
CBL-403	0.82 eB	0.88 dB	1.48 eA	4.12 gC	5.38 hB	7.63 hA	1.13 gB	1.49 eA	0.82 jC	0.55 hB	0.66 hB	3.72 cA	3.97 cB	4.48 cA	0.47 iC
CBL-404	0.86 eB	0.82 dB	1.24 gA	12.55 aC	14.85 aB	17.97 aA	0.50 kA	0.35 jB	0.37 lB	0.91 gA	0.81 gA	0.70 jA	1.49 gA	1.39 gA	1.24 gA
CBL-501	0.49 B	0.15 gC	1.16 gA	5.05 fB	4.70 hB	10.10 fA	0.61 jB	0.54 iB	0.90 iA	3.75 bB	3.72 bB	6.16 aA	0.00 lA	0.00 jA	0.17 jA
CBL-502	0.66 fB	0.45 fC	1.04 hA	8.10 dB	7.72 eB	13.65 bA	0.65 jA	0.50 iB	0.68 kA	0.80 gB	1.04 fA	0.88 iB	1.91 fA	1.31 gB	1.91 fA
CBL-503	0.32 gB	0.17 gC	0.65 jA	5.49 fB	5.86 gB	10.03 fA	1.09 gB	1.00 gB	1.35 gA	0.42 iA	0.39 iA	0.56 kA	0.30 jA	0.26 jA	0.31 jA
CBL-505	0.83 eB	0.68 eC	1.21 gA	5.33 fB	5.01 hB	7.43 hA	1.23 fA	1.16 fA	0.98 iB	0.61 hA	0.53 iA	0.00 mB	1.11 hA	0.92 hA	0.56 iB
CBL-507	0.94 dB	0.66 eC	1.64 dA	5.54 fC	6.76 fB	9.66 fA	0.98 hA	0.87 hA	0.87 iA	4.44 aC	5.34 aB	5.69 bA	0.49 iA	0.43 iA	0.59 iA
CBL-602	0.63 fB	0.10 gC	1.17 gA	5.70 fB	4.00 iC	11.65 dA	0.48 kB	0.20 kC	0.69 kA	1.56 eA	0.00 kC	0.31 lB	0.00 lC	0.89 hB	1.79 fA
CBL-603	0.71 fB	0.33 fC	1.03 hA	6.73 eB	6.91 fB	10.89 eA	0.58 kA	0.49 iA	0.61 kA	0.34 iA	0.25 jA	0.35 lA	6.48 bA	6.31 bA	5.75 bB
CBL-604	1.04 dB	0.77 dC	1.26 eA	7.41 dB	7.06 fB	9.69 fA	0.97 hA	0.83 hA	0.91 iA	2.73 cB	3.21 cA	3.20 dA	9.09 aB	10.40 aA	9.02 aB
CBL-605	0.90 eB	0.68 eC	1.42 eA	5.22 fB	5.11 hB	8.43 gA	0.65 jB	0.62 iB	0.79 jA	0.31 iA	0.18 jA	0.36 lA	3.75 cA	3.66 dA	2.27 eB
Mean	0.74	0.69	1.14	6.61	6.93	10.69	1.14	1.01	1.43	1.17	1.07	1.49	1.75	1.78	1.68
RRIo		924			931			970			989			1004	
RRIl		924			932			969			988			1002	
CV 1 (%)		9.52			7.05			7.12			9.30			16.28	
CV 2 (%)		9.11			4.75			6.49			8.38			11.85	

Compounds: (C1) α-thujene, (C2) α-pinene, (C3) sabinene, (C4) myrcene, and (C5) α-phellandrene. RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. (Continued)

Accession	Compound														
	C6			C7			C8			C9			C10		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CBL-101	1.23 iB	0.88 hC	1.75 jA	5.58 cB	4.87 eB	9.68 dA	0.00 iA	0.00 iA	0.00 hA	2.13 fB	1.32 gB	3.34 hA	2.50 jB	2.48 iB	3.65 hA
CBL-102	2.02 fC	2.45 cB	3.76 eA	1.56 eA	1.67 fA	0.00 eB	0.00 iA	0.00 iA	0.00 hA	6.17 cB	6.15 cB	13.12 cA	4.91 fB	5.03 dB	6.26 eA
CBL-103	0.99 jB	0.75 hC	1.36 kA	0.00 fA	0.00 gA	0.00 eA	7.51 eB	8.08 dB	12.10 cA	3.16 eB	2.93 fB	4.73 gA	2.61 jB	3.02 hA	2.91 jA
CBL-201	0.75 kA	0.46 iB	0.83 lA	10.86 aB	11.82 aB	17.34 aA	0.00 iA	0.00 iA	0.00 hA	2.78 eA	2.02 fA	2.56 iA	1.11 mA	1.03 kA	1.20 mA
CBL-202	2.34 eB	2.38 cB	4.40 dA	1.90 dA	1.61 fA	0.00 eB	0.00 iA	0.00 iA	0.00 hA	7.09 bB	5.70 cC	13.38 cA	5.00 fA	3.96 gB	4.82 gA
CBL 203	1.74 gB	1.33 gC	2.40 hA	0.00 fA	0.00 gA	0.00 eA	9.68 cB	9.39 cB	14.25 bA	0.00 gA	0.00 hA	0.00 jA	5.33 eB	4.92 dC	8.03 cA
CBL-204	2.69 dB	1.81 eC	3.24 gA	5.26 cA	1.85 fB	0.00 eC	0.00 iA	0.00 iA	0.00 hA	6.55 cC	8.19 bB	17.56 aA	8.24 aC	9.24 aB	10.68 aA
CBL-205	0.00 nA	0.00 jA	0.00 oA	0.92 eA	1.31 fA	0.00 eA	0.00 iA	0.00 iA	0.00 hA	6.15 cB	4.79 dC	12.61 cA	0.00 pA	0.00 nA	0.00 pA
CBL-206	1.58 hB	1.48 fB	2.43 hA	1.19 eA	0.83 fA	0.00 eA	0.00 iA	0.00 iA	0.00 hA	9.78 aB	9.98 aB	16.12 bA	2.11 kC	2.49 iB	3.33 iA
CBL-207	3.25 cB	2.38 cC	3.73 eA	7.95 bC	10.29 bB	15.19 bA	0.00 iA	0.00 iA	0.00 hA	4.42 dA	3.50 eA	4.62 gA	5.99 dC	7.76 bB	9.03 bA
CBL-301	3.07 cB	2.48 cC	3.46 fA	0.00 fA	0.00 gA	0.00 eA	6.61 fB	6.29 fB	8.23 fA	4.44 dB	4.46 dB	5.65 fA	4.93 fB	5.52 cA	5.64 fA
CBL-302	2.66 dA	1.47 fC	2.46 hB	0.00 fA	0.00 gA	0.00 eA	19.00 aB	15.70 aC	23.29 aA	0.00 gA	0.00 hA	0.00 jA	7.95 bA	4.76 eB	7.99 cA
CBL-304	0.87 kA	0.56 iB	0.97 lA	7.45 bB	7.67 cB	10.29 dA	0.00 iA	0.00 iA	0.00 hA	1.63 fA	1.51 gA	2.04 iA	0.71 nA	0.68 lA	0.85 nA
CBL-401	0.41 lB	0.34 iB	0.72 mA	0.00 fA	0.00 gA	0.00 eA	14.65 bB	14.72 bB	23.22 aA	0.64 gB	2.17 fA	0.00 jB	0.97 mA	0.82 lA	0.89 nA
CBL-402	0.00 nC	1.56 fB	1.96 iA	5.57 cB	6.31 dB	10.96 bA	0.00 iA	0.00 iA	0.00 hA	5.97 cB	5.46 cB	8.58 eA	3.39 iA	2.32 iB	1.04 nC
CBL-403	1.41 iB	1.28 gB	3.29 gA	6.89 bC	8.51 cB	11.91 bA	0.00 iA	0.00 iA	0.00 hA	4.79 dB	4.93 dB	6.47 fA	0.67 nA	0.67 lA	0.59 oA
CBL-404	0.66 kB	0.70 hB	1.95 iA	0.00 fA	0.00 gA	0.00 eA	9.19 dB	9.66 cB	11.06 dA	2.18 fA	0.84 gB	2.12 iA	5.17 eA	4.77 eB	3.44 iC
CBL-501	0.00 nB	0.00 jB	0.44 nA	1.04 eA	0.93 fA	0.00 eA	0.00 iA	0.00 iA	0.00 hA	5.45 cB	4.87 dB	11.28 dA	0.09 pB	0.00 nB	0.40 oA
CBL-502	0.57 lB	0.36 iC	1.36 kA	0.00 fA	0.00 gA	0.00 eA	7.72 eB	6.51 fC	11.28 dA	3.30 eA	2.61 fA	3.50 hA	0.44 oA	0.34 mA	0.41 oA
CBL-503	0.26 mB	0.00 jC	0.70 mA	2.42 dA	2.35 fA	1.25 eA	0.00 iA	0.00 iA	0.00 hA	5.74 cB	4.97 dB	10.72 dA	1.51 lA	1.29 jA	1.36 mA
CBL-505	1.83 gB	2.01 dB	4.51 dA	0.00 fA	0.00 gA	0.00 eA	6.84 fB	7.35 eB	9.84 eA	7.58 bA	7.47 bA	7.92 eA	4.36 gA	3.77 gB	1.98 lC
CBL-507	0.72 kB	0.43 iC	1.52 kA	0.00 fA	0.00 gA	0.00 eA	8.70 dB	9.02 cB	13.58 bA	6.13 cB	5.89 cB	7.97 eA	0.41 oA	0.20 nA	0.44 oA
CBL-602	2.14 fB	1.24 gC	3.58 eA	0.00 fA	0.00 gA	0.00 eA	4.18 gA	2.79 gB	3.13 gB	4.36 dB	2.74 fC	8.43 eA	6.83 cB	4.61 eC	7.70 dA
CBL-603	2.35 eB	1.57 fC	5.74 cA	0.00 fA	0.00 gA	0.00 eA	1.46 hA	1.26 hA	0.00 hB	4.29 dB	3.66 eB	8.54 eA	0.61 nA	0.47 mA	0.49 oA
CBL-604	4.82 bB	4.00 bC	7.34 bA	0.00 fA	0.00 gA	0.00 eA	1.98 hA	1.87 hA	0.00 hB	6.11 cB	5.25 dB	10.00 dA	0.90 mA	0.85 lA	0.76 nA
CBL-605	5.40 aB	4.69 aC	9.79 aA	2.10 dA	1.80 fA	0.00 eB	0.00 iA	0.00 iA	0.00 hA	5.91 cB	5.87 cB	13.13 cA	4.10 hA	4.20 fA	2.59 kB
Mean	1.68	1.41	2.83	2.33	2.38	2.95	3.75	3.56	5.00	4.49	4.13	7.48	3.11	2.89	3.33
RRIo		1022			1027			1029			1030			1087	
RRIl		1020			1024			1025			1026			1086	
CV 1 (%)		6.81			27.46			10.51			13.37			5.14	
CV 2 (%)		4.83			27.61			9.83			13.40			4.40	

