

UNIVERSIDADE FEDERAL DE ALFENAS

PAULA PIO DE OLIVEIRA SALEM

**ISOLAMENTO BIOGUIADO DE SUBSTÂNCIAS ANTIUROLÍTICAS E ANTI-
INFLAMATÓRIAS DE *Cissus gongylodes* (VITACEAE)**

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Química, pela Universidade Federal de Alfenas. Área de concentração: Química Orgânica.

Orientadora: Dra. Daniela Aparecida Chagas de Paula

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(VITACEAE)

A Presidente da banca examinadora abaixo assina a aprovação da Tese apresentada como parte dos requisitos para a obtenção do título de Doutora em Química pela Universidade Federal de Alfenas. Área de concentração: Química Orgânica

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RESUMO

A *Cissus gongylodes* tem sido tradicionalmente utilizada na dieta de povos indígenas no Brasil e na medicina tradicional para a remoção de cálculos renais e doenças inflamatórias. Este estudo teve como objetivo isolar, pela primeira vez, substâncias presentes na decocção das folhas de *C. gongylodes* responsáveis por suas propriedades etnofarmacológicas anti-inflamatórias e antiurolíticas. As frações mais ativas da decocção foram fracionadas utilizando C18 em sistema SPE, e as substâncias foram purificadas por HPLC-UV-DAD. A atividade anti-inflamatória foi avaliada utilizando um ensaio *ex vivo* em sangue humano induzido por LPS e ionóforo de cálcio, medindo mediadores inflamatórios, PGE2 e LTB4. A atividade antiurolítica foi avaliada utilizando um modelo experimental *in vitro* com urina humana para determinar a dissolução dos cristais de oxalato de cálcio. Além disso, a decocção foi caracterizada quimicamente pela análise metabolômica utilizando UHPLC-ESI-HRMS. As substâncias isoladas, incluindo rutina, eriodictyol 3'-O-glicosídeo e isoquercetina, demonstraram ações multi-alvo significativas. Esses componentes atuam como agentes anti-inflamatórios, inibindo a síntese dos principais mediadores inflamatórios, PGE2 e LTB4. Além disso, exibem propriedades antiurolíticas, promovendo a dissolução de cristais de oxalato de cálcio. A caracterização da decocção por UHPL-HRMS revelou um alto teor de flavonoides, principalmente flavonoides glicosilados. Os resultados sustentam o uso tradicional da decocção de *C. gongylodes*, identificando as substâncias responsáveis por seus efeitos anti-inflamatórios e antiurolíticos. A ação multi-alvo exibida por *C. gongylodes* é particularmente desejável no tratamento da urolitíase, uma vez que a inflamação e a produção de PGE2 precedem e contribuem para a formação de cristais de CaOx nos rins. Com base nessas ações, *C. gongylodes* se destaca como uma fonte potente de compostos ativos para o desenvolvimento de novos tratamentos para a urolitíase.

Palavras-chave: oxalato de cálcio; PGE2; LTB4; metabolômica.

ABSTRACT

Cissus gongylodes has been traditionally used in the diet of Indigenous peoples in Brazil and in traditional medicine for the treatment of kidney stones and inflammatory diseases. This study aimed to isolate, for the first time, compounds present in the decoction of *C. gongylodes* leaves responsible for its ethnopharmacological anti-inflammatory and anti-urolithiatic properties. The most active fractions of the decoction were fractionated using C18 in an SPE system, and the compounds were purified by HPLC-UV-DAD. The anti-inflammatory activity was evaluated using an *ex vivo* assay in human blood induced by LPS and calcium ionophore, measuring inflammatory mediators PGE2 and LTB4. The anti-urolithiatic activity was assessed using an *in vitro* experimental model with human urine to determine the dissolution of calcium oxalate crystals. Furthermore, the decoction was chemically characterized by metabolomic analysis using UHPLC-ESI-HRMS. The isolated compounds, including rutin, eriodictyol 3'-O-glucoside, and isoquercetin, demonstrated significant multi-target actions. These components act as anti-inflammatory agents by inhibiting the synthesis of key inflammatory mediators, PGE2 and LTB4. Additionally, they exhibit anti-urolithiatic properties by promoting the dissolution of calcium oxalate crystals. The characterization of the decoction by UHPLC-HRMS revealed a high content of flavonoids, primarily glycosylated flavonoids. The results support the traditional use of the *C. gongylodes* decoction, identifying the compounds responsible for its anti-inflammatory and anti-urolithiatic effects. The multi-target action exhibited by *C. gongylodes* is particularly desirable in the treatment of urolithiasis, as inflammation and PGE2 production precede and contribute to the formation of calcium oxalate crystals in the kidneys. Based on these actions, *C. gongylodes* stands out as a promising source of active compounds for the development of new treatments for urolithiasis.

Keywords: calcium oxalate; PGE2; LTB4; metabolomics.

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LISTA DE ABREVIATURAS E SIGLAS

AA	Ácido araquidônico
ACN	Acetonitrila
AcOET	Acetato de Etila
AIES	Anti-Inflamatório Esteroidais
AINEs	Anti-Inflamatórios Não Esteroidais
ANOVA	Análise de Variância
CaOx	Oxalato de Cálcio
CAP	Cloranfenicol
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
COD	Oxalato de Cálcio Dihidratado
COM	Oxalato de Cálcio Monohidratado
COX	Ciclooxigenases
COX-1	Ciclooxigenase-1
COX-2	Ciclooxigenase-2
EA	Extrato Aquoso
EAcOEt	Extrato Acetato de Etila
ECHCl ₃	Extrato Clorofórmio
EtOH	Etanol
EEtOH	Extrato Etanólico
EHex	Extrato Hexânico
EMeOH	Extrato Metanólico
DEC	Decocto
FoAc	Fração Acetato de Etila
FoCl	Fração Clorofórmica
FoHi	Fração Hidroalcoólica
HPLC	Cromatografia Líquida de Alta Performance (do inglês, <i>High Performance Liquid Chromatography</i>)
HPLC-UV-DAD	Cromatografia Líquida Acoplada a Detector de Ultravioleta com Arranjo de Diodos (do inglês, <i>Liquid Chromatography-Ultraviolet-Diode Array Detector</i>)

IL-1 β	Interleucina 1 β
IL-6	Interleucina 6
iNOS	Óxido Nítrico Sintase Induzível (do inglês, <i>Inducible Nitric Oxide Synthase</i>)
LOX	Lipoxigenase
LPS	Lipopolissacarídeo
LTB ₄	Leucotrienos B ₄
MDA	Malondialdeído
SRM	Monitoramento de reações selecionadas (do inglês, <i>Selected Reaction Monitoring</i>)
MS/MS	Espectrometria de massa/massa
NF- κ B	Fator Nuclear Kappa B
PGs	Prostaglandinas
PGE ₂	Prostaglandinas E ₂
ROS	Espécies Reativas do Oxigênio
TNF- α ;	Fator de Necrose Tumoral Alfa
UNIFAL	Universidade Federal de Alfenas
UPLC- HRMS	Cromatografia Líquida de Ultra Performance Acoplada à Espectrometria de Massas de Alta Resolução (do inglês, <i>Ultra Performance Liquid Chromatography high resolution Mass Spectrometry</i>)
5-LOX	5-Lipoxigenase

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1 INTRODUÇÃO GERAL

A urolitíase e a nefrolitíase, comumente conhecidas como cálculo renal, referem-se à formação de cálculos nos rins ou em qualquer parte do trato urinário. O cálculo renal afeta 10% da população mundial, podendo causar dor intensa, obstrução urinária, hematúria e, em casos graves, a perda da função renal (Bnaya *et al.*, 2024; Liu; Wang; Hao, 2024). A doença é considerada multifatorial devido aos fatores ambientais e genéticos, e tem uma alta taxa de recorrência, sendo de 60% em 10 anos (Khan, 2013). A formação dos cálculos envolve a nucleação dos cristais, crescimento, agregação e retenção deles nos rins. Cerca de 80% desses cálculos são formados por cálcio, incluindo oxalato de cálcio (CaOx) e fosfato de cálcio; o restante é composto de estruvita (fosfato de amônio e magnésio), ácido úrico e cistina (Singh *et al.*, 2022).

O acúmulo de deposição de cristais nos rins leva à superprodução de espécies reativas e à síntese de moléculas pró-inflamatórias, resultando em danos nos rins, perda de células renais, inflamação e até mesmo insuficiência renal crônica (Khan *et al.*, 2021). Mediadores químicos são recrutados para reduzir a inflamação como edema, dor, e a formação dos cálculos como mecanismo de defesa (Medzhitov, 2008). A enzima fosfolipase A2 (PLA2) presente nas membranas celulares catalisa a produção de ácido araquidônico (AA) (Nathan; Ding, 2010). O AA é metabolizado por enzimas como as ciclooxigenases (COXs), que produzem prostaglandinas como a prostaglandina E2 (PGE2), e as lipoxigenases (LOXs), que geram leucotrienos como o leucotrieno B4 (LTB4) (Chen *et al.*, 2018). A PGE2 promove vasodilatação, enquanto o LTB4 auxilia na quimiotaxia, recrutando neutrófilos para o local da lesão renal (Galli *et al.*, 2011; Medzhitov; Janeway, 2000).

Atualmente, o tratamento do cálculo renal inclui o uso de analgésicos, anti-inflamatórios e relaxantes musculares para alívio da dor e facilitação da eliminação de pequenos cristais. Agentes modificadores da constituição urinária são utilizados para evitar a supersaturação de sais formadores de cálculos (Khan; Canales; Dominguez-Gutiérrez, 2021). Em alguns casos, a intervenção cirúrgica e técnicas por ondas de choque são empregadas para a remoção dos cálculos, mas podem causar dor, sangramento, infecções e danos aos tecidos (Singh *et al.*, 2022). Apesar dos avanços farmacológicos e das técnicas sofisticadas, os procedimentos permanecem caros e podem causar hemorragias devido às técnicas invasivas

necessárias para a remoção dos cálculos. Assim, ainda não existem tratamentos totalmente eficazes para dissolver cálculos, reduzir sua recorrência e agir como anti-inflamatórios (Nimavat *et al.*, 2022).

