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DANIELE RODRIGUES DE LIMA

DOCUMENTAÇÃO QUÍMICA, TOXICIDADE E ATIVIDADES BIOLÓGICAS DE
***CHAMAECRISTA DUCKEANA* I & B E *CHAMAECRISTA ENSIFORMIS* I & B**

FORTALEZA

2022

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CHAMAECRISTA DUCKEANA I & B E *CHAMAECRISTA ENSIFORMIS* I & B

Tese de Doutorado submetida à avaliação da banca examinadora do Programa de Pós-Graduação em Biotecnologia da Rede Nordeste de Biotecnologia da Universidade Federal do Ceará como parte dos requisitos para obtenção do título de Doutora em Biotecnologia. Área de concentração: Biotecnologia de Produtos Naturais.

Orientadora: Profa. Dra. Maria Goretti de Vasconcelos Silva.

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RESUMO

Este trabalho apresenta os resultados obtidos pelo estudo inédito de duas plantas do gênero *Chamaecrista*, nativas da flora brasileira e que apresentam raros estudos sobre composição química e potencial biológico. A documentação química do perfil metabólico dos extratos de *Chamaecrista duckeana* I&B e *Chamaecrista ensiformis* I&B foi realizada empregando-se a técnica de UHPLC-MS (cromatografia líquida de ultra eficiência acoplada a espectrometria de massas). Os perfis químicos são dominados por flavonoides, mas com a presença de antraquinonas, chalconas, cumarinas, cromonas, fenilpropanoides e sesquiterpenos. Foram identificados trinta e quatro (34) compostos que estão sendo relatados pela primeira vez nas duas espécies. A atividade antioxidante dos extratos de folhas, caules e frutos de *C. duckeana* foi realizada utilizando ensaio *in vitro*, pelo sequestro do radical livre DPPH e na avaliação da atividade citotóxica dos extratos, foi utilizado ensaio MTT, sendo determinados valores de IC₅₀ contra as linhagens celulares HL60 e RAJI. Os extratos apresentaram potencial antioxidante, e a atividade do extrato do caule foi superior ao padrão BHT. No ensaio citotóxico, apenas a linhagem HL60 (leucemia) apresentou inibição de crescimento superior a 80%. O extrato do caule apresentou citotoxicidade mais expressiva com IC₅₀ de 137,3 (104,6-180,1) e 106,8 (96,52-118,3) $\mu\text{mol L}^{-1}$ para HL60 e RAJI, respectivamente. Extratos das folhas e flores de *C. ensiformis* foram investigados quanto a sua toxicidade frente a Zebrafish (*Danio rerio*) adulto. A atividade antioxidante foi realizada utilizando os ensaios com DPPH e ABTS e o potencial antiacetilcolinesterase foi realizado pelo método de Ellman. A citotoxicidade foi testada usando o método MTT. Os dados obtidos revelaram que os extratos não foram tóxicos frente ao Zebrafish (*Danio rerio*) adulto até 96 h de análise. Os extratos das folhas apresentaram boa atividade antioxidante com valor de IC₅₀ $5,516 \pm 0,027 \mu\text{g mL}^{-1}$ no método ABTS. O extrato das flores de *C. ensiformis* inibiu a enzima acetilcolinesterase (AChE), com halo de inibição de 0,8 cm de diâmetro e apresentou efeito citotóxico significativo contra as linhagens celulares HL60 e RAJI com IC₅₀ de 88,76 e 90,23 $\mu\text{g mL}^{-1}$, respectivamente. O presente trabalho apresenta um conhecimento abrangente e inédito sobre as espécies *C. duckeana* e *C. ensiformis*, ricas em metabólitos secundários bioativos com atividade citotóxica.

Palavras-chave: *Chamaecrista duckeana*; *Chamaecrista ensiformis*; UPLC-QTOF-MS; citotoxicidade; zebrafish; acetilcolinesterase.

ABSTRACT

This work presents the results obtained from the unpublished study of two plants of the genus *Chamaecrista*, native to the Brazilian flora and which present rare studies on chemical composition and biological potential. Chemical documentation of the metabolic profile of *Chamaecrista duckeana* I&B and *Chamaecrista ensiformis* I&B extracts was performed using the UHPLC-MS technique (ultra performance liquid chromatography coupled to mass spectrometry). The chemical profiles are dominated by flavonoids, but with the presence of anthraquinones, chalcones, coumarins, chromones, phenylpropanoids and sesquiterpenes. Thirty-four (34) compounds were identified that are being reported for the first time in both species. The antioxidant activity of the extracts of leaves, stems and fruits of *C. duckeana* was performed using an in vitro assay, by scavenging the free radical DPPH and in the evaluation of the cytotoxic activity of the extracts, the MTT assay was used, and IC₅₀ values were determined against the cell lines HL60 and RAJI cell lines. The extracts showed antioxidant potential, and the activity of the stem extract was superior to the standard BHT. In the cytotoxic assay, only the HL60 cell line (leukemia) showed growth inhibition greater than 80%. The stem extract showed the most expressive cytotoxicity with IC₅₀ of 137.3 (104.6-180.1) and 106.8 (96.52-118.3) $\mu\text{mol L}^{-1}$ for HL60 and RAJI, respectively. Extracts from leaves and flowers of *C. ensiformis* were investigated for their toxicity against adult Zebrafish (*Danio rerio*). Antioxidant activity was performed using the DPPH and ABTS assays and the antiacetylcholinesterase potential was performed using the Ellman method. Cytotoxicity was tested using the MTT method. The data obtained revealed that the extracts were not toxic against adult Zebrafish (*Danio rerio*) up to 96 h of analysis. The leaf extracts showed good antioxidant activity with an IC₅₀ value of $5.516 \pm 0.027 \mu\text{g mL}^{-1}$ in the ABTS method. The extract of *C. ensiformis* flowers inhibited the enzyme acetylcholinesterase (AChE), with an inhibition halo of 0.8 cm in diameter and showed a significant cytotoxic effect against cell lines HL60 and RAJI with IC₅₀ of 88.76 and 90.23 $\mu\text{g mL}^{-1}$, respectively. The present work presents a comprehensive and unprecedented knowledge about the species *C. duckeana* and *C. ensiformis*, rich in bioactive secondary metabolites with cytotoxic activity.

Keywords: *Chamaecrista duckeana*; *Chamaecrista ensiformis*; UPLC-QTOF-MS; cytotoxicity; zebrafish; acetylcholinesterase.

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LISTA DE ABREVIATURAS E SIGLAS

ABTS	2,2-azino-bis-3-etil-benzotiazolina-6-ácido sulfônico
ANOVA	Análise de variância
ANVISA	Agência Nacional de Vigilância Sanitária
ATCC	<i>American Type Culture Collection</i>
CLAE	Cromatografia líquida de alta eficiência
DAD	Detector de arranjo de diodos (Diode array detector)
DMSO	Dimetil sulfóxido
DP	Desvio-padrão
DPPH	2,2-difenil-1-picrilhidrazila
EAG	Equivalente ao Ácido Gálico
ER	Equivalentes de Rutina
EFS	Extração em fase sólida
FDA	Food and Drug Administration
HCT-116	Linhagem celular de cólon humano
HPLC	<i>High performance liquid chromatography</i>
HRMS	<i>High resolution mass spectrometry</i>
IP	Intraperitoneal
IV	Infravermelho
L929	Linhagem celular de fibroblasto de pulmão murinho
MTT	3-(4,5-Dimetiltiazol-2-il)2,5-Difenil Brometo de Tetrazolium
<i>m/z</i>	Razão massa/carga
MeOH	Metanol
MS/MS	Espectrometria de massas sequencial
PC3	Linhagem celular de câncer de próstata
RPMI	Meio de cultura de tecido animal desidratado
SF-295	Linhagem celular de glioblastoma humano
SNB-19	Linhagem celular de glioblastoma humano
tR	Tempo de retenção
UHPLC	<i>Ultra high performance liquid chromatography</i>
UV-Vis	Ultravioleta/Visível

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1 INTRODUÇÃO

As plantas são constituídas por metabólitos primários, compostos necessários a suas funções básicas vitais, e metabólitos secundários de natureza química diversificada, como polifenóis, saponinas, flavonoides, alcaloides e terpenoides, que são importantes na adaptação ao ambiente e, portanto, na sobrevivência da espécie. Historicamente, as plantas também representam uma fonte importante de substâncias farmacologicamente ativas, contribuindo assim na prevenção ou tratamento de diferentes enfermidades (ALARA *et al.*, 2018) e nesse sentido, há um crescente interesse em investigar produtos naturais como fonte de novas moléculas bioativas (PITCHAI; RAJARETINAM; FREEMAN, 2019, LUTFI *et al.*, 2017).

Dentre os vários produtos naturais utilizados na medicina contra diferentes doenças, destaca-se a busca de novos medicamentos contra o câncer, uma das principais causas de morte em todo o mundo. Como a maioria dos medicamentos antineoplásicos específicos para o alvo não alcançou o resultado esperado até o momento, torna-se urgente a busca de novos medicamentos anticâncer que sejam seletivos e pouco tóxicos (DUTTA *et al.*, 2019).

A família Fabaceae/Leguminosae representa a terceira maior família de angiospermas, com 770 gêneros e aproximadamente 19.500 espécies. Fabaceae compreende também a maior família de plantas do Brasil, com ampla distribuição e uma estimativa de 2.834 espécies distribuídas por todo o domínio fitogeográfico da Floresta Amazônica, Mata Atlântica, Caatinga, Cerrado, Pampa e Pantanal (WINK, 2013; SILVA e ARAÚJO, 2020). O gênero *Chamaecrista* se destaca nessa família por possuir distribuição pantropical, compreendendo mais de 330 espécies, distribuídas principalmente em toda a América tropical, com algumas espécies nativas da África, Ásia e Austrália. Na flora brasileira, ocorre na Caatinga, Cerrado, mas principalmente nos campos rupestres e ribeirinhos, com registro de 256 espécies sendo que 207 delas são endêmicas (SANTOS, *et al.*, 2017; ZEFERINO *et al.*, 2019).

Nas últimas décadas a evolução em técnicas analíticas permitiu a documentação química de plantas ainda não estudadas, e a contribuição efetiva para o conhecimento da composição química de várias espécies não exploradas. A impressão digital química (*fingerprint*), por exemplo, tem sido amplamente usada para o controle de qualidade de produtos naturais complexos, incluindo medicamentos tradicionais e fitomedicamentos (Li *et*

al., 2019a), permitindo a avaliação da constituição e do potencial de compostos ativos em extratos de plantas, possibilitando ainda relacionar impressões digitais químicas e atividades farmacológicas, tais como os efeitos analgésicos e anti-inflamatórios de extratos (SHEN *et al.*, 2018).

Além do conhecimento químico, a investigação de atividades biológicas se destaca e nesse sentido, o zebrafish (*Danio rerio*) se tornou um modelo animal importante para a farmacologia comportamental devido ao seu tamanho pequeno, rápido desenvolvimento e utilidade para exames de fármacos comportamentais de alto rendimento. Além disso, compostos solúveis em água podem ser administrados por imersão do peixe na solução do medicamento, o que fornece um método não invasivo de administração (TRAN *et al.*, 2017). Esse modelo também é mais barato e mais fácil de replicar em qualquer laboratório de pesquisa, tornando-o vantajoso na busca de novos fármacos (MAGALHÃES *et al.*, 2018).

Nesse cenário, o zebrafish é considerado um excelente modelo animal e vem sendo utilizado em estudos de atividade antinociceptiva orofacial (SANTOS *et al.*, 2019; SILVA *et al.*, 2020; SOARES *et al.*, 2019), ansiedade (SHYKEN *et al.*, 2019) análises de qualidade de água (PARK *et al.*, 2019), bem como toxicidade (LIN *et al.*, 2017; ZHOU *et al.*, 2019).

Este trabalho apresenta os resultados obtidos na prospecção química e avaliação do potencial biológico de *Chamaecrista duckeana* e *Chamaecrista ensiformis*.

A elaboração dessa tese obedece aos padrões estabelecidos pela coordenação do curso de Pós-Graduação em Biotecnologia da Universidade Federal do Ceará, bem como as regras e normas básicas da ABNT.

2. CONSIDERAÇÕES SOBRE O GÊNERO *CHAMAECRISTA* SPP. E SEUS METABÓLITOS SECUNDÁRIOS

2.1. Considerações Botânicas

2.1.1 O Gênero *Chamaecrista*

O gênero *Chamaecrista* (L.) Moench pertence a subfamília Caesalpinioideae da família das Fabaceae, é constituído por cerca de 330 espécies no mundo mais comumente encontradas de África para a Ásia e também na América do Sul. Ocorrem como árvores, arvoretas, arbustos e subarbustos, com folhas com 1 a muitos pares de folíolos e nectários extraflorais. As inflorescências são simples, terminais, axilares e as flores pentâmeras, assimétricas; sépalas livres, equilongas, pétalas amarelas, ou amarelas com base avermelhada, algumas vezes laranjas. O fruto é um legume, plano com sementes lustrosas e lisas. No Brasil, são encontradas 256 espécies do gênero, com a maioria (159) ocorrendo somente em Minas Gerais (ZEFERINO *et al.*, 2019). Espécies de *Chamaecrista* são utilizadas nos sistemas indígenas de saúde na Índia e em vários outros países (SEETHAPATHY *et al.*, 2015).

2.1.2 *Chamaecrista duckeana*

Chamaecrista duckeana (P. Bezerra & Afr.Fern.) H. S. Irwin & Barneby (Figura 1) com ocorrência relatada no Bioma da Caatinga e período de floração na estação chuvosa. Conhecida popularmente como palma-do-campo, é uma espécie subarborescente que pode atingir até 1 m de altura. Suas flores são de tamanho médio, amarelas com manchas avermelhadas e possuem anteras poricidas (MAIA-SILVA *et al.*, 2012).

Figura 1 – Registro fotográfico de *Chamaecrista duckeana* coletada em Massapê/CE



Fonte: MGVSilva

Segundo dados do Flora do Brasil (2020a) *Chamaecrista duckeana* é nativa e endêmica do Brasil, com relação a distribuição geográfica tem ocorrências confirmadas no Nordeste (Ceará) e possíveis ocorrências nos estados de Alagoas, Bahia, Paraíba, Pernambuco, Piauí, Rio Grande do Norte e Sergipe.

2.1.3. *Chamaecrista ensiformis*

Chamaecrista ensiformis (Vell.) H. S. Irwin & Barneby (Figura 2) diferencia-se das demais espécies, por ser a única que possui porte arbóreo (podendo atingir até 7 m de altura), a floração ocorre em períodos variados, porém, frequentemente, há predominância de flores nos meses de agosto a novembro (QUEIROZ e LOIOLA, 2009).

Segundo dados do Flora do Brasil (2020b) *Chamaecrista ensiformis* não é endêmica do Brasil e possui distribuição Geográfica com ocorrências confirmadas no Norte (Amazonas, Pará), Nordeste (Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe), Centro-Oeste (Goiás, Mato Grosso) e Sudeste (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo) em domínios fitogeográficos da Amazônia,

Caatinga, Cerrado, Mata Atlântica nas vegetações do tipo Floresta Ciliar ou Galeria, Floresta Ombrófila (= Floresta Pluvial) e Restinga.

Figura 2 – Registro fotográfico de *Chamaecrista ensiformis* coletada em Caucaia/CE



Fonte: DR Lima

2.2. *Chamaecrista* spp. e seus metabólitos secundários

Das 330 espécies de *Chamaecrista* registradas, apenas seis (7) apresentam estudos químicos: *Chamaecrista absus*, *Chamaecrista dyphilla*, *Chamaecrista nictitans*, *Chamaecrista nigricans*, *Chamaecrista duckeana*, *Chamaecrista flexuosa* e *Chamaecrista greggii*. A pesquisa realizada revelou o registro de 132 compostos (Tabela 1), presentes em extratos das cascas, folhas, sementes e raízes destas espécies, além de um único trabalho sobre a composição química do óleo essencial das raízes de *C. duckeana*.

Revisão na literatura sobre estudos químicos realizados com espécies do gênero *Chamaecrista*, revela a pouca quantidade de trabalhos relatada até a atualidade. O primeiro trabalho a ser publicado foi de Barba *et al.* (1992) que relata o isolamento e a determinação de estrutura de cinco diterpenos incomuns de esqueleto cassano, das raízes de *Chamaecrista flexuosa* (L.) denominadas de chamaetexanos A, B, C, D e E, que foram obtidos em pequenas

quantidades. A determinação da estrutura dependeu quase inteiramente de técnicas espectroscópicas e de difração de raios-X (Tabela 1).

Lopes; Campos; Craveiro, (1993) analisaram o óleo essencial das raízes de *Chamaecrista duckeana* coletada na região de Pentecostes, município do Ceará, Brasil. As raízes foram destiladas a vapor para produzir um óleo essencial amarelo composto majoritariamente por estragol (78,5%), seguido de metil eugenol (18,0%), eugenol (3,0%) e um constituinte em menor teor não identificado (0,5%). A identificação dos constituintes foi realizada utilizando comparação de índices de Kóvats com padrões, pesquisa em espectroscopias, seguidos pela interpretação visual dos espectros de massas. A identificação dos principais constituintes, estragol e metil eugenol, foi confirmada também por RMN de carbono-13.

Barba; Díaz; Herz, (1994) continuaram os estudos de Barba *et al.* (1992) e identificaram a partir das raízes e cascas de *Chamaecrista greggii* um diterpeno de esqueleto cassano conhecido e um novo, um conhecido e um inédito bis-norcassano, 16 antraquinonas, ácido betulínico 3-O-coumarato e resveratrol, além de outros constituintes mais comuns (Tabela 1).

Mateos-Martín *et al.* (2014) e Quirós-Guerrero *et al.* (2019) estudaram *Chamaecrista nictitans*. Mateos-Martín *et al.* (2014) usaram LC-ESI-MS/MS de alta resolução para identificar os componentes fenólicos do extrato das partes aéreas da planta, esses constituintes foram atribuídos como responsáveis pelas propriedades antivirais, atuando contra o vírus do herpes simplex inibindo a fixação do vírus à célula e a transcrição secundária do vírus dentro das células em uma concentração de 200 µg / mL do extrato. Quirós-Guerrero *et al.* (2019) descreveram um modelo multivariado baseado na impressão digital por LC-MS/MS capaz de discriminar subespécies e variedades de *Chamaecrista nictitans* distribuídas na Costa Rica. A determinação da impressão digital química foi realizada em LC-MS (ESI-QTOF) e os principais compostos detectados e identificados putativamente incluíram oligômeros de proantocianidina, vários glicosídeos C- e O-glicosídeos flavonoídicos, além de flavonoides acetilados.

As frações de esteróis e álcoois triterpênicos oriundos das sementes de *Chamaecrista absus* apresentaram atividade antibacteriana contra as cepas *Listeria ivanovii* e *Bacillus subtilis*, também foi encontrada uma considerável capacidade antirradicalar em relação à atividade sequestrante de DPPH (SEBEI *et al.*, 2014).

Análise por GC-MS do extrato metanólico das folhas de *Chamaecrista nigricans* permitiu identificar três compostos alifáticos, além de três ácidos graxos e um derivado de inositol. Esta publicação também relata o isolamento de três antraquinonas, emodina, crisofanol e fisciona, que foram identificadas usando espectros de RMN de UV-VIS, IR, ^1H e ^{13}C (TANGAVELOU *et al.*, 2018). Estas três antraquinonas são consideradas importantes agentes biologicamente ativos pois apresentam uma variedade de propriedades, tais como laxantes, diuréticas, hepatoprotetoras, imunoestimulante, antifúngicas, antipsoriática, antivirais, e agentes antitumorais, o que torna estas substâncias bastante interessante na busca de novos compostos bioativos, potencialmente úteis comprovando a importância da espécie para o desenvolvimento de medicamentos contra várias doenças (MACEDO, SILVA, SILVA, 2016).

O estudo mais recente de espécies do gênero, foi realizado por Gomes *et al.* (2021), que investigou a composição química das folhas de *Chamaecrista diphylla*. Utilizando técnicas de desreplicação por espectrometria de massas, e a estratégia conhecida como *molecular network*, que se caracteriza por comparar os dados MS/MS com base na similaridade química, trinta e cinco compostos foram anotados pela primeira vez em *C. diphylla*, sendo trinta e dois deles relatados pela primeira vez no gênero.

A Tabela 1 apresenta todas as substâncias isoladas e identificadas desde 1993 em espécies de *Chamaecrista*, a parte botânica das plantas estudadas, as características experimentais de massas utilizadas na identificação das substâncias como os íons MS/MS e a intensidade dos picos. Utilizou-se o Scifinderⁿ com a palavra-chave “*Chamaecrista*”, para obtenção dos dados.

Tabela 1- Substâncias identificadas no gênero *Chamaecrista* relatadas na literatura

	Substância	FM	Íons de produto MS/MS em modo negativo/positivo (intensidade relativa, %)	Partes da planta	Espécie	Referência
1.	(Epi)afz-(epi)afz-(epi)fis-(epi)gui	C ₆₀ H ₅₀ O ₁₉	937 (3), 817 (5), 707 (2), 665 (8), 545 (100), 409 (16), 393 (12), 272 (18), 137 (3)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
2.	(Epi)afz-(epi)afz-A-(epi)cat	C ₄₅ H ₃₆ O ₁₆	695, 679, 559, 423, 271	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
3.	(Epi)afz-(epi)cat-(epi)afz	C ₄₅ H ₃₈ O ₁₆	707 (3), 697 (6), 561 (30), 409 (47), 393 (13), 271 (91), 137 (15)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
4.	(Epi)afz-(epi)fis-(epi)fis	C ₄₅ H ₃₈ O ₁₅	707 (9), 681 (26), 665 (51), 545 (100), 435 (28), 409 (68), 393 (29), 271 (47), 137 (24)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
5.	(Epi)cat-(epi)cat	C ₃₀ H ₂₆ O ₁₂	451 (12), 425 (75), 289 (100), 161 (72), 137 (10)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
6.	(Epi)cat-(epi)fis-(epi)fis	C ₄₅ H ₃₈ O ₁₆	681 (25), 561 (100), 409 (33), 391 (60), 289 (87), 271 (30), 137 (13)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
7.	(Epi)cat-(epi)fis-A-(epi)afz	C ₄₅ H ₃₆ O ₁₆	695, 679, 543, 423, 271	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)

8.	(Epi)fis-(epi)afz	$C_{30}H_{26}O_{10}$	435, 409, 393, 273, 271, 137	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
9.	(Epi)fis-(epi)afz-(epi)gui	$C_{45}H_{38}O_{14}$	691 (4), 665 (60), 545 (100), 411 (18), 409 (70), 271 (56), 255 (37), 161 (53)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
10.	(Epi)fis-(epi)cat	$C_{30}H_{26}O_{11}$	451, 409, 391, 289, 271, 245, 161, 137	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
11.	(Epi)fis-(epi)cat-(epi)afz-(epi)gui	$C_{60}H_{50}O_{20}$	953 (20), 833 (40), 707 (60), 681 (30), 561 (85), 435 (40), 409 (100), 272 (95), 161 (70)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
12.	(Epi)fis-(epi)cat-(epi)fis	$C_{45}H_{38}O_{16}$	723 (1), 681 (2), 561 (16), 409 (46), 393 (16), 289 (75), 272 (90), 137 (10)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
13.	(Epi)fis-(epi)cat-(epi)gui	$C_{45}H_{38}O_{15}$	681 (2), 561 (14), 545 (10), 409 (36), 271 (55), 161 (57)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
14.	(Epi)fis-(epi)cat-A-(epi)fis	$C_{45}H_{36}O_{16}$	721, 679, 561, 559, 435, 407, 287, 271, 161	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
15.	(Epi)fis-(epi)fis-(epi)afz	$C_{45}H_{38}O_{15}$	707, 681, 665, 545, 435, 409, 393, 271, 161	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
16.	(Epi)fis-(epi)fis-(epi)cat	$C_{45}H_{38}O_{16}$	723, 681, 561, 451, 409, 289, 271	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)

17.	(Epi)fis-(epi)fis-(epi)cat-(epi)fis	$C_{60}H_{50}O_{21}$	953 (30), 935 (11), 833 (54), 681 (67), 543 (70), 433 (34), 391 (75), 271 (100), 161 (55)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
18.	(Epi)fis-(epi)fis-(epi)fis-(epi)fis	$C_{60}H_{50}O_{20}$	953 (1), 817 (10), 665 (15), 545 (100), 435 (7), 393 (36), 273 (73), 161 (20)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
19.	(Epi)fis-(epi)fis-(epi)gui	$C_{45}H_{38}O_{14}$	691 (7), 665 (20), 545 (100), 393 (50), 271 (30), 161 (28)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
			691, 665, 649, 545, 409, 393, 273, 161			Mateos-Martín <i>et al.</i> (2014)
20.	(Epi)fis-(epi)fis-A-(epi)afz	$C_{45}H_{36}O_{15}$	690 (44), 663 (10), 543 (77), 432 (54), 378 (43), 272 (100), 161 (96)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
			705, 679, 663, 545, 543, 527, 433, 419, 407, 273, 271, 161			Mateos-Martín <i>et al.</i> (2014)
21.	(Epi)fis-A-(epi)cat	$C_{30}H_{24}O_{11}$	449, 289, 287, 273, 271, 161	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
22.	(Epi)fis-A-(epi)fis-(epi)afz	$C_{45}H_{36}O_{15}$	689 (23), 679 (43), 663 (15), 543 (56), 433 (40), 272 (66)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
23.	(Epi)gui-(epi)afz	$C_{30}H_{26}O_9$	393, 273, 257, 255, 137	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
24.	(Epi)gui-(epi)afz-(epi)fis-(epi)cat	$C_{60}H_{50}O_{20}$	937 (15), 801 (30), 691 (10), 649 (15), 529 (25), 393 (76), 377 (31), 257 (21), 137 (15)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)

25.	(Epi)gui-(epi)cat	$C_{30}H_{26}O_{10}$	393 (7), 289 (8), 255 (11), 137 (36)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
26.	(Epi)gui-(epi)cat-(epi)cat	$C_{45}H_{38}O_{16}$	681 (1), 545 (18), 419 (35), 401 (8), 393 (4), 255 (82), 137 (7)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
27.	(Epi)gui-(epi)cat-(epi)fis	$C_{45}H_{38}O_{15}$	665 (2), 545 (15), 393 (71), 256 (84), 137 (4)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
28.	(Epi)gui-(epi)cat-(epi)gui	$C_{45}H_{38}O_{14}$	691 (2), 665 (1), 545 (12), 393 (26), 256 (100), 137 (14)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
29.	(Epi)gui-(epi)cat-(epi)gui-(epi)gui	$C_{60}H_{50}O_{18}$	921 (31), 801 (30), 649 (21), 545 (100), 393 (40), 289 (30), 255 (47), 137 (14)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
30.	(Epi)gui-(epi)fis-(epi)fis	$C_{45}H_{38}O_{14}$	649 (60), 529 (100), 419 (69), 377 (28), 271 (56), 255 (37), 137 (48)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
31.	(Epi)gui-(epi)fis-(epi)fis-(epi)fis	$C_{60}H_{50}O_{19}$	921 (21), 801 (17), 691 (23), 649 (15), 529 (100), 409 (25), 375 (41), 255 (41), 137 (9)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
32.	1,4,5,6,7-Pentametoxi-2- metilntraquinona	$C_{20}H_{20}O_7$	372 (M ⁺ , 100)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
33.	1,5-Diidroxi-4,6,7-trimetoxi-2- metilntraquinona	$C_{18}H_{16}O_7$	344 (M ⁺ , 100), 329 (12,1), 326 (61,7)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)