Compounds: (C6) *p*-cymene, (C7) limonene, (C8) β-phellandrene, (C9) 1,8-cineole, and (C10) terpinolene. RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. (Continued)

Accession	Compound														
	C11			C12			C13			C14			C15		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CBL-101	0.00 mC	0.31 jB	0.60 jA	0.86 lA	0.38 mB	0.87 mA	0.98 cC	2.14 bB	6.82 dA	1.10 gB	1.48 fA	1.52 gA	6.89 fA	6.42 fB	6.34 fB
CBL-102	0.00 mC	1.54 aB	1.60 cA	1.45 hA	1.19 fC	1.35 jB	1.05 bC	1.51 eB	5.28 fA	0.33 iA	0.40 hA	0.37 iA	5.34 iA	3.95 kB	4.28 iB
CBL-103	0.00 mC	0.33 jB	0.44 lA	1.12 jA	0.73 jB	1.09 kA	1.02 bC	1.98 cB	6.74 dA	1.77 eB	2.12 eA	2.12 fA	5.32 iA	4.52 jB	5.48 gA
CBL-201	0.00 mC	0.50 hB	0.84 gA	0.76 mA	0.40 mC	0.61 oB	0.92 cC	1.72 dB	5.67 eA	0.98 gA	1.10 gA	1.07 hA	4.44 kA	4.04 kB	3.47 jC
CBL-202	0.00 mC	0.69 gB	1.14 dA	2.46 cB	1.71 dC	2.56 dA	0.42 eC	0.94 gB	2.47 kA	6.19 bB	6.72 bA	5.25 bC	4.93 jA	4.80 iA	3.62 jB
CBL 203	1.42 cB	0.87 eC	1.63 cA	0.79 mA	0.48 lB	0.83 mA	1.15 bC	2.26 bB	7.52 cA	1.11 gA	1.27 gA	1.13 hA	2.71 nA	2.42 mA	2.04 mB
CBL-204	0.00 mC	1.32 cB	1.89 bA	3.09 bA	1.90 cB	3.09 bA	0.56 eC	1.29 fB	3.81 iA	1.71 eB	2.68 dA	2.51 eA	2.57 nA	2.52 mA	2.41 lA
CBL-205	0.00 mB	0.00 lB	0.20 mA	1.91 eB	1.13 fC	2.04 eA	1.73 aC	2.70 aB	10.50 aA	1.36 fA	1.56 fA	1.71 gA	3.56 lA	2.96 lB	3.59 jA
CBL-206	0.00 mC	0.38 iB	0.49 kA	3.34 aA	2.97 aC	3.19 aB	0.66 dC	1.03 gB	4.21 hA	0.46 iA	0.47 hA	0.57 iA	6.72 gA	5.38 hB	5.55 gB
CBL-207	0.00 mC	0.80 fB	1.10 eA	1.95 eA	1.33 eC	1.85 gB	0.53 eC	1.19 fB	4.24 hA	1.25 fB	1.46 fB	1.75 gA	4.43 kA	3.82 kB	3.70 jB
CBL-301	1.81 aB	1.13 dC	1.87 bA	1.52 gA	0.93 hC	1.03 kB	0.30 eC	0.76 hB	2.70 kA	2.08 dB	0.94 gC	2.78 dA	2.06 oA	2.22 mA	2.14 mA
CBL-302	1.10 dA	0.62 gC	0.90 fB	1.55 gA	0.99 hC	1.11 kB	0.52 eC	1.38 fB	4.47 gA	0.76 hB	1.20 gA	1.04 hA	2.19 oC	5.46 hA	2.75 kB
CBL-304	0.73 hB	0.48 hC	0.82 hA	0.71 mA	0.41 mB	0.69 nA	0.82 dC	1.82 dB	5.37 fA	2.43 cC	3.02 cB	3.48 cA	7.03 fA	6.49 fB	6.55 fB
CBL-401	0.26 lA	0.20 kB	0.24 mA	0.45 nA	0.26 nC	0.36 pB	0.90 cC	1.42 eB	4.61 gA	0.89 hA	0.77 hA	0.99 hA	12.86 aA	12.11 aB	10.62 bC
CBL-402	0.86 fA	0.49 hB	0.41 lC	1.57 gB	1.04 gC	1.69 hA	1.06 bC	1.91 cB	6.86 dA	1.42 fA	1.33 fA	0.83 hB	2.93 mB	3.01 lB	3.51 jA
CBL-403	0.59 iA	0.53 hB	0.58 jA	0.00 oC	0.86 iB	1.38 jA	1.06 bC	1.55 eB	3.49 jA	1.16 gA	1.34 fA	0.97 hA	10.42 cA	9.29 cB	9.63 cB
CBL-404	0.63 iA	0.46 hB	0.61 jA	0.77 mA	0.38 mC	0.60 oB	0.88 cC	1.25 fB	3.49 jA	0.33 iA	0.49 hA	0.25 iA	9.04 dA	8.03 eB	7.03 eC
CBL-501	0.45 jA	0.00 lB	0.45 kA	1.37 iB	0.82 iC	1.65 hA	1.62 aC	2.55 aB	9.12 bA	0.00 jA	0.00 iA	0.00 jA	5.80 hA	5.25 hB	4.99 hB
CBL-502	0.34 kB	0.23 kC	0.39 lA	1.58 gA	0.75 jC	1.43 iB	0.90 cC	1.47 eB	3.89 iA	1.49 fB	3.24 cA	0.78 hC	12.12 bA	10.68 bB	11.82 aA
CBL-503	0.35 kB	0.26 kC	0.50 kA	1.02 kA	0.56 kB	0.94 lB	0.94 cC	1.57 eB	5.14 fA	8.14 aB	8.82 aA	7.89 aB	3.11 mA	2.54 mB	3.18 jA
CBL-505	1.12 dA	0.66 gC	0.75 iB	2.53 cA	1.76 dB	1.45 iC	0.56 eB	0.71 hB	0.96 lA	0.00 jA	0.00 iA	0.00 jA	8.60 eA	8.51 dA	7.69 dB
CBL-507	0.43 jA	0.00 lC	0.23 mB	1.63 gA	0.00 oB	1.66 hA	1.18 bC	1.98 cB	5.51 eA	0.39 iA	0.56 hA	0.26 iA	7.01 fA	5.31 hC	5.76 gB
CBL-602	0.80 gB	0.45 hC	0.90 fA	2.03 dA	0.95 hC	1.94 fB	0.70 dC	1.12 gB	4.27 hA	0.30 iA	0.40 hA	0.34 iA	3.64 lB	4.14 kA	3.49 jB
CBL-603	1.04 aB	0.63 gC	1.18 dA	1.03 kA	0.56 kB	1.10 kA	1.21 bC	2.02 cB	5.57 eA	0.50 iB	1.08 gA	0.46 iB	6.61 gA	6.26 fB	5.32 hC
CBL-604	1.48 bB	1.36 cC	1.59 cA	1.74 fA	1.09 gC	1.38 jB	0.68 dC	1.07 gB	2.63 kA	1.03 gB	1.71 fA	1.00 hB	6.92 fA	6.03 gB	5.67 gC
CBL-605	1.75 aB	1.45 bC	2.18 aA	2.51 cB	2.03 bC	2.77 cA	0.54 eC	0.87 hB	1.17 lA	0.32 iB	0.33 hB	0.70 hA	5.11 iA	4.42 jB	3.67 jC
Mean	0.58	0.60	0.90	1.53	0.99	1.49	0.88	1.55	4.86	1.44	1.71	1.53	5.86	5.41	5.17
RRIo		1100			1188			1334			1389			1416	
RRIl		1095			1186			1335			1389			1417	
CV 1 (%)		5.93			4.03			5.24			11.41			3.34	
CV 2 (%)		4.61			3.24			5.94			12.51			3.80	

Compounds: (C11) linalool, (C12) α-terpineol, (C13) δ-elemene, (C14) β-elemene, and (C15) *(E)*-caryophyllene. RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. (Continued)