As plantas medicinais desempenham um papel complementar no tratamento do cálculo renal, sendo mais econômicas, eficazes e com menores efeitos colaterais. Os compostos naturais geralmente possuem estruturas químicas mais compatíveis com os sistemas biológicos humanos, o que pode resultar em uma melhor tolerabilidade e menor incidência de efeitos adversos. Diversas plantas medicinais têm demonstrado um papel promissor na inibição e dissolução dos cálculos, podendo ter potenciais diuréticos, antioxidantes, anti-inflamatórios, entre outros (Jebir; Mustafa, 2024). Sendo assim, produtos naturais derivados de plantas têm sido uma fonte valiosa na busca por substâncias bioativas no desenvolvimento de novos medicamentos (Newman; Cragg, 2020; Zhou *et al.*, 2018). Os extratos vegetais geralmente contêm uma grande variedade de metabólitos, e a bioatividade desses extratos pode ser atribuída a esses metabólitos. No entanto, isolar todos os metabólitos presentes em um extrato nem sempre é viável, devido ao tempo necessário, à complexidade química envolvida e à baixa concentração de muitos metabólitos especializados, além do isolamento de metabolitos já conhecidos na literatura (Aderemi *et al.*, 2021).

A metabolômica surgiu como uma estratégia indispensável para a análise de milhares de metabólitos presentes em um único extrato sob condições específicas. Essa abordagem utiliza diversas ferramentas, incluindo técnicas analíticas como a cromatografia líquida acoplada à espectrometria de massa (LC-MS), que possibilita a análise abrangente do perfil metabólico dos constituintes presentes nos extratos vegetais (Rochfort, 2005). Os dados obtidos podem ser utilizados para a identificação de biomarcadores em extratos biologicamente ativos e na descoberta de metabólitos possivelmente inéditos (Yuliana *et al.*, 2011).

Neste contexto, o decocto das folhas de *Cissus gongylodes* (Vitaceae) tem sido utilizado na medicina tradicional para a remoção de cálculos renais, além de problemas biliares e inflamatórios, sem a comprovação científica das substâncias envolvidas na atividade biológica associada à etnofarmacologia (Ahmed; Hasan, 2017; Ahmed; Hasan; Mahmood, 2016; Pirker *et al.*, 2012). Estudos anteriores mostraram que o decocto das folhas de *C. gongylodes* apresenta ação anti-inflamatória, inibindo as principais vias da inflamação, as COXs e LOXs, ação

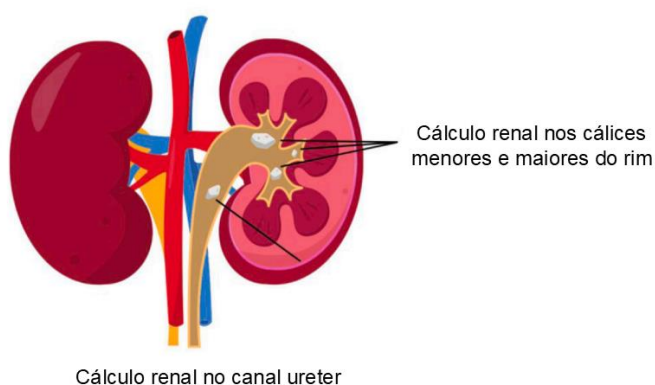
antiedematogênica e atividade antiurolítica, inibindo e dissolvendo cristais de CaOx, os mais prevalentes, e de estruvita (Salem *et al.*, 2020). Considerando o histórico etnofarmacológico da espécie *C. gongyloides* e as limitações dos tratamentos do cálculo renal, o presente estudo tem como objetivo avaliar pela primeira vez a atividade antiurolítica e anti-inflamatória das frações do decocto, a fim de bioguiar o isolamento de substâncias anti-inflamatórias e antiurolíticas. Além disso, visa compreender o perfil químico do decocto de *C. gongyloides* utilizando a cromatografia líquida acoplada a espectrometria de massas.

2 REVISÃO LITERATURA

2.1 UROLITÍASE

A urolitíase e a nefrolitíase, comumente chamadas de cálculo renal, referem-se à formação de cálculos nos rins e no sistema urinário, podendo causar episódios de dor intensa e até mesmo o comprometimento da função renal (Sakhaee; Maalouf; Sinnott, 2020). Os cálculos renais são depósitos minerais que podem ser encontrados nos cálices renais e na pelve de forma livre ou presos às papilas renais (Figura 1) (Khan, 2016). A urolitíase é considerada uma doença multifatorial, resultante da interação entre fatores ambientais e genéticos. A doença está associada a condições relacionadas ao estilo de vida, incluindo doenças cardiovasculares, hipertensão, doença renal crônica, diabetes e síndrome metabólica (Liu *et al.*, 2022). A urolitíase afeta cerca de 13% da população mundial, e sua ocorrência é comparável à do diabetes. A doença apresenta uma alta taxa de recorrência, sendo de 50% entre 5 e 10 anos e 75% dentro de 20 anos, tanto em homens quanto em mulheres (Ghale-Salimi *et al.*, 2018).

Figura 1 – Representação dos cálculos renais no interior do rim



Fonte: Zakaria *et al.* (2023).

Os cálculos são formados de cálcio 80%, especificamente oxalato de cálcio (CaOx) e fosfato de cálcio sendo os mais ocorrentes, 10% de estruvita (fosfato de amônio e magnésio), enquanto os 10% restantes são formados por ácido úrico e

cistina. Os cálculos de CaOx podem ser de dois tipos monohidratado (COM) e dihidratado (COD) (Lotan *et al.*, 2017). Os fatores metabólicos como a hipercalcúria e hiperoxalúria contribuem para a formação de cristais de CaOx devido ao aumento da concentração dos níveis séricos normais de cálcio e oxalato de cálcio na excreção urinária, levando a formação dos cálculos urinários (Alelign; Petros, 2018; Tiselius, 2011).

Os cálculos de estruvita são originados de infecções no trato urinário causadas por bactérias como *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Corynebacterium*, entre outras. As bactérias produtoras da enzima urease promovem a decomposição da ureia em amônia e dióxido de carbono (Sharma; Khan; Ahmad, 2016). Esse processo, catalisado pela enzima urease, torna a urina alcalina, aumentando a concentração dos componentes que formam os cristais de estruvita, o que, por fim, propicia a formação desses cristais (Das *et al.*, 2017). Os cristais de ácido úrico decorrem do metabolismo das purinas, resultando no excesso do ácido úrico e contribuindo para a formação dos cristais (Khan, 2014). Já os cálculos de cistina ocorrem devido ao aumento da concentração desse aminoácido, geralmente causado por uma condição hereditária chamada de cistinúria (Duan *et al.*, 2013; Sharma; Khan; Ahmad, 2016).

O mecanismo de formação dos cálculos envolve uma série de processos complexos, incluindo nucleação, crescimento de cristais insolúveis, agregação e retenção desses cristais nos rins (Liu; Wang; Hao, 2024). O processo de nucleação dos cristais é o primeiro estágio da cristalização e se inicia pela supersaturação de minerais formadores de cálculos, como cálcio e oxalato, presentes na urina (Gomes *et al.*, 2005). A nucleação pode ocorrer de forma homogênea ou heterogênea, na qual os íons minerais se combinam na presença de proteínas e outros tipos de minerais (Velu *et al.*, 2017). A precipitação dos cristais em urina supersaturada pode ocorrer durante a formação da urina nos túbulos renais (Mulay; Anders, 2014). Uma vez formado, o núcleo de um cristal pode crescer devido à presença de urina supersaturada, resultando em crescimento e aglomeração no interior dos rins, especificamente nos epitélios tubulares (Kumar *et al.*, 2016).

A formação e o crescimento dos cristais podem ser influenciados pela composição da urina, incluindo a saturação de minerais formadores de cálculos e a presença de modificadores inorgânicos e orgânicos, como glicoproteínas e glicosaminoglicanos (Muvhulawa *et al.*, 2022). Modificadores conhecidos como

inibidores de cristalização e nucleação, como citrato, magnésio e zinco, atuam reduzindo a disponibilidade de íons oxalato e cálcio, impedindo, por exemplo, a formação desses cristais (Gupta *et al.*, 2011). As glicoproteínas, produzidas pelas células epiteliais dos túbulos renais, como nefrocalcina, osteopontina, uropontina, bicunina e Tamm-Horsfall (uromodulina), presentes na matriz do cálculo, podem exercer efeitos inibitórios na formação, nucleação e agregação dos cálculos. Essas proteínas se ligam aos cristais nas fases iniciais, alterando suas superfícies e dificultando o crescimento e a adesão dos cálculos ao epitélio renal. Contudo, a proteína polimerizada Tamm-Horsfall (uromodulina), em certas condições, pode promover o crescimento de cristais (Argade *et al.*, 2015; Kaleeswaran *et al.*, 2018).

Entre as condições que favorecem a retenção dos cálculos nos rins, destacam-se a interação entre o tipo de cristal e os epitélios renais, as condições iônicas, as macromoléculas e a quantidade de minerais precipitados (Chewcharat; Curhan, 2021). Os cristais de oxalato de cálcio di-hidratado (COD) interagem menos com os epitélios renais em comparação aos cristais de oxalato de cálcio monohidratado (COM). Essa diferença se deve à morfologia dos cristais: os cristais COM possuem uma superfície mais complexa, que favorece a adesão celular, enquanto os cristais COD são mais lisos e menos propensos a interagir com as células renais (Romero *et al.*, 2010). A superfície dos cristais COM oferece mais sítios de ligação e maiores interações com as proteínas presentes no epitélio renal, facilitando sua adesão e retenção nos rins. A maior adesão dos cristais COM sugere uma maior probabilidade de esses cálculos causarem dano celular, inflamação e apresentarem menor propensão à eliminação (Saha; Verma, 2013).

A adesão dos cristais de COM é inibida por diversas moléculas aniônicas solúveis encontradas na urina humana normal, como hialuronano, bicunina e osteopontina, e ânions solúveis específicos no fluido tubular podem revestir a superfície do cristal, bloqueando assim sua adesão às células renais próximas, enquanto os ânions da superfície celular competem e se ligam a esses cristais (Sansores-Spain *et al.*, 2022). O resultado dessa competição determina se o cristal é eliminado do néfron na corrente de fluido tubular ou é retido no rim e se desenvolve em um cálculo (Youssef *et al.*, 2020).

O cálculo renal pode ser sintomático e assintomático e geralmente o indivíduo apresenta cólica no reno-ureteral, dor na lombar e até mesmo associações de hematúria visíveis (Dinnimath *et al.*, 2017). O diagnóstico envolve uma combinação

de história clínica apoiado por exames de imagem como a ultrassonografia renal e a tomografia computadorizada (Liu *et al.*, 2022). Essas técnicas apresentam sensibilidade e especificidade para identificar a presença e o tamanho dos cálculos renais. Os exames laboratoriais também contribuem para avaliar a composição química e fatores predisponentes (Khan *et al.*, 2021).