34.	1,7-Dimetoxi-4-hidroxi-5,6-metilenodioxi-2-metilantraquinona	$C_{18}H_{14}O_7$	342 (M^+ , 100), 314 (17,7) 313 (13,3), 311 (14,0)	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994)
35.	1H-2-benzopirán-1-ona,6,8-dihidroxi-3-metil	$C_{10}H_8O_4$	176, 149	Folhas	Cdp	Gomes <i>et al.</i> (2021)
36.	2(3H)-Furanona, 4-acetil-3-(4-acetiloxi-3-metoxifenil)-5-(4-acetiloxifenil) [3S-(3 α ,4 β ,5 α)]	$C_{23}H_{22}O_9$	414 (M^+ - CO,10,3), 382 (M^+ -HOAc, 24,3), 354 (M^+ -OHOAc,100), 322 [M^+ -2HOAc, 73,1) ⁺ , 312 (53,1), 311 (52,8), 281 (45,7), 269 (31,2)	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994)
37.	2(3H)-Furanona, diidro-4-hidroxi-3-(4-hidroxi-3-metoxifenil)-5-(4-hidroxifenil)-, [3S-(3 α ,4 β ,5 α)]	$C_{17}H_{16}O_6$	317 (M^+ , 15,5), 289 (M^+ 11- CO, 100), 259 (82,5), 257 (33,3), 251 (55,1), 246 (64,1)	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994)
38.	2,4-Diacetil floroglucinol	$C_{10}H_{10}O_5$	191, 165, 123	Folhas	Cdp	Gomes <i>et al.</i> (2021)
39.	24-Metilenocicloartenol	$C_{31}H_{52}O$	440 (22), 425 (5), 422 (67), 407 (70), 379 (33), 353 (10), 300 (35), 216 (17), 203 (12), 187 (15), 175 (31), 161 (25), 147 (35), 135 (51), 107 (71), 95 (100)	Sementes	Cab	Sebei <i>et al.</i> (2014) Gunawan <i>et al.</i> (2006)
40.	3-O-Ácido betulínico <i>p</i> -coumarato	$C_{39}H_{54}O_5$	602 (M^+ , 3,7), 438 (14,1), 423 (9,3), 395 (10,3), 189 (40,4), 164 (20,6), 147 (100)	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994)
41.	4,5-Diidroxi-1,6,7-trimetoxi-2-metilantraquinona	$C_{18}H_{16}O_7$	344 (M^+ , 100), 329(23,0), 313 (50,8), 300 (26,6)	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994)

42.	4,6-Diidroxi-1,5,7-trimetoxi-2-metilntraquinona	C ₁₈ H ₁₆ O ₇	344 (100), 329 (13,5), 315 (15,8), 313 (16,6), 310 (15,4) 301(12,7)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
43.	4,6-Diidroxi-2-hidroximetil-1,5,7-trimetoxiantraquinona	C ₁₈ H ₁₆ O ₈	360 (M ⁺ , 100), 345 (26,5), 342 (38,9), 327 (29,6) 311 (20,3)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
44.	4,6-Diidroxi-5,7-dimetoxi-2-metilntraquinona	C ₁₇ H ₁₄ O ₆	314 (M ⁺ , 100), 296 (49,0), 267 (47,0)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
45.	4,7-Diidroxi-1,5,6-trimetoxi-2-metilntraquinona	C ₁₉ H ₁₈ O ₇	358 (M ⁺ , 100), 343 (21,3), 329 (15,0) 327 (17,6), 325 (14,2);	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
46.	4,7-Diidroxi-2-hidroximetil-1,5,6-trimetoxiantraquinona	C ₁₈ H ₁₆ O ₈	360 (M ⁺ , 100), 345 (24,0), 342 (32,0), 326 (67,9), 299 (63,6)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
47.	4-Hidroxi-1,5,6,7-tetrametoxi-2-metilntraquinona	C ₁₉ H ₁₈ O ₇	358 (M ⁺ , 100), 343 (21,3), 329 (15,0), 327 (17,6), 325 (14,2)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
48.	4-Hidroxi-2-hidroximetil-1,5,6,7-tetrametoxiantraquinona	C ₁₉ H ₁₈ O ₈	374 (M ⁺ , 100), 359(31,9), 356(29,7), 346(47), 325 (228), 313 (31,2);	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
49.	4-Hidroxi-5,6,7-trimetoxi-2-metilntraquinona	C ₁₈ H ₁₆ O ₆	328 (100), 313 (68,8), 310 (23,9)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
50.	5,6-Dimetoxi- 1,4,7-triidroxi-2-metilntraquinona	C ₁₇ H ₁₄ O ₇	330 (M ⁺ , 100), 312(16,8), 149 (7,1)	Raíces e cascás	Cgr	Barba, Diaz e Herz, (1994)
51.	5,7-Diidroxi-8-[3,4,5-triidroxi-6-(hidroximetil)oxan-2-il]cromen-4-ona	C ₂₂ H ₂₂ O ₁₃	447 (M – H) ⁻ , 369, 357, 327, 299, 285	Folhas	Cdp	Gomes <i>et al.</i> (2021)
52.	5-[4-[(2 <i>S</i> ,3 <i>R</i> ,4 <i>S</i> ,5 <i>S</i> ,6 <i>R</i>)-3,4,5-triidroxi-6-(hidroximetil) oxan-2- il]oxifenil]cromen-4-ona	C ₂₂ H ₂₂ O ₁₃	446, 299	Folhas	Cdp	Gomes <i>et al.</i> (2021)

53.	5-Hidroxi-1,4,6,7-tetrametoxi-2-metilantraquinona	$C_{19}H_{18}O_7$	358 (M^+ , 100), 343 (20,3), 327 (18,1), 325 (17,6);	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994)
54.	7-Hidroxi-2,5-dimetil-4H-cromen-4-ona	$C_{11}H_{10}O_3$	174, 161, 149	Folhas	Cdp	Gomes <i>et al.</i> (2021)
55.	9,12,13-Triidroxi-10(<i>E</i>),15(<i>Z</i>)-ácido octadecadienóico	$C_{18}H_{32}O_5$	291, 229, 211, 183, 171	Folhas	Cdp	Gomes <i>et al.</i> (2021)
56.	9,12,13-Triidroxi-octadec-10-enóico	$C_{18}H_{34}O_5$	311, 293, 229, 211, 183, 171	Folhas	Cdp	Gomes <i>et al.</i> (2021)
57.	Ácido 2-metil-butanóico	$C_5H_{10}O_2$	57, 102, 41, 74, 85	Folhas	Cni	Tangavelou <i>et al.</i> (2018) Gao <i>et al.</i> (2009)
58.	Ácido azeláico	$C_9H_{16}O_4$	181, 125	Folhas	Cdp	Gomes <i>et al.</i> (2021)
59.	Ácido betulínico	$C_{30}H_{48}O_3$	455,5	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994) Rhourri-Frih (2009)
60.	Ácido butanodióico 2-(4,4-dimethyl-2-metilenopentil)	$C_{12}H_{20}O_4$	209, 183, 165	Folhas	Cdp	Gomes <i>et al.</i> (2021)
61.	Ácido elágico	$C_{14}H_6O_8$	283, 257, 229, 173	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
62.	Ácido elágico pentosídeo	$C_{19}H_{14}O_{12}$	301	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)

63.	Ácido hidroxiocetadecatrienóico	$C_{18}H_{30}O_3$	275, 256, 224, 195	Folhas	Cdp	Gomes <i>et al.</i> (2021)
						Sebei <i>et al.</i> (2014)
64.	Ácido linoleico	$C_{18}H_{32}O_2$	279, 155 $[M-C_6H_{12}-H]^-$	Sementes	Cab	Golias <i>et al.</i> (2019)
						Songsong <i>et al.</i> (2015)
						Sebei <i>et al.</i> (2014)
65.	Ácido linolênico	$C_{18}H_{30}O_2$	277	Sementes	Cab	Kaanin-Boudraa <i>et al.</i> (2021)
66.	Ácido n-hexadecanóico	$C_{16}H_{32}O_2$	256 ($M^+ 56,9$), 228 (15,5), 213 (19,4), 185 (22,7), 157 (17,5), 129 (58,9), 97 (39,2), 83 (44,6), 73 (94,7), 60 ($C_2H_4O_2^+$ 100)	Folhas	Cni	Tangavelou <i>et al.</i> (2018)
						Badria <i>et al.</i> (2003)
67.	Ácido octadecanóico	$C_{18}H_{36}O_2$	257, 228, 214, 186, 157, 130, 97.	Folhas	Cni	Tangavelou <i>et al.</i> (2018)
						Liu <i>et al.</i> (2002)
68.	Ácido 3- β -O- <i>trans</i> -cafeoilbetulinico (Ácido piracênico)	$C_{39}H_{54}O_6$	641,4 ($M^+ Na^+$)	Raízes e cascas	Cgr	Barba, Diaz e Herz, (1994)
						Cho <i>et al.</i> (2013)
69.	Ácido sinápico	$C_{11}H_{12}O_5$	208, 193, 179, 164, 149	Folhas	Cdp	Gomes <i>et al.</i> (2021)
70.	7-hidroxi-2-(2-hidroxiopropil)-5-metilcromen-4-ona (Aloesol)	$C_{13}H_{14}O_4$	215, 189, 174 161, 149	Folhas	Cdp	Gomes <i>et al.</i> (2021)
71.	Apigenina	$C_{15}H_{10}O_5$	225, 183, 159, 151, 149, 117, 107	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
72.	Apigenina hexosídeo	$C_{21}H_{20}O_{10}$	269	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)

73.	Apigenina-6-C-hexosil-(1→2) ramnosídeo	C ₂₇ H ₃₀ O ₁₄	431(2), 413(70), 311(18), 293(100), 283(3), 174(1)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
74.	Apigenina-C-hexosídeo	C ₂₁ H ₂₀ O ₁₀	341(40), 311(100), 283(18), 269(5)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
75.	β-Amirina	C ₃₀ H ₅₀ O	426 [C ₃₀ H ₅₀ O, M ⁺] (16), 411 [M -Me] ⁺ (17), 408 [M - H ₂ O] ⁺ (18), 393 [M - Me-H ₂ O] ⁺ (34), 257 [M - C ₁₁ H ₂₁ O] ⁺ , (20), 218 [M- C ₁₄ H ₂₄ O] ⁺ (100), 207 [M - C ₁₆ H ₂₇ O] ⁺ (11), 203 [M - C ₁₅ H ₂₇ O] ⁺ (46), 189 [M - C ₁₆ H ₂₉ O] ⁺ (58)	Sementes	Cab	Sebei <i>et al.</i> (2014) Saeed <i>et al.</i> (2003)
76.	Sitosterol	C ₂₉ H ₅₀ O	397 (100), 413 (1,3), 411 (0,5)	Sementes	Cab	Sebei <i>et al.</i> (2014) Rozenberg <i>et al.</i> (2003)
77.	Campesterol	C ₂₈ H ₄₈ O	383 (100), 399 (1,6), 397 (0,9)	Sementes	Cab	Sebei <i>et al.</i> (2014) Rozenberg <i>et al.</i> (2003)
78.	Carviolina	C ₁₆ H ₁₂ O ₆	284, 256, 227, 199	Folhas	Cdp	Gomes <i>et al.</i> (2021)
79.	Cassiaoccidentalina A	C ₂₇ H ₂₈ O ₁₃	457(8), 395(37), 377(5), 367(15),321(69), 310(25), 293(100), 282(17),269(35), 151(3)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
80.	Cassiaoccidentalina B	C ₂₇ H ₂₈ O ₁₄	473(34), 471(2), 429(1), 411(48) 557, 531, 429, 411, 285	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019) Mateos-Martín <i>et al.</i> (2014)
81.	Chamaetexanes C-7β, 16-diidroxi-12-oxocassa-13,15-dieno	C ₂₀ H ₃₀ O ₃	318 (69), 300 (23), 289 (100) 271 (41)	Raízes	Cfl	Barba <i>et al.</i> (1992)

82.	Chamaetexanes D- metil 7 β -12 β -diidroxicass-13-en-15-in-17-oato	C ₂₁ H ₃₀ O ₄	347 (M ⁺ , 53), 329 (100)	Raízes	Cfl	Barba <i>et al.</i> (1992)
83.	Chamaetexanes E-15,16-Bis-nor-12-oxocassan-8 α H,17-olideo	C ₁₈ H ₂₄ O ₃	288 (M ⁺ , 100), 269 (23)	Raízes	Cfl	Barba <i>et al.</i> (1992)
84.	Chamaetexano A-15,17-epoxi-7 β -hidroxi-12-oxocassa-13 (15), 14 (17)-dieno	C ₂₀ H ₂₈ O ₃	316 (100)	Raízes	Cfl	Barba <i>et al.</i> (1992)
85.	Chamaetexano B - 7 β ,16-diidroxi-15,17-epoxi-12-oxocassa-13 (15), 14 (17)-dieno	C ₂₀ H ₂₈ O ₄	333 (M ⁺ , 100), 315 (26), 273 (26)	Raízes	Cfl	Barba <i>et al.</i> (1992)
86.	Chamaetexano C acetonídeo	C ₂₃ H ₃₄ O ₃	359 (M ⁺ H, 100), 301 (20) 283 (7);	Raízes e cascas	Cgr	Barba, Diaz e Herz, (1994)
87.	Cicloartenol	C ₃₀ H ₅₀ O	426 (52), 411 (60), 393 (43), 365 (28), 339 (31), 339 (10) 315 (14), 286 (61), 203 (15), 187 (15), 175 (35), 95 (62), 69 (100)	Sementes	Cab	Sebei <i>et al.</i> (2014) Gunawan <i>et al.</i> (2006)
88.	Colesterol	C ₂₇ H ₄₆ O	369 (100) 385 (2,0) 383 (1,5)	Sementes	Cab	Sebei <i>et al.</i> (2014) Rozenberg <i>et al.</i> (2003)
89.	Crisofanol	C ₁₅ H ₁₀ O ₅	270 (M ⁺ , 100), 239 (8), 226 (10), 197 (9), 152 (6), 127 (4)	Folhas	Cni	Tangavelou <i>et al.</i> (2018)
90.	δ -5-24-Estigmastadienol	C ₂₉ H ₄₈ O	395 [M+H-H ₂ O] ⁺	Sementes	Cab	Sebei <i>et al.</i> (2014) Lerma-García <i>et al.</i> (2011)

91.	δ -5-Avenasterol	C ₂₉ H ₄₈ O	395, 411, 409	Sementes	Cab	Sebei <i>et al.</i> (2014) Rozenberg <i>et al.</i> (2003)
92.	Diidrocaempferol	C ₁₅ H ₁₂ O ₆	269, 259, 243, 201, 151	Folhas	Cdp	Gomes <i>et al.</i> (2021)
93.	Éster 1, 2-benzenodicarboxílico, diisooctila	C ₂₄ H ₃₈ O ₄	149 (100), 279 (18), 43 (12)	Folhas	Cni	Tangavelou <i>et al.</i> (2018) Chen <i>et al.</i> (2005)
94.	Emodina	C ₁₅ H ₁₀ O ₅	270 (M ⁺ , 100), 242 (12), 213 (14), 185 (8), 155 (8), 141 (10).	Folhas	Cni	Tangavelou <i>et al.</i> (2018)
95.	Estragol	C ₁₀ H ₁₂ O	77, 117, 133	Raízes	Cdc	Lopes, Campos e Craveiro, (1993), Holzinger <i>et al.</i> (2005) Martins (2010)
96.	Eugenol	C ₁₀ H ₁₂ O ₂	164 (M ⁺ , 100), 149(34), 137(15), 131(20), 121(14), 104(14), 103(16), 91(15), 77(17)	Raízes	Cdc	Lopes, Campos e Craveiro, (1993) Nishida <i>et al.</i> (2004)
97.	Fisciona	C ₁₆ H ₁₂ O ₅	284 (M ⁺ , 100), 260 (18), 240 (12), 226 (11), 189 (6), 167 (7), 139 (10), 111 (4)	Folhas	Cni	Tangavelou <i>et al.</i> (2018)
98.	Fisetina	C ₁₅ H ₁₀ O ₆	239 (3), 228 (8), 162 (6), 149 (10) 239, 229, 162, 149, 135, 121	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019) Mateos-Martín <i>et al.</i> (2014)
99.	Isoorientina	C ₂₁ H ₂₀ O ₁₁	429, 369, 357, 327, 299, 285	Folhas	Cdp	Gomes <i>et al.</i> (2021)

100.	Isovitexina	$C_{21}H_{20}O_{10}$	413, 353, 341, 311, 283, 269	Folhas	Cdp	Gomes <i>et al.</i> (2021)
101.	Isovitexina-O-pentosídeo	$C_{30}H_{26}O_{12}$	431, 413, 353, 341, 311, 283, 269	Folhas	Cdp	Gomes <i>et al.</i> (2021)
102.	Caempferol-6-C-hexosil-(1→2)-ramnosídeo	$C_{27}H_{30}O_{15}$	473 (38), 447 (1), 429 (10), 357 (28), 327 (60), 298 (100), 285 (2) 255 (5)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
103.	Caempferol-O-(acetil) hexosídeopentosídeo	$C_{28}H_{30}O_{16}$	561 (8), 285 (61), 255 (22), 227 (6)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
104.	Caempferol-O-hexosídeo-O-ramnosil- hexosídeo	$C_{33}H_{40}O_{20}$	635 (3), 593 (100), 431 (1), 285 (35), 255 (13), 227 (3)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
105.	Caempferol-O-ramnosilhexosídeo- O- acetato	$C_{29}H_{32}O_{16}$	593 (1), 431 (2), 285 (100), 255 (5), 227 (4)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
106.	Luteolina	$C_{15}H_{10}O_6$	217, 199, 175, 151, 133, 107	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
107.	Luteolina dihexosídeo	$C_{27}H_{30}O_{16}$	447, 285	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
108.	Luteolina hexosídeo	$C_{21}H_{20}O_{11}$	285	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)
109.	Luteolina-6-C-hexosil-(1→2)-ramnosídeo	$C_{27}H_{30}O_{15}$	575 (24), 557 (1), 473 (28), 411 (100), 337 (46), 327 (10), 298 (9), 285 (17), 151 (6), 133 (4)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
110.	Luteolina-7-O-glicosídeo	$C_{21}H_{20}O_{11}$	357, 339, 327, 311, 299, 285, 255, 151, 133	Folhas	Cdp	Gomes <i>et al.</i> (2021)

111.	Luteolina-C-hexosídeo	$C_{21}H_{20}O_{11}$	429 (15), 411(2), 357 (90), 327 (100), 298 (13), 285 (10)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
112.	Octadecatrien (Z, Z, Z)-9, 12,15) noato de metila.	$C_{19}H_{32}O_2$	236, 108	Folhas	Cni	Tangavelou <i>et al.</i> (2018) Hejazi et al (2009)
113.	Metil eugenol	$C_{11}H_{14}O_2$	178 (M ⁺ , 100), 163 (30), 147 (29), 107 (19), 103 (18), 91 (19), 77 (8)	Raízes	Cdc	Lopes, Campos e Craveiro, (1993) Nishida <i>et al.</i> (2004)
114.	Metil-15,16-bisnor-7β-acetoxi- 12-oxocassan-8(14)-en-17-oato	$C_{21}H_{30}O_5$	363 (M ⁺ , 100)	Raízes e cascas	Cgr	Barba, Diaz e Herz, (1994)
115.	Naringenina	$C_{15}H_{12}O_5$	253, 227, 199, 177, 151	Folhas	Cdp	Gomes <i>et al.</i> (2021)
116.	Nitrato de n-nonila	$C_9H_{19}NO_3$	76, 46	Folhas	Cni	Tangavelou <i>et al.</i> (2018) Yang <i>et al.</i> (2019)
117.	1,3,6-triidroxi-8-metilxanten-9-ona (norlichexantona)	$C_{14}H_{10}O_5$	213, 171, 159, 137	Folhas	Cdp	Gomes <i>et al.</i> (2021)
118.	Orientina	$C_{21}H_{20}O_{11}$	429, 369, 357, 327, 299, 285	Folhas	Cdp	Gomes <i>et al.</i> (2021)
119.	Orientina-O-hexosídeo	$C_{30}H_{26}O_{13}$	575, 447, 429, 369, 357, 327	Folhas	Cdp	Gomes <i>et al.</i> (2021)
120.	Quercetina pentosídeo	$C_{20}H_{18}O_{11}$	301, 300, 271	Partes aéreas	Cnc	Mateos-Martín <i>et al.</i> (2014)

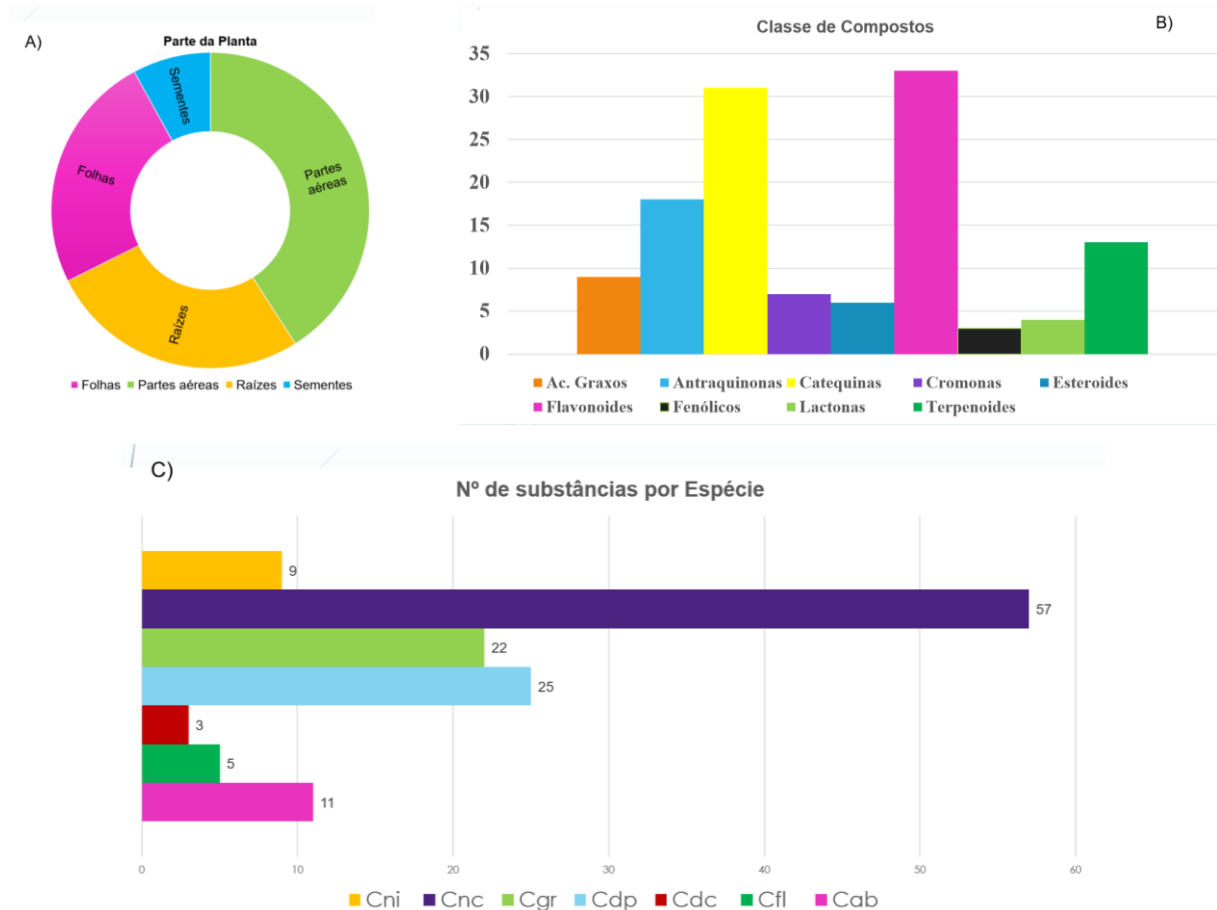
121.	Quercetina- <i>O</i> -ramnosil-(1→6)-hexosídeo	$C_{27}H_{30}O_{16}$	535 (4), 505 (6), 463 (13), 403 (5), 343 (8), 329 (10), 301 (66), 285 (100), 255 (17), 179 (9), 151 (10)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
122.	Quercetina- <i>O</i> -hexosídeo	$C_{21}H_{20}O_{12}$	343 (10), 301 (100), 285 (12), 255 (8), 243 (5), 151 (6) 300, 271, 179	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019) Mateos-Martín <i>et al.</i> (2014)
123.	Quercetina- <i>O</i> -hexosídeo- <i>O</i> -acetato	$C_{23}H_{22}O_{13}$	463 (4), 343 (0,8), 300 (100), 271 (18), 255 (8), 151(4)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
124.	Quercetina- <i>O</i> -ramnosídeo- <i>O</i> -ramnosilpentosídeo	$C_{32}H_{38}O_{19}$	593 (2), 463 (14), 389 (1), 301 (9), 284 (27), 255 (7), 243 (0,5), 151 (2)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
125.	Ramnetina- <i>O</i> -(acetil)hexosídeo	$C_{24}H_{24}O_{13}$	459 (0,6), 357 (3), 314 (100), 299 (18), 285 (50), 271 (53), 257 (16), 243 (41), 151 (2)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
126.	Ramnetina- <i>O</i> -hexosilramnosídeo	$C_{28}H_{32}O_{16}$	609 (30), 447 (5), 343 (6), 315 (35), 300 (2), 299 (22), 285 (5), 151 (1)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
127.	Ramnetina- <i>O</i> -pentosídeo-hexosídeo- <i>O</i> -Acetato	$C_{29}H_{32}O_{17}$	609 (5), 519 (2), 314 (100), 299 (38), 285 (3), 255 (1), 151 (3)	Partes aéreas	Cnc	Quirós-Guerrero <i>et al.</i> (2019)
128.	Resveratrol	$C_{14}H_{12}O_{13}$	(56), 165 (18), 107 (10), 85 (100), 57 (8)	Raíces e cascas	Cgr	Barba, Diaz e Herz, (1994) Polunin e Schmalz 2004
129.	Rutaretina	$C_{14}H_{14}O_5$	246, 231, 217, 203	Folhas	Cdp	Gomes <i>et al.</i> (2021)
130.	Estigmasterol	$C_{29}H_{48}O$	412 (M ⁺ , 19), 379 (5), 351 (9), 300 (12), 255 (30), 213 (12), 83 (64), 55 (100)	Sementes	Cab	Sebei <i>et al.</i> (2014) Itoh <i>et al.</i> (2003)

131.	Toracrisona-8-hexosilpentosídeo isômero	$C_{25}H_{32}O_{13}$	245, 230, 215	Folhas	Cdp	Gomes <i>et al.</i> (2021)
132.	Vitexina	$C_{21}H_{20}O_{10}$	413, 353, 341, 311, 283, 269	Folhas	Cdp	Gomes <i>et al.</i> (2021)

Cab; *Chamaecrista absus*; Cdp: *Chamaecrista dyphilla*; Cnc: *Chamaecrista nictitans*; Cni: *Chamaecrista nigricans*; Cdc: *Chamaecrista duckeana*; Cfl: *Chamaecrista flexuosa*; Cgr: *Chamaecrista greggii*

A análise dos dados sumarizados da Tabela 1 produziu o gráfico abaixo.

Gráfico 1 - Parte da planta estudada (A); Classe de compostos identificadas em *Chamaecrista* spp. (B) e nº de substâncias por espécie (C).