Accession	Compound														
	C16			C17			C18			C19			C20		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CBL-101	0.68 kA	0.73 iA	0.40 jB	1.65 fA	1.46 fB	1.28 dC	1.69 bB	1.85 bA	1.37 bC	5.62 cB	8.45 bA	4.34 aC	0.29 cA	0.32 dA	0.25 cA
CBL-102	0.53 lA	0.44 jA	0.28 jA	1.23 hA	0.86 iB	0.87 fB	1.36 dA	1.13 eB	1.08 cB	4.47 dA	4.77 eA	2.39 bB	0.00 cA	0.00 dA	0.00 cA
CBL-103	0.68 kA	0.44 jB	0.32 jB	1.12 iA	0.81 jB	1.11 eA	1.26 dA	1.11 eB	0.00 gC	5.65 cB	7.27 cA	3.64 aC	0.51 cA	0.49 dA	0.41 cA
CBL-201	0.78 kA	0.70 iA	0.44 jB	0.79 kA	0.67 kB	0.54 hC	1.49 cA	1.45 cA	1.15 cB	2.07 fB	2.85 gA	1.49 cB	0.31 cA	0.26 dA	0.24 cA
CBL-202	0.20 mB	0.26 jB	0.95 hA	0.96 jA	0.88 iA	0.63 hB	0.63 fB	0.82 fA	0.67 eB	2.18 fA	3.01 gA	1.26 cB	0.95 cA	0.99 cA	0.62 cA
CBL 203	1.40 iA	1.30 gA	0.83 hB	0.52 lA	0.45 lA	0.37 iA	1.35 dA	1.38 cA	1.05 cB	1.39 gA	1.83 hA	1.01 dA	0.41 cA	0.10 dA	0.20 cA
CBL-204	0.51 lA	0.41 jA	0.25 jA	0.61 lA	0.52 lA	0.47 iA	1.00 eA	0.95 eA	0.75 B	1.05 gA	1.90 hA	0.94 dA	0.37 cA	0.31 dA	0.35 cA
CBL-205	1.35 iA	1.08 hB	0.85 hC	0.73 kA	0.61 kB	0.71 gA	2.00 aA	1.89 bA	1.66 aB	6.99 bB	8.76 bA	4.98 aC	0.43 cA	0.27 dA	0.47 cA
CBL-206	0.63 kA	0.44 jB	0.38 jB	1.63 fA	1.22 gB	1.15 eB	1.25 dA	1.09 eB	1.07 cB	4.54 dB	5.59 eA	3.04 bC	0.07 cA	0.00 dA	0.00 cA
CBL-207	1.09 jA	0.97 hA	0.71 iB	1.11 iA	0.93 iB	0.80 fC	1.26 dA	1.13 eA	0.96 dB	1.13 gA	1.62 hA	0.88 dA	0.51 cA	0.32 dA	0.35 cA
CBL-301	0.29 mA	0.28 A	0.20 jA	0.31 mA	0.23 mA	0.35 iA	0.67 fA	0.61 gA	0.73 eA	7.47 bA	6.50 dA	4.32 aB	0.68 cB	3.58 aA	0.49 cB
CBL-302	0.47 lB	0.94 hA	0.37 jB	0.53 lB	1.15 hA	0.00 jC	0.97 eB	1.24 dA	0.55 fC	0.86 gB	2.35 gA	0.95 dB	0.07 cA	0.63 dA	0.00 cA
CBL-304	0.59 kA	0.53 jA	0.43 jA	1.81 eA	1.53 fB	1.38 dC	0.99 eA	1.03 eA	0.92 dA	3.64 eB	5.08 eA	2.68 bC	0.91 cA	0.75 cA	0.94 cA
CBL-401	2.06 hB	2.33 dA	1.20 gC	3.60 aA	3.23 aB	2.31 aC	1.06 eB	1.22 dA	0.95 dB	2.37 fA	1.16 iB	1.35 cB	1.18 cA	0.00 dB	0.83 cA
CBL-402	1.39 iC	1.80 eB	2.13 eA	0.51 lB	0.65 kA	0.71 gA	1.23 dB	1.51 cA	1.55 aA	7.19 bA	7.69 cA	2.60 bB	0.40 cA	0.54 dA	0.46 cA
CBL-403	1.85 hA	1.63 eA	1.15 gB	2.33 cA	2.15 cB	1.83 cC	0.96 eB	1.10 eA	0.88 dB	8.30 aB	10.41 aA	3.82 aC	1.18 cA	1.12 cA	0.94 cA
CBL-404	3.26 eA	3.10 cA	3.19 bA	2.18 dA	1.96 dB	1.42 dC	1.05 eB	1.25 dA	1.07 cB	2.00 fA	1.75 hA	0.49 dB	0.79 cA	0.94 cA	1.28 bA
CBL-501	4.99 cC	5.21 aA	4.51 aB	1.36 gA	1.32 gA	1.07 eB	1.92 aB	2.30 aA	1.66 aC	2.78 fB	3.98 fA	1.44 cC	0.00 cA	0.00 dA	0.17 cA
CBL-502	2.75 fA	2.26 dB	1.90 fC	2.86 bA	2.50 bB	2.12 bC	1.31 dA	1.39 cA	1.00 dB	2.49 fB	3.80 fA	1.36 cC	1.96 bA	1.48 cA	1.80 bA
CBL-503	1.52 iA	1.45 fA	1.24 gB	0.94 jA	0.77 jB	0.84 fB	1.07 eA	1.08 eA	1.10 cA	2.80 fA	3.22 gA	1.64 cB	3.54 aA	3.17 aA	2.88 aA
CBL-505	2.37 gA	2.51 dA	2.37 dA	1.91 eA	1.82 eA	1.34 dB	1.34 dA	1.43 cA	1.43 bA	2.12 fA	2.50 gA	0.50 dB	0.26 cA	0.26 dA	0.22 cA
CBL-507	3.48 eB	3.80 bA	3.33 bB	1.71 fA	1.23 gB	1.17 eB	1.64 bA	1.52 cA	1.34 bB	1.64 gA	1.84 hA	0.60 dB	0.98 cA	0.98 cA	0.89 cA
CBL-602	4.51 bA	3.98 bB	3.26 bC	0.92 jA	0.92 iA	0.71 gB	1.68 bB	1.89 bA	1.37 bC	0.71 gA	1.16 iA	0.47 dA	1.37 bA	1.33 cA	1.23 bA
CBL-603	5.02 aA	3.98 bC	4.29 aB	1.80 eA	1.47 fB	1.15 eC	1.82 aA	1.85 bA	1.45 bB	0.70 gA	1.03 iA	0.35 dA	1.61 bA	1.63 cA	1.95 bA
CBL-604	1.41 iA	1.26 gA	1.33 gA	1.35 gA	1.15 hB	1.03 eC	0.68 fA	0.69 gA	0.72 eA	4.00 eB	5.04 eA	1.93 cC	1.88 bA	2.12 bA	1.93 bA
CBL-605	3.80 dA	3.12 cB	2.64 cC	1.27 hA	1.09 hB	0.81 fC	1.56 cA	1.31 dB	1.15 cC	0.38 gA	0.47 iA	0.00 dA	1.21 cA	1.08 cA	1.10 cA
Média	1.83	1.73	1.50	1.37	1.21	1.01	1.28	1.32	1.06	3.25	4.00	1.86	0.84	0.87	0.77
RRIo		1434			1449			1456			1478			1482	
RRIl		1439			1452			1458			1480			1489	
CV 1 (%)		7.58			5.81			6.64			19.97			73.01	
CV 2 (%)		7.98			5.77			7.62			19.23			72.05	

Compounds: (C16) aromadendrene, (C17) α-humulene, (C18) allo-aromadendrene, (C19) germacrene D, and (C20) β-selinene. RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. (Continued)