O tratamento do cálculo renal geralmente depende do tamanho, tipo e localização do cálculo. Pode variar desde a observação em casos de cálculos pequenos (cerca de 4 mm) até a intervenção cirúrgica para cálculos maiores (acima de 7 mm) (Pachaly; Baena; Carvalho, 2016). O manejo da dor e a prevenção de novos cálculos e infecções urinárias são aspectos essenciais do tratamento (Evan, 2010). Em alguns casos, o aumento da ingestão hídrica e a restrição alimentar podem ser suficientes para expelir e prevenir a formação de cálculos renais (Frochot; Daudon, 2016). As modificações alimentares incluem uma dieta balanceada em sódio, oxalato, cálcio e proteína animal, além do aumento do consumo de alimentos cítricos. A ingestão excessiva de proteínas pode aumentar a reabsorção renal do citrato, diminuindo sua excreção e efeito inibitório, além de elevar a concentração de constituintes de cálculos, como cálcio e ácido úrico. Acredita-se que os alimentos cítricos aumentam os níveis de citrato, contribuindo para a inibição da formação de cristais (Gul; Monga, 2014).

O uso de medicamentos alcalinizantes, relaxantes musculares e reguladores de distúrbios metabólicos pode facilitar a expulsão dos cálculos renais, reduzir a recorrência e a supersaturação de minerais urinários, contribuindo assim para a inibição da formação de novos cálculos (Scardelato; Legramandi; Sacramento, 2013). Além disso, o uso de analgésicos e anti-inflamatórios é essencial para aliviar a dor associada à cólica renal, que é causada pela obstrução urinária provocada pelo cálculo, sua passagem pelo ureter, ou pela obstrução do fluxo urinário no rim ou no trato urinário (Mikawlawng; Kumar; Vandana, 2014).

A intervenção cirúrgica é necessária em casos de comprometimento da função renal, obstrução ureteral, com ou sem quadros infecciosos, e em cálculos obstrutivos acima de 7 mm (Ahn; Harper, 2021). Os cálculos obstrutivos podem levar a hidronefrose devido à obstrução do fluxo urinário, causando dilatação do rim e, eventualmente, perda da função renal (Khan *et al.*, 2021). Entre as técnicas utilizadas para a remoção cirúrgica estão a litotripsia extracorpórea por ondas de choque e a nefrolitotomia percutânea, entre outras (Albert *et al.*, 2017). Apesar dos

avanços na medicina moderna, as técnicas empregadas na remoção dos cálculos renais permanecem caras, podem causar hemorragias e, a longo prazo, danos renais e hipertensão, além de não reduzirem a recorrência dos cálculos renais (Pearle *et al.*, 2014; Xu *et al.*, 2013) (Geraghty; Wood; Sayer, 2020).

Atualmente, não existem técnicas ou medicamentos satisfatórios capazes de dissolver completamente os cálculos renais, reduzir sua recorrência e atuar como anti-inflamatórios (Forbes *et al.*, 2022). Por essa razão, as plantas medicinais desempenham um papel importante na busca por substâncias e extratos que possam oferecer uma ação multialvo no tratamento da urolitíase de forma eficaz, econômica e com menos efeitos colaterais (Afshar *et al.*, 2010; Mulay; Evan; Anders, 2014).

2.2 PROCESSO INFLAMATÓRIO

A fixação e o acúmulo e de cristais de CaOx nos rins são condições importantes para o desenvolvimento da nefrocalcinose. Os cristais de COM, por exemplo se ligam as células epiteliais renais, resultando na proliferação celular e na inflamação (Khan, 2004). Além disso, ocorre a sinalização de espécies reativas do oxigênio (ROS), causando lesão, inflamação intrarrenal, formação, retenção dos cristais e até mesmo a insuficiência renal crônica (Singh *et al.*, 2022). Essa condição resulta na superprodução de ROS, ocasionado a redução da capacidade antioxidante endógena, o aumento do estresse oxidativo e a síntese de moléculas pró-inflamatórias pela ativação da enzima PIA2 e conseqüentemente a síntese de PGE2 (Khan *et al.*, 2021; Thongboonkerd; Yasui; Khan, 2021).

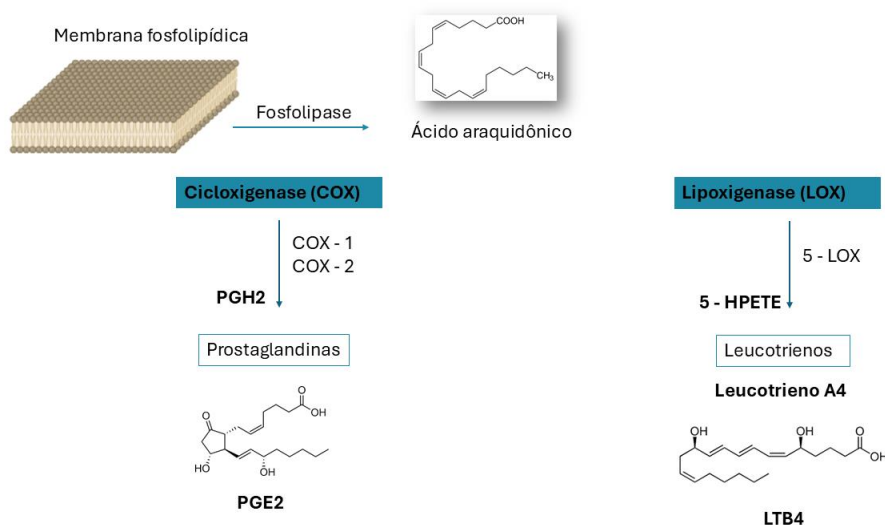
A inflamação é uma resposta complexa do sistema imunológico (Cardinal *et al.*, 2016). Quando ocorre uma mineralização iminente, o corpo reage produzindo macromoléculas que inibem a cristalização (Calixto; Otuki; Santos, 2003). Após a formação dos cristais, o corpo atrai células inflamatórias para eliminá-los (Khan, 2014; Meng; Liu; Lai, 2015). As macromoléculas conhecidas como mediadores químicos desempenham um papel crucial na regulação da resposta inflamatória, coordenando uma série de eventos que incluem vasodilatação, aumento da permeabilidade vascular e migração de células imunológicas para o local da lesão (Abdulkhaleq *et al.*, 2018; Medzhitov, 2010)

A regulação do processo inflamatório inicia na ativação de enzimas fosfolipases, especialmente a PLA2 presentes nas membranas celulares que por sua vez, catalisa a síntese do AA (Medzhitov, 2008; Nathan; Ding, 2010). O AA é regulador fundamental na inflamação, devido à sua posterior metabolização por diversas vias metabólicas. O AA geralmente é metabolizado por enzimas como as COXs sendo composta por duas isoformas ciclooxigenase-1 (COX-1) e ciclooxigenase-2 (COX-2), e as LOXs (Meirer; Steinhilber; Proschak, 2014). A isoforma COX-1 está presente nos vasos sanguíneos, plaquetas, estômago, intestino e rins, sendo denominada de enzima construtiva. A COX-1 também resulta em diversos efeitos fisiológicos, como proteção gástrica, agregação plaquetária, homeostase vascular e manutenção do fluxo sanguíneo renal (Medzhitov; Janeway, 2000). Enquanto a COX-2 é induzida durante a inflamação, sendo denominada de enzima indutiva e facilita a resposta inflamatória pela expressão de células como macrófagos e monócitos. A via das COXs é responsável pela síntese das prostaglandinas (PGs), como a PGE2, tromboxanos, prostaciclina e a via das LOX produz os leucotrienos como o LTB4 (Chen *et al.*, 2018). A PGE2 promove a vasodilatação, aumentando a permeabilidade vascular, enquanto o LTB4 desempenha a função de quimiotaxia, recrutando os neutrófilos para o local lesionado (Galli *et al.*, 2011). E as vias COX e LOX produzem os mediadores mais expressivos do processo inflamatório, destacando-se a PGE2 e o LTB4 (Fig. 2) (Lambeau; Gelb, 2008; Dobrian *et al.*, 2011).

A presença de AA e a sua conversão em PGE2 desempenha um papel importante na formação de cristais de CaOx devido à sua influência na excreção renal de cálcio e oxalato, o que contribui para a formação de cálculos renais de oxalato de cálcio (Miller; Evan; Lingeman, 2007). Além disso, a PGE2 pode alterar a interação entre a superfície da matriz do cálculo de COM e as células epiteliais renal, dificultando a eliminação dos cálculos renais. a ligação de cristais de COM à superfície das células epiteliais renais (Baggio *et al.*, 2000; Rodgers *et al.*, 2018).

Dessa forma, os anti-inflamatórios são usados para reduzir a dor, inibir os mediadores químicos e seus efeitos pró-inflamatórios e o estresse oxidativo ocasionado pela nefrocalcinose (Geraghty *et al.*, 2020). Além disso, a inibição do AA e da PGE2 pode favorecer a formação de cálculo renais sendo importante a sua inibição.

Figura 2 – Representação do processo inflamatório



Fonte: Do Autor (2024).

Dessa forma, os anti-inflamatórios são usados para reduzir a dor, inibir os mediadores químicos e seus efeitos pró-inflamatórios e o estresse oxidativo ocasionado pela nefrocalcinose (Geraghty *et al.*, 2020). Além disso, a inibição do AA e da PGE2 pode favorecer a formação de cálculo renais sendo importante a sua inibição. Dentre as classes dos anti-inflamatórios existem os anti-inflamatórios não esteroidais (AINEs) e esteroidais (AIEs) atuando de forma distintas no processo inflamatório. Os anti-inflamatórios (AINEs) podem ser seletivos para as COXs como a COX-2 ou não seletivos (Pallio *et al.*, 2016). Os AINEs, como a indometacina, inibem as COXs, impedindo a conversão do AA pela COXs em PGE2 (Bindu *et al.*, 2020). A inibição da COX-1 pode levar a efeitos gastrointestinais, toxicidade renal e insuficiência renal quando administrado em altas doses. Na tentativa de minimizar os efeitos adversos da inibição da COX-1, foram produzidos medicamentos inibidores seletivos da via COX-2. No entanto, esses inibidores seletivos da COX-2 estão associados a efeitos cardiovasculares e fenômenos trombóticos (Domingos *et al.*, 2019).