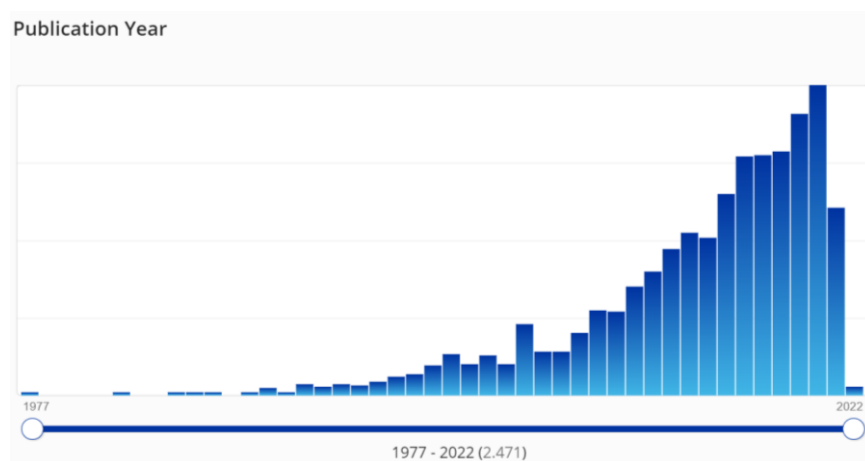
Fonte: DR Lima

Observou-se que a espécie que tem mais estudos relatados é *C. nictitans* e que a classe de compostos mais frequente no gênero são os flavonoides, seguidos de catequinas. O gênero apresenta uma classe rara de diterpenos, com esqueleto cassano, denominados de chamaetexanos e com ocorrência bastante limitada a certos subgrupos da família Leguminosae. Para a composição química do óleo essencial neste gênero, existe apenas um relato, em que o óleo foi extraído das raízes, indicando que o gênero não é rico em voláteis. Em relação a parte da planta estudada, apesar de espécies de *Chamaecrista* apresentarem flores abundantes, não foi encontrado nenhum relato para estudo químico ou biológico, para esta parte da planta isoladamente, embora nos trabalhos que envolvem a parte aérea os autores podem ter utilizado também as flores.

3 ZEBRAFISH COMO MODELO DE EXPERIMENTAÇÃO ANIMAL

Em busca, realizada até 25 de janeiro de 2022 na base de dados Scifinderⁿ, pelos conceitos “zebrafish and natural product” foram encontradas 2471 referências (Figura 3), com a primeira de 1977. As publicações estão distribuídas nos seguintes tipos de documentos da base de dados: Journal (2.390), Patent (68), Review (164), Clinical Trial (1), Commentary (3), Conference (8), Dissertation (3), Letter (3), Preprint (12) e Report (6) Nessa busca as seções com maior número de trabalhos associados foram: Toxicology (621), Pharmacology (431), Nonmammalian Biochemistry (408), Biochemical Genetics (190), Pharmaceuticals (79), Biochemical Methods (73), Mammalian Pathological Biochemistry (68), Immunochemistry (66), Water (44), Plant Biochemistry (40), Microbial, Algal, and Fungal Biochemistry (38), Mammalian Biochemistry (37), General Biochemistry (28) e Enzymes (26).

Figura 3 – Pesquisa bibliográfica na base de dados Scifinderⁿ sobre “zebrafish and natural product”



Fonte: Scifinderⁿ

3.1 Zebrafish como modelo animal para investigar atividades de compostos bioativos

Nas últimas décadas, o uso do Zebrafish tem se revelado um modelo animal extremamente interessante para experimentos em diversas atividades farmacológicas devido ao seu tamanho, rápido desenvolvimento e baixo custo. A evolução nos relatos científicos como apresentada na Figura 3, foi muito significativa e diversificada. Alguns recortes destes relatos são comentados em seguida.

O Eugenol [2-metoxi-4-(2-propenil)-fenol] além de vastas aplicações farmacológicas, tem amplo uso como anestésico para organismos aquáticos devido ao seu baixo preço e disponibilidade. Para testar a eficácia analgésica pós-exposição de baixas doses

de eugenol em zebrafish, Baldisserotto, Parodi e Stevens (2018) analisaram o comportamento da natação em peixes (velocidade mediana, tempo de imobilidade, natação em alta velocidade e distância movida na direção vertical) foi registrado em uma arena de vídeo de 1,6 L antes e após a exposição ao eugenol (0, 1, 2, 5, 10 e 20 mg/L). Em um segundo experimento, os peixes foram anestesiados com 2-fenoxi-etanol e tratados com uma injeção de ácido acético a 5% (estímulo nocivo), e depois expostos a 0, 1, 2 e 5 mg/L de eugenol. O comportamento de natação dos peixes também foi registrado. As doses mais altas (10 e 20 mg/L) reduziram a velocidade mediana, natação em alta velocidade e distância movidas na direção vertical e aumentaram o tempo de imobilidade. O comportamento dos peixes não foi alterado pelo eugenol (1, 2 e 5 mg/L) após estimulação nociva. Com este estudo concluiu-se que a mudança no comportamento de zebrafish associada a um estímulo nocivo pode ser monitorada e é um bom modelo para o estudo da analgesia. A resposta após um estímulo nocivo não foi afetada pela exposição a doses mais baixas, não sendo recomendado seu uso como analgésico.

Agatisflavona, um biflavonoide isolado das folhas de *Schinus polygamus* (Cav.) Cabrera é relatado pelas várias atividades biológicas como propriedades anti-inflamatórias, promovendo a cognição e prevenindo o câncer. Dumitru *et al.* (2019) testaram a hipótese de que ansiedade, amnésia e estresse oxidativo cerebral induzido pela escopolamina poderiam ser neutralizados por agatisflavona em modelo de zebrafish. A ansiedade e o comprometimento da memória foram induzidos com escopolamina e medidos com o teste de mergulho em tanque (NTT) e o teste do labirinto em Y. O estado oxidativo do cérebro e a atividade da acetilcolinesterase (AChE) também foram investigados. Os resultados demonstraram que agatisflavona reverteu significativamente a alteração comportamental induzida pela escopolamina nos testes de NTT e labirinto em Y. Consequentemente, a agatisflavona promoveu a inibição da atividade da AChE promoveu a ação antioxidante e melhorou os déficits de ansiedade e memória induzidos pela escopolamina.

Psoraleno, um composto ativo de ervas chinesas como *Psoralea corylifolia* L., amplamente utilizado na medicina tradicional para tratamento de psoríase, vitiligo, osteoporose, osteossarcoma, fratura óssea e osteomalácia. A toxicidade do psoraleno é uma questão de grande preocupação. No entanto, não havia um ensaio para a toxicidade induzida por este composto. Assim, Xia *et al.* (2018) tentaram desenvolver um ensaio de toxicidade induzido por psoraleno em embriões/larvas de zebrafish. O tratamento com psoraleno causou uma diminuição na taxa de eclosão e comprimento do corpo e um aumento significativo na taxa de malformação do peixe-zebra. Edema pericárdico, deficiência de bexiga natatória e

formato corporal curvo também foram observados após o tratamento. Outras experiências indicaram que o psoraleno exerceu efeitos tóxicos no coração, fígado, fagócitos e sistema nervoso em desenvolvimento. O aumento da geração de espécies reativas de oxigênio, a inibição da atividade total da superóxido dismutase e o aumento das concentrações de malondialdeído indicaram inibição da capacidade antioxidante e presença de estresse oxidativo. Além disso, os resultados da análise de expressão gênica mostraram que o psoraleno induziu toxicidade no desenvolvimento por meio de estresse oxidativo, apoptose e anormalidades no metabolismo energético. Esses resultados foram úteis no entendimento da toxicidade induzida por psoraleno e deverão ser verificadas em outros modelos animais.

O diabetes mellitus é uma doença metabólica caracterizada por hiperglicemia crônica que induz outras patologias, incluindo retinopatia diabética e doença óssea. *Danio rerio* adulto (peixe-zebra) representa um modelo poderoso para estudar o metabolismo da glicose e do osso. O objetivo deste estudo foi avaliar os efeitos da liquiritigenina (LTG) no nível de glicose no sangue e nas complicações do diabetes em zebrafish adulto hiperglicêmico. LTG é um flavonoide extraído de *Glycyrrhiza glabra* raízes que possui importantes propriedades antioxidantes, anti-inflamatórias e antidiabéticas. Durante quatro semanas de tratamento com glicose, o LTG impediu significativamente o aparecimento da hiperglicemia em zebrafish adulto. Além disso, peixes hiperglicêmicos apresentaram níveis avançados de produtos finais de glicação avançada (AGEs) e paratormônio, enquanto o LTG impediu completamente essas duas alterações metabólicas. Grandes áreas de perda óssea foram encontradas nas escamas de peixes tratados com glicose, enquanto apenas pequenas lacunas de reabsorção foram detectadas após o tratamento com glicose/LTG. Ensaios bioquímicos e histológicos de fosfatase ácida resistente a tartarato (TRAP) confirmaram que o LTG impediu o aumento da atividade osteoclástica em peixes hiperglicêmicos. A análise da expressão gênica mostrou que o LTG impede a alteração em genes reguladores ósseos cruciais. Este estudo confirmou que a LTG é uma abordagem terapêutica natural muito promissora para a redução da glicose no sangue e para contrastar o desenvolvimento de complicações ósseas correlacionadas à hiperglicemia crônica (CARNOVALI *et al.*, 2019).

Os frutos de *Garcinia xanthochymus* são comestíveis e também utilizados na medicina tradicional. Os produtos naturais isolados de *G. xanthochymus* apresentam atividade antioxidante e citotoxicidade nas células cancerígenas do cólon. Por esse motivo, Li *et al.* (2017) combinaram ensaios de zebrafish com um método estatístico quimiométrico baseado em UPLC-QTOF-MS, para identificar possíveis compostos de atividade antiangiogênica de

G. xanthochymusfrutas. Nos resultados, tanto amentoflavona quanto a fukugetina, que são biflavonoides, pela primeira vez, exibiram efeitos antiangiogênicos em zebrafish, demonstrando uma estratégia rápida e eficaz para identificar produtos naturais para a atividade antiangiogênese.

3.2 Toxicidade frente a Zebrafish

Zhou *et al.* (2019) avaliaram a toxicidade e os efeitos no desenvolvimento de captan (usado como fungicida para o tratamento de doenças foliares, transmitidas pelo solo e por sementes) em diferentes estágios da vida (embrião e adulto em Zebrafish). Os resultados mostraram que a concentração letal de 96 horas (CL_{50}) dos peixes embrião e adulto (expostos a captan) foi de 0,81 (0,75-0,87) mg/L e 0,65 (0,62-0,68) mg/L, respectivamente. Os resultados mostraram que captan pode diminuir significativamente os batimentos cardíacos e inibir a taxa de eclosão e o crescimento de embriões de zebrafish. Além disso, a exposição ao captan pode induzir uma série de deformidades. Os resultados forneceram uma base científica para a correta aplicação e avaliação de risco do captan em ambientes.

A toxicidade do paracetamol [acetaminofeno (APAP)] é a base universal da hepatotoxicidade aguda induzida por drogas. No trabalho de Swarnalatha (2017) foi induzido a hepatotoxicidade no modelo de zebrafish usando acetaminofeno e estudado o papel protetor da heptametoxiflavona, isolada da erva daninha *Sphaeranthus amaranthoides* Burm. O acetaminofeno esgotou os antioxidantes, como os níveis de GST, SOD e CAT, elevou as enzimas marcadoras hepáticas (ALT e AST), induziu apoptose e causou necrose de hepatócitos. O tratamento com heptametoxiflavona mostrou papel protetor em embriões de peixe-zebra e em adultos. Aumentaram os níveis de enzimas antioxidantes e normalizou os marcadores hepáticos comparando significativamente os grupos controle e induzido por acetaminofeno. Os resultados deste trabalho confirmaram o papel protetor da heptametoxiflavona isolada de *Sphaeranthus amaranthoides* contra a hepatotoxicidade induzida pelo acetaminofeno no modelo testado (zebrafish).

O glioblastoma é o tumor cerebral primário mais comum e mais maligno, com sobrevida mediana de 15 meses. Lazari *et al.* (2017) procurou determinar se N-(*p*-coumaroil) serotonina (CS), um alcaloide indólico com efeitos antioxidantes e cardioprotetores após isquemia e atividade antitumoral, poderia exercer efeitos citotóxicos e citostáticos em células de glioma *in vitro*. Neste estudo, zebrafish foi utilizado para avaliar a possível toxicidade da exposição de embriões ao N-(*p*-coumaroil) serotonina, para isso, mediram a mortalidade

durante um período de observação contínuo e como resultado, a mortalidade dos animais no quinto dia pós-fertilização foi zero em todas as concentrações de CS testadas, até 1 mM, sendo assim, o tratamento de linhagens celulares de glioblastoma com CS pode representar uma nova estratégia para direcionar o glioblastoma.

4 POTENCIAL CITOTÓXICO DE PRODUTOS NATURAIS

O câncer é o principal problema de saúde pública no mundo e já está entre as quatro principais causas de morte prematura (antes dos 70 anos) na maioria dos países. No Brasil, a estimativa para cada ano do triênio 2020-2022 indica que haverá 625 mil novos casos de câncer. Ao analisar leucemia, esse número chega a 5.920 novos casos para homens e 4.890 para mulheres, correspondendo a um risco estimado de 5,67 casos novos a cada 100 mil homens e 4,56 para cada 100 mil mulheres. Na Região Nordeste, a incidência do câncer do colo do útero e de estômago tem impacto importante, apesar de apresentarem os cânceres de próstata e mama feminina como principais na população (INCA, 2019).

Em busca, realizada até 27 de fevereiro de 2022 na base de dados Scifinderⁿ, pelo conceito “*phytochemicals*” e refino por “*câncer*”, 20.017 biocompostos diferentes que podem ter efeitos potenciais contra vários tipos de câncer são relatados em 11.211 referências.

Produtos naturais de plantas são fontes para terapia do câncer, principalmente pela capacidade antioxidante, inibindo processos oxidativos (CHOUDHARI *et al.*, 2020). Além disso, eles são reconhecidos como “captadores de radicais livres”, formando espécies radicalares mais estáveis. Os antioxidantes podem reduzir a ocorrência de diferentes doenças como: envelhecimento, câncer, distúrbios neurodegenerativos, diabetes, inflamação, doença hepática, doença cardiovascular, catarata e nefrotoxicidade. Antioxidantes naturais ou sintéticos podem ser usados como ferramentas para prevenção precoce dessas doenças (NEHA *et al.*, 2019). Entre as principais classes de compostos quimiopreventivos encontrados em frutas e vegetais estão carotenoides, polifenólicos, vitaminas, catequinas, flavonoides, além das substâncias curcumina, resveratrol, genisteína e quercetina.

Os polifenólicos são os componentes bioativos com ação antioxidante que ocorrem naturalmente em frutas e vegetais estando presente de forma abundante na dieta humana. Estudos estão sugerindo que a ingestão de polifenóis pode ser útil para melhorar síndromes metabólicas que podem contribuir na prevenção de várias doenças crônicas como diabetes, obesidade, hipertensão e câncer (SHABBIR *et al.*, 2021). A catequina denominada 3-galato-epigallocatequina (EGCG), um composto ativo do chá verde (*Camellia sinensis*), é um exemplo de polifenol com papel na cura e prevenção de doenças já comprovado. Seu papel no manejo de doenças pode ser atribuído às suas propriedades antioxidantes e anti-inflamatórias. O EGCG provou possuir um efeito quimiopreventivo por meio da inibição do processo de carcinogênese, como iniciação, promoção e progressão. Além disso, esta catequina provou seu papel no tratamento do câncer através da modulação de várias vias de

sinalização celular, como regulação da proliferação, apoptose, angiogênese e morte de vários tipos de células cancerígenas. O efeito aditivo ou sinérgico da epigallocatequina com agentes quimiopreventivos reduz as toxicidades e potencializa os efeitos anticancerígenos (ALMATROODI *et al.*, 2020). O resveratrol, um composto fenólico natural, com excelente capacidade antioxidante e antienvhecimento, tem citotoxicidade significativa em linhagens celulares cancerígenas, podendo desempenhar um papel tóxico através da indução da apoptose da célula cancerosa de maneira dependente do tempo e da concentração (WU *et al.*, 2019).

Em uma recente revisão da literatura Greco *et al.* (2021) descreve criticamente o potencial anticancerígeno das antraquinonas, seu potencial genotóxico e mutagênico. Algumas antraquinonas, como emodina e fisiona são citadas como um novo recurso no arsenal para combater o câncer. Aloe-emodina, é um importante ingrediente antraquinônico de vários medicamentos tradicionais chineses com uma variedade de atividades biológicas benéficas *in vitro*, incluindo citotoxicidade e genotoxicidade (LI *et al.*, 2019b).

Terpenos e terpenoides também se constitui em uma classe de substâncias conhecidas por demonstrar uma ampla variedade de atividades biológicas em vários modelos de doenças humanas (KIM *et al.*, 2020) incluindo atividade antitumoral.

Os flavonoides, amplamente distribuídos no reino vegetal, possuem uma ampla gama de atividades biológicas, tais como: agentes antitumorais, anti-inflamatórios e anti-Alzheimer e têm sido apontados como potenciais quimioterápicos porque são tóxicos para as células cancerígenas, mas não prejudiciais às células saudáveis (BISOL, CAMPOS e LAMERS, 2021; JUCÁ *et al.*, 2020).

Erazua *et al.* (2019) avaliaram as atividades anticancerígenas de onze flavonoides: luteolina, apigenina, crisina, quercetina, galangina, hesperetina, naringenina, taxifolina, daidzeína, kaempferol e genisteína. Esses flavonoides foram testados contra linhagem celular de leucemia usando o método químico quântico através da teoria do funcional da densidade (DFT) e abordagem de docagem molecular. Esses flavonoides foram acoplados à linhagem de células de leucemia (PDB: 1AOI) e a correlação entre os descritores calculados e sua afinidade de ligação com células de leucemia foi examinada. Os resultados mostraram que a taxifolina apresentou a maior eficiência de inibição contra a linhagem de células leucêmicas entre os compostos estudados, enquanto a daidzeína apresentou a menor eficiência de inibição. Esses resultados reforçam os flavonoides como potenciais agentes terapêuticos.

Caempferol-3-O- β -D-glicopiranosídeo isolado de *Wedelia chinensis* por Zhong *et al.* (2018), foi relatado com ação anti-inflamatória comparável ao do resveratrol, que foi

extensivamente estudado por pesquisadore. Esse composto é uma tetrahidroxiflavona natural que também pode ser isolada da casca de *Harungana madagascariensis*. Em estudo recente, foi listado como um potente composto citotóxico que pode ser utilizado para quimioterapia como agente anticancerígeno (SIVAS, KARAOSMANOGLU e KUETE, 2019).

O sesquiterpeno eupalinolideo A, isolado de *Psiadia punctulata* tem sido relatado na literatura por sua potente citotoxicidade. Este composto foi testado por Yang *et al.* (2007), quanto à citotoxicidade contra a célula de câncer de pulmão A549, célula de câncer de glândula gástrica BGC-823, célula de câncer de fígado SMMC-7721 e célula leucêmica HL60. Os resultados mostraram a potente citotoxicidade dessa substância contra as linhagens tumorais A-549, BGC-823 e HL60. Eupalinolide A também tem sido relatado como um agente anti-inflamatório natural e apresentou potente citotoxicidade contra as linhagens celulares de leucemia NB4 e K562, pelo método MTT (WU *et al.*, 2012).

O composto siringina detectado por Lall *et al.* (2015) no extrato etanólico de *Foeniculum vulgare* exibiu toxicidade significativa contra as linhagens cancerígenas MCF-7, HeLa e DU145. Anteriormente, o extrato também exibia toxicidade significativa contra a linhagem HeLa. Sendo assim, a atividade citotóxica foi associada a presença dessa substância. Em outra pesquisa, o mesmo composto foi anotado no extrato das flores de *Centaurea ragusina* L. usando cromatografia líquida de alta eficiência, o extrato exibiu atividade contra linhagens celulares de câncer de bexiga humano (T24) e glioblastoma humano (A1235) (RADAN *et al.*, 2017).

Procianidina B2 foi relatada por Quirós Guerrero *et al.* (2019) em 40 amostras de *Chamaecrista nictitans* coletadas em diferentes locais do Vale Central e da costa do Pacífico da Costa Rica. Essa substância mostra uma ampla atividade anticancerígena em várias células cancerígenas humanas. No câncer gástrico, por exemplo, esse composto exerce efeitos antiproliferativos e apoptóticos e induz autofagia sendo considerada potencial para o desenvolvimento de fármaco terapêutico para esse câncer (LI *et al.*, 2021).

As diferentes classes de metabólitos secundários estudadas evidenciam a importância de compostos com potencial citotóxico oriundos de produtos naturais. Sendo assim, os compostos já conhecidos e extensivamente relatados na literatura por suas diversas propriedades são a chave para a investigação e aplicação biotecnológica de espécies pouco estudadas como *Chamaecrista duckeana* e *Chamaecrista ensiformis*.

5 OBJETIVOS

5.1 Objetivo geral

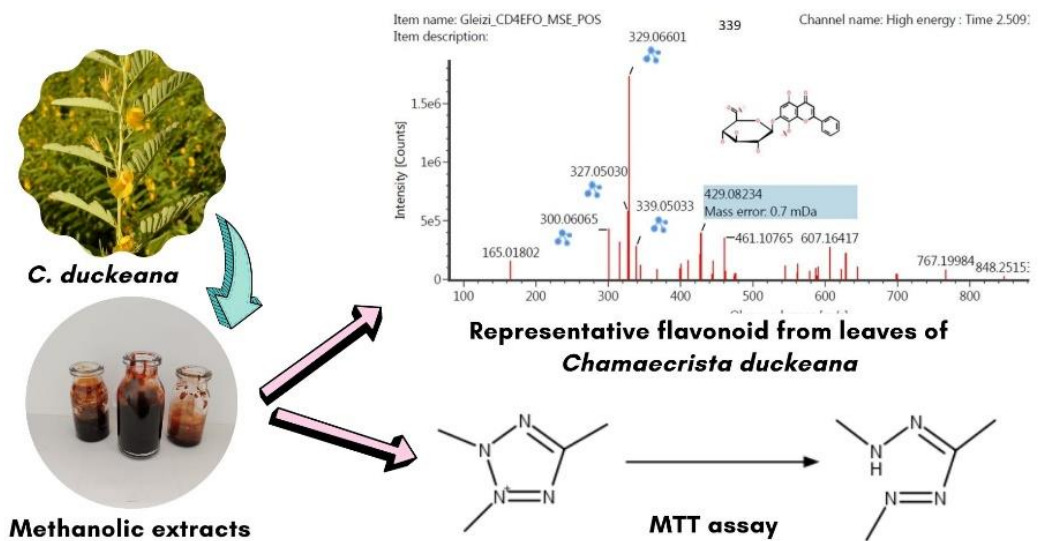
Realizar a prospecção química e avaliação do potencial biológico de *Chamaecrista duckeana* e *Chamaecrista ensiformis*.

5.2 Objetivos específicos

- Caracterizar e documentar o perfil cromatográfico das espécies *Chamaecrista duckeana* e *Chamaecrista ensiformis*;
- Identificar novas fontes de agentes antioxidantes;
- Investigar atividades antiacetilcolinesterásica e citotóxica dos extratos.
- Utilizar o modelo do zebrafish para avaliar a toxicidade de extratos de *Chamaecrista ensiformis*;
- Colaborar para o desenvolvimento da biotecnologia de recursos naturais, ainda insipiente no Brasil;
- Contribuir para a valorização e conservação da biodiversidade brasileira.

6 METABOLIC PROFILING AND CYTOTOXIC ACTIVITY OF METHANOL EXTRACTS FROM *CHAMAECRISTA DUCKEANA* (P. BEZERRA & A. FERN.) H. S. IRWIN & BARNEBY (LEGUMINOSAE, CAESALPINIOIDEAE)

Aceito na Revista Química Nova em 10 de fevereiro de 2022



Representative identification of flavonoid from *Chamaecrista duckeana*

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Química Nova - Decision on Manuscript ID QN-2021-0348.R1

Jorge David <onbehalf@manuscriptcentral.com>
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Para: mgvsilva@ufc.br

10 de fevereiro de 2022 08:51

10-Feb-2022

Dear Prof. Silva:

It is a pleasure to accept your manuscript entitled "METABOLIC PROFILING AND CYTOTOXIC ACTIVITY OF METHANOL EXTRACTS FROM *CHAMAECRISTA DUCKEANA* (P. BEZERRA & A. FERN.) H. S. IRWIN & BARNEBY (LEGUMINOSAE, CAESALPINIOIDEAE)" in its current form for publication in the *Química Nova*.

You will receive soon an e-mail from QN Office (quimicanova@sbq.org.br) to proceed the fee payment.

The proofing production will be proceeded only after payment verification by QN Office.

Thank you for your fine contribution. On behalf of the Editors of the *Química Nova*, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Jorge David
Associate Editor, *Química Nova*
jorgemaucio.david@gmail.com

**METABOLIC PROFILING AND CYTOTOXIC ACTIVITY OF METHANOL
EXTRACTS FROM *CHAMAECRISTA DUCKEANA* (P. BEZERRA & A. FERN.) H. S.
IRWIN & BARNEBY (LEGUMINOSAE, CAESALPINIOIDEAE)**

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(X) Manuscrito com material suplementar

() Manuscrito sem material suplementar

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**METABOLIC PROFILING AND CYTOTOXIC ACTIVITY OF METHANOL
EXTRACTS FROM *CHAMAECRISTA DUCKEANA* (P. BEZERRA & A. FERN.) H. S.
IRWIN & BARNEBY (LEGUMINOSAE, CAESALPINIOIDEAE)**

Abstract

The genus *Chamaecrista* comprises more than 330 species, with only a few studies on their chemical composition and biologic activities. In this study, the phytochemical profile of leaf, stems, and fruits extracts of the *C. duckeana* were examined by UPLC-ESI-HRMS analysis to determine possibly bioactive constituents. The antioxidant activity was carried out through in vitro assay, by the sequestration of the free radical DPPH. To evaluate the cytotoxic activity of the extracts, an MTT assay was used and the IC₅₀ was determined against HL60 and RAJI cell lines. The metabolic profiles of the botanical parts are dominated by flavonoid class, highlighting isoflavonoids such as daidzin and ononin. All these compounds are reported for the first time in *C. duckeana*. The extracts presented antioxidant potential, and the activity of the stems extract was higher than the standard butylated hydroxytoluene. In the cytotoxic assay, only HL60 line (leukemia) had growth inhibition over 80 %. The stems presented more expressive cytotoxicity with IC₅₀ of 137.3 (104.6-180.1) and 106.8 (96.52-118.3) $\mu\text{mol. L}^{-1}$ for HL60 and RAJI, respectively. In conclusion, the present work provides an in-depth knowledge about the chemical profile of *C. duckeana*, a species rich in bioactive secondary metabolites with cytotoxic activity.

Keywords: *Chamaecrista duckeana*, UPLC-ESI-HRMS, cytotoxicity, isoflavonoids.

INTRODUCTION

Since ancient times, natural products have been used to treat human ailments. After recent developments and technological advances, natural products have received great attention as a source of new drug candidates, since they have the potential to discover new supports for therapeutic targets for the treatment of various diseases, including cancer.¹ Flavonoid class compounds, frequently identified in plants, are proposed as potential chemotherapeutics since flavonoids have a dual role, acting as antioxidants under normal conditions and as powerful pro-oxidants in cancer cells.² Mass spectrometry (MS) is a widely used method for detection, identification, and structural elucidation of flavonoids which, associated with the Ultra Performance Liquid Chromatography (UPLC), is an efficient tool for flavonoid analysis.³

Chemotherapy remains to be one of the main approaches in the clinical treatment of acute leukemia. This treatment strategy still has some limitations, such as side effects and multidrug resistance, which limits the therapeutic efficacy. Therefore, drugs for leukemia with lower toxicity and higher effectiveness are expected. Natural products are a source of inspiration in medicinal chemistry for biological activity, including antileukemic potential.⁴⁻⁷

The genus *Chamaecrista* has a pantropical distribution, comprising more than 330 species which are mainly distributed across tropical America, with a few species native to Africa, Asia, and Australia. It is well represented in the Brazilian flora, mainly in rupestrian and riparian fields where 256 species occur, with 207 of them being endemic.⁸ Species of this genus are widely used in traditional medicine in Africa, Asia, and Americas and they have been reported to be used for important pharmacological activities such as cholinesterase inhibitory activity, antitrypanosomal and anticonvulsant activity for *Chamaecrista mimosoides*,⁹⁻¹¹ and activity against bacterial cell lines of *Shigella sonnei* for *Chamaecrista desvauxii*.¹² *Chamaecrista diphylla*, *Chamaecrista repens* var. *multijuga*, and *Chamaecrista mimosoides* presented good antioxidant properties.^{10,13-15}

Chamaecrista duckeana is popularly known as “palma-do-campo”, and it is a subshrub species that can reach up to 1 m in height.¹⁶ In the only chemical study of the species, the GC-MS analysis of the essential oil extracted from the roots of the Brazilian *C. duckeana*, reported the presence of methyl chavicol, methyl eugenol, and eugenol.¹⁷ In this context, this work characterizes the chemical composition of *C. duckeana*, using dereplication methods by UPLC-ESI-HRMS, which is an efficient and rapid analytical tool to obtain the chemical

fingerprinting of the sample.¹⁸⁻²⁰ Furthermore, the evaluation of the antioxidant and cytotoxic properties against tumor cells of these extracts was performed.