Accession	Compound														
	C21			C22			C23			C24			C25		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CBL-101	16.23 eB	24.69 dA	7.77 cC	0.32 hA	0.18 hB	0.00 gC	1.24 fA	1.31 eA	0.89 eB	0.00 dA	0.00 bA	0.00 dA	0.32 fA	0.33 fA	0.00 dB
CBL-102	17.00 eA	17.82 gA	5.70 eB	0.00 jA	0.00 iA	0.00 gA	1.20 gA	1.08 gB	0.83 eC	0.00 dA	0.00 bA	0.00 dA	0.36 fA	0.34 fA	0.00 dB
CBL-103	16.87 eB	23.08 eA	7.60 cC	0.50 fA	0.34 gB	0.00 gC	1.01 hA	1.07 gA	0.75 fB	0.00 dA	0.00 bA	0.00 dA	0.33 fA	0.16 hB	0.00 dC
CBL-201	17.03 eB	22.34 eA	6.70 dC	0.21 iA	0.07 hB	0.00 gB	1.82 bB	2.24 aA	1.32 bC	0.11 cA	0.00 bB	0.00 dB	0.00 hA	0.00 iA	0.06 cA
CBL-202	9.29 iB	11.69 jA	3.08 fC	0.54 eA	0.27 gB	0.25 eB	0.75 kA	0.81 iA	0.48 iB	0.00 dB	0.00 bB	1.24 aA	1.84 bA	1.58 bB	0.11 cC
CBL 203	20.18 cB	26.40 cA	8.30 cC	2.93 aA	2.75 aB	1.22 aC	0.53 lA	0.58 jA	0.36 jB	0.00 dA	0.00 bA	0.00 dA	0.00 hB	0.00 iB	0.22 bA
CBL-204	11.43 hB	17.33 gA	4.67 eC	0.50 fA	0.44 fA	0.00 gB	1.27 fA	1.14 gB	0.73 fC	0.00 dA	0.00 bA	0.00 dA	0.00 hA	0.00 iA	0.00 dA
CBL-205	27.39 aB	35.74 aA	11.84 aC	0.35 hA	0.00 iB	0.00 gB	1.17 gA	1.21 fA	0.90 eB	0.00 dA	0.00 bA	0.00 dA	0.00 hB	0.00 iB	0.32 aA
CBL-206	12.62 gA	13.26 iA	4.76 eB	0.00 jA	0.00 iA	0.00 gA	0.94 iA	0.86 iA	0.66 gB	0.00 dA	0.00 bA	0.00 dA	0.00 hA	0.07 iA	0.00 dA
CBL-207	10.44 iB	15.65 hA	4.91 eC	2.33 bA	2.14 bB	1.10 bC	0.75 kA	0.59 jB	0.45 iC	0.00 dA	0.00 bA	0.00 dA	0.00 hA	0.00 iA	0.06 cA
CBL-301	6.86 jB	11.40 jA	3.50 fC	0.53 fA	0.45 fB	0.00 gC	1.39 eA	1.31 eA	0.89 eB	0.00 dA	0.00 bA	0.00 dA	0.24 gA	0.11 hB	0.00 dC
CBL-302	10.68 iB	16.53 hA	5.11 eC	0.25 iA	0.14 hB	0.00 gC	0.58 lB	1.19 fA	0.49 B	0.00 dA	0.00 bA	0.00 dA	0.00 hA	0.00 iA	0.00 dA
CBL-304	13.63 gB	20.32 fA	6.47 dC	0.74 eA	0.57 eB	0.00 gC	0.87 jA	0.83 iA	0.57 hB	0.00 dA	0.00 bA	0.00 dA	0.59 dA	0.62 eA	0.22 bB
CBL-401	15.13 fB	16.76 hA	6.00 eC	0.29 hA	0.27 gA	0.00 gB	1.30 fB	1.43 dA	0.73 fB	0.00 dA	0.00 bA	0.00 dA	0.60 dA	0.61 eA	0.00 dB
CBL-402	18.16 dB	23.12 eA	8.08 cC	0.42 gA	0.16 hB	0.00 gC	1.53 dA	1.53 dA	1.22 cB	0.00 dA	0.00 bA	0.00 dA	0.25 gA	0.26 gA	0.00 dB
CBL-403	15.59 fB	17.14 gA	4.67 eC	2.01 cA	1.76 cB	0.83 cC	1.66 cA	1.49 dB	1.11 dC	0.05 dB	0.00 bA	0.00 dA	0.75 cA	0.69 eA	0.00 dB
CBL-404	19.51 cB	20.85 fA	5.37 eC	0.00 jA	0.00 iA	0.00 gA	1.14 gA	1.08 gA	0.90 eB	0.00 dA	0.00 bA	0.00 dA	0.00 hB	0.00 iB	0.20 bA
CBL-501	27.12 aB	35.53 aA	10.59 bC	0.00 jA	0.00 iA	0.00 gA	1.51 dB	1.70 cA	1.12 dC	0.00 dA	0.00 bA	0.00 dA	0.41 eA	0.00 iB	0.35 aA
CBL-502	18.12 dB	20.31 fA	6.49 dC	0.26 iA	0.00 iB	0.00 gB	2.53 aA	2.09 bB	1.92 aC	0.20 bA	0.00 bB	0.00 dB	0.47 eB	1.31 cA	0.06 cC
CBL-503	18.65 dB	23.38 eA	7.93 cC	1.12 dA	0.80 dB	0.49 dC	1.17 gA	1.21 fA	0.85 eB	2.97 aA	2.90 aB	0.25 bC	3.08 aA	2.76 aB	0.13 cC
CBL-505	9.96 iA	9.73 kA	1.78 gB	0.00 jA	0.00 iA	0.00 gA	1.16 gB	1.29 eA	0.96 eC	0.00 dA	0.00 bA	0.00 dA	0.34 fA	0.36 fA	0.00 dB
CBL-507	23.27 bB	30.02 bA	7.22 dC	0.00 jB	0.00 iB	0.17 fA	0.78 kB	0.97 hA	0.67 gC	0.00 dA	0.00 bA	0.00 dA	0.00 hB	0.00 iB	0.24 bA
CBL-602	15.13 fB	22.52 eA	5.97 dC	0.00 jA	0.00 iA	0.00 gA	0.96 iA	1.04 gA	0.67 gB	0.00 dA	0.00 bA	0.00 dA	0.26 gA	0.00 iB	0.29 aA
CBL-603	23.20 bB	29.04 bA	8.01 cC	0.00 jA	0.00 iA	0.00 gA	1.00 hA	0.91 hA	0.77 fB	0.00 dA	0.00 bA	0.00 dA	0.00 hB	0.00 iB	0.31 aA
CBL-604	10.68 iB	13.40 iA	4.85 eC	0.27 iA	0.33 gA	0.16 fB	1.21 gB	1.35 eA	1.06 dC	0.00 dA	0.00 bA	0.09 cB	0.82 cA	0.90 dA	0.06 cB
CBL-605	11.89 hB	13.43 iA	3.22 fC	0.00 jA	0.00 iA	0.00 gA	0.86 jA	0.78 iA	0.56 hB	0.00 dA	0.00 bA	0.00 dA	0.00 hA	0.00 iA	0.00 dA
Mean	16.00	20.44	6.18	0.52	0.41	0.16	1.17	1.20	0.84	0.13	0.11	0.06	0.41	0.39	0.10
RRIo		1496			1501			1521			1529			1552	
RRIl		1500			1501			1522			1533			1559	
CV 1 (%)		5.15			14.15			4.87			29.20			14.53	
CV 2 (%)		4.99			13.21			5.59			28.66			17.21	

Compounds: (C21) bicyclogermacrene, (C22) aciphyllene, (C23) δ-cadinene, (C24) trans-cadina-1,4-diene, and (C25) germacrene B. RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. (Continued)