Por outro lado, os anti-inflamatórios esteroides (AIEs), também conhecidos como glicocorticoides, inibem a PLA2 reduzindo a disponibilidade de AA e, conseqüentemente, diminuindo a produção de PGE2 e LTB4, como ocorre com o fármaco dexametasona (Medzhitov, 2021). O uso prolongado de AIEs pode resultar

em efeitos colaterais graves, incluindo irritação gástrica, osteoporose e hipertensão (Domingos *et al.*, 2019; Sohail *et al.*, 2023). Portanto, é crucial desenvolver agentes anti-inflamatórios que visem tanto as vias COX quanto LOX, inibindo o AA na inflamação renal, enquanto minimizam os efeitos adversos (Hotamisligil, 2020).

2.3 GÊNERO *CISSUS*

A família Vitaceae compreende 15 gêneros e 950 espécies de lianas, distribuídas principalmente nos trópicos e regiões temperadas (Cervantes *et al.*, 2021). Entre estes, o gênero *Cissus* é o maior da família, com 300 espécies, a maioria das quais são lianas conhecidas como trepadeiras. Essas lianas possuem grande diversidade morfológica e estão amplamente distribuídas em regiões tropicais da África (135 espécies), das Américas (77 espécies), do sul da Ásia (85 espécies) e da Austrália (12 espécies) (Lombardi, 2000; Rodrigues *et al.*, 2014). Na América do Sul, podem ser encontradas 65 espécies do gênero *Cissus* sp., sendo que, no Brasil, cerca de 50 espécies desse gênero estão presentes nos biomas da Amazônia, Caatinga, Cerrado, Mata Atlântica e Pantanal (Souza; Lorenzi, 2005).

O gênero *Cissus* sp. é utilizado na medicina popular em diversos países devido às suas propriedades antimicrobiana, antiparasitária, anti-inflamatória, anti-hipertensiva, antitérmica, antiproliferativa, antiviral, anticonvulsivante, antiurólítica, hipoglicêmica, diurética, antidiabética e para tratar queimaduras, feridas, infecções cutâneas, hematomas, fraturas ósseas, anemia, dor de garganta, reumatismo, artrite, problemas gastrointestinais, diarreia, controle dos níveis de colesterol, entre outros (Asem *et al.*, 2014; Fernandes; Banu, 2012; Jainu; Devi, 2005; Lekshmi *et al.*, 2015; Madaleno, 2015; Ningombam *et al.*, 2022; Salgado *et al.*, 2009; Srissok *et al.*, 2011; Yi-Jie *et al.*, 2016; Zamora-Martínez; Pola, 1992).

Diversos estudos científicos vêm comprovando o uso etnofarmacológico desse gênero, incluindo a atividade antiurólítica e anti-inflamatória. As espécies como *C. adnata* e *C. gongylodes* demonstram atividade antiurólítica, enquanto *C. multistriata*, *C. repens*, *C. quadrangularis*, *C. sicyoides*, *C. repanda* e *C. gongylodes* exibem ação anti-inflamatória (Quadro 1-2).

O gênero *Cissus* sp. apresenta uma diversidade de metabólitos secundários encontrados em diferentes partes de plantas tais como, flores, caules, partes aéreas, folhas, raízes e frutos (Quadro 3). As classes mais expressivas são flavonoides,

flavonoides glicosilados, triterpenos, alcaloides, fitosteróis, saponinas, terpenos, estilbenos, ligninas, quinonas, cumarinas e, isoladas das espécies *C. sicyoides*, *C. pteroclada*, *C. rheifolia*, *C. javana* e *C. quadrangularis* (Fig. 3) Ahmadu; Onanuga; Aquino *et al.*, 2010; Ansarali *et al.*, 2016; Beltrame *et al.*, 2002; Fernandes; Banu, 2012; Méndez-López *et al.*, 2020).

Quadro 1 – Atividade antiurolítica do gênero *Cissus* sp.

Espécie	Extrato	Mecanismo de ação	Referência
<i>C. adnata</i> Roxb.	EEtOH ^a	Diminuição do pH urinário; decristalização de urinas coletadas de pacientes pré-operatório com histórico de nefrolitíases e a dissolução de cristais de estruvita sintética*	Laikangbam <i>et al.</i> (2010)
	ECHCl ₃ ^a	Inibição da nucleação e agregação de cristais de CaOx**	Ningombam <i>et al.</i> (2022)
<i>C. gongylodes</i> (Burch. Ex. Baker) Planch	EA ^a	Inibição e dissolução de CaOx e estruvita**	Salem <i>et al.</i> (2020)
<i>C. discolor</i> Blume	ECHCl ₃ ^a	Inibição da nucleação e agregação de cristais de CaOx**	Ningombam <i>et al.</i> (2022)
<i>C. javana</i> DC	EMeOH ^a	Dissolveu cálculos renais de COM**	Singh; Devi (2016)

Fonte: Autor (2024).

Legenda: Extrato clorofórmio (ECHCl₃), Extrato metanólico (EMeOH), Extrato etanólico (EEtOH), Extrato aquoso (EA), a folhas, * avaliação in vivo e ** avaliação in vitro.

A espécie *C. gongylodes* (Burch Ex. Baker) Planch., sinônimo de *Vitis gongylodes*, e *C. sulcicaulis* é utilizada como planta medicinal em forma de infusões e decocções no tratamento de cálculos renais, processos inflamatórios e biliares por populações da Índia, Áustria e Austrália (Ahmed; Hasan; Mahmood, 2016; Ahmed; Hasan, 2017; Pirker *et al.*, 2012). A espécie é comumente conhecida como cupá, cipó de uvas, cipó mãe-boá, uva do mato, videira brava, batata de cobra, entre outros nomes (Rodrigues *et al.*, 2014). A *C. gongylodes* não é uma planta endêmica do Brasil, podendo ser encontrada no Acre, Pará, Ceará, Maranhão, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo e Paraná (Lombardi, 1997).

Quadro 2 – Atividade anti-inflamatória do gênero *Cissus* sp.

Espécie	Extrato	Mecanismo de ação	Referência
<i>C. multistriata</i> L.	EMeOH ^a	Inibiu o edema de pata em ratos induzido por injeção de ácido acético *	James <i>et al.</i> (2013)
<i>C. repens</i> Lamk.	EMeOH ^c	Inibiu o edema de pata induzido por λ -carragenina diminuindo os níveis de MDA e NO; e aliviou os níveis de citocinas pró-inflamatórias, como IL-1 β , IL-6, TNF α , NF κ B, iNOS e COX-2*	Chang <i>et al.</i> (2012)
<i>C. repanda</i> Vahl.	EMeOH ^b	Inibiu o edema de pata de rato induzido por carragenina por 2-5h*	Kumar <i>et al.</i> (2013)
	EAcOEt ^b	Ação anti-inflamatória em ratos diabéticos induzidos por STZ*	Lekshmi <i>et al.</i> (2015)
<i>C. quadrangularis</i> Linn.	EMeOH ^c	Inibição da via da COX e 5-LOX avaliados no modelo de edema de pata induzido por carragenina e ácido araquidônico; e edema de orelha induzido por etil fenil propionato em ratos*	Panthong <i>et al.</i> (2007)
<i>C. sicyoides</i> L.	EA ^b	Redução do edema de pata induzido por carragenina em ratos e edema de orelha em camundongos via inflamação tópica usando acetato de tetradecanoilforbol *	Garcia <i>et al.</i> (2000)
	EEtOH ^a	Inibição do edema induzido por xileno e AA e diminuição dos níveis de PGE ₂ pela administração oral do extrato *	Beserra <i>et al.</i> (2016)
<i>C. gongylodes</i> (Burch. Ex. Baker) Phanch	EA ^a	Inibição do edema de orelha em camundongos induzido por óleo de cróton e inibição da quantificação de PGE ₂ e LTB ₄ *	Salem <i>et al.</i> (2020)
<i>Cissus pteroclada</i> Hayata	Bergenina-11-O- α -D-galactopiranosídeo ^e (10)	Inibiu a produção de mediadores pró-inflamatórios NO e PGE ₂ e a expressão de NF- κ B, TNF- α , IL-1 β , iNOS e COX-2**	Yi-Jie <i>et al.</i> (2016)

Fonte: Autor (2024).

Legenda: Extrato metanólico (EMeOH), extrato etanólico (EEtOH), extrato aquoso (EA), fração acetato de etila (AcOEt), ^a folhas, ^b caule/haste, ^c planta inteira, ^e partes aéreas, * avaliação *in vivo* e ** avaliação *in vitro*.

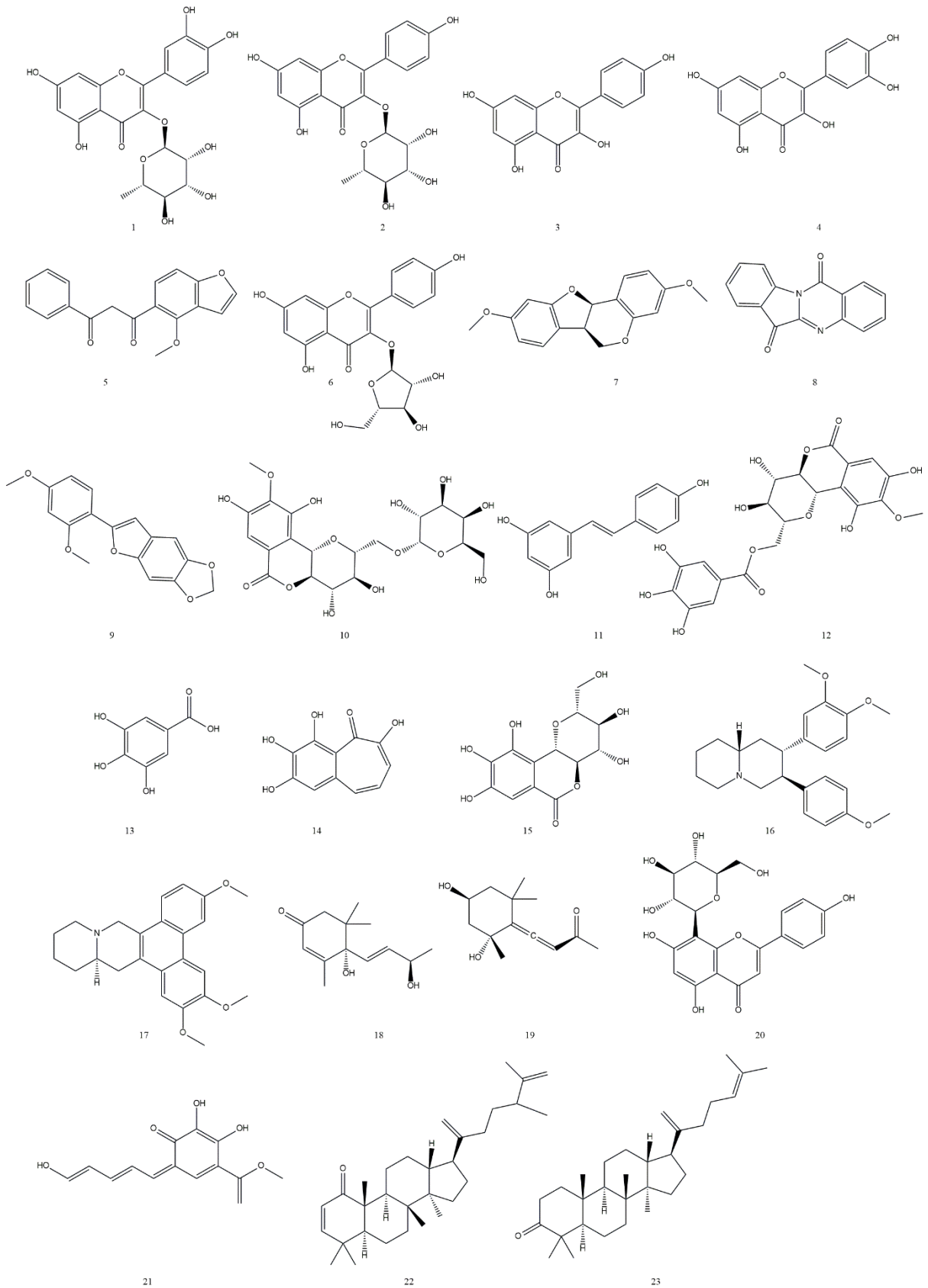
Quadro 3 – Substâncias isoladas do gênero *Cissus* sp