EXPERIMENTAL

Chemicals and reagents

Solvent methanol (J. T. Baker, USA) was of chromatographic grade. The formic acid (Synth, Brazil) was of analytical grade (ACS). Ultrapure water was obtained using a Millipore water purification system (Millipore, USA). BHT, quercetin, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Sigma (USA).

Plant material collection

Leaves, stems, and fruits of *C. duckeana* (P. Bezerra & A. Fern.) H. S. Irwin & Barneby were collected in September 2018, in Massapê, Ceará, Brazil. *C. duckeana* was authenticated by the Federal University of Ceará Herbarium Prisco Bezerra (Ceara State, Brazil) as number 61466. The plant is registered in the National Genetic Heritage Management System (SISGEN) under the number AB06D11. After collection, leaves, stems, and fruits were kept in an oven at 40 °C until drying (from 12 to 24 h, depending on the collected material).

Plant material extraction

The dried and grounded samples were extracted with ultrasound assistance (UAE) in a ratio of 1 g of plant material to 30 mL of methanol three times, and then dried on a rotary evaporator. The dried extracts were weighed and stored at -18 °C until analysis.

Sample preparation for UPLC-ESI-HRMS analysis

The extract solutions were prepared at concentrations of 1 mg mL⁻¹ for UPLC-ESI-HRMS analysis and filtered on Chromafil®Xtra RC-20/25 membranes (0.20 µm pores) before analysis.

UPLC-ESI-HRMS analysis settings

The chromatography was performed on a 100×2.1 mm Waters® ACQUITY UPLC® 1.8 µm HSS T3 column in an ACQUITY Ultra Performance LC™ system equipped with a QTOF

mass spectrometer LC-MS/MS platform. The column was kept at 40 °C. Injection volume was 0.8 μL . The flow rate was set at 0.580 $\mu\text{L min}^{-1}$ and gradient elution was carried out with a binary system consisting of [A] 0.1 % aqueous formic acid (Synth, Brazil) and [B] 0.1 % formic acid (Synth, Brazil) in methanol (J. T. Baker, USA). The gradient elution was programmed as follows: 5–95% (B) from 0 to 4.31 min, 100 % (B) from 4.31 to 5.75 min. Data was collected in centroid mode, with a lock spray frequency of 10 s, and the data was averaged over 10 scans. Calibration with sodium formate (reference mass 860.8467 uma) was performed in positive mode within an m/z range between 100 to 1200 in positive ionization mode. MassLynx software (version 4.1, Waters) was used for data acquisition and processing. Experimental m/z and MS/MS spectra obtained at both higher and lower energy were used for compound matching across UNIFI platform from Waters Corporation, a database with more than 6000 compounds.

Cytotoxic assay

Cell lines culture

The human tumor cell lines SNB19 (central nervous system), HCT116 (human colon), PC3 (prostate), HL60, and RAJI (leukemia) were gently provided by the National Cancer Institute (Bethesda, MD, USA). The cells were maintained as monolayer cultures in appropriate media. The RPMI 1640 was supplemented with 10 % fetal bovine serum (FBS), 1 % antibiotic at 37 °C in a humidified atmosphere of 5 % CO_2 .

Determination of cytotoxicity

The cytotoxic effect of the samples, fruits, stems and leaves were determined by MTT assay.²¹ The three extracts were first tested against four human cancer cell lines: SNB19 (central nervous system), HCT116 (human colon), PC3 (prostate), and HL60 (leukemia). In this round of cytotoxicity evaluation, cell growth inhibition was estimated for all cell lines treated with the different extracts at a single concentration (100.0 $\mu\text{g mL}^{-1}$) for 72 hrs to determine the percentage of cell growth inhibition (GI %). In the second round, the half-maximal inhibitory concentration (IC_{50}) against HL60 and RAJI cell lines was determined. The cells were plated at concentrations of 0.3×10^6 cells mL^{-1} . After the incubation, the supernatant was replaced by a fresh medium containing MTT (100 μL). Three hours later, the formazan product was dissolved in 100 μL of DMSO, and the absorbance was measured at 595 nm (DTX-880

Multimode Detector, Beckman Coulter®). The samples were diluted in DMSO to stock concentrations of 20 mg mL⁻¹. To perform the single concentration test, all samples were tested on a concentration curve that ranged from 3.13 to 200 µg mL⁻¹.

Statistical analysis:

Single concentration experiments were analyzed according to the mean ± standard deviation (SD) of the percentage of cell growth inhibition from three experiments carried out in triplicate, using the GraphPadPrism 6 program.

Antioxidant assay

The antioxidant activity was determined using the 1,1-diphenyl-2-picryl- hydrazyl (DPPH) method, according to Yepes et al.²² In a test tube, 3.9 mL of a 6.5 × 10⁻⁵ mol L⁻¹ methanol solution of DPPH was mixed with 0.1 mL of each methanolic extract solution. After 60 min, the absorbance was read using a spectrophotometer at 515 nm at concentrations of 10,000, 5,000, 1,000, 500, 100, 50, 10 and 5 ppm of the samples. The inhibition percentage (IP) was calculated in relation to the UV absorption of the initial DPPH solution by the equation: IP (%) = [(AbsDPPH – AbsSAMPLE)/AbsDPPH] × 100. Linear regression analysis of the inhibition percentage of the various concentrations was used to find a linear equation to obtain the IC₅₀, the effective concentration of the sample that inhibits 50 % of the DPPH radical.

RESULTS AND DISCUSSION

UPLC-HRMS-ESI (+) and HRMS/MS analysis

Table 1S presents the chemical composition of leaf, stem, and fruit extracts analyzed by UPLC-ESI-HRMS regarding their molecular formula, retention times (Rt), exact mass detected (positive ionization mode), as well as the MS/MS fragment ions and bibliographic references used to confirm characterization. The analysis showed that flavonoids were the most prevalent constituent, representing 79 % of the components identified in the extracts. The main components were kaempferol-3-O-β-D-glucopyranoside (**7**, *m/z* 449.1080) at Rt 1.98 min and eupalinolide A (**20**, *m/z* 501.1543) at Rt 2.70 min, present in extracts of fruits and stems. Analysis of *C. duckeana* samples provided the tentative identification of 21 compounds (Figure 1S). Putatively identified compounds correspond to seventeen flavonoids (**1-3**, **5-10**, **12-13**, **15-19**, **21**), including glycosides of flavones, flavonols, flavononols,

catechins, acylated flavonol glycosides and isoflavonoids, one anthraquinone (**11**), one sesquiterpenoid lactone (**20**), one thiazinedione (**14**), and one semi-quinone chalcone (**4**).

Fabaceae (Leguminosae) represent the third largest plant family, with 770 genera and approximately 19,500 species distributed among the subfamilies. In Brazil is the largest plant family with wide distribution and an estimated 2,834 species. Flavonoids quercetin and kaempferol have a wide distribution in this family, however, others occur in a limited number, including isoflavones, catechins and terpenoids.^{23,24} Therefore, it was interesting to observe the presence of daidzin (6, m/z 417.1173) and ononin (19, m/z 431.1324) in the stems of *C. duckeana*, once these metabolites are almost exclusively found in the Papilionoideae (Fabaceae) family. They are associated with many biological activities, including the treatment of osteoporosis, cardiovascular diseases, menopausal symptoms, and cancer prevention. Studies indicate antioxidant and estrogenic activities in addition to antifungal and insecticidal properties.²⁴⁻²⁶

Erazua²⁷ evaluated anticancer activities of eleven flavonoids: luteolin, apigenin, chrysin, quercetin, galangin, hesperetin, naringenin, taxifolin, daidzein, kaempferol, and genistein. These flavonoids were tested against leukemia cell line using quantum chemical method through density functional theory (DFT) and molecular docking approach. These flavonoids were docked against leukemia cell line (PDB: 1AOI) and the correlation between the calculated descriptors and their binding affinity with leukemia cell line was examined. The results showed that taxifolin had the highest inhibition efficiency against leukemia cell line among the studied compounds, while daidzein had the least inhibition efficiency. These results reinforce flavonoids as potential therapeutic agents.

Kaempferol-3-O- β -D-glucopyranoside (**7**) was isolated from *Wedelia chinensis* and this compound displayed moderate inhibitory effects on LPS-stimulated NO production. The values were comparable with that of resveratrol which has been extensively studied by previous researchers. In addition, the compound did not display any obvious cytotoxicity.²⁸ Kaempferol-3-O- β -D-glucopyranoside is a natural tetrahydroxyflavone that can be isolated from the bark of *Harungana madagascariensis*. In a recent study, it is listed as a potent cytotoxic compound that can be used for cancer chemotherapy as an anticancer agent.²⁹ The presence of this compound in extracts of fruits and stems of *C. duckeana* can be an important indicator of cytotoxicity, being able to partially justify the results found in this study.

Procyanidin B2 (**21**, m/z 579.1504) at Rt 2.75 min was previously reported in 40 samples of *Chamaecrista nictitans* collected in different sites of the Central Valley and the Pacific coast of Costa Rica.¹⁸ To the best of our knowledge, it is reported for the first time for

C. duckeana. Procyanidin B2 shows a wide anticancer activity in various human cancer cells. In gastric cancer, for example, this compound exerts anti-proliferative and apoptotic effects and induces autophagy; therefore, it may be developed as a potential therapeutic drug for this cancer.³⁰

Antioxidant assay

The values of the antioxidant activities of the studied extracts are informed by median inhibitory concentrations (IC₅₀), as shown in Table 1. The highest antioxidant activity obtained was from stem extracts, with an IC₅₀ value of 165.71. Stems presented a higher antioxidant activity in comparison to the standard butylated hydroxytoluene (BHT, IC₅₀ = 175.18 µg mL⁻¹). All extracts presented lower antioxidant activity in comparison to standard rutin (IC₅₀ = 133.01 µg mL⁻¹) or quercetin (IC₅₀ = 57.07 µg mL⁻¹). In the past two decades, a great number of flavonoid derivatives - belonging to the class of chalcones, flavones, flavanones, isoflavones, and other complex structures - has been studied for their potential as antioxidant agents.³¹ Comparing the results of the antioxidant activity with the data obtained in the UPLC-ESI-HRMS analysis, it was possible to verify that the predominance of flavonoids in the extracts may be associated with a greater antioxidant capacity. Stems had a higher number of flavonoids compounds, nine in total, and they had the highest antioxidant activity.

Table 1. Antioxidant activity of methanol extracts from *C. duckeana* by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method

Methanolic Extracts	IC ₅₀ ± SD (µg mL ⁻¹)
Leaves	283.48 ± 4.19
Stems	165.71 ± 6.94
Fruits	261.08 ± 2.53
BHT ^{a,b}	175.18 ± 6.41
Rutin ^b	133.01 ± 3.11
Quercetin ^b	57.07 ± 0.39

^abutylated hydroxytoluene; ^b standard synthetic antioxidant; IC₅₀: median inhibitory concentration; SD: standard deviation.

Cytotoxic assay

The growth inhibition percentage (GI %) of all cells treated with each extract is described in Table 2. The samples exhibited cell growth inhibition only against HL60 (leukemia), with a GI% ranging from 86.62% to 89.32%. The three extracts showed a GI % below 58.17 % against SNB19 (central nervous system), HCT116 (human colon), and PC3 (prostate). Only HL60 (leukemia) had a growth inhibition higher than 80 %.

Table 2. Cytotoxic effect of extracts from *C. duckeana* at single concentration (100.0 $\mu\text{g mL}^{-1}$) against four human tumor cell lines after 72 h of incubation using MTT assay.

Sample	SNB19	HCT116	PC3	HL60
Fruits	28.10 \pm 4.62	4.16 \pm 8.53	30.44 \pm 0.74	88.28 \pm 3.57
Leaves	27.31 \pm 0.89	58.17 \pm 0.70	38.35 \pm 4.60	89.32 \pm 3.07
Stems	15.38 \pm 1.37	28.67 \pm 4.81	31.55 \pm 1.94	86.62 \pm 3.66

Tumor cell lines: SNB19 (central nervous system); HCT116 (human colon); PC3 (prostate); HL60 (leukemia).

In a second round of MTT assays, with extract-treated cells, the half-maximal inhibitory concentration (IC_{50}) was estimated in comparison to untreated cells. Two tumor cell lines, HL60 and RAJI, were tested at 72 hrs of incubation (Table 3). Extract of stems displayed a cytotoxic effect against two tumor cell lines, with IC_{50} values of 137.3 (104.6-180.1) and 106.8 (96.52-118.3) $\mu\text{mol L}^{-1}$ for the HL60 and the RAJI, respectively. Among the tumor cell lines used, HL60 cells were more sensitive to extract of fruits treatment, showing IC_{50} values of 133.4 (114.2-155.7) $\mu\text{mol L}^{-1}$. Sample of leaves did not show cytotoxic activity at a concentration of 200 $\mu\text{g mL}^{-1}$ in any of the tested cell lines.

Table 3. Cytotoxic activity of extracts on cancer cell lines after 72 hrs:

Sample	HL60	RAJI
IC_{50} (Confidence interval)		
Fruits	133.4 (114.2 -155.7)	> 200
Leaves	>200	>200
Stems	137.3 (104.6-180.1)	106.8 (96.52-118.3)

For human cells, data is presented as IC50 values and 95% confidence intervals; Tumor cell lines; HL60 and RAJI = leukemia;

The sesquiterpene eupalinolide A (**20**, m/z 501.1543) isolated from *Psiadia punctulata* has been reported in the literature for its potent cytotoxicity. This compound was tested for cytotoxicity against lung cancer cell A549, gastric gland cancer cell BGC-823, liver cancer cell SMMC-7721 and leukemia cell HL60 tumour cell. The results showed the potent cytotoxicity of sesquiterpene lactone Eupalinolide A against A-549, BGC-823, and HL60 tumor cell lines.³² Eupalinolide A has been related as a natural anti-inflammatory agent and this compound also presented potent cytotoxicity against NB4 and K562 leukemia cell lines using the MTT method.³³ In our results, this compound is present in extracts of fruits and stems of *C. duckeana* and can be associated with the cytotoxic activity of extracts.

CONCLUSION

The UPLC-ESI-HRMS techniques led to putatively identified of 21 compounds of several classes of metabolites including flavonoid, anthraquinone, sesquiterpenoid lactone, thiazinedione, and semi-quinone chalcone. Of the flavonoid classes present, two are isoflavonoids considered rare for the Leguminosae family. To the best of our knowledge, the cytotoxicity of the crude extracts of *C. duckeana* towards cancer lines is being reported herein for the first time, and cytotoxic capacity of *C. duckeana* against cancer cell lines HL60 and RAJI was identified. These extracts are potential natural products that deserve more investigation to develop novel anticancer agents. In conclusion, results demonstrate that *C. duckeana* is a species rich in bioactive secondary metabolites with antioxidant capacity and cytotoxic activity.

SUPPLEMENTARY MATERIAL

Information about structural representation and chromatographic data of compounds annotated in *C. duckeana* extracts by UPLC-ESI-HRMS is given in the supplementary material.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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SUPPLEMENTARY MATERIAL

Table 1S. Compounds annotated in *C. duckeana* extracts by the UNIFI software platform

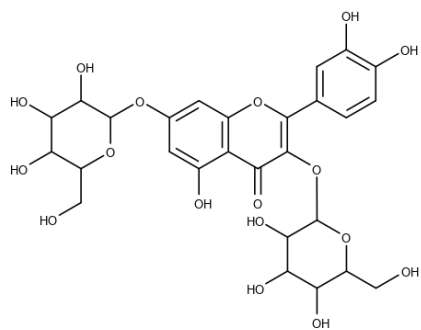
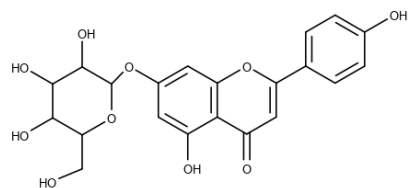
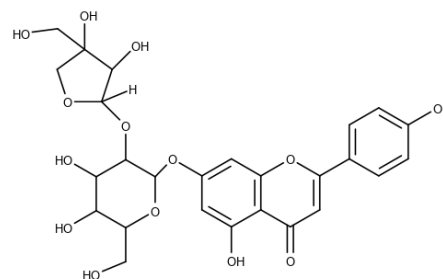
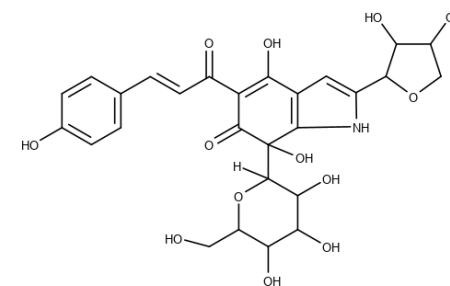
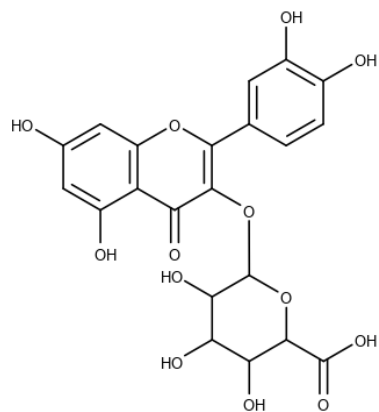
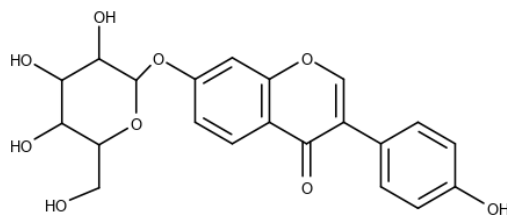
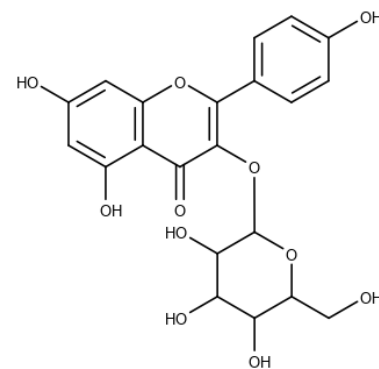
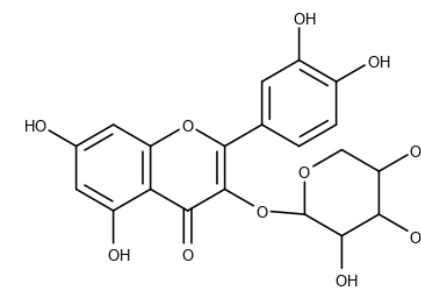
	Component name	MF/MW	Observed RT (min)	Observed <i>m/z</i>	Adducts	Fragments	Error (ppm)	Reference	Sample
1.	Quercetin-3-galactoside-7-glucoside	C ₂₇ H ₃₀ O ₁₇ 626.520	1.64	627.1557	+H	299.05470 303.04992 379.09890	0.3	1	stems
2.	Apigenin-7-O-galactopyranoside	C ₂₁ H ₂₀ O ₁₀ 432.381	1.81	433.1123	+H	195.02819 313.07018 355.07941	-1.6	2	fruits
3.	Apiin	C ₂₆ H ₂₈ O ₁₄ 564.496	1.89	565.1552	+H	329.06571 353.06556 465.13597	0.2	3	stems
4.	Cartormin	C ₂₇ H ₂₉ NO ₁₃ 575.523	1.90	576.1708	+H	329.06590 449.10806	-0.7	4	fruits
5.	Quercetin 3-O-β-D-glucopyranosiduronic acid	C ₂₁ H ₁₈ O ₁₃ 478.362	1.94	479.0813	+H	201.04656 303.04969 359.07681	0.6	5–7	fruits
6.	Daidzin	C ₂₁ H ₂₀ O ₉ 416.382	1.96	417.1173	+H	195.02807 283.05976 313.07038 339.08580 343.08066	-0.1	8	stems
7.	Kaempferol-3-O-β-D-glucopyranoside	C ₂₁ H ₂₀ O ₁₁ 448.380	1.98	449.1080	+H	287.05461 329.06563	0.2	2,4,6	stems fruits
8.	Quercetin-3-O-α-L-arabinopyranoside	C ₂₀ H ₁₈ O ₁₁ 434.353	2.06	435.0928	+H	329.06605 417.08266	-0.3	9	leaves

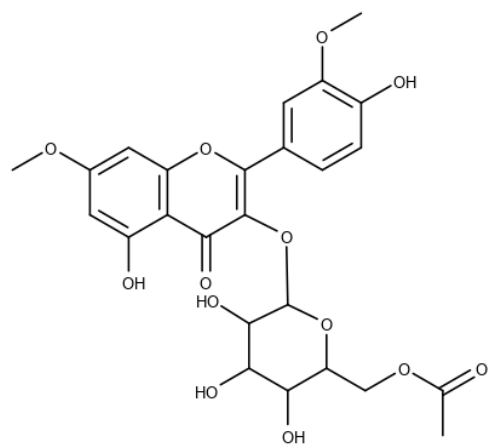
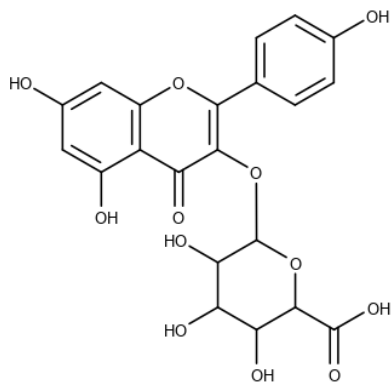
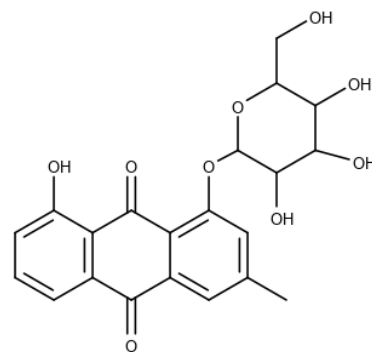
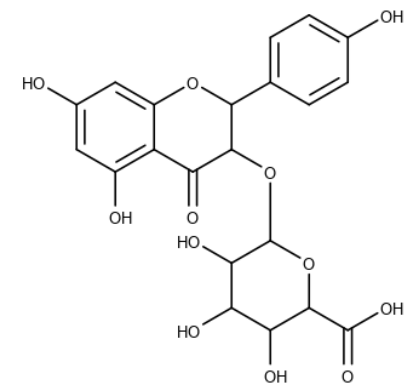
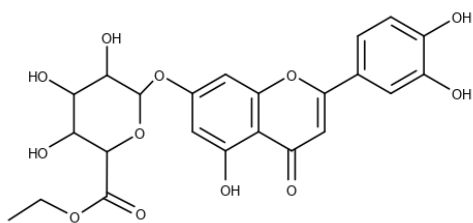
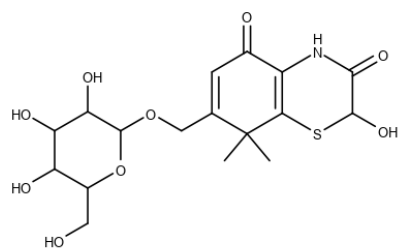
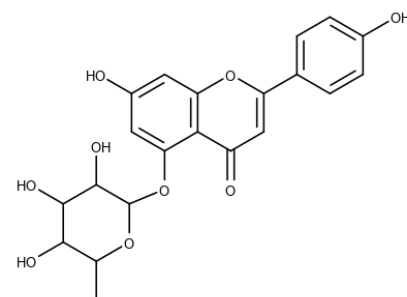
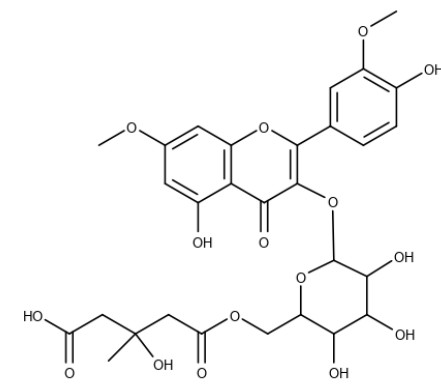
9.	Viscumneoside II	C ₂₅ H ₂₆ O ₁₃ 534.470	2.08	535.1443	+H	273.07554 315.04916 463.12264	-0.4	10	fruits
10.	Kaempferol-3-O-β-D-glucuronide	C ₂₁ H ₁₈ O ₁₂ 462.363	2.12	463.0874	+H	287.05533 303.05001 449.10880	-0.9	7,11	fruits
11.	Chrysophanol glucoside	C ₂₁ H ₂₀ O ₉ 416.382	2.33	417.1170	+H	287.05536 297.07617 299.05563 313.07095	-2.5	12	fruits
12.	Dihydrokaempferol-3-glucuronide	C ₂₁ H ₁₈ O ₁₂ 464.379	2.35	465.1025	+H	201.04640 299.05517 329.06582 431.09746	0.0	6	stems
13.	Luteolin-7-O-glucuronide ethyl ester	C ₂₃ H ₂₂ O ₁₂ 490.417	2.39	491.1178	+H	178.9968 313.0356	-1.9	13	leaves
14.	2-Hydroxyxanthoside	C ₁₇ H ₂₃ NO ₉ S 417,43	2.41	440.0979	+Na	205.01258 255.06542 299.05555	-1.6	14	fruits
15.	Apigenin-5-rhamnoside	C ₂₁ H ₂₀ O ₉ 416.382	2.48	417.1173	+H	283.06038 313.07100	-3.4	15	stems
16.	Viscumneoside IV	C ₂₉ H ₃₂ O ₁₆ 636.559	2.49	637.1760	+H	313.07085 415.10262 561.15958 593.15084	0.7	16	leaves
17.	Wogonoside	C ₂₂ H ₂₀ O ₁₁ 460.391	2.51	461.1079	+H	300.06065 327.05030 329.06601 339.05033 429.08234	-0.8	17	leaves

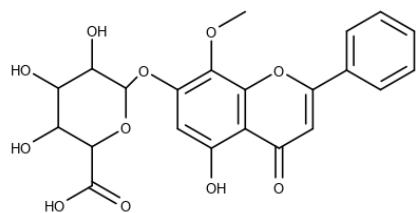
18	Isorhamnetin-3-O-β-D-galactoside	C ₂₂ H ₂₂ O ₁₂ 478.406	2.53	501.1024	+Na	201.04663 259.06029 329.06604 413.08730	0.4	18	stems
19	Ononin	C ₂₂ H ₂₂ O ₉ 430.409	2.57	431.1324	+H	283.06017 285.07522 377.10153	-2.5	8, 19	stems
20	Eupalinolide A	C ₂₄ H ₃₀ O ₉ 462.495	2.70	501.1543	+K	287.05542	0.3	10, 20	stems fruits
21	Procyanidin B2	C ₃₀ H ₂₆ O ₁₂ 578.526	2.75	579.1504	+H	271.06031 279.09363 421.09258	0.1	21, 22	fruits

Rt: retention time; MF: molecular formula; MW: molecular weight; BP: base peak;