Accession	Compound														
	C26			C27			C28			C29			C30		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CBL-101	19.20 cA	16.08 eC	17.85 dB	2.28 cA	2.17 bA	1.37 dB	3.22 aA	1.04 eC	1.91 bB	0.79 dA	0.83 cA	0.55 cB	1.54 cA	1.54 bA	1.10 bB
CBL-102	19.66 cA	19.92 cA	18.61 dB	2.02 cA	1.63 cA	0.97 dB	2.88 bA	0.82 fC	1.75 bB	0.61 eB	0.87 cA	0.57 cB	1.38 cA	1.46 bA	0.92 cB
CBL-103	15.99 eA	13.60 fB	14.25 fB	2.69 bA	2.34 bB	2.03 cB	2.74 bA	0.82 fC	1.85 bB	0.94 dA	0.79 cA	0.72 cA	1.51 cA	1.29 cA	1.06 bB
CBL-201	12.28 gA	10.73 hB	10.22 hB	1.90 dA	1.51 cB	1.19 dB	2.03 cA	0.75 fC	1.51 cB	1.18 cA	1.17 bA	0.83 bB	0.82 eA	0.83 dA	0.00 eB
CBL-202	12.07 gC	14.84 eA	13.46 fB	2.41 bA	2.13 bA	1.77 cB	1.29 eB	1.54 cA	1.03 dC	0.37 fA	0.00 eB	0.32 dA	1.08 eA	1.29 cA	0.00 eB
CBL 203	18.79 cA	16.27 eB	15.16 eB	1.79 dA	1.56 A	1.09 dB	2.35 cA	0.81 fC	1.76 bB	0.52 fA	0.48 dA	0.37 dA	1.25 dA	1.22 cA	0.00 eB
CBL-204	17.73 dA	15.09 eB	12.93 fC	1.38 dA	1.20 cA	1.03 dA	2.75 bA	0.84 fC	1.84 bB	0.90 dA	0.94 cA	0.57 cB	1.24 dA	1.41 bA	0.00 eB
CBL-205	18.33 dA	14.42 fB	17.44 dA	2.33 cA	2.02 bA	1.57 dB	2.82 bA	0.77 fC	2.36 aB	0.86 dA	0.86 cA	0.75 cA	1.79 bA	1.74 bA	0.00 eB
CBL-206	19.73 cA	19.58 cA	17.48 dB	2.50 bA	2.23 bA	1.17 dB	2.39 cA	1.19 dC	1.70 bB	0.89 dA	0.88 cA	0.58 cB	1.40 cA	1.56 bA	0.00 eB
CBL-207	21.63 bA	17.50 dB	15.38 eC	1.89 dA	1.55 cA	1.18 dB	2.54 bA	1.00 eC	1.67 bB	0.48 fA	0.46 dA	0.29 dA	1.26 dA	1.29 cA	0.21 dB
CBL-301	15.17 fA	14.97 eA	14.87 eA	1.80 dA	1.36 cB	1.10 dB	1.82 dA	0.66 fC	1.60 bB	1.61 bA	1.37 aB	1.12 aC	1.82 bA	1.67 bA	1.31 aB
CBL-302	17.91 dA	15.19 eB	15.63 eB	1.70 dA	1.56 cA	1.13 dB	2.02 dA	0.77 fC	1.74 bB	0.68 eA	0.69 cA	0.57 cA	0.85 eA	0.88 dA	0.73 cA
CBL-304	18.03 dA	13.54 fB	13.41 fB	2.60 bA	2.15 bB	1.74 cB	2.32 cA	1.14 dC	1.70 bB	0.80 dA	0.77 cA	0.63 cA	1.51 cA	1.48 bA	1.37 aA
CBL-401	8.04 iB	7.63 iB	9.72 hA	2.47 bB	1.84 cC	2.88 aA	0.93 fA	0.73 fA	0.89 dA	2.33 aA	1.52 aB	0.00 eC	0.73 fB	0.67 eB	1.09 bA
CBL-402	14.70 fB	15.60 eB	17.83 dA	2.10 cA	1.56 cB	1.38 dB	1.89 dA	1.21 dB	1.79 bA	1.16 cA	0.83 cB	0.44 dC	1.32 dA	1.20 cA	0.06 eB
CBL-403	10.21 hB	8.42 iC	11.57 gA	2.22 cA	1.59 cB	2.34 bA	1.33 eA	0.70 fB	1.34 cA	0.98 dA	0.36 dB	0.40 dB	1.17 dA	1.10 dA	0.00 eB
CBL-404	9.19 iC	10.53 hB	16.33 eA	2.07 cA	1.97 bA	1.92 cA	0.86 fB	1.02 eB	1.55 bA	1.25 cA	0.85 cB	0.56 cC	0.48 fA	0.47 eA	0.00 eB
CBL-501	16.36 eA	12.53 gB	15.61 eA	3.21 aA	2.47 bB	2.12 cB	1.48 eB	1.28 dB	1.71 bA	0.84 dA	0.00 eC	0.26 dB	0.57 fA	0.61 eA	0.29 dB
CBL-502	8.92 iB	9.01 iB	10.74 gA	2.05 cA	2.02 bA	2.15 cA	0.76 fB	0.84 fB	1.32 cA	1.33 cA	0.71 cB	0.56 cB	0.86 eB	1.21 cA	0.38 dC
CBL-503	10.68 hB	8.76 iC	14.29 fA	2.14 cA	1.80 cA	1.72 cA	0.98 fB	0.88 eB	1.83 bA	0.43 fA	0.58 dA	0.18 eB	2.47 aB	2.98 aA	0.17 dC
CBL-505	18.34 dC	22.89 bB	26.68 aA	3.15 aA	2.59 bB	2.76 aB	1.89 A	1.95 bA	1.81 bA	0.82 dA	0.40 dB	0.00 eC	0.64 fA	0.72 eA	0.33 dB
CBL-507	12.60 gB	9.06 iC	13.86 fA	2.87 aA	2.16 bB	2.32 bB	1.18 eB	0.92 eC	1.43 cA	1.05 cA	0.48 dB	0.32 dB	0.64 fA	0.65 eA	0.00 eB
CBL-602	21.45 bB	25.79 aA	20.08 cC	2.97 aA	3.17 aA	1.98 cB	2.13 cB	2.43 aA	1.74 bC	1.02 dA	0.37 dB	0.31 dB	1.01 eA	1.21 cA	0.00 eB
CBL-603	14.37 fB	13.94 fB	19.67 cA	2.55 bA	2.40 bA	1.75 cB	1.40 eB	1.35 dB	1.82 bA	0.84 dA	0.15 eB	0.25 dB	0.76 fA	0.86 dA	0.00 eB
CBL-604	13.02 gA	11.33 hB	13.23 fA	2.56 bA	1.99 bB	2.02 cB	1.78 dA	1.01 eC	1.40 cB	0.61 eA	0.43 dB	0.36 dB	1.34 dB	1.61 bA	0.00 eC
CBL-605	23.32 aB	25.38 aA	24.84 bA	2.88 aA	3.10 aA	2.22 cB	1.96 dB	2.37 aA	1.97 bB	1.06 cA	0.92 cA	0.68 cB	0.67 fA	0.85 dA	0.00 eB
Mean	15.68	14.72	15.81	2.33	2.00	1.73	1.91	1.11	1.66	0.94	0.68	0.47	1.16	1.22	0.35
RRIo		1578			1581			1625			1643			1650	
RRIl		1577			1582			1630			1645			1651	
CV 1 (%)		5.18			16.61			11.80			17.74			19.17	
CV 2 (%)		4.46			11.73			8.34			17.78			15.88	

Compounds: (C26) spathulenol, (C27) caryophyllene oxide, (C28) muurola-4,10(14)-dien-1-β-ol, (C29) cubenol, and (C30) pogostol. RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 3. (Continued)

Accession	Compound					
	C31			TI		
	1	2	3	1	2	3
CBL-101	2.98 cA	3.03 eA	2.13 dB	86.43	90.20	87.43
CBL-102	4.08 aA	3.74 bB	2.25 cC	88.00	88.89	88.30
CBL-103	3.10 cA	2.91 fB	2.08 dC	86.87	89.51	85.16
CBL-201	2.19 eA	2.04 hA	1.33 hB	83.70	86.82	85.68
CBL-202	1.56 gB	1.77 jA	1.24 hC	82.09	85.49	86.67
CBL 203	3.45 bA	3.46 cA	2.31 cB	92.00	93.30	90.12
CBL-204	3.95 aB	4.39 aA	2.59 bC	86.41	87.56	87.29
CBL-205	3.95 aA	3.83 bA	3.07 aB	90.72	91.76	86.74
CBL-206	3.06 cA	2.80 fB	1.90 eC	89.10	89.36	87.51
CBL-207	3.55 bA	3.28 dB	2.74 bC	88.81	90.78	89.90
CBL-301	2.57 dB	2.77 fA	2.01 eC	81.92	83.92	82.36
CBL-302	2.94 cA	2.55 gB	2.26 cC	91.44	90.65	91.68
CBL-304	2.17 eA	1.80 jB	1.75 fB	85.90	88.43	83.90
CBL-401	0.77 iA	0.53 mB	0.78 jA	86.82	86.35	89.47
CBL-402	1.76 fA	1.58 jA	1.14 iB	85.27	90.44	85.01
CBL-403	0.75 iB	0.62 mB	0.99 iA	88.91	92.14	84.29
CBL-404	0.62 iB	0.59 mB	1.00 iA	89.56	91.13	87.33
CBL-501	1.16 hA	0.82 lB	0.84 jB	89.45	91.26	88.57
CBL-502	0.74 iB	0.73 lB	1.08 iA	88.18	86.89	85.92
CBL-503	0.65 iA	0.59 mA	0.24 kB	85.34	86.37	80.35
CBL-505	1.12 hC	1.36 kB	1.79 fA	87.96	90.35	87.26
CBL-507	0.77 iA	0.58 mB	0.77 jA	91.60	91.68	89.70
CBL-602	1.05 hB	1.27 kA	0.85 jC	88.52	90.69	88.30
CBL-603	1.07 hA	1.26 kA	1.20 iA	90.09	91.67	89.98
CBL-604	0.81 iB	0.69 lB	1.10 iA	89.34	88.78	85.72
CBL-605	1.41 gA	1.46 jA	1.50 gA	90.85	91.28	89.96
Mean	2.01	1.94	1.57	87.90	89.44	87.10
RRIo		1666		-	-	-
RRIl		1668		-	-	-
CV 1 (%)		6.64		-	-	-
CV 2 (%)		5.89		-	-	-

Chemical classes of the compounds	Relative percentage (%)		
	Harvest time		
	1 (Feb. 2024)	2 (Jun. 2024)	3 (Oct. 2024)
Monoterpene hydrocarbons (C1-C8 e C10)	22.28	21.72	30.54
Oxygenated monoterpenes (C9, C11 e C12)	6.60	5.71	9.87
Sesquiterpene hydrocarbons (C13-C25)	34.99	40.34	25.10
Oxygenated sesquiterpenes (C26-31)	24.03	21.67	21.59
Total monoterpenes (C1-C12)	28.88	27.43	40.41
Total sesquiterpenes (C13-C31)	59.02	62.01	46.69
Total	87.90	89.44	87.10

Compounds: (C31) 14-hydroxy-9-epi-(*E*)-caryophyllene. TI: total relative percentage of compounds (only those with a concentration ≥ 2% in at least one sample were included in the statistical analysis); RRIo: observed relative retention index; RRIl: literature relative retention index; CV: coefficient of variation (%). Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ significantly from each other according to the Scott–Knott test ($p \leq 0.05$).

Table 4. Antioxidant activity of essential oils from 20 accessions of *Croton blanchetianus*, corresponding to Harvest 1 (February 2024), from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe, Brazil.

Accession	DPPH Inhibition (%)	ABTS Inhibition (%)	FRAP (mg mL^{-1})
CBL-101	59.54 ± 0.82 c	89.62 ± 0.43 b	0.52 ± 0.03 b
CBL-201	24.12 ± 0.82 k	61.80 ± 0.22 e	0.39 ± 0.03 c
CBL-203	72.86 ± 0.82 a	61.37 ± 5.19 e	0.46 ± 0.05 b
CBL-205	28.66 ± 0.97 i	87.82 ± 1.48 b	0.48 ± 0.00 b
CBL-206	25.77 ± 0.54 j	75.31 ± 1.14 c	0.29 ± 0.04 d
CBL-207	58.32 ± 0.78 c	67.63 ± 0.16 d	0.43 ± 0.05 c
CBL-301	66.24 ± 0.54 b	92.51 ± 0.16 a	0.57 ± 0.09 a
CBL-302	54.07 ± 2.33 d	48.82 ± 0.51 g	0.29 ± 0.01 d
CBL-303	27.65 ± 1.32 i	57.30 ± 0.67 f	0.46 ± 0.10 b
CBL-304	19.01 ± 0.22 l	64.17 ± 1.14 e	0.34 ± 0.03 d
CBL-401	19.15 ± 0.54 l	59.00 ± 2.21 f	0.36 ± 0.03 d
CBL-402	51.12 ± 0.76 e	87.73 ± 1.40 b	0.63 ± 0.09 a
CBL-403	29.09 ± 0.66 i	89.05 ± 1.61b	0.35 ± 0.07 d
CBL-502	27.86 ± 0.78 i	69.24 ± 4.08 d	0.40 ± 0.00 c
CBL-503	67.02 ± 0.99 b	68.48 ± 2.00 d	0.56 ± 0.04 a
CBL-505	48.52 ± 1.59 f	71.00 ± 1.13 d	0.44 ± 0.05 c
CBL-507	31.97 ± 1.30 h	63.32 ± 0.86 e	0.47 ± 0.01 b
CBL-603	25.41 ± 0.90 j	51.75 ± 2.00 g	0.48 ± 0.10 b
CBL-604	35.13 ± 0.90 d	76.49 ± 1.99 c	0.39 ± 0.06 c
CBL-605	22.68 ± 2.12 k	59.24 ± 1.32 f	0.44 ± 0.04 c
CV (%)	2.79	2.75	12.69
Mean	39.71	70.08	0.44

Means followed by the same letter do not differ from each other according to the Scott-Knott test ($p \leq 0.05$); values are expressed as mean ± standard deviation; CV: coefficient of variation (%).