<i>C. sicyoides</i> Linn.			
1	Quercitrina	EMeOH ^e	Beltrame <i>et al.</i> (2001)
2	Afzelin	EMeOH ^e	Beltrame <i>et al.</i> (2001)
3	Kaempferol	EMeOH ^e	Xu <i>et al.</i> (2009)
4	Quercetina	EMeOH ^e	Xu <i>et al.</i> (2009)
5	Pongamol	EMeOH ^e	Xu <i>et al.</i> (2009)
6	Juglanina	EMeOH ^e	Xu <i>et al.</i> (2009)
7	Homopterocarpina	EMeOH ^e	Xu <i>et al.</i> (2009)
8	Triptantrina	EMeOH ^e	Xu <i>et al.</i> (2009)
9	6-(2,4-dimetoxifenil)furo[2,3-f][1,3]benzodioxol	EMeOH ^e	Xu <i>et al.</i> (2009)
<i>C. pteroclada</i> Hayata			
10	bergenina-11-O- α -d-galactopiranosideo	EEtOH ^e	Yi-Jie <i>et al.</i> (2016)
11	Resveratrol	EEtOH ^e	Yi-Jie <i>et al.</i> (2016)
12	11-o-Galoilbergenina	EEtOH ^e	Yi-Jie <i>et al.</i> (2016)
13	Ácido gálico	EEtOH ^e	Yi-Jie <i>et al.</i> (2016)
14	Purpurogalina	EEtOH ^e	Yi-Jie <i>et al.</i> (2016)
15	Norbergenina	EEtOH ^e	Yi-Jie <i>et al.</i> (2016)
<i>C. rheifolia</i> Planch.			
16	(2S,3S,9aR)-2-(3,4-dimetoxifenil)-3-(4-metoxifenil)-2,3,4,6,7,8,9,9a-octahidro-1H-quinolizina	ECHCl ₃ ^a	Saifah <i>et al.</i> (1983)
17	Criptopleurina	ECHCl ₃ ^a	Saifah <i>et al.</i> (1983)
18	Vomifoliol	ECHCl ₃ ^a	Saifah <i>et al.</i> (1983)
19	(R)-4-((2R,4S)-2,4-dihidroxi-2,6,6-trimetilciclohexilideno)but-3-en-2-ona	ECHCl ₃ ^a	Saifah <i>et al.</i> (1983)
20	Vitexina	EMeOH ^a	Saifah <i>et al.</i> (1983)
<i>C. javana</i> DC			
21	Cissus javanol	EMeOH ^a	Asem <i>et al.</i> (2014)
<i>C. quadrangularis</i> Linn.			
22	24-metil-dammara-2,20,25-trieno-1-ona	EHex ^b	Pathomwichaiwat <i>et al.</i> (2015)
23	24-metil-dammara-20,25-dieno-3-ona	EHex ^b	Pathomwichaiwat <i>et al.</i> (2015)

Fonte: Autor (2024).

Legenda: Extrato clorofórmio (ECHCl₃), Extrato metanólico (EMeOH), Extrato etanólico (EEtOH), Extrato hexânico (EHex), ^afolhas, ^bCaule/haste, e ^e Partes aéreas.

Figura 3 – Representação dos metabólitos secundários isolados no gênero *Cissus* sp.



Fonte: Autor (2024).

Mesmo sendo encontrado em uma extensa distribuição geográfica, a *C. gongylodes* não apresenta diferenças morfológicas notáveis (Lombardi, 1995). Seu ciclo de vida é do tipo liana-volúvel, germina no solo, e suas raízes permanecem no solo durante toda a sua vida, necessitando de um suporte para se manter ereto e normalmente cresce em direção à luz ultravioleta abundante. A *C. gongylodes* floresce de janeiro a maio, frutifica de fevereiro a agosto e apresenta adaptação a ambientes áridos, devido aos seus caules carnosos (Lombardi, 2000). A planta é caracterizada por folhas trifolioladas, inflorescências com flores verde-amareladas, caules grossos e carnosos, e seus frutos são do tipo baga (Fotografia 1) (Santo *et al.*, 1987).

Fotografia 1 – Folhas, caules e inflorescência de *C. gongylodes*



Fonte: Autor (2024).

A *C. gongylodes* demonstrou ser eficiente na remoção de poluentes do ar, como benzeno e tolueno (Yoo *et al.*, 2006). Os caules de *C. gongylodes* são utilizados como fonte de alimentação pelos índios Kayapós no Brasil, demonstrando seu baixo potencial toxicológico (Lombardi, 2007; Prance, 1991). A decocção dos caules e a infusão das folhas de *C. gongylodes* não apresentaram toxicidade nem alterações morfológicas em rins, fígado e coração quando administradas em uma dose de 5000 mg/kg em ratos por sete dias. Além disso, não exibiram ação antimicrobiana contra as cepas de *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* e *Candida albicans* (Navarro, 2009). O extrato

aquoso das folhas de *C. gongylodes* apresenta uma importante alternativa para a obtenção de micronutrientes essenciais, como cobre (0,95 mg), ferro (3,46 mg) e zinco (1,69 mg) em 1g de planta, quando comparado com outras plantas alimentícias apreciadas na alimentação, que contêm cobre (1,37 mg), ferro (5,13 mg) e zinco (2,96 mg) (Andrade *et al.*, 2005).

Até o momento, não havia relatos na literatura sobre a composição química de *C. gongylodes* e a relação dos metabólitos associados ao seu potencial farmacológico. Assim como o gênero, a espécie *C. gongylodes* mostrou ser uma planta promissora e atóxica, necessitando ser estudada para comprovar os metabólitos e o seu mecanismo de ação associados às atividades anti-inflamatória e antiurólítica. As plantas com histórico etnofarmacológico têm grande potencial de serem farmacologicamente ativas e pode fornecerem substâncias de grande importância terapêutica e farmacêutica (Verpoorte; Choi; Kim, 2005). Portanto, essas plantas podem revelar substâncias líderes para o desenvolvimento de novos candidatos a fármacos para o tratamento da urolitíase, por exemplo (Maier, 2015; Pinto *et al.*, 2002).

2.4 METABOLÔMICA

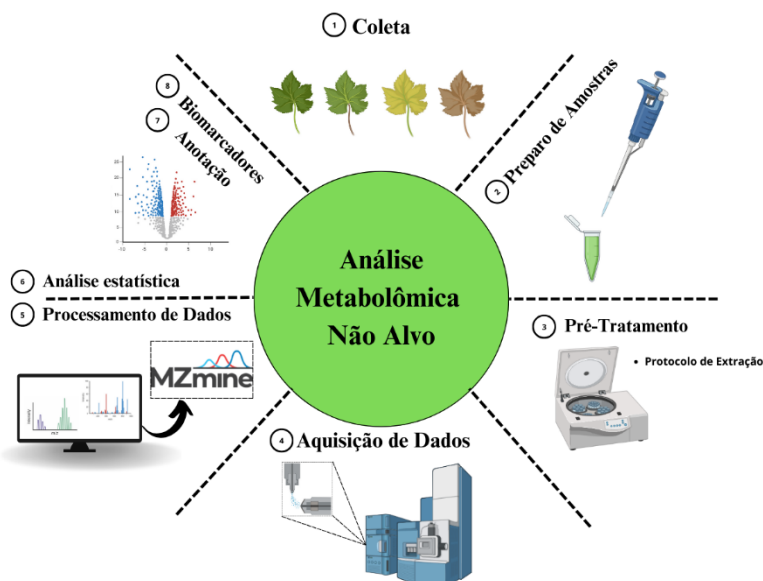
As plantas produzem diversos metabólitos que desempenham funções cruciais para o crescimento, desenvolvimento e adaptação ao ambiente, que são divididos em primários e especializados. Os metabólitos primários exercem a função de crescimento e desenvolvimento da planta. Em contraste, os metabólitos especializados podem desempenhar um papel significativo na proteção e defesa contra os fatores bióticos contra herbívoros, insetos e patógenos e abióticos no aumento da resistência da planta em fatores que incluem temperatura, luz solar, umidade, pH do solo, pressão atmosférica e composição química do ar e da água (Gobbo-Neto; Lopes, 2007). Embora seu papel inicial dos metabólitos secundários seja voltado para atender às necessidades ecológicas e de defesa dos organismos que as produzem, muitos desses metabólitos também exibem atividades farmacológicas relevantes para a saúde humana. Assim, a diversidade e complexidade estrutural dos metabólitos secundários tornam essas moléculas importantes fontes para a descoberta de novos agentes terapêuticos (Prinsloo; Nogemane, 2018).