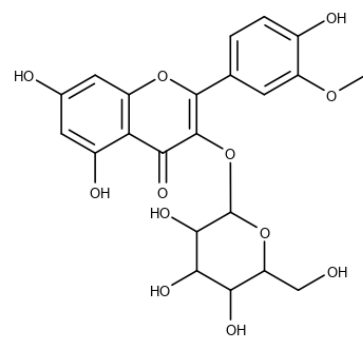
Figure 1S. Structural representation of compounds annotated in C. duckeana methanolic extracts

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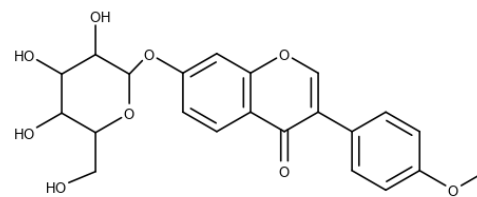
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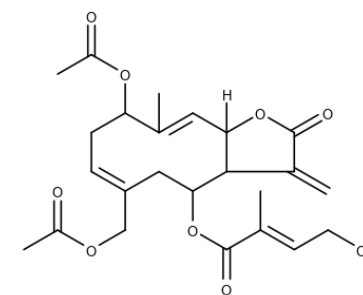
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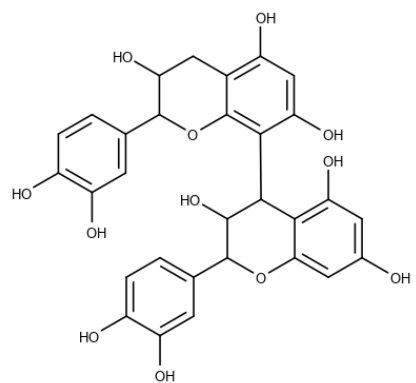
(18)



(19)



(20)



(21)

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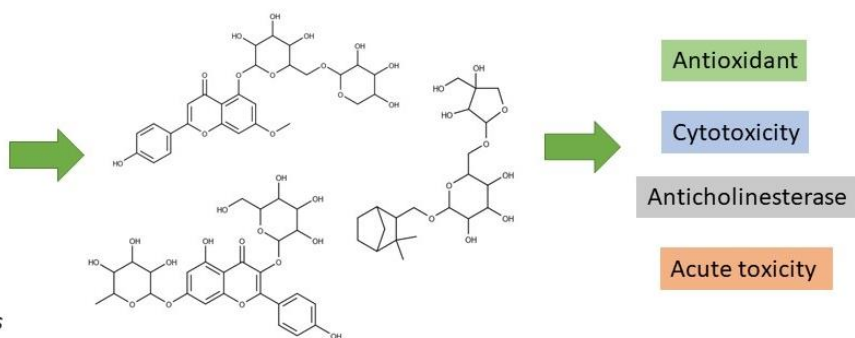
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7 CHEMICAL PROFILING AND BIOLOGICAL PROPERTIES OF LEAVES AND FLOWERS EXTRACTS OF *CHAMAECRISTA ENSIFORMIS* IRWIN & BARNEBY

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Chamaecrista ensiformis
(Vell.) H.S.Irwin & Barneby



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Chemical profiling and biological properties of leaves and flowers extracts of *Chamaecrista ensiformis* Irwin & Barneby

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The aim of this study was to evaluate chemical composition, antioxidant content, *in vitro* cytotoxic and antiacetylcholinesterase potentials, and *in vivo* toxicity in adult zebrafish (aZF) of leaves and flowers extracts of *Chamaecrista ensiformis*. The chemical profile of the extracts was obtained by UPLC-ESI-HRMS analysis. The antioxidant activity was carried out by scanning the free radical DPPH and ABTS and antiacetylcholinesterase potential was performed using the method of Ellman. Cytotoxicity was tested using the MTT method. aZF were used to assess the acute toxicity of the extract. The chemical composition of the both extracts by UPLC-QTOF-MS provided the annotation of 13 compounds, classes of flavonoids and terpenoids were predominant. The leaves extracts showed good antioxidant activity with IC₅₀ value $5.516 \pm 0.027 \mu\text{g mL}^{-1}$ in the ABTS method. The flowers extract inhibited AChE, with an inhibition halo of 0.8 cm in diameter and showed cytotoxic significant effect against HL60 and RAJI cell lines with IC₅₀ of 88.76 and 90.23 $\mu\text{g mL}^{-1}$ respectively. The results showed that the extracts were not toxic against adult zebrafish (aZF). These results suggest that extracts of *C. ensiformis* are promising source of natural antioxidant, showing a positive relationship between chemical composition and activities studied.

Keywords: dereplication, cytotoxicity, adult zebrafish.

RESUMO

O objetivo deste estudo foi avaliar a composição química, conteúdo antioxidante, potencial citotóxico e antiacetilcolinesterásico in vitro e toxicidade in vivo em zebrafish adulto (aZF) de extratos de folhas e flores de *Chamaecrista ensiformis*. O perfil químico dos extratos foi obtido por análise UPLC-ESI-HRMS. A atividade antioxidante foi realizada por varredura dos radicais livres DPPH e ABTS e o potencial antiacetilcolinesterásico foi realizado pelo método de Ellman. A citotoxicidade foi testada pelo método MTT. aZF foram usados para avaliar a toxicidade aguda do extrato. A composição química de ambos os extratos por UPLC-QTOF-MS proporcionou a anotação de 13 compostos, predominando as classes de flavonóides e terpenóides. Os extratos das folhas apresentaram boa atividade antioxidante com valor de IC_{50} $5,516 \pm 0,027 \mu\text{g mL}^{-1}$ pelo método ABTS. O extrato das flores inibiu a AChE, com halo de inibição de 0,8 cm de diâmetro e apresentou efeito citotóxico significativo contra as linhagens HL60 e RAJI com IC_{50} de 88,76 e 90,23 $\mu\text{g mL}^{-1}$ respectivamente. Os resultados mostraram que os extratos não foram tóxicos para zebrafish adulto. Esses resultados sugerem que os extratos de *C. ensiformis* são fontes promissoras de antioxidantes naturais, mostrando uma relação positiva entre a composição química e as atividades estudadas.

Palavras-chave: desrepliação, citotoxicidade, zebrafish adulto.

1. INTRODUCTION

Cancer is the main public health problem in the world and is already among the four main causes of premature death (before 70 years of age) in most countries. In Brazil, the estimate for each year of the 2020-2022 triennium indicates that there will be 625,000 new cases of cancer. When analyzing leukemias, this number reaches 5920 new cases for men and 4890 for women [1]. Natural products from plants are sources for cancer therapy, specially by antioxidant capacity, inhibiting oxidative processes [2]. Furthermore, they are recognized as “free radical scavengers” as they form smaller reactive species through the radicals. Antioxidants can reduce the occurrence of different diseases such as: aging, cancer, neurodegenerative disorders, diabetes, inflammation, liver disease, cardiovascular disease, cataract and nephrotoxicity. Natural or synthetic antioxidants can be used as tools for early prevention of these diseases [3]. Antioxidants also act to preserve acetylcholine, improve cognitive function, inflammatory function and brain flow, essential properties in the therapeutic effectiveness of Alzheimer's Disease (AD), bringing improvements in cognition, memory and delaying neurodegenerative progression [4].

In addition to chemical knowledge, the investigation of biological activities stands out and in this sense, zebrafish has been drawing attention with animal model for behavioral

pharmacology due to its characteristics, including small size, rapid development and usefulness for high-performance behavioral drug testing [5]. Adult zebrafish (*Danio rerio*) is a test organism that allows the investigation of effects on a wide range of biological levels, such as molecular level and behavioral parameters. Being considered a reliable model, since it shares physiological, morphological and histological similarities with mammals, zebrafish have been used as a tool for the discovery of anticonvulsant compounds present in plants [6] it is also considered an attractive model for research aimed at understanding toxicity [7, 8], of anti-inflammatory drug [9] and plants used in traditional medicine [10].

The genus *Chamaecrista* is notable for its pantropical distribution, including more than 330 species distributed mainly in tropical. In the Brazilian flora, 256 species are registered, of which 207 are endemic [11]. There are few chemical and biological studies on the species of *Chamaecrista*, making it an attractive genus in the search for therapeutic alternatives. In literature have a few works on genus *Chamaecrista*, phenolic compounds, terpenoids, anthraquinones and flavonoids are reported in *C. absus* [12], *C. dyphilla* [13], *C. duckeana* [14], *C. flexuosa* [15], *C. greggii* [16], *C. nictitans* [17, 18] and *C. nigricans* [19].

In this sense, this work aimed to investigate the action of methanolic extract of *C. ensiformis* leaves and flowers, using dereplication methods to characterize chemical composition, as well as to evaluate the antioxidant, cytotoxicity, anti-acetylcholinesterase and toxicity potential against adult zebrafish (*Danio rerio*).

2. MATERIALS AND METHODS

2.1 Chemicals

For the development of this study, the following reagents and drugs were used: methanol from J. T. Baker (USA). ethanol, acetic acid, dimethylsulfoxide (DMSO) and Folin–Ciocalteu from Dinamica. Formic acid and dimethyl sulfoxide from Synth. Formaldehyde was purchased from Dinamica. Gallic acid, aluminium chloride $AlCl_3$, rutin, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), were purchased from Sigma (USA).

2.2 Plant material

Leaves and flowers of the *Chamaecrista ensiformis* (Vell.) H. S. Irwin & Barneby plant were collected in Caucaia, Ceará, Brazil, on September 28, 2018, and certified in the Department of Biology at the Federal University of Ceará (UFC). The samples were authenticated and deposited at the Herbário Prisco Bezerra, UFC, Brazil, under the number of exsiccate 61617. The plant is registered in the National Genetic Heritage Management System (SISGEN-AB06D11).

2.3 Preparation of methanol extracts

After collection, the plant material was dried in an oven at 40 °C for 7 days or complete drying. The dried and crushed botanical material (leaves and flowers) was extracted using the solvent methanol P.A. (99.8%) in the proportion of 1 g of plant material to 30 mL of solvent followed by sonication for 15 min [20]. 0.1503 g (7.51% of extraction yield) of the methanol extract of the leaves of *Chamaecrista ensiformis* (MetECeL) and 0.4682 g (23.39% of extraction yield) of the methanol extract of the flowers of *C. ensiformis* (MetECeF) were obtained.

2.4 Dereplication of extracts

2.4.1 Sample preparation for UPLC-ESI-HRMS analysis

Around 100 mg of the samples, accurately weighed, were submitted to UAE with 10 mL of methanol, for 15 min. The extracts were dried, weighed, and stored at -18 °C until analysis. The extract solutions were prepared at concentrations of 1 mg/mL for the UPLC-ESI-HRMS analysis. They were filtered on Chromafil® Xtra RC-20/25 membranes (0.20 µm pores) before the analysis.

2.4.2 Chromatographic and Mass Spectrometry conditions

Chromatographic profiles were performed on a 100 × 2.1 mm Waters® ACQUITY UPLC® 1.8 µm HSS T3 column, in an ACQUITY Ultra Performance LC™ system equipped with a Q-ToF Premier mass spectrometer LC/MS/MS platform. The column was kept at 40 °C. Injection volume was 0.8 µL. The flow rate was set at 0.580 µL/min, and gradient elution was carried out with a binary system consisting of [A] 0.1% aqueous formic acid (Synth, Brazil) and [B] 0.1% formic acid (Synth, Brazil) in methanol (J. T. Baker, USA). The gradient elution was programmed as follows: 5–95% (B) from 0 to 4.31 min, 100% (B) from 4.31 to

5.75 min. The data were collected in centroid mode, with a lock spray frequency of 10 s, and the data were averaged over 10 scans. The Synapt was calibrated in positive mode with sodium formate (reference mass 860.8467 uma) with an m/z range between 100 to 1200 in positive ionization mode. MassLynx software (version 4.1, Waters) was used for acquisition and data processing.

2.5 Determination of total phenolic and flavonoid compounds, and antioxidant activity

The determination of the total phenolic and concentration of flavonoids content in the methanolic extracts of *Chamaecrista ensiformis* was established spectrometric methods according to [21]. The absorbance of the samples was read using a UV-Vis FEMTON 700 plus spectrophotometer. The tests were performed in triplicate. The results were presented as mean \pm standard deviation (SD).

The total phenol content for *C. ensiformis* extracts was expressed as mg of GAE (gallic acid equivalents) per g of extract from the calibration curve of the standard gallic acid solution in the range (0.01-0.36 mg/mL), described by the equation $y = 0.0008x + 0.0043$ and $R = 0.9999$. After obtaining the standard curve of gallic acid, it was possible to determine the concentration of phenolic compounds, corresponding to gallic acid.

The amount of total flavonoids was determined based on the formation of a flavonoid-aluminum complex. The results were compared with a standard rutin curve in the range of (0-0.04 mg/mL), with equation ($y = 0.016x - 0.0161$; $R^2 = 0.9947$).

The antioxidant activity was determined by the DPPH and ABTS free radical methods. Solutions of DPPH (3.9 mL, 6.5×10^{-5} M) (Morais et al., 2019) or ABTS (5 mL; 7 mM) [22] were added to the solutions containing the samples (5–10,000 $\mu\text{g/mL}$). The tests were performed in triplicate. The absorbance values were measured spectrophotometrically at 515 and 734 nm, respectively. The antioxidant capacity was compared with the standard rutin and IC_{50} was determined.

2.6 *In vitro* anti-acetylcholinesterase activity

The inhibitory activity of the enzyme acetylcholinesterase (AChE) was performed using the method of [23], adapted for thin layer chromatography by [24]. As a positive control, the eserina at 30 mg/mL was used.

2.7 *In vitro* cytotoxicity

The tumor lines HCT116 (Colon - Human), SNB-19 (Glioblastoma), PC3 (Prostate), HL60 and RAJI (Leukemia) were used. The first four were provided by the National Cancer Institute (USA) and the RAJI was acquired from the Rio de Janeiro Cell Bank (BCRJ), having been grown in RPMI 1640 medium, supplemented with 10% fetal bovine serum, and 1% antibiotics. All cells were kept in an oven at 37 °C and an atmosphere containing 5% CO₂. The samples were diluted in DMSO to stock concentrations of 20 mg/mL. To perform the single concentration test, they were all tested at a concentration of 100 µg/mL. Cytotoxicity analysis was performed by the MTT method [25], and the cells were plated at concentrations of 0.7 x 10⁵, 0.1 x 10⁶, 0.3 x 10⁶ cells/mL for the HCT116, SNB-19, PC3 and HL60, respectively. The plates were incubated with the samples for 72 hours, in an oven at 5% CO₂, at 37 °C. Afterwards, they were centrifuged and the supernatant was removed. Then, 150 µL of the MTT solution (tetrazolium salt) was added and the plates were incubated for 3 hours. After incubation, the plates were again centrifuged to remove the MTT solution. The absorbance was read after dissolving the formazan precipitate with 150 µL of pure DMSO, in a plate spectrophotometer at 595 nm. The single concentration experiments were analyzed according to the mean ± standard deviation (SD) of the percentage of cell growth inhibition using the GraphPadPrism 6.0 program. In the second round, the half maximal inhibitory concentration (IC₅₀) was determined against HL60 and RAJI cell lines. The cells were plated at concentrations of 0.3 x 10⁶ cells/mL. After the incubation, the supernatant was replaced by fresh medium containing MTT (100 µL). Three hours later, formazan product was dissolved in 100 µL of DMSO, and absorbance was measured at 595 nm (DTX-880 Multimode Detector, Beckman Coulter®). The samples were diluted in DMSO to stock concentrations of 20 mg/mL. To perform the single concentration test, all were tested on a concentration curve that ranged from 3.13 to 200 µg/mL.

2.8 Toxicity

2.8.1 *Zebrafish*

Wild zebrafish (*Danio rerio*) (aZF), aged 60-90 days, both sexes, sizes of 3.5 ± 0.5 cm and weight 0.4 ± 0.1 g, obtained from Comércio de Produtos Veterinário LTDA, a supplier located in Fortaleza (Ceará, Brazil). Groups of 50 fish were acclimatized for 24 h in glass aquariums (40 x 20 x 25 cm) containing dechlorinated water (ProtecPlus[®] anti-chlorine) and air pumps with submerged filters, at 25 °C and pH 7.0, with a circadian cycle of 14: 10 h of light/dark. The fish were fed (Spirulina[®]) ad libitum 24 h before the experiments. After the experiments, the animals were sacrificed by immersion in cold water (2-4 °C), for 10 minutes, until the loss of opercular movements (Concea, 2018). All experimental procedures were approved by the animal ethics committee of the State University of Ceará (CEUA-UECE), under protocol No. 3344801/2017. The zebrafish tests were performed based on methodologies proposed by [26]. On the day of the experiments, the fish were selected randomly, transferred to a wet sponge and treated with the test or control samples via intraperitoneal (*i.p.*). Afterwards, individually the animals were placed to rest in glass cups of 250 mL containing 150 mL of aquarium water. For treatments via *i.p.*, an insulin syringe (0.5 mL; Ultra FinaTM BD) with a 30G needle was used.

2.8.2 Acute toxicity 96 h

The study of acute toxicity was accomplished against adult zebrafish (*Danio rerio*) in accordance with OECD guidelines [27] and [28]. The animals (n = 6/each) were treated with 20 μ L, via *i.p.*, with a test sample (0.1 or 0.5 or 1.0 mg/mL) or Control (3% DMSO, vehicle) and left at rest for the mortality rate to be analyzed. After 96 hours of treatments, the number of dead fish in each group was recorded and the lethal dose capable of killing 50% of the animals (LD₅₀) was determined using the mathematical method Trimmed Spearman-Kärber with a 95% confidence interval [29].

3. RESULTS

3.1 Dereplication of extracts

The chemical composition of the MetECeF and MetECeL by UPLC-ESI-HRMS provided the annotation of 13 new compounds for the species (Table S1 - Annex), 6 in the MetECeF and 7 in the MetECeL. The analysis showed that the classes of terpenoids and flavonoids were predominant, with 3 (picroside III, shionoside A and shionoside C) and 5

compounds (isovitexin-3''-O-glucopyranoside, 7-O- α -L-rhamnosyl-3-O- β -D-glucopyranosyl kaempferol, genistein-7,4'-di-O- β -D-glucoside, yuankanin and kaempferitrin), respectively. An anthraquinone (physcion 8-glucoside), coumarin (3'R-hydroxymarmesin-4'-O- β -D-glucopyranoside), anthrone (isoaloeresin A), phenylpropanoid (syringin), and polyoxypregnane (marstenacigenin A) have also been putatively identified.

3.2 Determination of total phenolic compounds (TPC), total flavonoid content (TFC) and antioxidant activity

The MetECeF e MetECeL showed TP of 131.656 ± 7.906 and 212.718 ± 3.953 mg EAG/g of extract, as well TFC of 120.243 ± 1.761 and 68.252 ± 0.459 mg ER/g of extract. Regarding the antioxidant activity, of the two tested extracts, MetECeL showed greater antioxidant activity with IC_{50} values 9.330 ± 0.186 μ g/mL and 5.516 ± 0.027 μ g/mL against DPPH and ABTS, respectively. The standard rutin are IC_{50} DPPH = 4.647 μ g/mL and IC_{50} ABTS = 2.176 ± 0.024 μ g/mL (Table 1).

Table 1. *Antioxidant activity, content of total phenols and total flavonoids in the methanolic extracts of Chamaecrista ensiformis*

Samples	DPPH	ABTS	TP	TF
	IC_{50} μ g/mL (SD)	IC_{50} μ g/mL (SD)	mg EAG/g of extract (SD)	mg ER/g of extract (SD)
MetECeF	14.862 ± 0.618	11.011 ± 0.162	131.656 ± 7.906	120.243 ± 1.761
MetECeL	9.330 ± 0.186	5.516 ± 0.027	212.718 ± 3.953	68.252 ± 0.459
Rutin*	4.647 ± 0.01	2.176 ± 0.024	-	-

*standard antioxidant; IC_{50} : median inhibitory concentration; MetECeL (leaves); MetECeF (flowers); SD: standard deviation; ER: Rutin equivalent; IC_{50} : content of the extract capable of inhibiting 50% of DPPH/ABTS radicals. TP (total phenol) expressed in mg of gallic acid equivalent per gram of the extract. TF (total flavonoid) expressed as rutin equivalent per gram of the extract.

3.3 *In vitro* anti-acetylcholinesterase activity

Of the two extracts tested, only MetECeF inhibited AChE, with an inhibition halo of 0.8 cm in diameter. The positive control eserine inhibited AChE with a 1.0 cm diameter halo of inhibition.

3.4 *In vitro* cytotoxicity

In the evaluation of *in vitro* cytotoxicity, the two samples tested, MetECeL and MetECeF, showed greater inhibition of cell growth against HL60 (leukemia) of 56.60 ± 1.82 and 71.58 ± 4.97 , respectively (Table 2). In a second sequence of MTT assays with extracts-treated cells, the half maximal inhibitory concentration (IC_{50}) was determined in comparison to untreated cells. Two tumor cell lines HL60 and RAJI were tested at 72 h of incubation (Table 3). Only MetECeF showed cytotoxic effect against two tumor cell lines, having IC_{50} values of 88.76 (71.61 -110.0) and 90.23 (81.86-99.46) μM for HL60 and RAJI, respectively.

Table 2. Cytotoxic effect of extracts of *Chamaecrista ensiformis* at single concentration (100.0 $\mu\text{g}/\text{mL}$) against four human tumor cell lines after 72 h of incubation using MTT assay

Sample	SNB-19		HCT116		PC3		HL60	
	GI%	DP	GI %	DP	GI %	DP	GI %	DP
MetECeL	12.77	7.87	16.96	1.66	7.91	1.4	56.60	1.82
MetECeF	26.21	2.23	10.12	7.70	37.02	1.33	71.58	4.97

Percentage of growth inhibition (GI%) \pm standard deviation (DP) from three experiments performed in triplicate calculated by Prism 6.0 (GraphPad/Intuitive Software for Science, San Diego, CA). Viability data were normalized to control group (DMSO 0.5% in media). MetECeL (leaves); MetECeF (flowers). (SNB-19, HCT116, PC3 and HL60) SNB-19 (glioblastoma), HCT-116 (colon - human), PC3 (prostate), HL60 (leukemia).

Table 3. Mean Inhibitory Concentration (IC_{50}) with confidence interval (CI) of samples with maximum concentration of 200 $\mu\text{g}/\text{mL}$ using MTT assay

Sample	HL60	RAJI
	IC_{50} ($\mu\text{mol.L}^{-1}$) (Confidence interval)	
MetECeL	>200	>200
MetECeF	88.76 (71.61 -110.0)	90.23 (81.86-99.46)

MetECeL (leaves); MetECeF (flowers); cell lines HL60 and RAJI (leukemia).

3.5 Acute toxicity 96h

The samples were not toxic against adult zebrafish (*Dano rerio*) within 96 h of analysis (Table 4).

Table 4. Results of the acute toxicity tests of the test samples against adult zebrafish

Mortalities of the Adult Zebrafish	96h
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Sample	CN	C1	C2	C3	LD ₅₀ (mg/kg; <i>i.p.</i>)/IV
MetECeL	0	3	3	2	> 40
MetECeF	0	2	2	2	> 40

MetECeL (leaves); MetECeF (flowers); CN- Negative control group: DMSO 3%. C1 – concentration 1 (4 mg/kg; *i.p.*); C2 – concentration 2 (20 mg/kg; *i.p.*). C3 – concentration 3 (40 mg/kg; *i.p.*). LD₅₀- lethal dose to kill 50% of adult zebrafish; IV – confidence interval;

4. DISCUSSION

Flavonoids are largely distributed in the plant kingdom, arousing large interest in research, as they have a wide range of biological activities, such as: Anti-tumor, anti-inflammatory and anti-Alzheimer agents. Flavonoids have been suggested as potential chemotherapeutics because they are toxic to cancer cells but not harmful to healthy cells [30, 31]. Terpenes and terpenoids are also substances known to demonstrate a wide variety of biological activities in various models of human disease [32].

Genkwanin 5-O-β-D-primeveroside (yuankanin), flavonoid present in MetECeF (5, *m/z* 579.1692) RT 2.31 min, may be one of the responsible factors for the antioxidant activity of the extract. This substance reported in the literature regarding its anti-inflammatory and antioxidant effect present in the medicinal plant *Daphne genkwa*, which is used in traditional Chinese medicine [33]. It is also one of the three main bioactive constituents of *Aquilaria crassna* leaves, demonstrated that this glycoside plays a decisive role in the prevention of cellular oxidative stress, increasing mRNA expression of antioxidant enzymes [34]. On the other hand, shionoside A (11, *m/z* 471.2199) RT 4.22 min also detected in MetECeF, was isolated by [35] from *Aster tataricus L.* and showed no antioxidant activity. This result can suggest that the substance is not responsible for the antioxidant activity of the *C. ensiformis* extract.

MetECeL presented the most expressive total phenol content, with FT equals to 212.72 ± 3.95 mg of GAE/g extract. The flower extract was also considered rich in phenolic compounds, with FT equals to 131.66 ± 7.91 mg of GAE/g extract, because, according to [36], extracts considered rich in phenolic compounds are those with concentrations above 100 mg of GAE/g of extract. In our tests, the leaves extract had a higher antioxidant activity than that of the flowers, probably due to the higher content of total phenols, since phenolic compounds have a high antioxidant capacity and, therefore, are beneficial to human health [37]. The results of antioxidant activity suggest that the compounds noted in the extracts are

hydrophilic and/or lipophilic in nature, as they reduced DPPH and ABTS lowers can be associated with this result, on this account it has already been reported in plant extract that has antioxidant activity [39].

In acetylcholinesterase test, the eserine pattern presented a 1.0 cm diameter halo. The flower extract had a diameter smaller than the standard eserine, with 0.8 cm. This inhibition of AChE makes the sample a candidate for determining the IC₅₀. The leaf extract showed no halo, resulting in negative activity. These results improve research and its translation into target drugs for people with dementia, major cause of disability and dependency amongst the elderly [40] The presence of the compound 7-O- α -L-rhamnosyl-3-O- β -D-glucopyranosyl kaempferol (3, m/z 595.1663) RT 2.17 min, present in MetECeL, may be responsible for the inhibitory activity of AChE, according to [41] who report the presence of this substance in the root extract of *Dichocarpum auriculatum*, showing a strong ability to inhibit AChE activity by 95.83%.

The most promising result for cytotoxicity was that of the MetECeF, which may be associated with its high content of phenolic compounds, as well as in a study by [42] with syringin compound detected in ethanolic extract of *Foeniculum vulgare* exhibited significant toxicity against MCF-7, HeLa, and DU145 cancer cell line. Previously the extract have also exhibited significant toxicity against HeLa cells. In other research the flower extract of *Centaurea ragusina* L. exhibited activity against human bladder (T24) and human glioblastoma (A1235) cancer cell lines and syringin compound was tentatively identified using high performance liquid chromatography [43]. In our results, syringin was putately identify (6, m/z 411.1072) RT 2.31 min and can be responsible for the cytotoxicity of the *C. ensiformis* extract.

The search for natural products guided by the use of zebrafish as a vertebrate model platform has methodically identified bioactive natural products. In modern biomedicine, the growing requirement to develop experimental models to gain a detailed understanding of disease conditions and to represent pioneering treatments has resulted in the application of zebrafish to close the gap between in vitro and in vivo assays. Thus, in pharmacogenetics and neuropharmacology, the zebrafish is becoming a widely used organism [44]. As a result, it was found that the samples were safe, as they were not toxic against zebrafish and these

results indicate the possible development of herbal medicines from these extracts, as pointed out by [28].

5. CONCLUSION

The methanolic extracts of leaves (MetECeL) and flowers (MetECeF) of *Chamaecrista ensiformis* are rich in flavonoids, terpenoids, anthraquinone, coumarin, chromone, phenylpropanoid and polyoxypregnane, showed expressive antioxidant activity against DPPH and ABTS, potential cytotoxicity against the tested cell lines, with more expressive cytotoxicity of MetECeF against HL60 and RAJI cell lines (leukemia), and did not exert acute toxicity against zebrafish. These results encourage the continuity of research aimed at isolating and characterizing the active agents responsible for research activities. Therefore, this study proves the pharmacological potential of *C. ensiformis* leaves and flowers in the development of plant drugs with cytotoxic properties.

6. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

7. FUNDING

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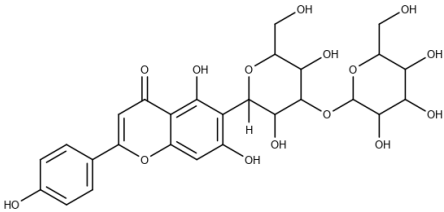
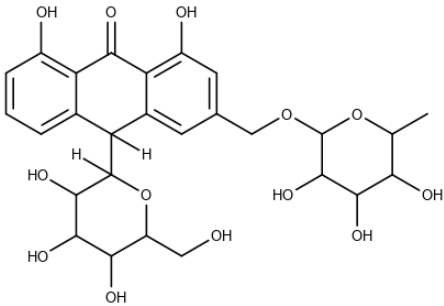
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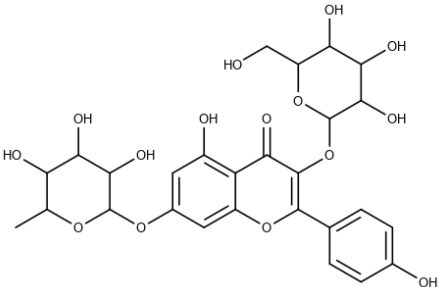
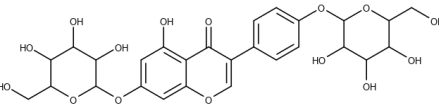
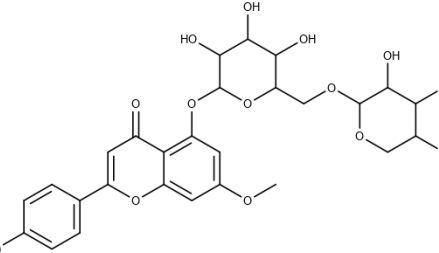
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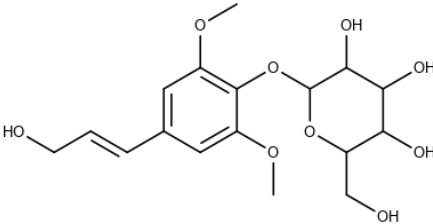
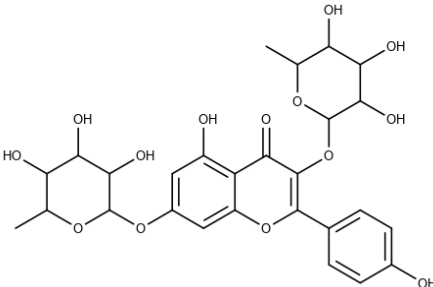
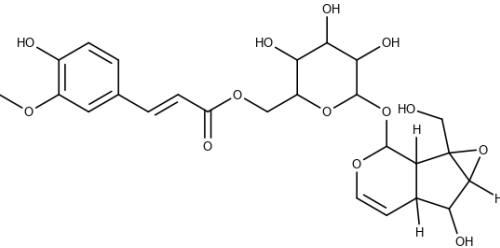
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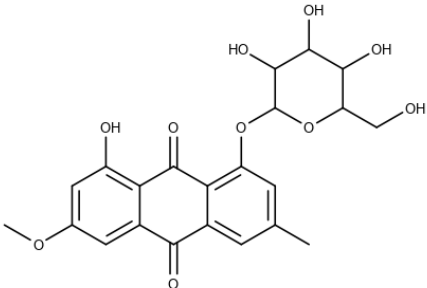
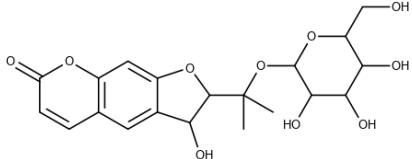
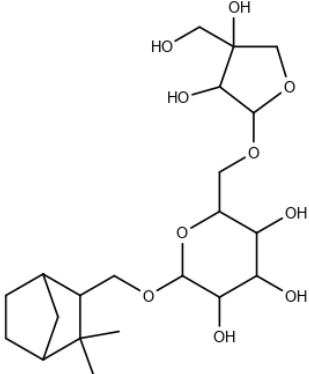
ANNEX

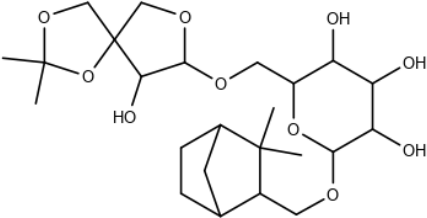
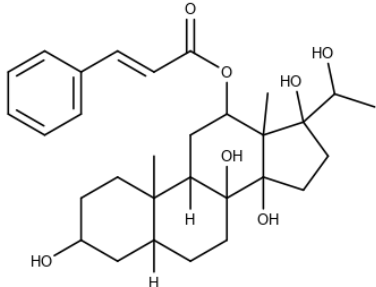
Table S1. Compounds annotated in methanolic extracts of *Chamaecrista ensiformis* leaves (MetECeL) and flowers (MetECeF) by UPLC-ESI-HRMS

Component name	MF/MW	Observed RT MS (min)	Observed m/z	Adducts	Metabolite Class/Reference	Fragments	Structure	MetECe
1 Isovitexin-3"-O-glucopyranoside	C ₂₇ H ₃₀ O ₁₅ 594.522	1.89	595.1660	+H, +Na	Flavonoid [45,46]	165.01808		L
						283.06005		
						299.05543		
						329.06603		
						353.06589		
2 Isoaloesin A	C ₂₇ H ₃₂ O ₁₃ 564.540	2.06	563.1509	+Na	Anthrone [47]	198.09120		L
						311.05512		
						329.06598		
						446.14198		

3	7- <i>O</i> - α -L-Rhamnosyl-3- <i>O</i> - β -D-glucopyranosyl kaempferol	C ₂₇ H ₃₀ O ₁₅ 594.522	2.17	595.1663	+H	Flavonoid [48]	201.04711		L
							299.05574		
							329.06625		
							341.06608		
							359.07705		
4	Genistein-7,4'-di- <i>O</i> - β -D-glucoside	C ₂₇ H ₃₀ O ₁₅ 594.522	2.24	617.1476	+Na	Flavonoid [48]	177.05454		L
							201.04693		
							361.07203		
							397.09058		
							433.11143		
5	Yuankanin	C ₂₇ H ₃₀ O ₁₄ 578.523	2.31	579.1692	+H	Flavonoid [49, 50]	301.07142		F
							377.10185		

6	Syringin	C ₁₇ H ₂₄ O ₉ 372.370	2.36	411.1072	+k	Phenylpropanoid [51]	187.03856 258.05240		F
7	Kaempferitrin	C ₂₇ H ₃₀ O ₁₄ 578.523	2.39	579.1697	+H	Flavonoid [52, 53]	313.03182 330.06943 411.07210 429.08239 475.12356		L
8	Picroside III	C ₂₅ H ₃₀ O ₁₃ 538.502	2.49	561.1598	+Na	Noriridoide [54, 55]	361.07096 377.10217		F

9	Physcion 8-glucoside	C ₂₂ H ₂₂ O ₁₀ 446.408	2.6	447.1281	+H	Anthraquinone [56]	271.06044 283.06045 379.08263		L
10	3'R-Hydroxymarmesin-4'-O-β-D-glucopyranoside	C ₂₀ H ₂₄ O ₁₀ 424.402	2.69	445.1126	+Na	Coumarin [57]	201.04686 283.06029 287.05553		L
11	Shionoside A	C ₂₁ H ₃₆ O ₁₀ 448.509	4.22	471.2199	+Na	Monoterpenoid [58]	201.04643 333.15159		F

12	Shionoside C	$C_{24}H_{40}O_{10}$ 488.574	4.22	511.2518	+Na	Monoterpenoid [59]	201.04643 333.15159		F
13	Marstenacigenin A	$C_{30}H_{42}O_7$ 514.659	4.37	537.2826	+Na	Steroid [39]	184.07347 437.20406		F

MetECe - methanolic extracts of *Chamaecrista ensiformis*; **L** -leaves; **F**- flowers

8 CONSIDERAÇÕES FINAIS

Este trabalho relatou a documentação química e ensaios biológicos com extratos de *Chamaecrista duckeana* e *Chamaecrista ensiformis*, espécies essas com pouquíssima literatura referente à sua composição química e atividades farmacológicas. As técnicas UPLC-ESI-HRMS levaram à anotação de 21 compostos em *C. duckeana* de várias classes de metabólitos, das classes de flavonoides presentes, dois compostos são isoflavonoides restritos à subfamília Papilionoideae. A citotoxicidade dos extratos brutos de *C. duckeana* para linhagens de câncer está sendo relatada pela primeira vez, sendo identificada a capacidade citotóxica contra linhagens de células leucêmicas HL60 e RAJI. Os resultados demonstraram que esta espécie é rica em metabólitos secundários bioativos com capacidade antioxidante e atividade citotóxica. O artigo resultante desse trabalho foi publicado na *Revista Química Nova*. Um segundo artigo produzido, apresenta o potencial farmacológico dos extratos metanólicos das folhas e flores de *C. ensiformis*. Os extratos se mostraram ricos em flavonoides, terpenoides, antraquinonas, cumarinas, aurona e fenilpropanoides sendo anotados 13 compostos, apresentando expressiva atividade antioxidante contra DPPH e ABTS, potencial citotoxicidade, sobretudo para o extrato metanólico das flores contra as linhagens celulares HL60 e RAJI, e não exerceu toxicidade aguda contra zebrafish. Esses resultados foram submetidos na *Revista Scientia Plena*. Por fim, esse estudo contribuirá para o conhecimento e a valorização das espécies estudadas, bem como colaborará para o desenvolvimento da biotecnologia de recursos naturais e na descoberta de novos fármacos.

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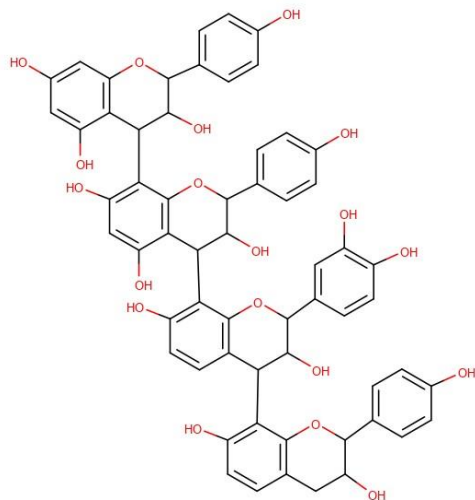
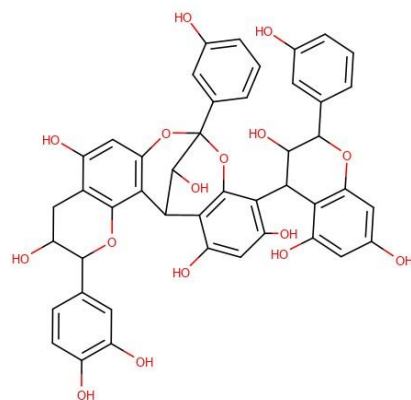
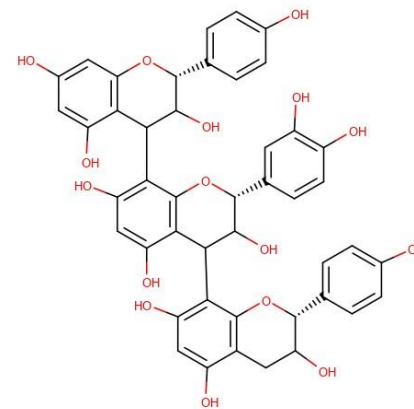
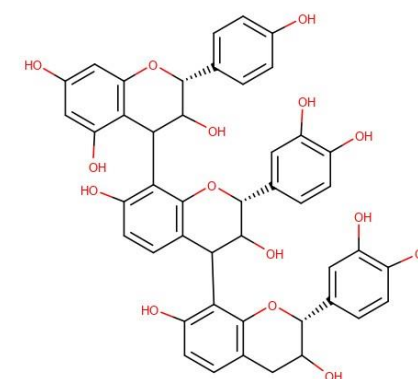
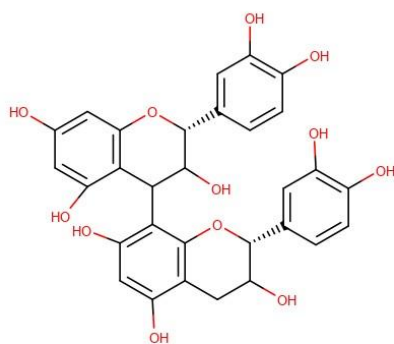
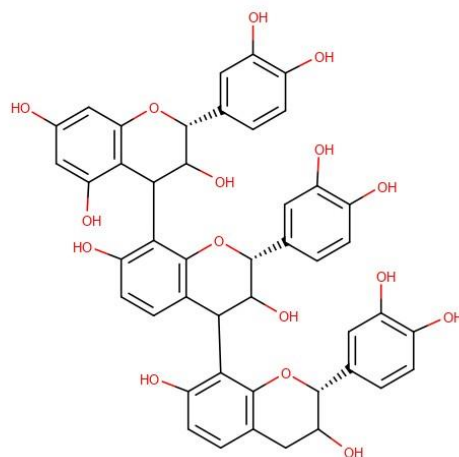
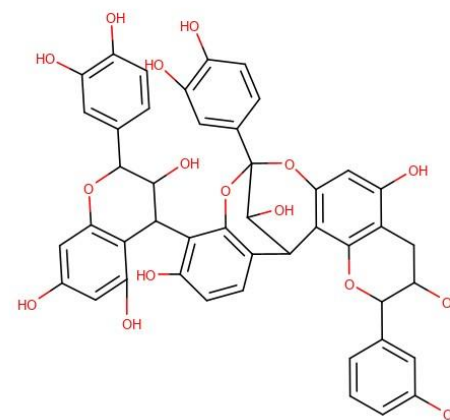
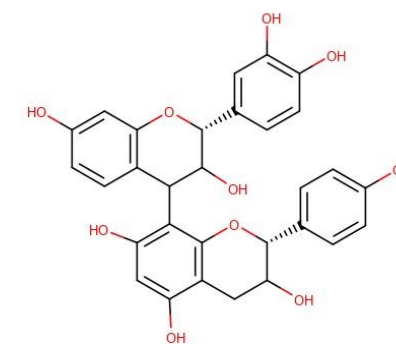
YANG, N. Y.; QIAN, S. H.; DUAN, J. A.; LI, P.; TIAN, L. J. Cytotoxic sesquiterpene lactones from *Eupatorium lindleyanum*. **Journal of Asian natural products research**, v. 9, n. 4, p. 339-345, 2007.

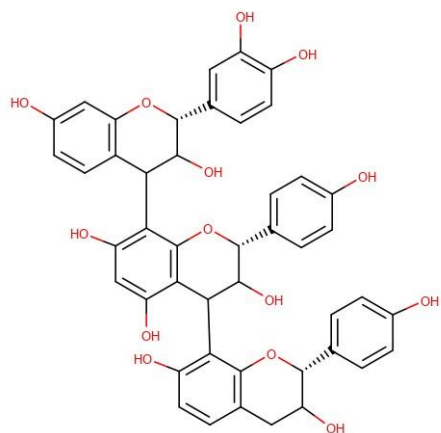
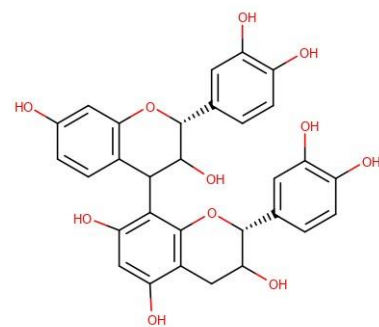
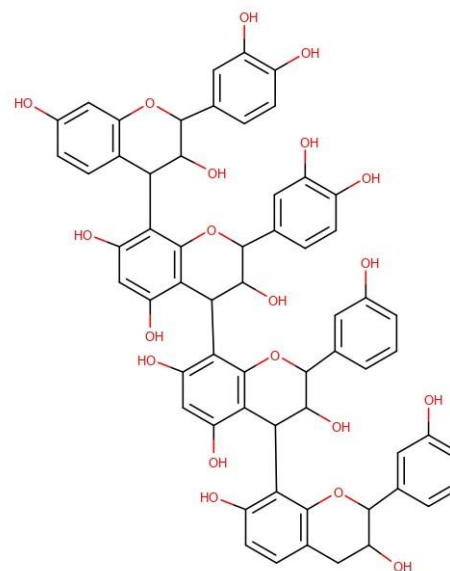
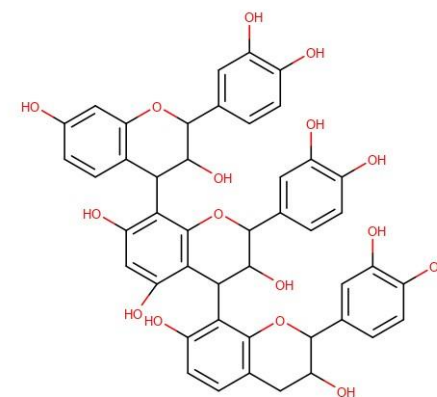
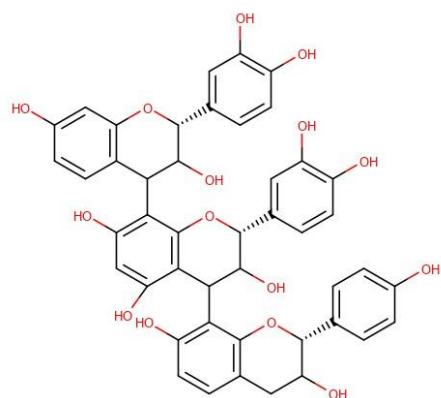
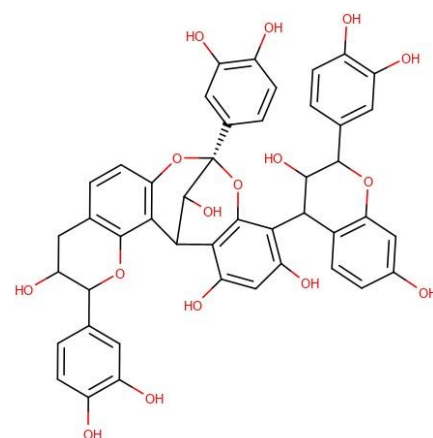
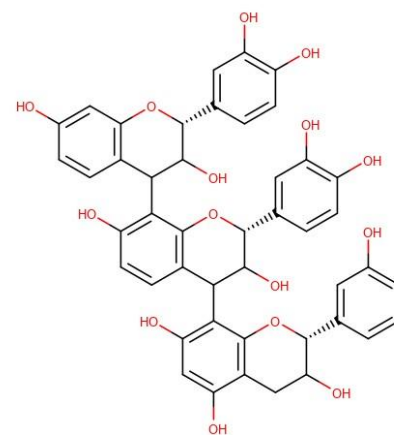
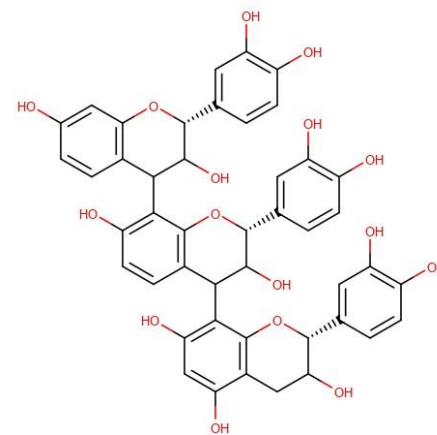
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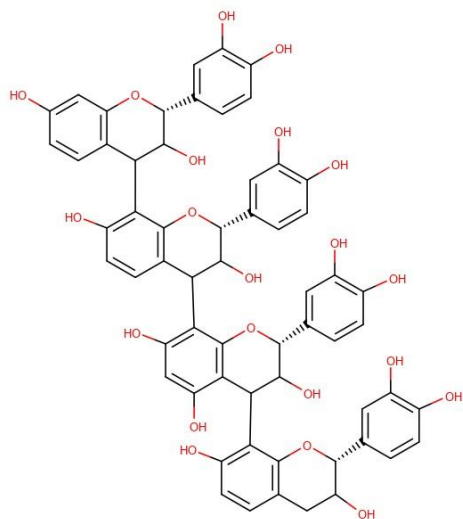
ZEFERINO, L. C.; QUEIROZ, R. T.; RANDO, J. G.; COTA, M. M. T.; FANTINI, I. F.; CAETANO, A. P. de S.; PEREZ, A. P. F. O gênero *Chamaecrista* (Leguminosae: Caesalpinioideae) no Parque Estadual do Rio Preto, São Gonçalo do Rio Preto, Minas Gerais, Brasil. **Rodriguésia**, v. 70, 2019.

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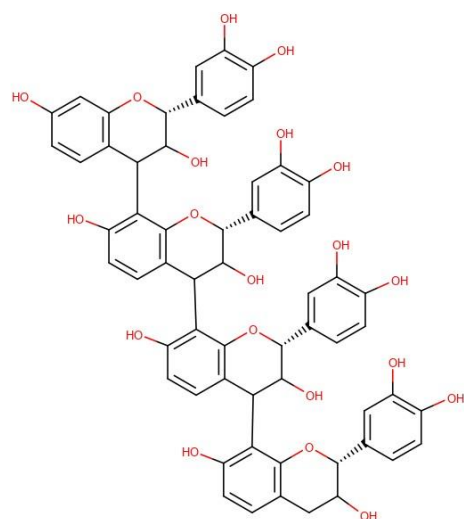
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APÊNDICE A - REPRESENTAÇÃO ESTRUTURAL DE SUBSTÂNCIAS IDENTIFICADAS NO GÊNERO *CHAMAECRISTA***(1)****(2)****(3)****(4)****(5)****(6)****(7)****(8)**

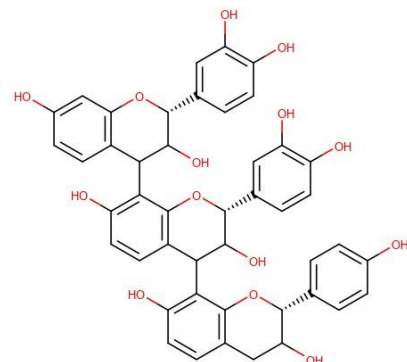
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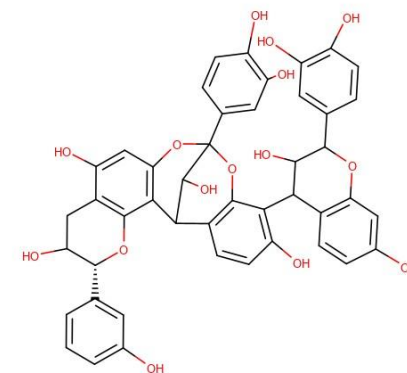
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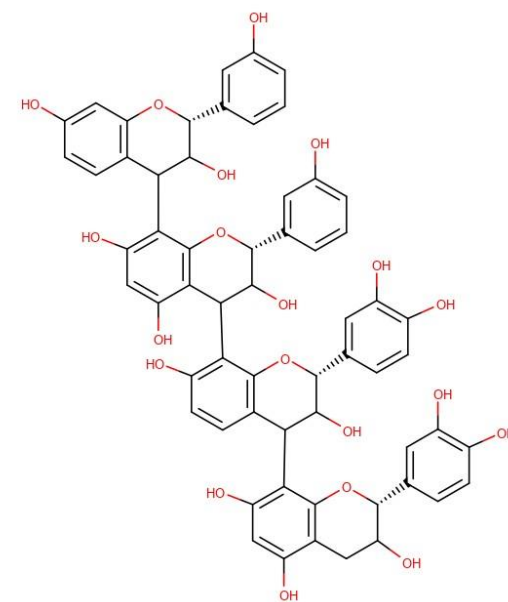
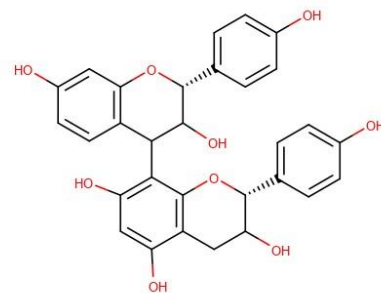
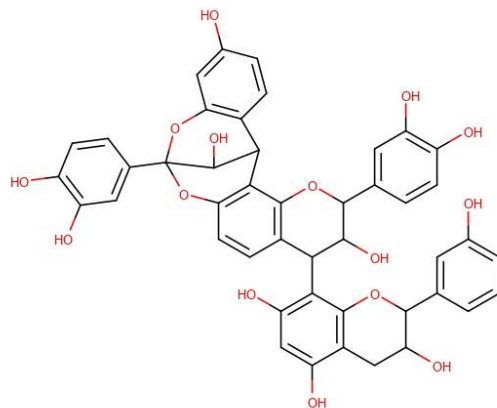
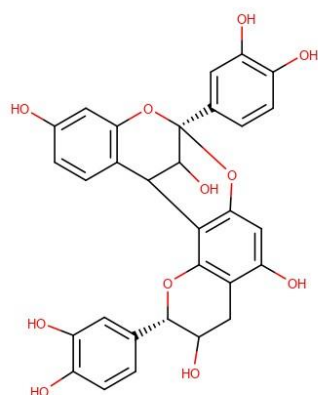
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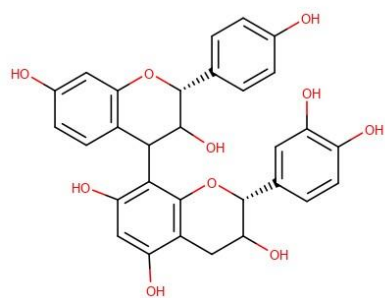
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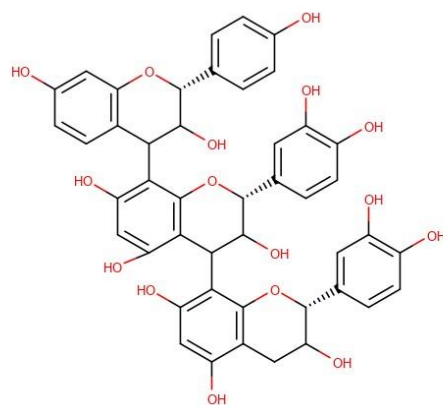
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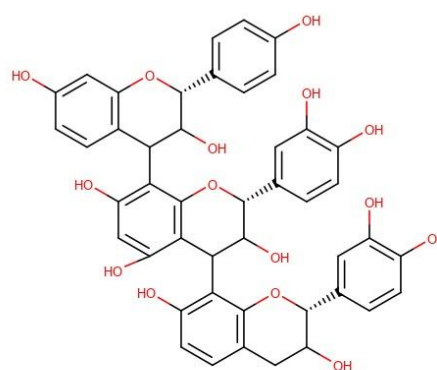
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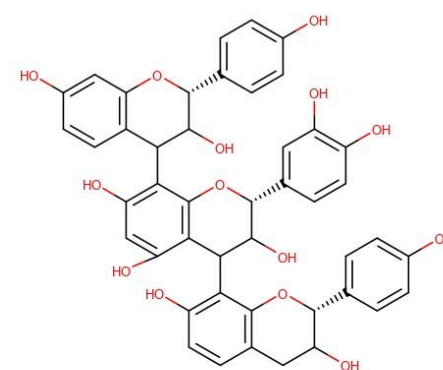
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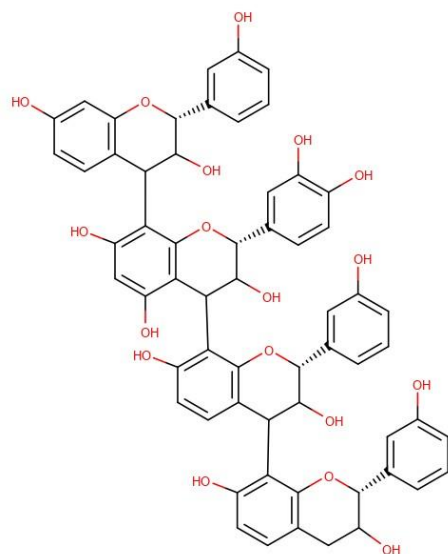
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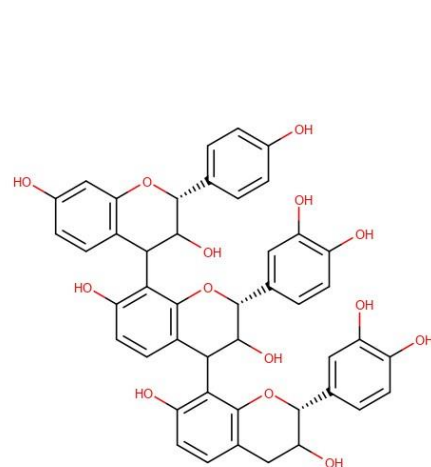
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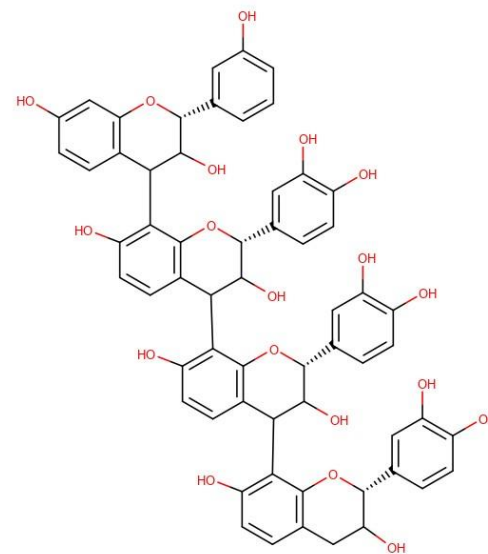
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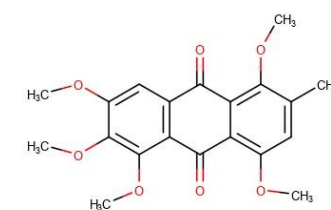
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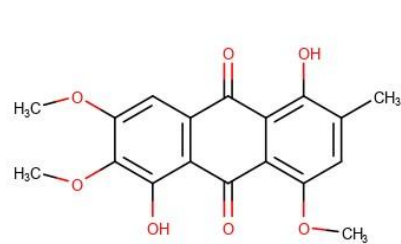