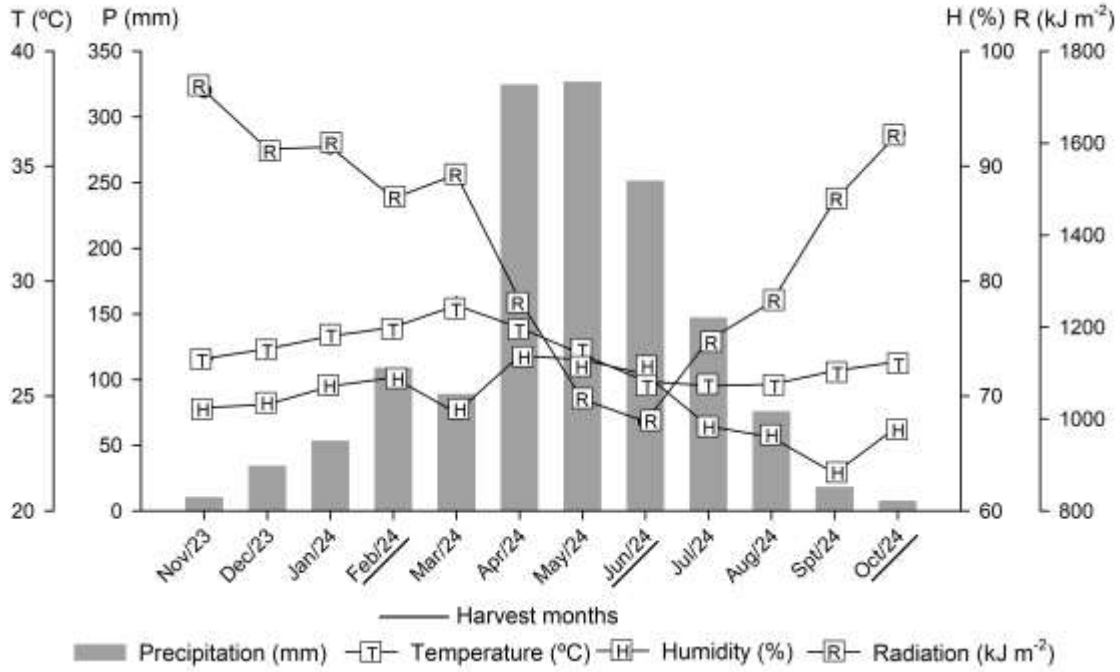

Figure 1. Climatological parameters of precipitation, mean temperature, relative humidity, and solar radiation in Aracaju, SE, Brazil, from November 2023 to October 2024.

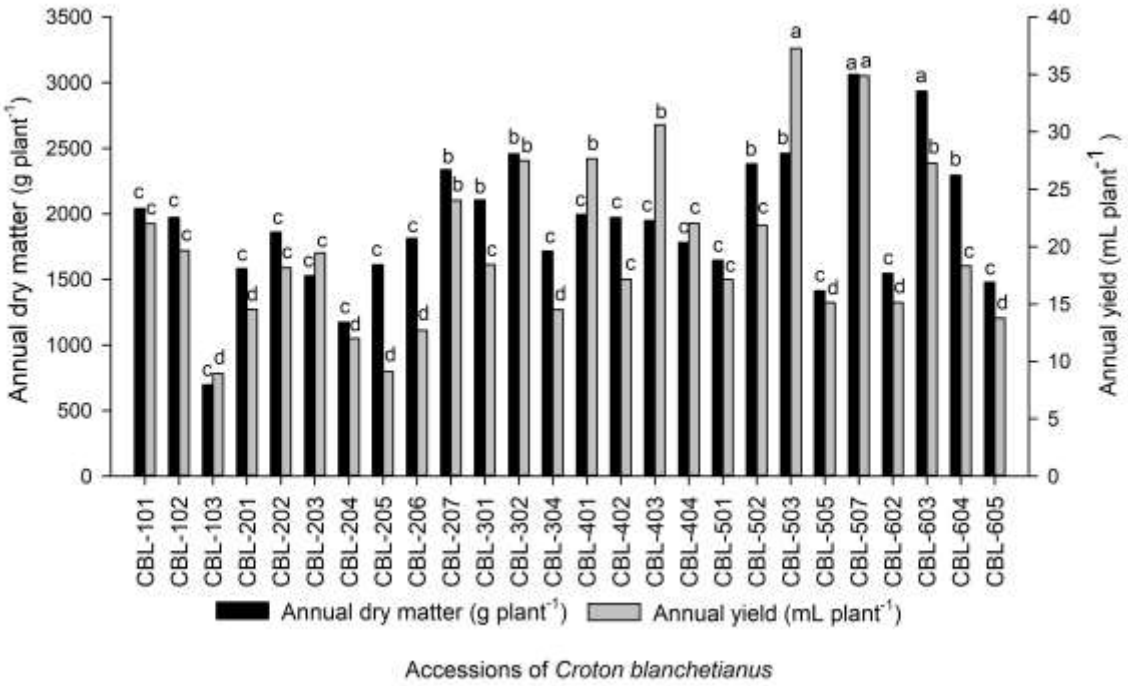

Figure 2. Annual leaf dry mass and annual essential oil yield of *Croton blanchetianus* accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe. Mean values followed by the same letter do not differ significantly according to the Scott-Knott test ($p \leq 0.05$).

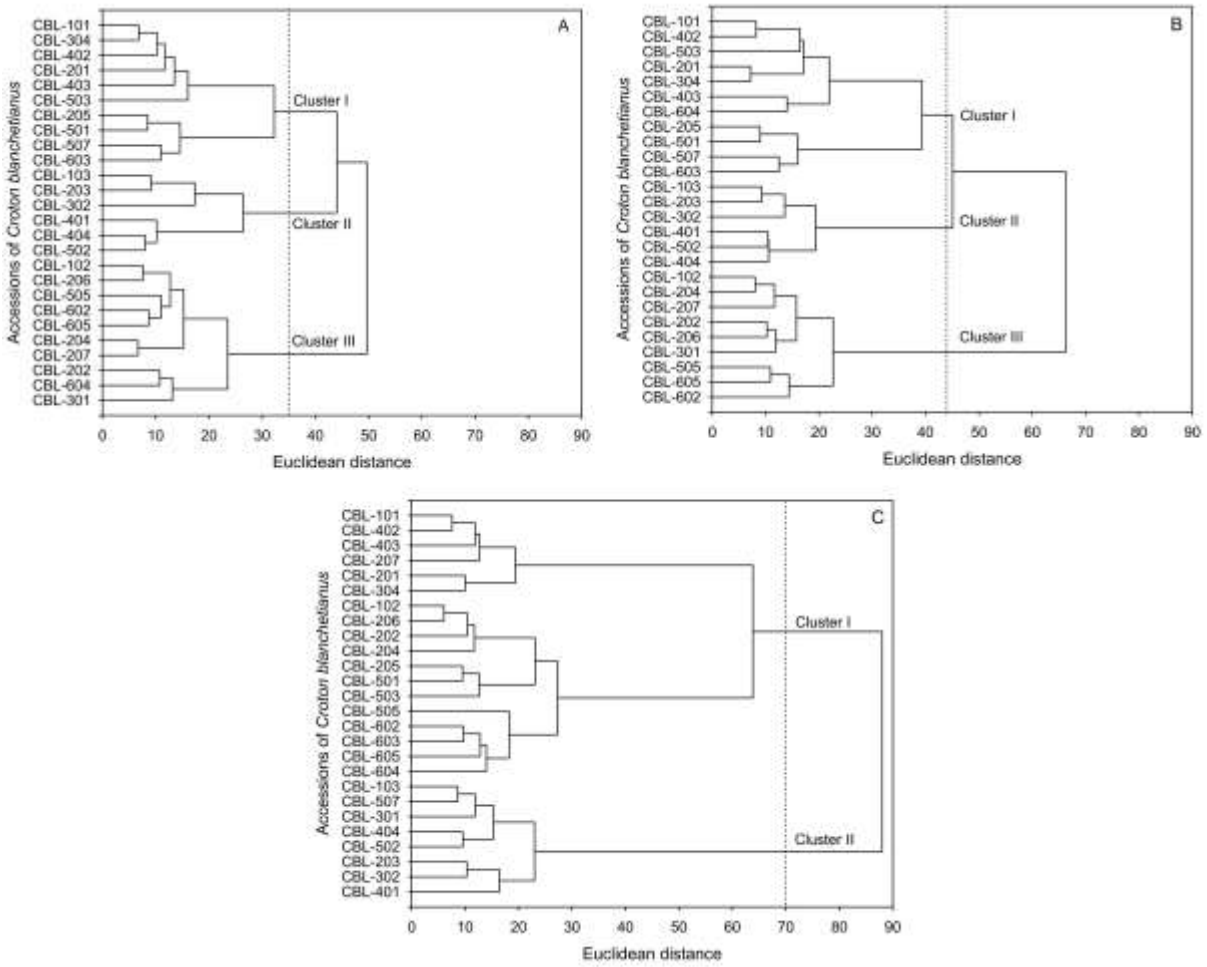

Figure 3. Two-dimensional dendrogram representing the similarity of essential oil composition among 26 *Croton blanchetianus* accessions from the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe for Harvests 1 (A), 2 (B), and 3 (C).