Sendo assim, a metabolômica é uma poderosa estratégia no estudo de produtos naturais, especialmente na identificação e caracterização de metabólitos secundários com potenciais atividades biológicas e em resposta as diferentes condições ambientais (Lunić *et al.*, 2022). A identificação está relacionada à determinação da identidade de uma substância, especialmente útil para confirmar substâncias conhecidas, enquanto a caracterização é indispensável para elucidar sua estrutura e conformação espacial principalmente em estudos com substâncias inéditas (Chagas-Paula *et al.*, 2015). A metabolômica permite uma análise abrangente dos perfis químicos de plantas, por exemplo possibilitando a identificação de substâncias específicas em resposta a estímulos bióticos e abióticos. Isso é essencial para entender como as plantas produzem determinadas substâncias bioativas e como esses metabólitos podem ser modulados em diferentes condições ambientais (Xiao *et al.*, 2022).

Diante disso, a metabolômica com abordagem não direcionada é uma ferramenta sistematizada e poderosa para identificar e quantificar pequenos metabólitos (<1500 Da) em amostras biológicas de plantas (Liu *et al.*, 2013). Utilizando técnicas analíticas como a cromatografia líquida acoplada à espectrometria de massa de alta resolução (LC-HRMS), a LC promove a separação dos compostos com base na polaridade, tamanho molecular ou interação com a fase estacionária. A HRMS, por sua vez, analisa os compostos separados, determinando a massa molecular e os padrões de fragmentação, possibilitando a identificação e a caracterização dos metabólitos por meio de bancos de dados. Essa abordagem ortogonal proporciona uma análise abrangente e eficiente do perfil metabólico de plantas em diferentes condições biológicas (Zhang *et al.*, 2012).

Em geral, o fluxo de trabalho da metabolômica não direcionada consiste em planejamento experimental, preparo de amostras, aquisição de dados, processamento, análise estatística multivariada, identificação de marcadores e interpretação significativa dos resultados (Fig. 4) (Ren *et al.*, 2018). O preparo de amostras é uma etapa fundamental, pois influencia diretamente a qualidade e a confiabilidade dos dados obtidos. Nesse contexto, a técnica LC-HRMS proporciona uma análise abrangente do metaboloma, destacando-se pela sua robustez, alta sensibilidade e eficiência na detecção de uma vasta gama de metabólitos (Canuto *et al.*, 2018).

Figura 4 – Fluxo de trabalho da metabolômica não direcionada



Fonte: Autor (2024).

Além disso, estudos recentes destacam as vantagens das técnicas de aquisição independente de dados (DIA), que fragmentam indiscriminadamente todos os íons precursores detectáveis dentro de uma ampla faixa m/z com múltiplos ciclos de baixa e alta energia (Katchborian-Neto *et al.*, 2024). Essa abordagem amplia a cobertura de dados espectrais de fragmentos LC-MS^E em uma única corrida cromatográfica, permitindo a captura de metabolitos majoritários e minoritários. A fragmentação dos íons em intervalos de massa específicos, sem a necessidade de pré-seleção com base na intensidade do sinal. A aquisição DIA não realiza uma separação seletiva do MS¹ e MS² e em vez disso, alterna entre níveis de energia para coletar fragmentos de todos os íons presentes (como no MS^E), criando um perfil de fragmentação de toda a amostra (Alves *et al.*, 2024). No entanto, a DIA é ideal para abordagens não direcionadas, especialmente em estudos exploratórios de plantas com perfis químicos complexos e ainda pouco investigados, onde a identificação de novos metabolitos secundários é necessária. (Katchborian-Neto *et al.*, 2023; Wang *et al.*, 2019).

Em contraste, o método de aquisição dependente de dados (DDA), amplamente utilizada na metabolômica, adquire dados MS² ao selecionar íons com base na intensidade do sinal. Para isso, o DDA identifica primeiro os íons intactos no

espectro MS¹ e, em seguida, escolhe aqueles com maior intensidade para fragmentação e aquisição dos espectros MS², fornecendo informações estruturais detalhadas dos metabólitos de maior abundância na amostra (Fenaille *et al.*, 2017). Uma limitação desse método é a utilização de uma janela espectral relativamente estreita para a fragmentação, o que significa que ele foca em um pequeno número de íons de alta intensidade em cada ciclo de aquisição. Essa abordagem acaba deixando muitos metabólitos de baixa abundância sem dados MS², ou seja, sem fragmentação detalhada para análise estrutural. Isso reduz a abrangência da análise e pode limitar a identificação de substâncias menos concentradas na amostra, especialmente aqueles com potencial atividade biológica que podem estar presentes em quantidades reduzidas (Schmid *et al.*, 2021). Nesse sentido, a DDA pode ser menos eficaz em análises não direcionadas, onde o objetivo é explorar e identificar o maior número possível de metabólitos (Tsugawa *et al.*, 2015).

Na metabolômica, softwares como *Global Natural Products Social Molecular Networking* (GNPS), MS-DIAL e MZmine desempenham um papel essencial no processamento e análise de dados espectrométricos de massa, cada um oferecendo características distintas que facilitam a interpretação e a anotação dos dados obtidos (Silva *et al.*, 2021). O GNPS é plataforma baseada na web que permite a análise colaborativa e compartilhamento de dados de espectrometria de massa (MS/MS) (Wang *et al.*, 2016). Através de ferramentas como *networking molecular*, GNPS ajuda a organizar espectros em famílias, facilitando a descoberta de novos metabolitos e a comparação de dados com bibliotecas de fragmentação públicas. Essa rede colaborativa torna o GNPS uma ferramenta valiosa para a identificação de metabólitos conhecidos e a inferência de dados contextuais de metabolitos em experimentos de metabolômica (Ramos *et al.*, 2019). Além disso, tanto o MS-DIAL quanto o MZmine suportam análise não direcionada, o que é fundamental para a exploração de perfis metabólicos complexos. Eles fornecem ferramentas para o alinhamento de dados e a deconvolução de picos, essenciais para a correta identificação de metabólitos em amostras biológicas (Li *et al.*, 2018). Ambos também incluem funcionalidades de quantificação, ajudando na avaliação das concentrações relativas dos metabólitos identificados. Além disso, esses softwares permitem a integração com bancos, se tornando crucial para a identificação e anotação precisas de metabólitos. Essa conexão possibilita a comparação de espectros obtidos em suas análises com espectros de referência armazenados em bibliotecas públicas,

aumentando a confiabilidade dos resultados (Katchborian-Neto *et al.*, 2020; Kontou *et al.*, 2023).

De acordo com *Metabolomics Standards Initiative* (MSI), a identificação dos metabólitos em metabolômica compreende níveis de desreplicação que variam de 0 a 4 (Sumner *et al.*, 2007) O nível 0 envolve a identificação completa de substâncias isoladas, incluindo a determinação de suas estruturas. O nível 1 baseia-se na comparação com padrões autênticos, usando análises espectroscópicas e espectrométricas. No nível 2, a estrutura é proposta sem padrões, enquanto o nível 3 permite identificar a classe do composto. Por fim, o nível 4 se limita à presença ou ausência do metabólito, sem informações estruturais detalhadas (Pilon *et al.*, 2020).

O estudo metabolômico é um campo emergente, impulsionado pelo aprimoramento das ferramentas de aquisição de dados e tratamento analítico (Segers *et al.*, 2019). A diversidade química dos metabólitos e suas concentrações variadas tornam desafiadora a captura do metaboloma completo. Assim, a combinação de múltiplas plataformas analíticas, incluindo técnicas de alta resolução como LC-MS e espectroscopia, tem se mostrado essencial para expandir a cobertura das substâncias detectadas, resultando em uma compreensão mais robusta dos processos biológicos e metabólicos dos organismos em estudo (Gonzalez-Dominguez *et al.*, 2017).

2.5 ISOLATION OF ANTI-INFLAMMATORY AND ANTI-UROLITHIATIC ACTIVE COMPOUNDS FROM THE DECOCTION OF *CISSUS GONGYLODES* LEAVES

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Bioguided isolation of anti-inflammatory and anti-urolithiatic active compounds from the decoction of *Cissus gongylodes* leaves

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ABSTRACT

Ethnopharmacological relevance: The *Cissus gongylodes* has traditionally been used in the diet of indigenous people in Brazil and in traditional medicine for kidney stone removal and inflammatory diseases. The active compounds responsible for these pharmacological activities are unknown.

Aim of the study: This study aims to isolate, for the first time, the compounds in the decoction of *C. gongylodes* leaves responsible for their anti-inflammatory and anti-urolithiatic ethnopharmacological properties.

Materials and methods: The most active fractions of the *C. gongylodes* leaf decoction were fractionated using SPE-C18 and the compounds were purified through HPLC-UV-DAD. The decoction fractions and isolated compounds were evaluated for their anti-inflammatory and anti-urolithiatic activities. The anti-inflammatory activity was assessed using an *ex vivo* assay in human blood induced by LPS and calcium ionophore, measuring inflammatory mediators, PGE2 and LTB4. The anti-urolithiatic activity was evaluated using an *in vitro* experimental model with human urine to determine the dissolution of the most recurrent calcium oxalate (CaOx) crystals. Additionally, the decoction was chemically characterized through metabolomic analysis using UHPLC-ESI-HRMS.

Results: The isolated compounds from the decoction of *C. gongylodes*, including rutin, eriodictyol 3'-O-glycoside, and isoquercetin, have demonstrated significant multi-target actions. These components act as anti-inflammatory agents by inhibiting the release of main inflammatory mediators, PGE2 and LTB4. Additionally, they exhibit anti-urolithiatic properties, promoting the dissolution of calcium oxalate (CaOx) crystals. Furthermore, the characterization of the decoction by UHPLC-ESI-HRMS revealed a high content of flavonoids, mainly glycosylated flavonoids.

Conclusions: The results support the traditional use of *C. gongylodes* decoction, identifying the compounds responsible for its anti-inflammatory and anti-urolithiatic effects. The decoction fractions and isolated compounds exhibited dual anti-inflammatory activity, effectively inhibiting key inflammatory pathways and potentially presenting fewer adverse effects while also promoting the dissolution of CaOx crystals associated with urolithiasis. The multi-target action displayed by *C. gongylodes* is particularly desirable in the treatment of urolithiasis, as inflammation and PGE2 production precede and contribute to the formation of CaOx crystals in the kidneys. Based on these actions, *C. gongylodes* emerges as a potent source of active compounds for the development of new treatments for urolithiasis.