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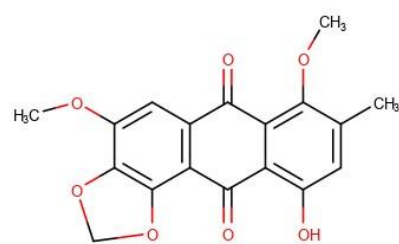
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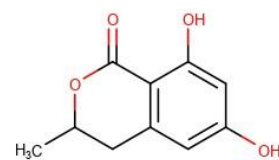
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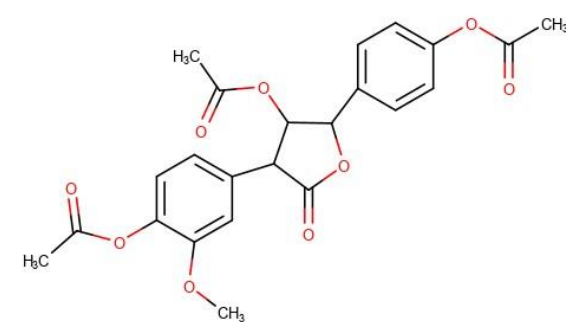
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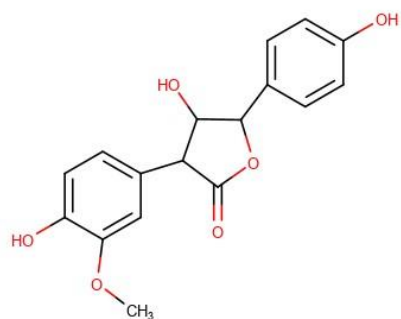
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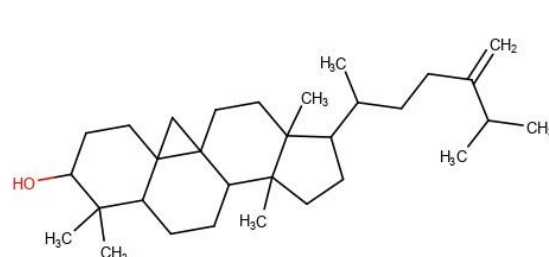
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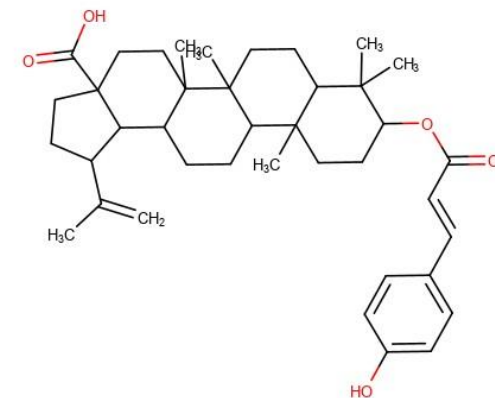
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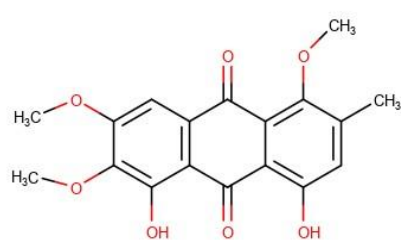
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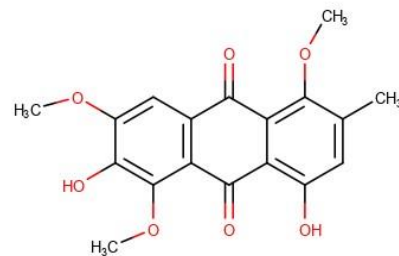
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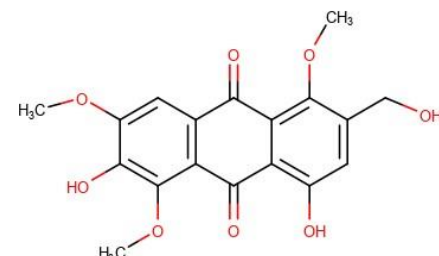
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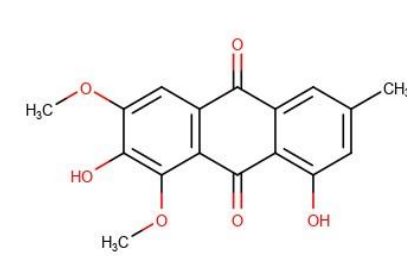
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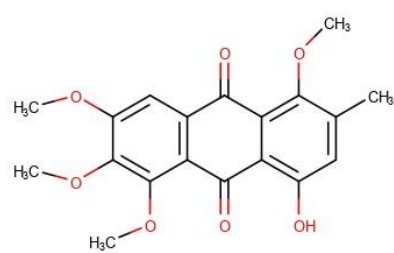
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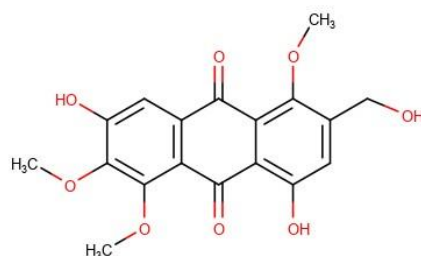
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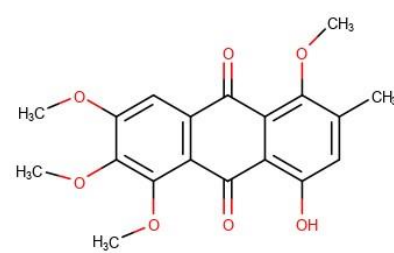
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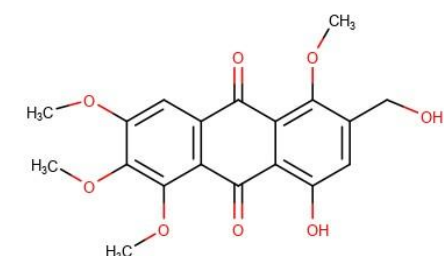
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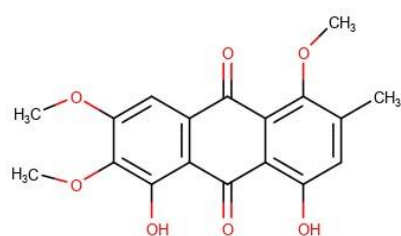
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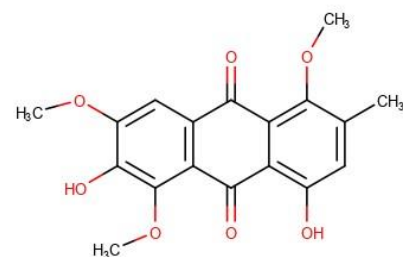
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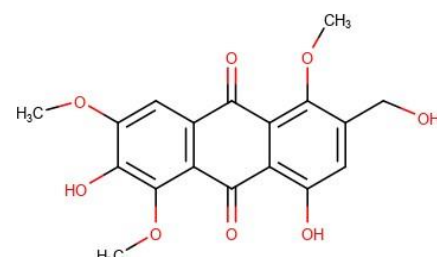
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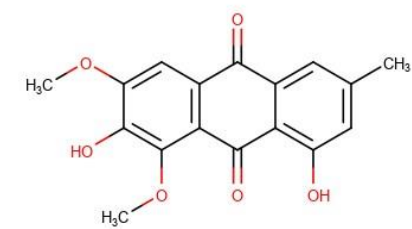
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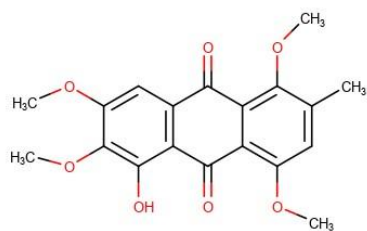
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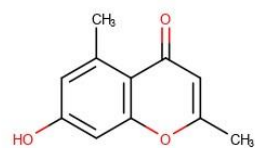
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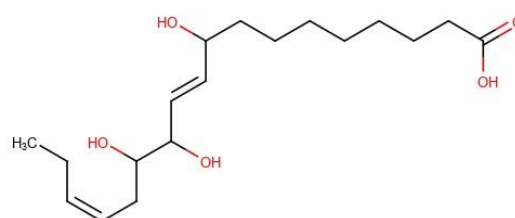
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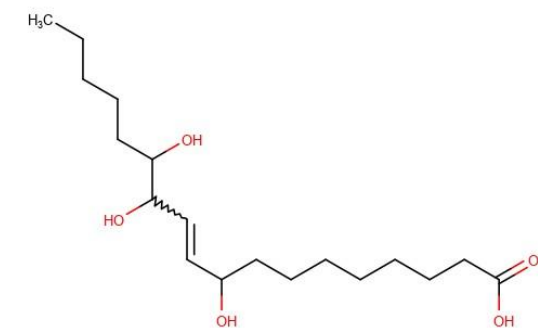
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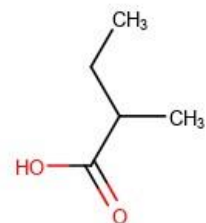
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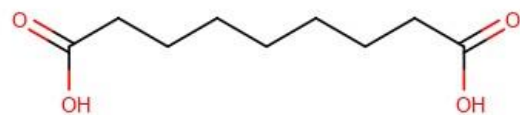
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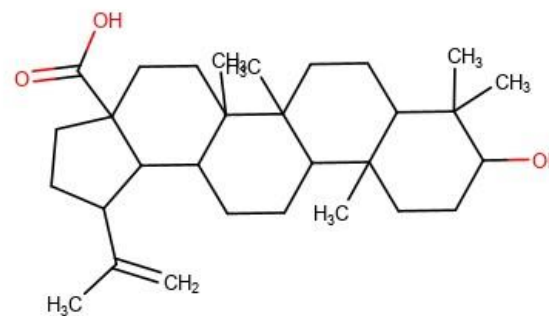
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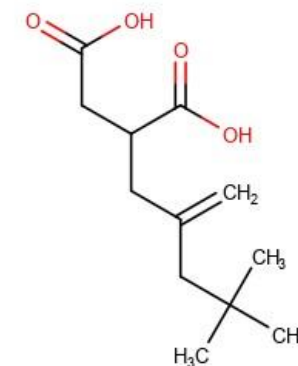
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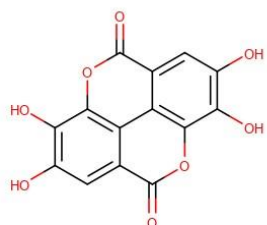
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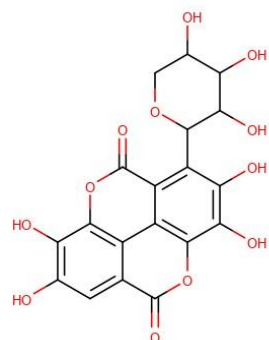
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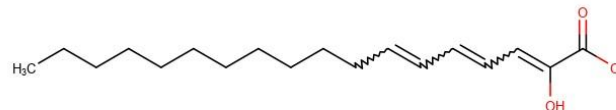
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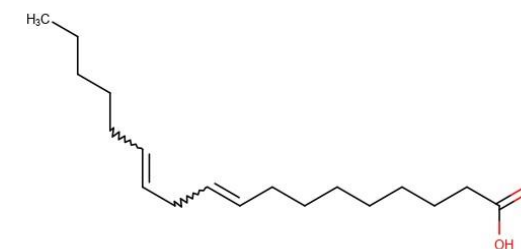
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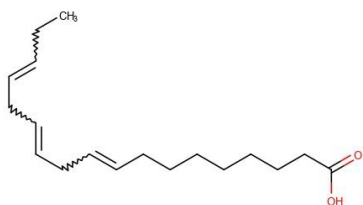
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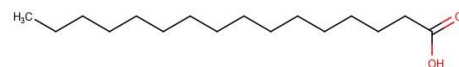
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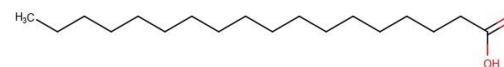
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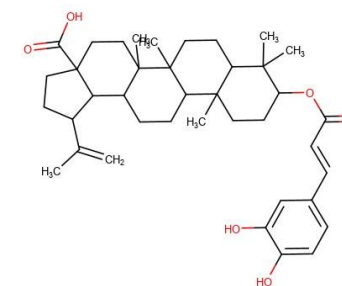
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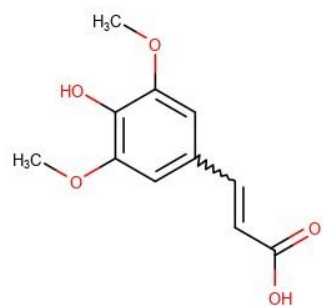
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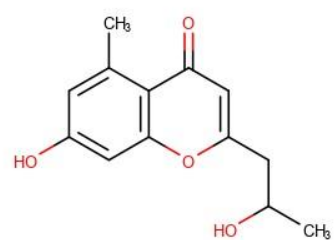
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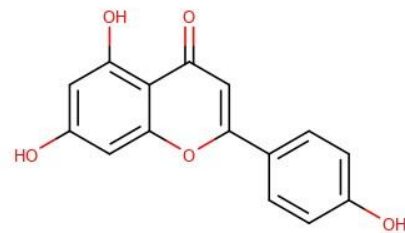
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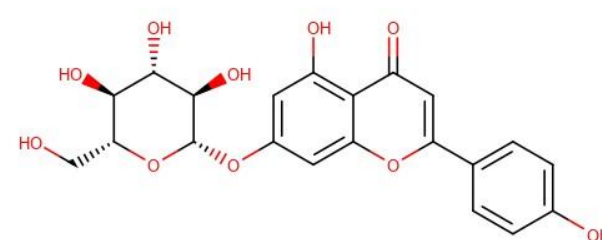
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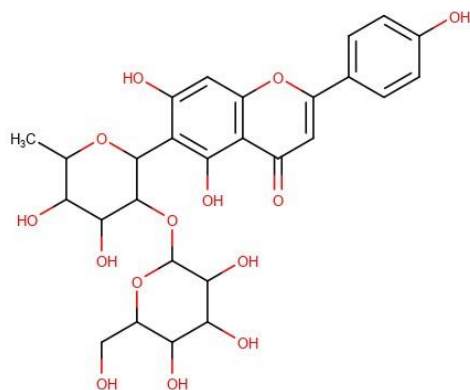
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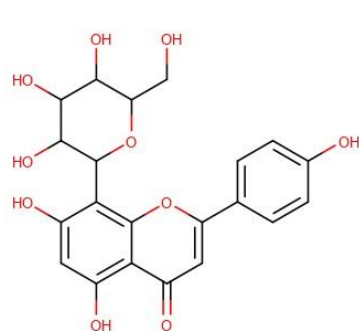
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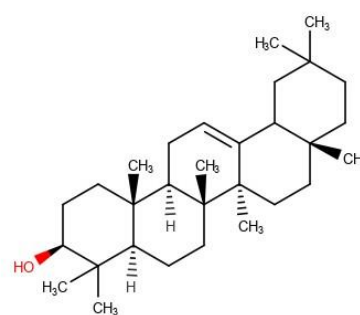
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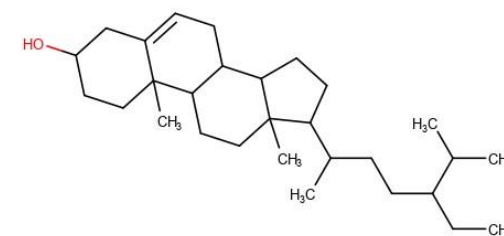
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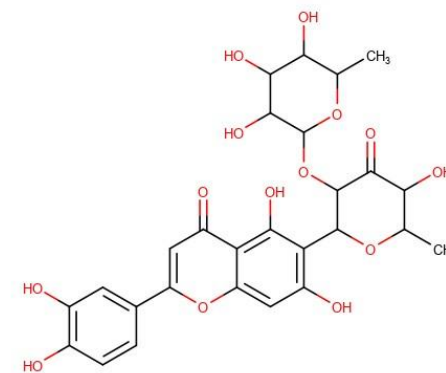
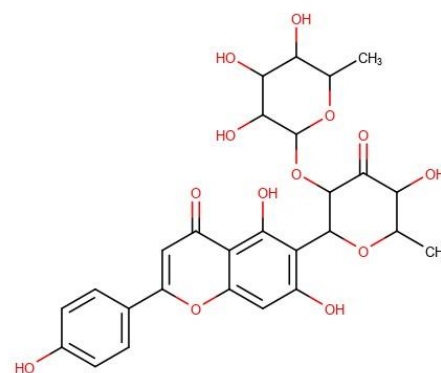
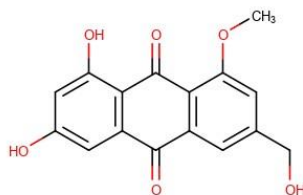
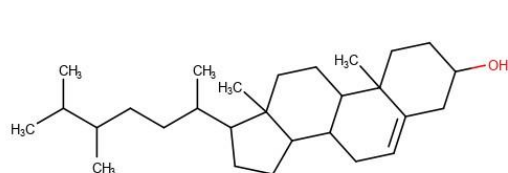
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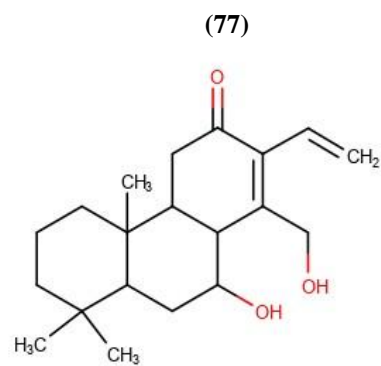


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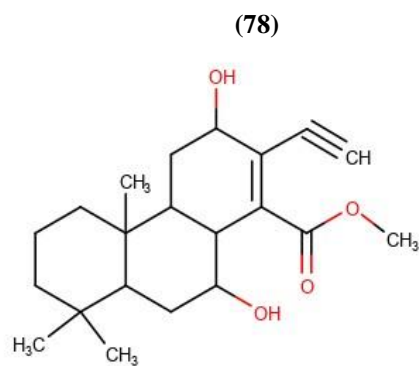


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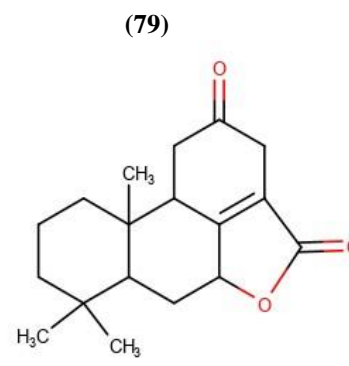




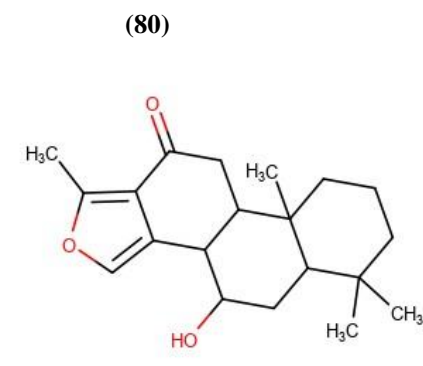
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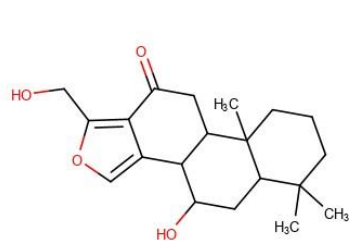
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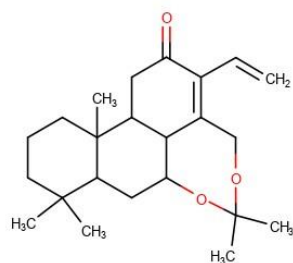
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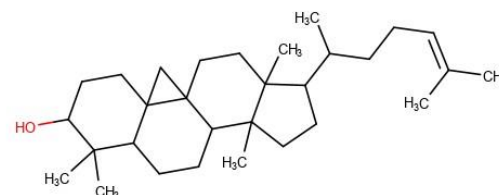
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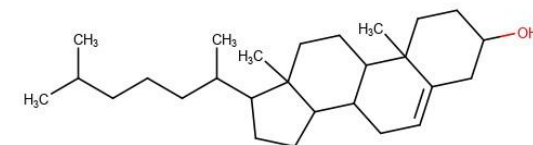
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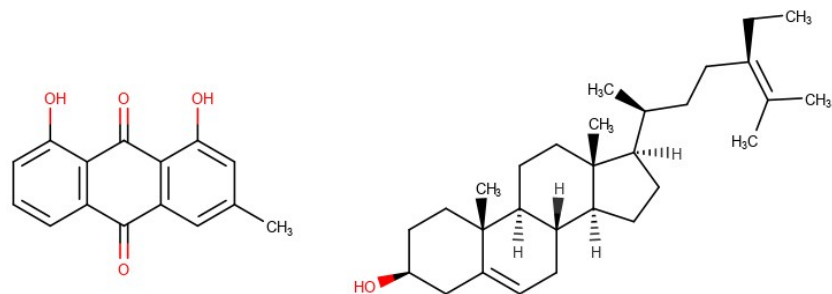
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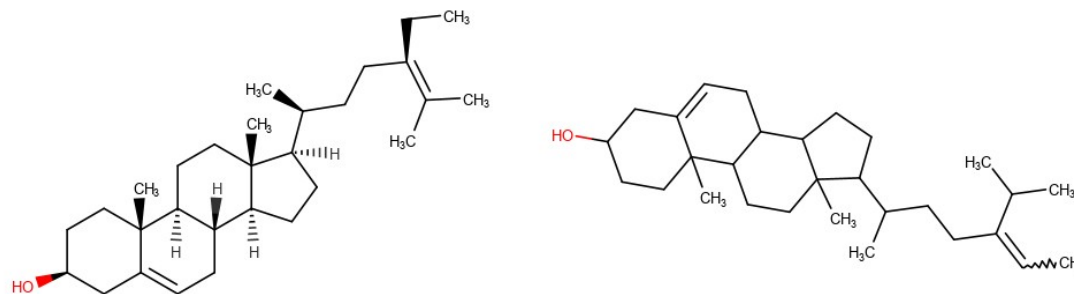
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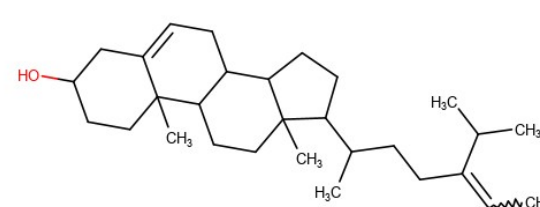
(88)



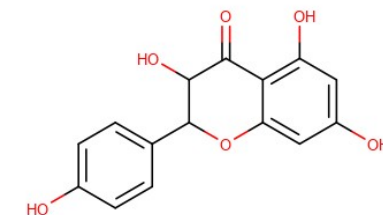
(89)



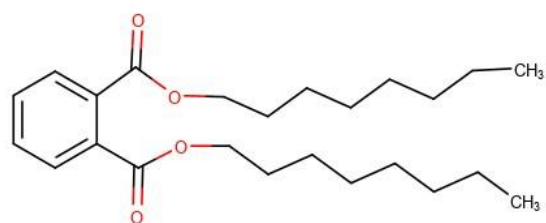
(90)



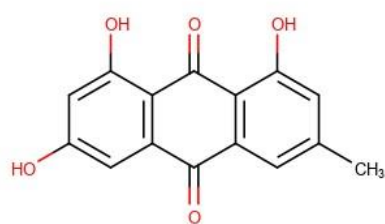
(91)



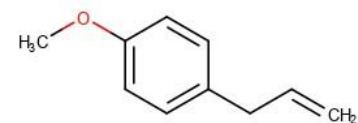
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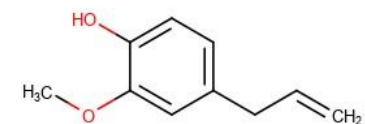
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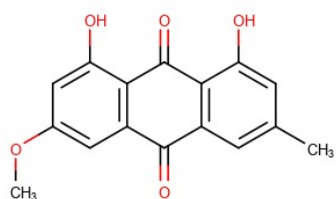
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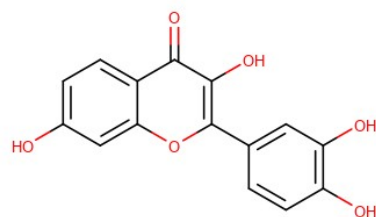
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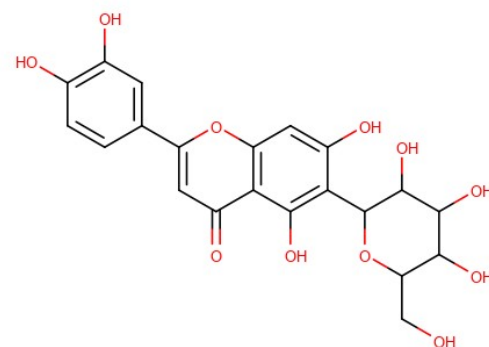
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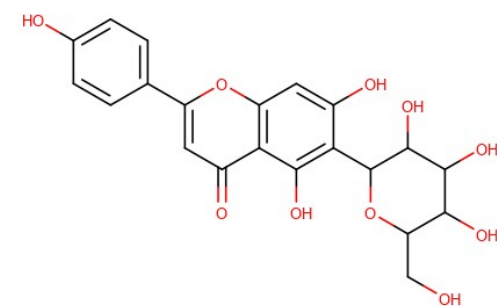
(97)