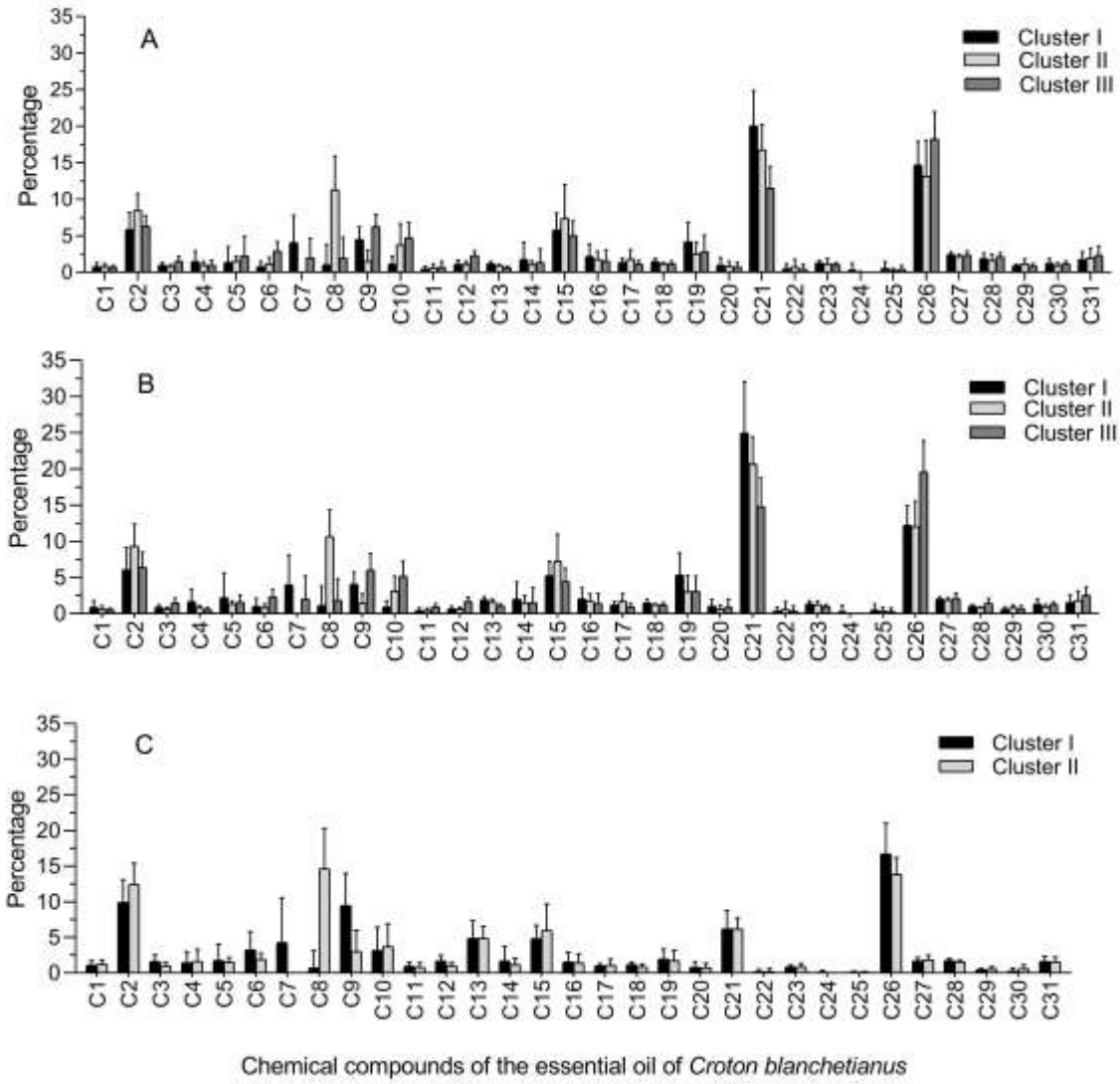

Figure 4. Mean values of the groups formed in the cluster analysis of essential oil chemical compounds from 26 *Croton blanchetianus* accessions of the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe, for Harvests 1 (A), 2 (B), and 3 (C). Compounds are as follows: (C1) α-thujene, (C2) α-pinene, (C3) sabinene, (C4) myrcene, (C5) α-phellandrene, (C6) p-cymene, (C7) limonene, (C8) β-phellandrene, (C9) 1,8-cineole, (C10) terpinolene, (C11) linalool, (C12) α-terpineol, (C13) δ-elemene, (C14) β-elemene, (C15) *(E)*-caryophyllene, (C16) aromadendrene, (C17) α-humulene, (C18) allo-aromadendrene, (C19) germacrene D, (C20) β-selinene, (C21) bicyclogermacrene, (C22) aciphyllene, (C23) δ-cadinene, (C24) trans-cadina-1,4-diene, (C25) germacrene B, (C26) spathulenol, (C27) caryophyllene oxide, (C28) muurola-4,10(14)-dien-1-β-ol, (C29) cubenol, (C30) pogostol, and (C31) 14-hydroxy-9-epi-(*E*)-caryophyllene.

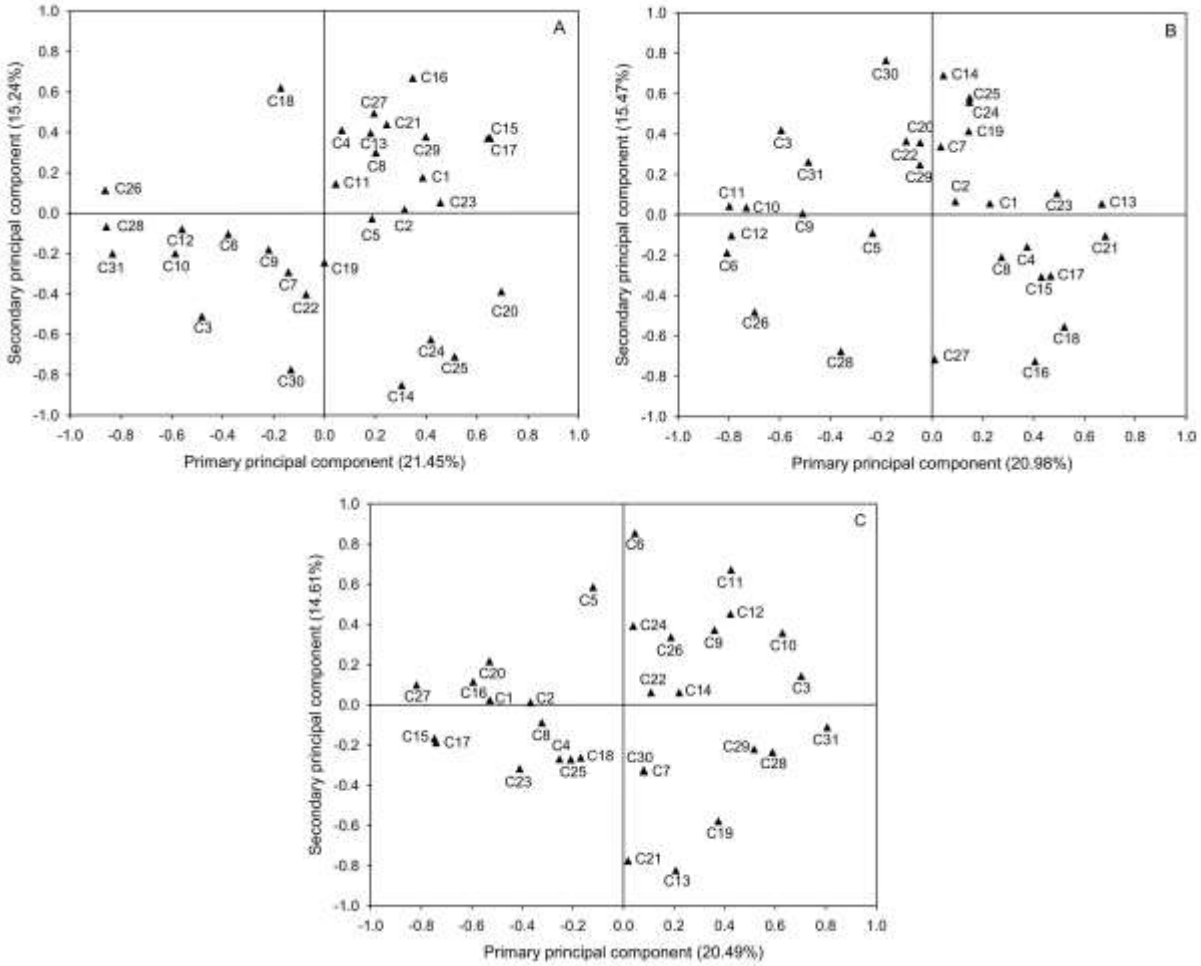

Figure 5. Distribution of chemical compounds in the essential oil of *Croton blanchetianus* in relation to the two principal components through principal component analysis (PCA) for Harvest 1 (A), 2 (B), and 3 (C). Compounds are as follows: (C1) α-thujene, (C2) α-pinene, (C3) sabinene, (C4) myrcene, (C5) α-phellandrene, (C6) p-cymene, (C7) limonene, (C8) β-phellandrene, (C9) 1,8-cineole, (C10) terpinolene, (C11) linalool, (C12) α-terpineol, (C13) δ-elemene, (C14) β-elemene, (C15) (*E*)-caryophyllene, (C16) aromadendrene, (C17) α-humulene, (C18) allo-aromadendrene, (C19) germacrene D, (C20) β-selinene, (C21) bicyclogermacrene, (C22) aciphyllene, (C23) δ-cadinene, (C24) trans-cadina-1,4-diene, (C25) germacrene B, (C26) spathulenol, (C27) caryophyllene oxide, (C28) muurola-4,10(14)-dien-1-β-ol, (C29) cubenol, (C30) pogostol, and (C31) 14-hydroxy-9-epi-(*E*)-caryophyllene.