1. Introduction

Urolithiasis, or kidney stones, involves the formation of stones in the

kidneys and urinary system, leading to urinary obstruction and impaired renal function (Sakhaee and Maalouf, 2020). Urolithiasis is a condition with high prevalence and recurrence, associated with factors such as diet, fluid intake, sedentary lifestyle, metabolic disorders, and genetic

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Glossary:

AC	Arachidonic acid
ANOVA	Variance analyses
CaOx	Calcium oxalate
COX	Cyclooxygenase enzyme
DEX	Dexamethasone
GNPS	Global natural product social molecular networking
G	G Force
HPLC	High-performance liquid chromatography
HPLC-UV-DAD	High-performance liquid chromatography coupled to diode-array ultraviolet detector
ID	Identification number
IND	Indomethacin
LOX	Lipoxygenase
LPS	Lipopolysaccharides
LTB4	Leukotrienes B4
MF	Molecular formula
MS	Mass spectrometry

NMR	Nuclear magnetic resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
PBS	Phosphate-buffered saline
PGE2	Prostaglandin E2
PLA2	Enzyme phospholipase A2
PTFE	Polytetrafluoroethylene
QTOF	Quadrupole time-of-flight mass
Rpm	Rotations per minute
Rt	Retention time
SAIDs	Steroidal anti-inflammatory drugs
SD	Standard deviation
SisGen	National system of genetic heritage and associated traditional knowledge
SPE	Solid-phase extraction
UHPLC-ESI-HRMS	Ultra-high-performance liquid chromatography coupled to electrospray and high-resolution mass spectrometry
UHPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometry

predisposition (Liu et al., 2022; Romero et al., 2010; Sansores-España et al., 2022). Low urinary volume, resulting from insufficient water intake, increases the concentration of lithogenic components in the urine, promoting stone crystallization (Chewcharat and Curhan, 2021). Excessive consumption of sodium and animal proteins raises urinary levels of calcium and uric acid, while dietary calcium restriction may exacerbate the condition by increasing intestinal absorption and urinary excretion of oxalate, thereby heightening the risk of stone formation (Lotan et al., 2017). Dietary and lifestyle changes are thus essential for preventing kidney stones and promoting urinary health (Lin et al., 2020). Stone formation is a multifactorial process that includes supersaturation, nucleation, growth, and crystal aggregation (Kumar et al., 2016). The composition of kidney stones is closely linked to the individual characteristics of each person, with calcium oxalate (CaOx) stones, particularly in the monohydrate (COM) and dihydrate (COD) forms, being the most common (Evan, 2010; Lieske et al., 2014). Metabolic disorders, such as hypercalciuria and hyperoxaluria, increase the excretion of precursors of calcium oxalate (CaOx) stone formation (Youssef et al., 2020). Hypercalciuria and hyperoxaluria may be linked to elevated levels of arachidonic acid (AA) and prostaglandin E2 (PGE2), which increase calcium and oxalate excretion and contribute to the formation of CaOx stones (Rodgers et al., 2018).

Nephrocalcinosis resulting from the deposition of CaOx crystals in the kidneys leads to the overproduction of reactive species and the synthesis of pro-inflammatory molecules, causing kidney damage, loss of kidney cells, inflammation, and even chronic renal failure (Khan et al., 2021). Chemical mediators are recruited to reduce inflammation and kidney stone formation. Phospholipase A2 in cell membranes catalyzes the production of arachidonic acid (AA) (Medzhitov, 2008; Nathan and Ding, 2010). AA is metabolized by enzymes such as cyclooxygenases (COXs), which produce prostaglandins such as PGE2 and lipoxygenases (LOXs), which generate leukotrienes such as LTB4 (Chen et al., 2018). PGE2 promotes vasodilation, while LTB4 assists in chemotaxis, recruiting neutrophils to the site of renal injury (Galli et al., 2011; Medzhitov and Janeway Jr, 2000).

Treatment of kidney stones includes analgesics, anti-inflammatory drugs, muscle relaxants for pain relief and stone elimination, and invasive procedures for their removal (Forbes et al., 2022; Wang et al., 2022). However, pharmacological therapies may have adverse effects, and surgical procedures are costly and can lead to complications such as bleeding (Pearle et al., 2014; Xu et al., 2013). Despite advancements, these treatments are often ineffective in dissolving stones and do not reduce the recurrence of stone formation (Nimavat et al., 2022; Singh

et al., 2022).

Anti-inflammatory drugs are used to reduce inflammation resulting from nephrocalcinosis (Geraghty et al., 2020). Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COXs, blocking the conversion of arachidonic acid into prostaglandins such as PGE2 (Bindu et al., 2020). However, COX-1 inhibition can lead to gastrointestinal effects and renal toxicity, while selective COX-2 inhibitors are associated with cardiovascular effects. Conversely, steroidal anti-inflammatory drugs (SAIDs) inhibit PLA2, reducing the availability of arachidonic acid and consequently decreasing the production of PGE2 and LTB4 (Medzhitov, 2021). Long-term use of SAIDs can result in severe side effects, including gastric irritation, osteoporosis, and hypertension (Domingos et al., 2019; Sohail et al., 2023). Therefore, it is crucial to develop anti-inflammatory agents that target both COX and LOX pathways, thus inhibiting arachidonic acid in renal inflammation while minimizing adverse effects (Hotamisligil, 2020).

Decoction of *Cissus gongyloides* (Baker) Burch. ex Baker (Vitaceae) leaves are used in traditional medicine to treat kidney stones and inflammation (Ahmed et al., 2016; Bieski et al., 2012; Pirker et al., 2012). In Brazil, the plant is part of the diet of the Kayapó indigenous people, demonstrating its therapeutic and nutritional potential, and it is considered non-toxic due to its consumption (Kerr et al., 1978). Toxicity studies of the decoction of the plant's leaves have shown no toxic effects in animals at a concentration of 5000 mg/kg (Navarro, 2009). Our previous research shows that the decoction of *C. gongyloides* leaves dissolves calcium oxalate crystals in human urine and exhibits *in vivo* anti-edematogenic activity, reducing inflammatory mediators PGE2 and LTB4 (Salem et al., 2020). Considering the ethnopharmacological history of *C. gongyloides* and the need for effective multi-target therapies for urolithiasis, this study aims to isolate anti-inflammatory and anti-urolithiatic substances from decoctions of *C. gongyloides* leaves. The study reports, for the first time, the active compounds isolated from this species, which also support its traditional use. Additionally, it includes the chemical characterization of the decoction using UHPLC-ESI-HRMS.

2. Materials and Methods

2.1. Reagents and equipment

The indomethacin was acquired from Eurofarma® (São Paulo, SP, Brazil). The internal standard limaprost, along with the lipopolysaccharide inducers (LPS) from *Escherichia coli* O26, calcium ionophore, and the reference drug, dexamethasone, were acquired from Sigma

Aldrich® (St Louis, MO, USA). All solvents used were of high-performance liquid chromatography (HPLC) grade, including hexane, methanol, ethanol, formic acid, glacial acetic acid, and acetonitrile from Sigma Aldrich® (St Louis, MO, USA). Ultrapure water was purified using a Millipore Milli-Q® water purification system (Bedford, MA, USA). The deuterated solvent methanol (CD₃OD) was obtained from Sigma-Aldrich® (St Louis, MO, USA). Penicillin-Streptomycin was acquired from Gibco™ (Grand Island, NY, USA), sodium oxalate from Synth® (Diadema, SP, Brazil), and polyherbal Cystone® was acquired from Himalaya Herbal Healthcare (Makali, BLR, India). The chromatographic profile and isolation of substances were conducted by a high-performance liquid chromatography coupled to diode-array ultraviolet detector - HPLC-UV-DAD (Shimadzu Prominence) in equipment consisting of two LC-20AD pumps, a DGU-20A 3R degasser, a SIL-20A HT autoinjector, a CTO-20A column oven, a UV-Vis detector with diode array (SPD-M20A, DAD), and a CBM-20 communicator. Metabolomic data of the decoction and spectrometric data of the isolated compounds were obtained on an UHPLC-ESI-HRMS with a quadrupole time-of-flight mass as analyzer (QTOF, Xevo-G2-XS-QTOF/MS, Waters Corp., Milford, MA, USA). The plasma samples were purified using solid-phase extraction (SPE) with a Supelco Manifold System and Supelclean™ LC-18 cartridges (SPE, 100 mg adsorbent, #504270). The quantification of PGE₂ and LTB₄ was performed by ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) using a model 8030 instrument (Shimadzu®, Kyoto, Japan) equipped with a triple quadrupole mass analyzer and electrospray ionization operating in negative mode. ¹H and ¹³C NMR spectra were obtained using a Bruker Avance III spectrometer operating at 7.05 T (300 MHz for ¹H and 75 MHz for ¹³C).

2.2. Plant material

The leaves of *C. gongylodes* were collected by P. P. O. Salem in March 2020, in the city of Alfenas, MG, Brazil, according to the geographic coordinates S 21° 25' 04.7"; W 45° 57' 26.3"; altitude 846 m. The species was cataloged and deposited in the herbarium of the Federal University of Alfenas (# voucher 2987). The leaves were dried in an oven with an air circulator at 40 °C for 72 h and kept in conditions free from light and humidity until the extraction process was conducted. The species of *C. gongylodes* was identified by Professor J. A. Lombardi from the State University of São Paulo (UNESP). The study had access registered on the National System for Governance of Genetic Heritage and Associated Traditional Knowledge (SisGen # A3D5C99).

2.3. Obtaining the decoction and fractions of *C. gongylodes*

The decoction of *C. gongylodes* (Dec) was prepared from 200 g of powdered dried and crushed leaves in a knife mill, to which distilled water (1:10, g/mL) was added to the plant powder and brought to a boil for 20 min. The Dec (58 g) was filtered under reduced pressure, frozen, and freeze-dried. The ethyl acetate (FoAc), chloroform (FoCl), and hydroalcoholic (FoHi) fractions were obtained from the dried Dec by liquid-liquid extraction using the organic solvents ethyl acetate (EtOAc) and chloroform (CHCl₃). The Dec (45 g) was suspended in H₂O/EtOH, 2:1 (v/v) to a final volume of 720 mL, after which the hydroalcoholic fraction was acidified to pH 2 with a 5% (v/v) hydrochloric acid solution, and the acidified fraction was partitioned using the EtOAc solvent to obtain the EtOAc fraction. The hydroalcoholic fraction was then basified to pH 8 with a 5% (v/v) sodium hydroxide solution and subsequently partitioned with the CHCl₃ solvent to obtain the CHCl₃ fraction (Katchborian-Neto et al., 2020). Because there are reports indicating the presence of alkaloids in species of the *Cissus* spp, the acid-base extraction methodology was procedure (Bafna et al., 2021). The resulting hydroalcoholic fraction (FoHi) was then neutralized. The FoAc (5.6 g), FoCl (2.5 g), and FoHi (10.8 g) fractions were then rotary evaporated under reduced pressure at 30 °C and 100 rpm, frozen at

–20 °C, and freeze-dried.