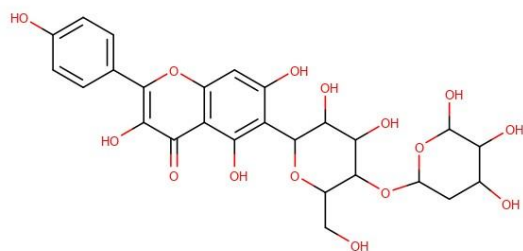
(98)



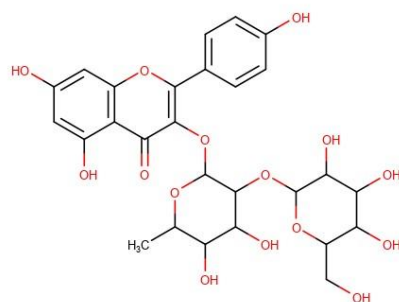
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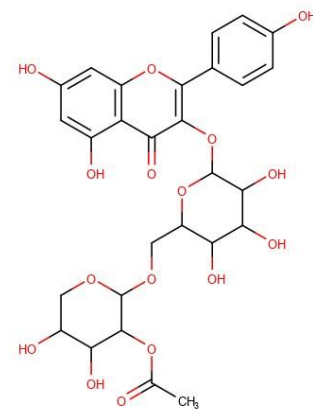
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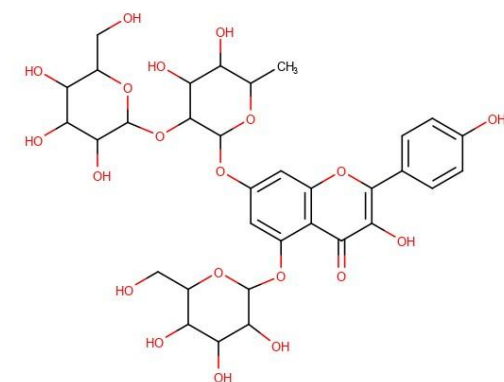
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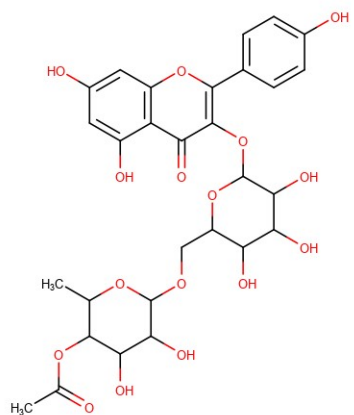
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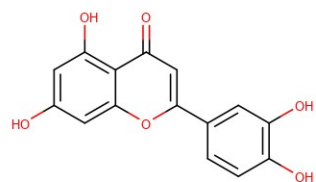
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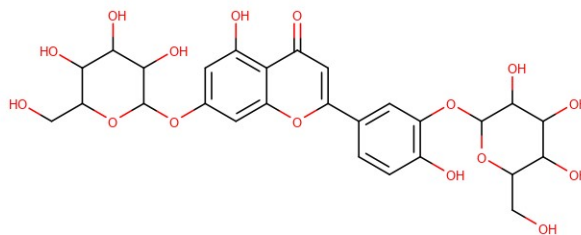
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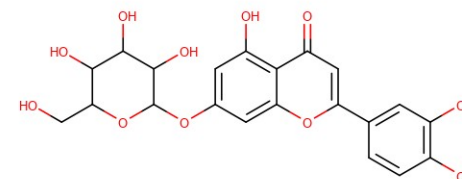
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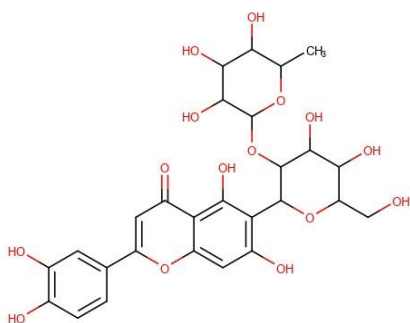
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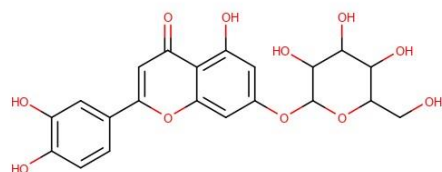
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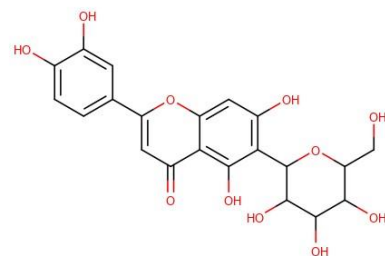
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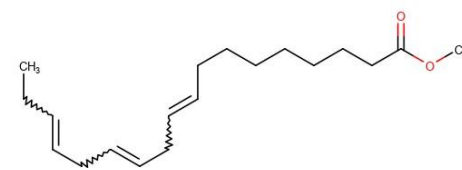
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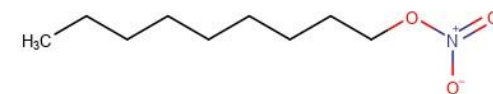
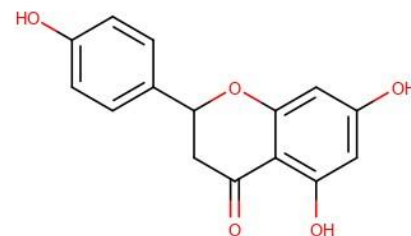
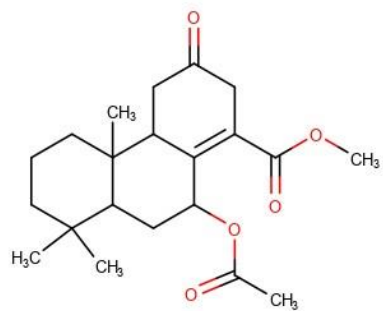
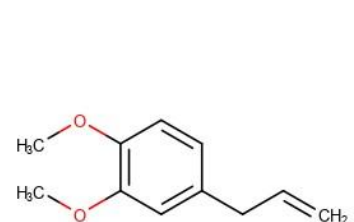
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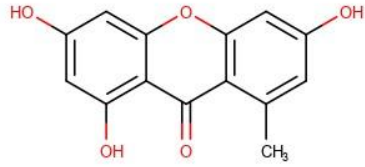
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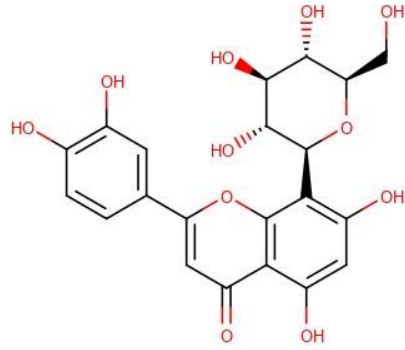
(112)



(113)



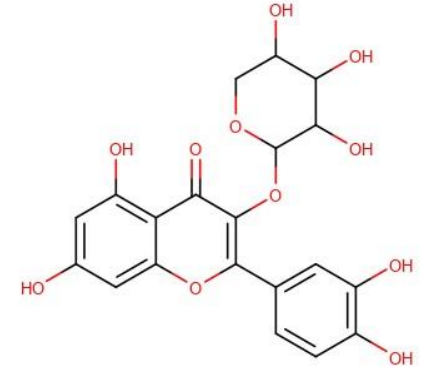
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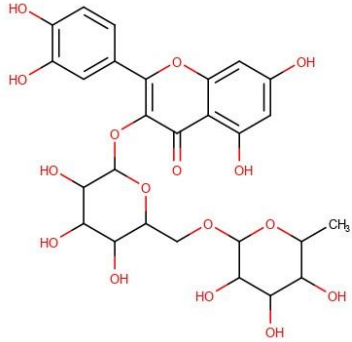
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(116)

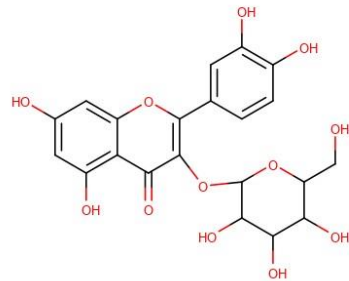


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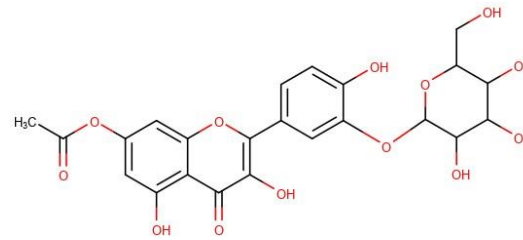
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(118)



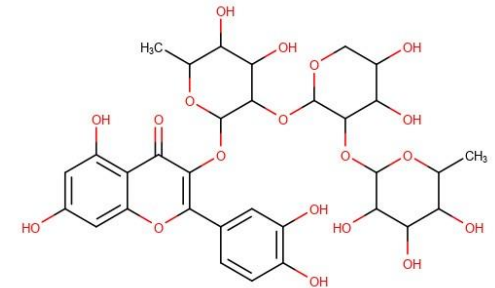
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(119)

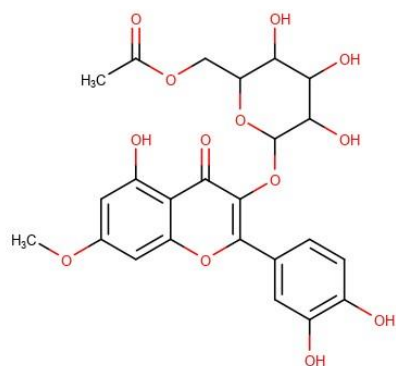


(123)

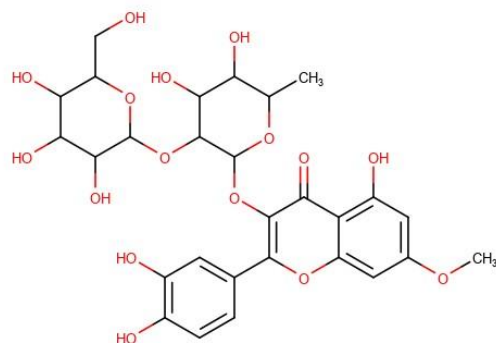
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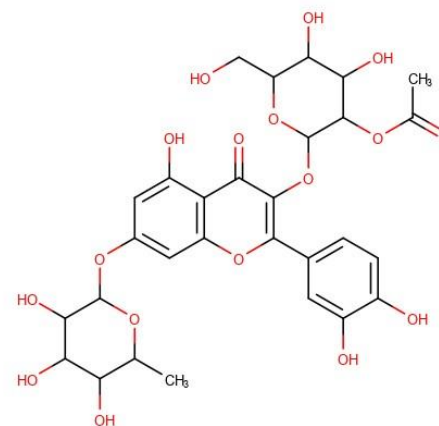
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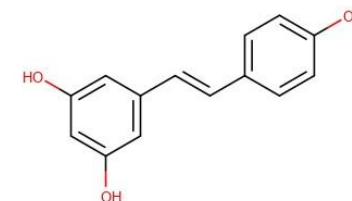
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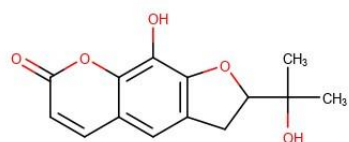
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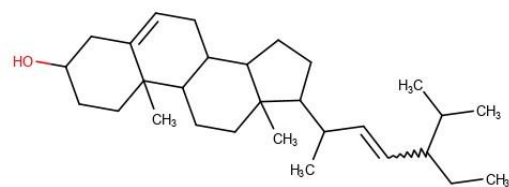
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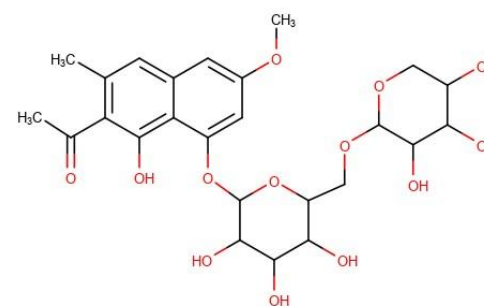
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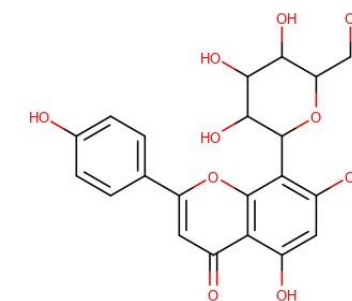
(129)



(130)



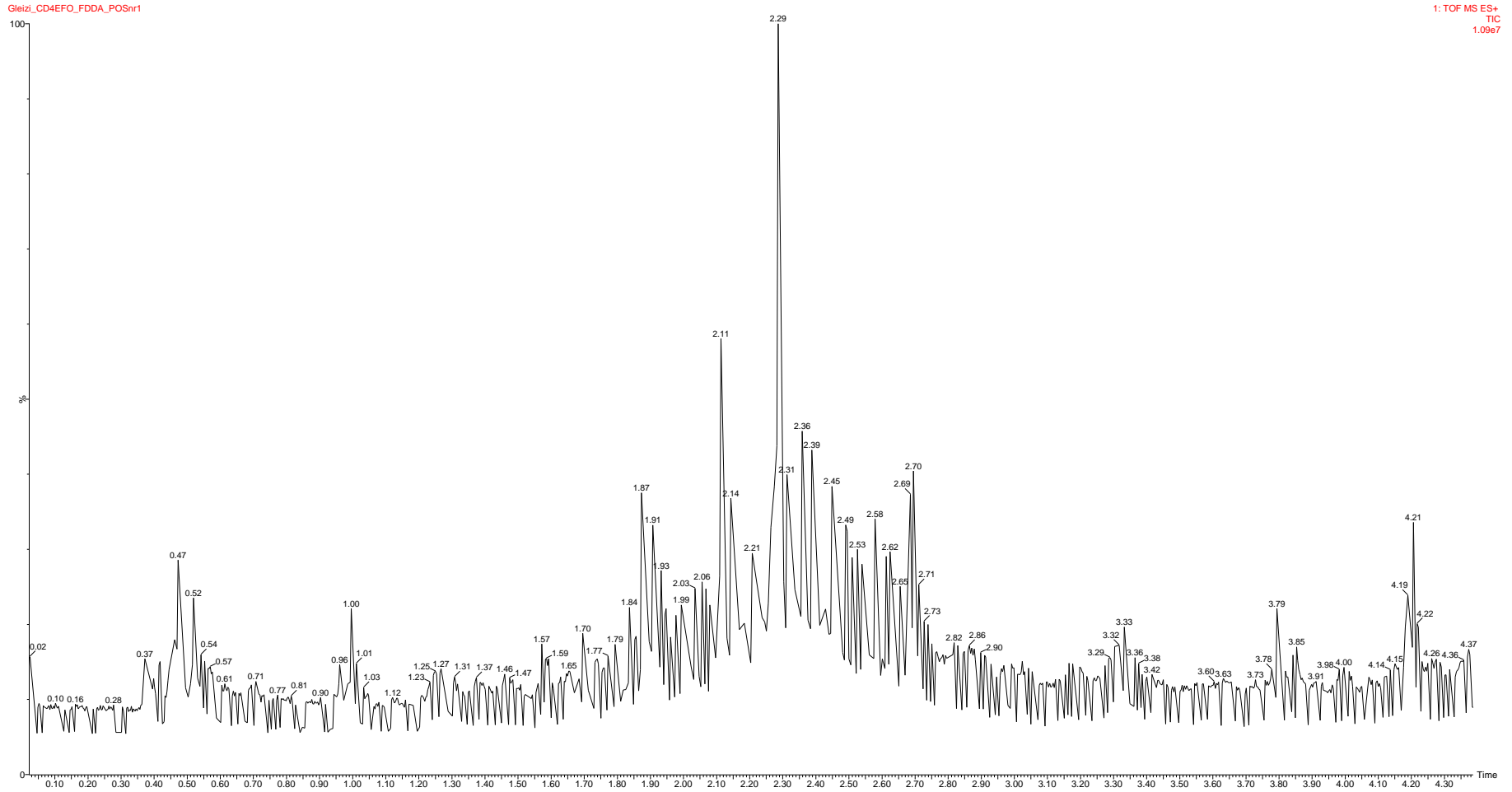
(131)



(132)

APÊNDICE B - CROMATOGRAMAS

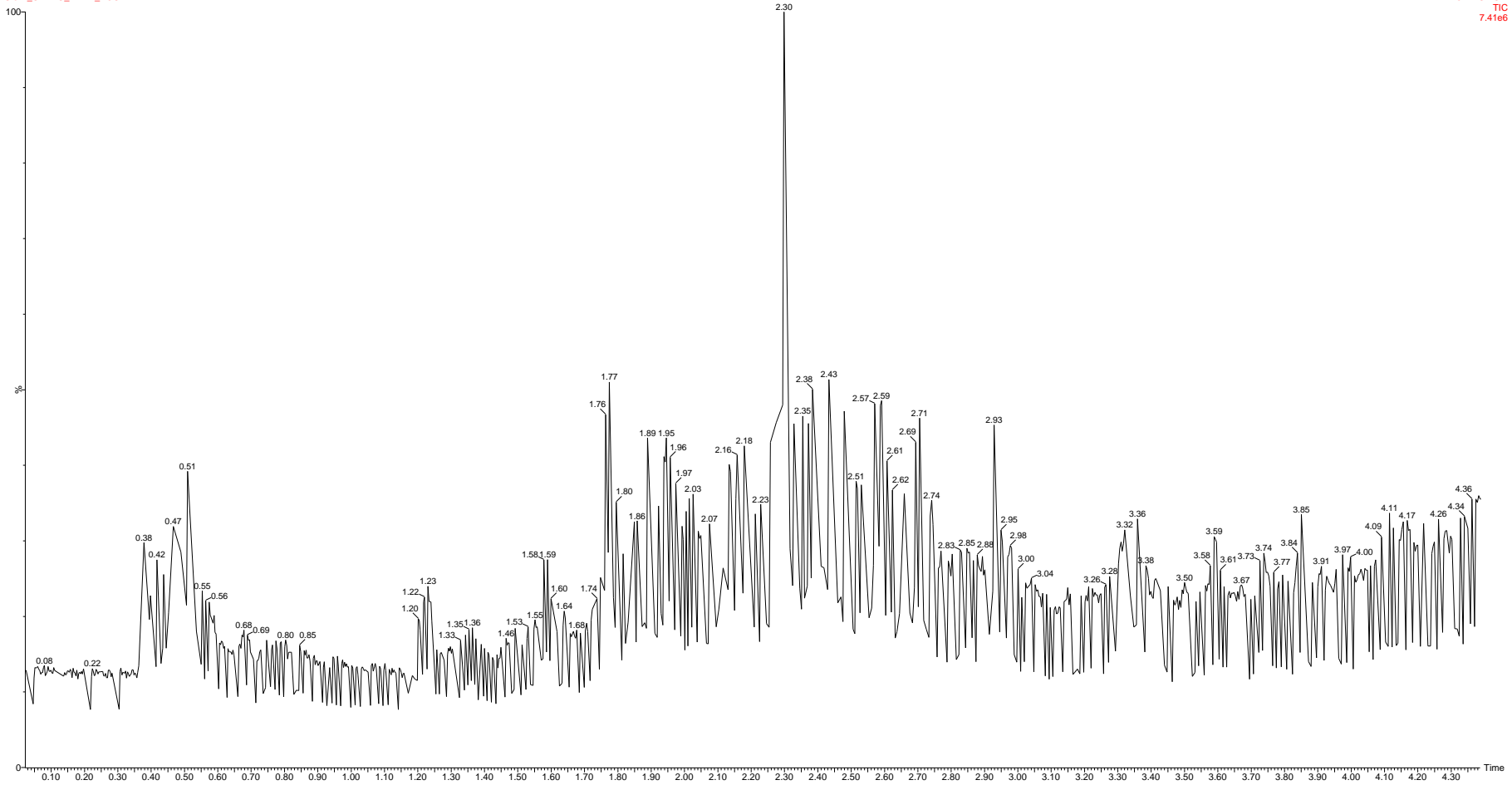
Folhas – *C. duckeana*



Galhos – *C. duckeana*

Gleizi_CD4EG_FDDA_POShr1

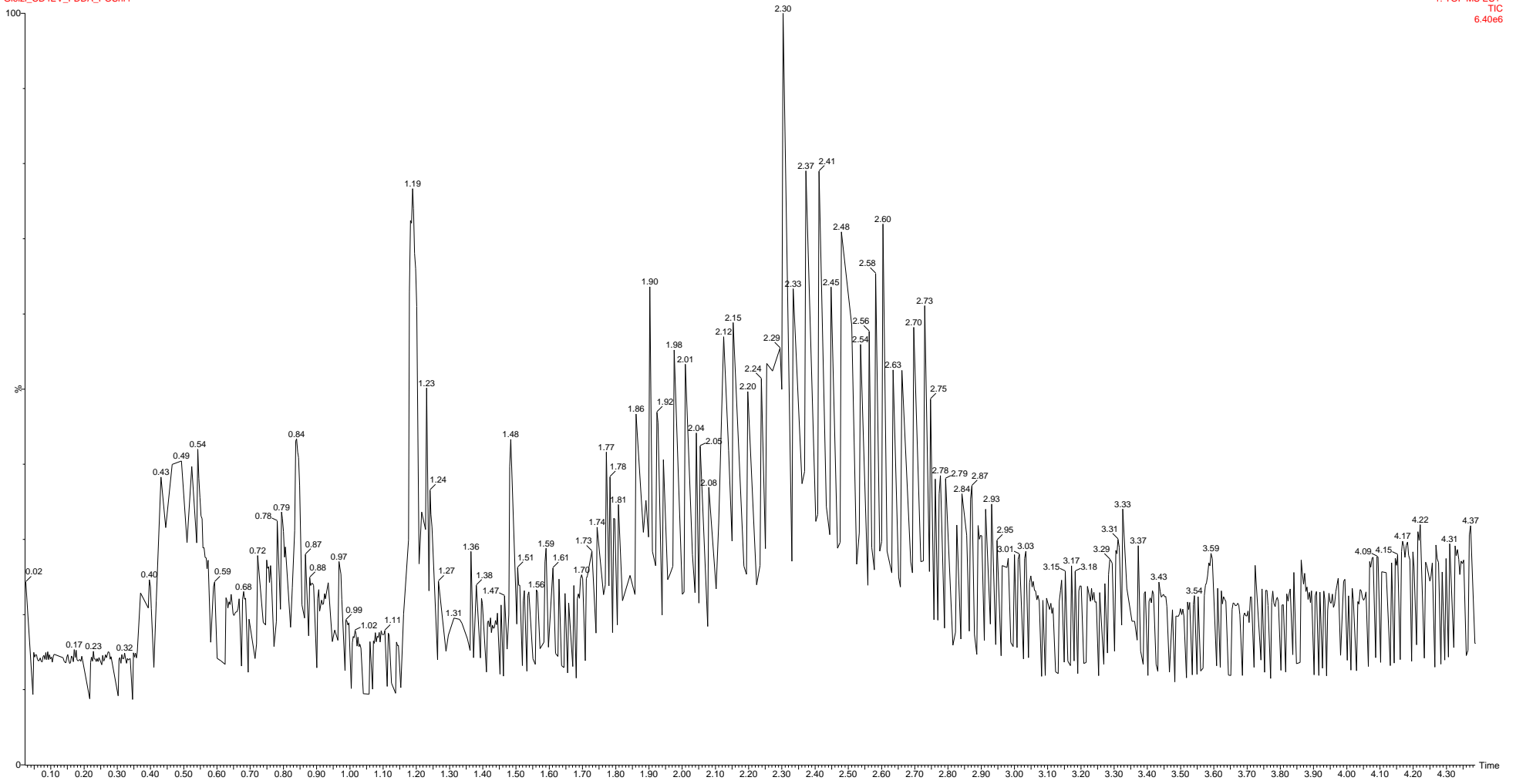
1: TOF MS ES+
TIC
7.41e6



Vagens – *C. duckeana*

Gleizi_CD4EV_FDDA_POSnr1

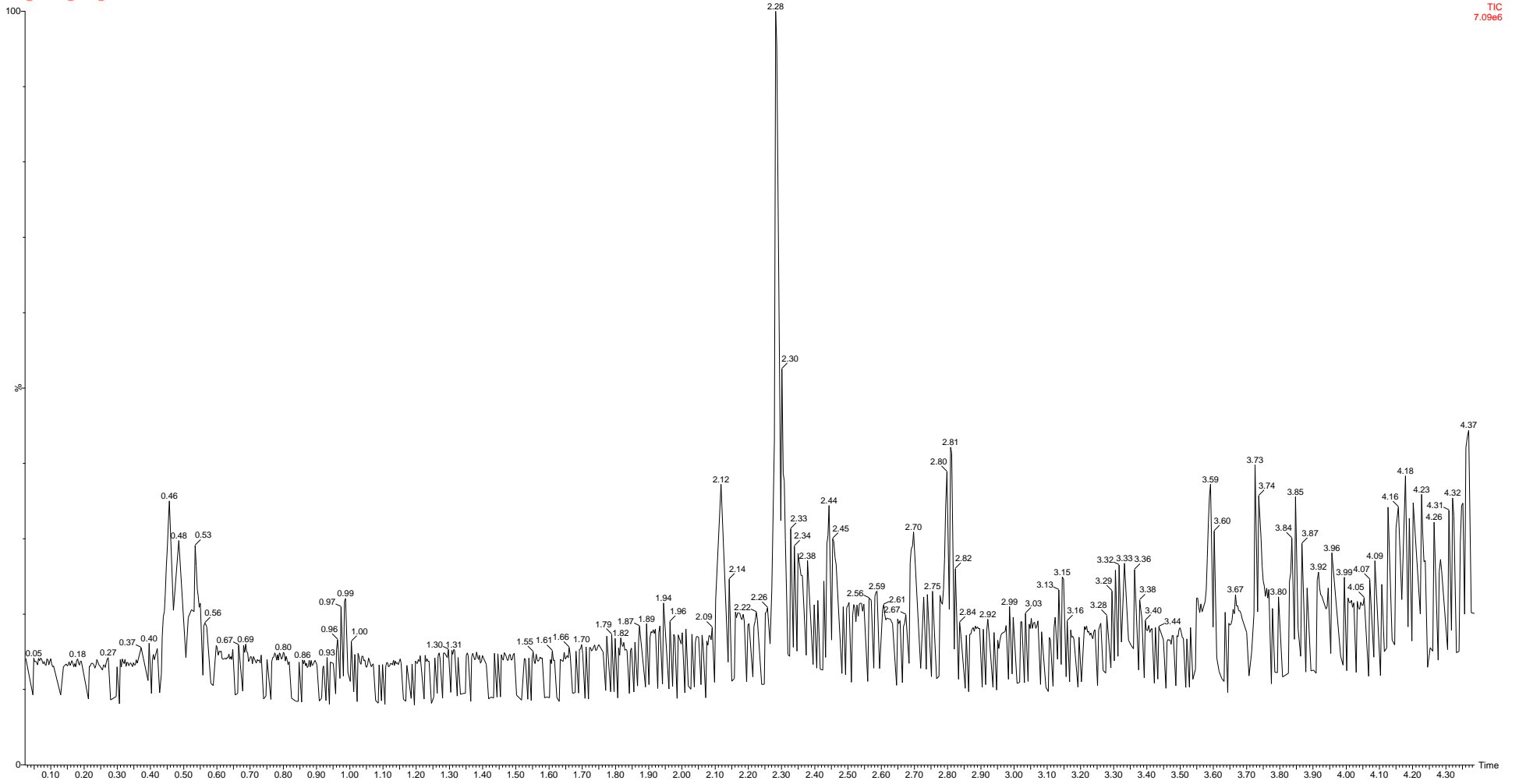
1: TOF MS ES+
TIC
6.40e6



Flores – *C. ensiformis*

Gleizl_CESEFL_FDDA_POSnr1

1: TOF MS ES+
TIC
7.09e6



Folhas – *C. ensiformis*