9. CONSIDERAÇÕES FINAIS

Os estudos desenvolvidos nesta tese ampliaram o conhecimento sobre *Croton blanchetianus* Baill., espécie medicinal e aromática endêmica da Caatinga, por meio de uma abordagem integrada envolvendo prospecção, conservação e caracterização genética, química, morfoagronômica e sazonal. Os resultados, obtidos a partir de cinco artigos científicos, evidenciam a complexidade e o potencial da espécie, tanto em populações naturais quanto em acessos conservados em Banco Ativo de Germoplasma.

A análise da diversidade genética revelou baixa diferenciação entre populações naturais, indicando elevada similaridade genética independentemente da origem geográfica. Esse padrão sugere a ocorrência de fluxo gênico e reforça a importância da conservação *in situ* associada à conservação *ex situ*, uma vez que a redução de áreas de ocorrência naturais da espécie pode comprometer a variabilidade ainda existente.

Em contraste, os estudos químicos demonstraram elevada diversidade na composição dos óleos essenciais, com ampla variação na concentração dos principais mono e sesquiterpenos, possibilitando a identificação de diferentes perfis químicos e potenciais quimiotipos. Esses resultados destacam o potencial bioativo da espécie e sua relevância para futuras aplicações tanto na agropecuária quanto na área medicinal.

A caracterização morfoagronômica dos acessos conservados evidenciou alta variabilidade fenotípica, especialmente para caracteres relacionados ao crescimento vegetativo, à produção de biomassa e ao teor e rendimento de óleo essencial. Esses resultados demonstram que o Banco Ativo de Germoplasma da Universidade Federal de Sergipe constitui uma base genética estratégica para a seleção de materiais promissores e para futuros programas de melhoramento genético.

A análise comparativa entre folhas e frutos evidenciou diferenças qualitativas e quantitativas na composição química dos óleos essenciais, indicando que o órgão vegetal influencia diretamente o perfil metabólico da espécie. A identificação exclusiva de determinados compostos nos frutos, como o acetato de mirtenila, reforça o potencial desse metabólito como marcador químico, além de abrir perspectivas para usos específicos conforme o órgão explorado.

A avaliação da sazonalidade demonstrou que fatores ambientais influenciam significativamente a produção e a composição química dos óleos essenciais. A variação entre épocas de colheita, associada ao desempenho superior de determinados acessos, evidencia a importância da definição do período ideal de colheita e da seleção de genótipos promissores para maximizar o rendimento e o potencial bioativo dos óleos essenciais, bem como o potencial antioxidante da espécie.

De forma geral, os resultados confirmam que *C. blanchetianus* apresenta variabilidade genética, morfoagronômica e química, com influência sazonal e potencial antioxidante. Essas informações subsidiam estratégias de conservação, seleção e uso sustentável da espécie, além de fornecerem bases para estudos futuros voltados ao melhoramento genético, à padronização da produção e à exploração racional de seus recursos naturais. Assim, esta tese pode contribuir para a valorização científica e a conservação de uma espécie nativa da Caatinga, promovendo o uso consciente da biodiversidade brasileira.

ANEXOS

Tabela 1A. Resumo da análise de variância dos fatores acessos e época de colheita para as variáveis massa seca, teor e rendimento de óleo essencial de *Croton blanchetianus* Baill. Universidade Federal de Sergipe, São Cristóvão - SE, 2025.

FV	GL	QM		
		MS	TOE	ROE
Acessos (A)	2	266334,91**	0,52**	54,27**
Bloco	25	8099,83^{ns}	0,01 ^{ns}	2,28 ^{ns}
Erro a	50	51067,38	0,01	5,15
Época de colheita (E)	2	55090994,24**	1,44**	553,83**
A x E	50	46617,60**	0,04**	9,67**
Erro b	104	172660,75	0,01	2,25
Total	233	-	-	-
CV 1 (%)	-	35,39	7,30	34,07
CV 2 (%)	-	20,58	7,67	22,54

MS: massa seca; TOE: teor de óleo essencial; ROE: Rendimento de óleo essencial; CV: coeficiente de variação (%); ns, **, *: não significativo, significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

Tabela 2A. Resumo da análise de variância dos fatores acessos e épocas de colheita para porcentagem relativa (%) dos compostos químicos do óleo essencial de acessos de *Croton blanchetianus* Baill. do Banco Ativo de Germoplasma de Plantas Medicinais e Aromáticas da Universidade Federal de Sergipe em três colheitas anual (1 (02/2024), 2 (06/2024) e 3 (10/2024)).

FV	GL	QM															
		C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	C13	C14	C15	C16
Acessos (A)	25	1,84**	67,12**	3,78**	13,42**	38,24**	21,89**	145,98**	309,52**	93,03**	64,08**	1,90**	4,45**	9,12**	30,12**	62,96**	17,17**
Erro a	52	0,01	0,32	0,01	0,01	0,08	0,02	0,49	0,19	0,51	0,02	0,00	0,00	0,02	0,03	0,03	0,02
Épocas (E)	2	4,88**	402,07**	3,64**	3,68**	0,20**	44,65**	9,12**	47,49**	263,35**	3,68**	2,52**	7,09**	355,45**	1,46**	9,67**	2,28**
A x E	50	0,51**	3,12**	0,19**	0,71**	0,96**	1,54**	7,09**	6,86**	9,93**	2,16**	0,35**	0,25**	3,36**	0,49**	1,04**	0,23**
Erro b	104	0,01	0,15	0,01	0,01	0,04	0,01	0,50	0,16	0,52	0,02	0,00	0,00	0,02	0,04	0,04	0,02
Total	233	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CV 1 (%)	-	9,52	7,05	7,12	9,30	16,28	6,81	27,46	10,51	13,37	5,14	5,93	4,03	5,24	11,41	3,34	7,58
CV 2 (%)	-	9,11	4,75	6,49	8,38	11,85	4,83	27,61	9,83	13,40	4,40	4,61	3,24	5,94	12,51	3,80	7,98

FV	GL	QM															
		C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	-
Acessos (A)	25	3,82**	1,13**	40,58**	5,06**	203,81**	3,26**	1,24**	1,47**	1,70**	149,58	1,79**	1,09**	0,68**	1,32**	9,39**	-
Erro a	52	0,00	0,01	0,37	0,36	0,53	0,00	0,00	0,00	0,00	0,64	0,11	0,03	0,01	0,03	0,01	
Épocas (E)	2	2,66**	1,46**	91,62**	0,22^{ns}	4157,06**	2,64**	3,02**	0,90**	2,31**	27,96	7,08**	13,14**	4,27**	18,61**	4,24**	-
A x E	50	0,12**	0,09**	2,28**	0,46^{ns}	21,69**	0,18**	0,05**	0,35**	0,49**	13,33	0,23**	0,65**	0,20**	0,40**	0,40**	-
Erro b	104	0,00	0,90	0,34	0,36	0,50	0,00	0,00	0,00	0,00	0,47	0,06	0,02	0,01	0,02	0,01	-
Total	233		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CV 1 (%)	-	5,81	6,67	19,97	73,01	5,15	14,15	4,87	29,20	14,53	5,18	16,61	11,80	17,74	19,17	6,64	
CV 2 (%)	-	5,77	7,62	19,23	72,05	4,99	13,21	5,59	28,66	17,21	4,46	11,73	8,34	17,78	15,88	5,89	-

Compostos: (C01) α-tujeno, (C02) α-pineno, (C03) sabineno, (C04) mirceno, (C05) α-felandreno, (C06) *p*-cimeno, (C07) limoneno, (C08) β-felandreno, (C09) 1,8-cineol, (C10) terpinoleno, (C11) linalol, (C12) α-terpineol, (C13) δ-elemeno, (C14) β-elemeno, (C15) (*E*)-cariofileno, (C16) aromadendreno, (C17) α-humuleno, (C18) *alo*-aromadendreno, (C19) germacreno D, (C20) β-selineno, (C21) biciclogermacreno, (C22) acifileno, (C23) δ-cadineno, (C24) trans-cadina-1,4-dieno, (C25) germacreno B, (C26) espatulenol, (C27) óxido de cariofileno, (C28) muurola-4,10(14)-dien-1-β-ol, (C29) cubenol, (C30) pogostol e (C31) 14-hidroxi-9-epi-(*E*)-cariofileno. CV: coeficiente de variação (%); ns, **, *: não significativo, significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.