2.4. Isolation of substances from the active fractions of *C. gongylodes* leaves

The bioactive fractions FoAc and FoHi were subjected to solid-phase extraction (SPE) fractionation using Supelclean™ LC-18 cartridges (SPE, 1 g adsorbent) with a manifold system at a flow rate of 3.15 mL/min and a pressure of –5 Kpa. The FoHi (3.1088 g) and FoAc (2.9634 g) fractions were eluted in LC-18 cartridges with different variable ratios of the eluents water and methanol in the volume of 60 mL (v/v) per aliquot, resulting in 20 subfractions (Table S1). The FoAc fraction obtained 10 subfractions: Ac-1 (1293 mg), Ac-2 (102 mg), Ac-3 (117 mg), Ac-4 (272 mg), Ac-5 (60 mg), Ac-6 (89 mg), Ac-7 (66 mg), Ac-8 (18 mg), Ac-9 (21 mg), and Ac-10 (49 mg). The FoHi fraction obtained 10 subfractions: Hi-1 (2756 mg), Hi-2 (115 mg), Hi-3 (65 mg), Hi-4 (22 mg), Hi-5 (11 mg), Hi-6 (7 mg), Hi-7 (5 mg), Hi-8 (2.5 mg), Hi-9 (1 mg) and Hi-10 (5 mg). The chemical profile of the subfractions was characterized using HPLC-UV-DAD, with chromatograms recorded at 254 nm. The subfractions Hi-5, Hi-6, and Hi-7 were grouped into Hi-5a due to their similar chemical profile. Their composition is the same, but there are differences in their peak area and intensity. The 20 subfractions obtained from SPE were frozen and lyophilized. The subfractions, Ac-5 (60 mg) and Hi-5a (8.5 mg) showed lower chemical complexity and significant activity in both anti-inflammatory and anti-urolithiatic assays. Therefore, they were subjected to purification by HPLC-UV-DAD. The active subfractions were dissolved in MeOH: H₂O (v/v) and filtered through a 0.45 µm PTFE syringe filter.

Compounds were separated on a semipreparative reversed phase (C18) Inertsil® (ODS-4) column, with a particle size of 5 µm and dimensions of 7.6 mm × 250 mm. The solvent system was acetonitrile (solvent B) and ultrapure water acidified with 0.1% AcOH (solvent A) and a flow rate of 1.5 mL/min. Isocratic elution with 20% solvent B was maintained for 30 min chromatograms were monitored and recorded at a wavelength of 254 nm, obtained by Labsolutions® software. The compounds rutin (P1) 6.5 mg, eriodictyol 4'-O-glucoside (P2) 2.5 mg, and isoquercetin (P3) 3.2 mg, have times of 9.5, 10.8 and 11.6 min, respectively. All compounds were identified by analysis of NMR and MS data.

2.5. Metabolomic analysis

2.5.1. UHPLC-ESI-HRMS

The Dec was resuspended in a (1:1, v/v) mixture of ACN/H₂O at a concentration of 1 mg/mL, and for the blanks, only the solvent mixture was used. Subsequently, the samples underwent clean-up through liquid-liquid partitioning with hexane (2 × 300 µL) followed by filtration in 0.45 µm pore size PTFE filters. Analyses were conducted using UHPLC-ESI-HRMS in data-independent acquisition mode, with electrospray ionization and a quadrupole-time-of-flight analyzer (UHPLC/ESI-QTOF-MS) in both positive and negative ionization modes. The equipment used was the Xevo® G2-XS-QTOF, equipped with an ACQUITY UHPLC® CSHT C18 column (2.1 × 100 mm, 1.7 µm particle size). The mobile phase consisted of ultra-purified water (solvent A) and acetonitrile (solvent B), both acidified with 0.1% formic acid. A gradient method was applied from 1% to 99% acetonitrile over 10 min: 10% B to 99% B in 7.5 min (0–7.5 min), maintained at 99% B for 0.5 min (7.5–8.0 min), returned to the initial mobile phase condition of 10% B over 0.1 min (8.0–8.10 min), followed by a 1.9-min (8.10–10.0 min) re-equilibration at 10% B. The flow rate was constant at 0.4 mL/min, with an injection volume of 10 µL per sample and a column oven temperature of 50 °C. The scanning range was from 50 to 1200 Da for the centroid data-independent acquisition (MS^E) in both positive and negative modes. The instrument settings in positive mode included a capillary voltage of 3.0 kV, a source temperature of 130 °C, a desolvation temperature of 450 °C, a cone gas flow rate of 100 L/h, a

desolvation gas flow rate of 900 L/h, and a collision energy of 3.0 eV. In negative mode, the settings were a capillary voltage of 3.0 kV, a source temperature of 130 °C, a desolvation temperature of 500 °C, a cone gas flow rate of 50 L/h, a desolvation gas flow rate of 800 L/h, and a collision energy of 15.0 eV, with a mass range of 70–1000 *m/z*.

2.5.2. Metabolites annotation

Data processing was carried out using MZMine 3 software, including steps such as mass detection, deconvolution, deisotoping, alignment, and gap filling, which were crucial to ensure a proper understanding and visualization of the obtained data. Data were processed separately for each ionization mode according to the following steps: mass detection, chromatogram construction followed by deconvolution, noise removal, and sample alignment. The processing parameters were established according to the general aspects of the chromatograms for each ionization mode. Thus, for the negative mode, the following parameters were used: mass detection using the exact mass algorithm, noise level: 3.0E1; minimum time interval, 0.1 min; minimum height, 8.0 E¹; *m/z* tolerance, 0.01 *m/z* or 10.0 ppm; peak height, 2.0 E²; peak duration, 0.1–1.0 min; baseline, 2.0 E¹; isotope removal, *m/z* tolerance, 0.003 *m/z* or 5 ppm; retention time tolerance, 0.5 min; maximum charge, 1; representative isotope, most intense; filtering, retention time: 0.05–10 min; peak duration range: 0.1–1 min; alignment using the join aligner module, *m/z* tolerance, 0.001 *m/z* or 10 ppm; retention time tolerance, 0.045 min; and gap filling, *m/z* tolerance, 0.005 *m/z* or 7 ppm; intensity tolerance, 10%; *m/z* from 0.005 to 7.0. For the positive mode, the following parameters were used: mass detection using the exact mass algorithm, noise level: 6.0E2; minimum time interval, 0.1 min; minimum height, 1.2 E³; *m/z* tolerance, 0.005 *m/z* or 10.0 ppm; peak height, 2.0 E³; peak duration, 0.1–1.0 min; baseline, 1.2 E³; deisotoping, *m/z* tolerance: 0.001 *m/z* or 10 ppm; retention time tolerance, 0.1 min; maximum charge, 1; representative isotope, most intense; filtering, retention time: 0.03–10.0 min; peak duration range: 0.1–1 min; alignment using the join aligner, *m/z* tolerance, 0.001 *m/z* or 10 ppm; weight for *m/z*, 15; retention time tolerance, 0.5 min; and gap filling, *m/z* tolerance, 0.005 *m/z* or 12 ppm; intensity tolerance, 10%; *m/z*, 0.005 to 12. The chemical annotation was completed using *in-house* databases with isolated compounds from the *Cissus* genus and the *Br.Flav* Ms¹ and MS² flavonoid database (Alves et al., 2024). Precursor annotation (MS¹) was performed, followed by online spectral databases (Mass Bank, PubChem, MoNA, and Global Natural Product Social Molecular Networking – GNPS) for MS² fragment annotation.

2.6. Identification of compounds from bioactive fractions by UHPLC-QTOF MS/MS and NMR

The mass spectra of the isolated compounds were obtained using a UHPLC instrument, equipped with an ACQUITY UHPLC® CSHT C18 column (2.1 × 100 mm, 1.7 μm particle size) and coupled to a QTOF mass spectrometer through an ionization interface electrospray positive ionization mode (ES⁺). The mobile phase consisted of water containing 0.1% (v/v) formic acid (A) and acetonitrile containing 0.1% formic acid (B). Elution was 0–5 min, 1–40% B (0–2 min), 40–98% B (2–3 min), 98% B (3–3.5 min), 98–1% B (3.5–3.6 min) and 1% B (3.6–5 min). The injection volume was 20 μL. The ESI-MS scan range was *m/z* 100–1200 Da. The ESI voltage was 6.0 kV in positive ion mode. The mass spectra were analyzed using Masslynx software, version 4.1, and Mzmine 3.9.0.

The isolated compounds P1, P2, and P3 were dissolved in 550 μL of the deuterated solvent CD₃OD at concentrations ranging from 3 to 6.0 mg/mL at room temperature. Molecular structural characterization was performed using nuclear magnetic resonance (NMR) spectroscopy techniques, including one-dimensional (¹H and ¹³C) and two-dimensional (COSY, HSQC, and HMBC) experiments, and on a 300 MHz spectrometer operating at ¹H and 75 MHz for ¹³C nuclei, NMR spectra were processed using Bruker's Topspin software, version 4.1.

2.7. Ex-vivo anti-inflammatory evaluation

The *ex vivo* anti-inflammatory experiment on human blood was conducted according to Katchborian-Neto et al. (2023) and Rosa et al. (2021), with modifications. The experiment was approved by the research ethics committee of the Federal University of Alfenas (89.325.818.10000.5142, approved on August 15, 2022), and all donors provided written consent before blood collection. Fresh blood samples were collected in vacuum tubes containing sodium heparin immediately before the *ex-vivo* assay incubation. The positive controls dexamethasone (DEX) and indomethacin (IND) were tested at 1 μg/mL (final well concentration) according to Katchborian-Neto et al. (2020) and Rosa et al. (2021). The Dec and its fractions FoAc, FoCl, and FoHi, and the subfractions Ac-1 to Ac-10 and Hi-1 to Hi-10, were evaluated at 10 μg/mL (final well concentration) according to Ferreira et al. (2024) and Gonçalves et al. (2023). Only the subfractions H-8 and H-9 could not be evaluated due to their low yield. The isolated substances (P1, P2, and P3) and positive controls were evaluated at concentrations of 0.5, 1, 2, 4, and 10 μg/mL (final well concentration) according to Silva et al. (2023), including the inhibitory concentration range expected for the isolated substances to allow IC₅₀ calculation. The samples were resuspended in a solution of EtOH/H₂O (1:1, v/v), and 1x phosphate-buffered saline (PBS - pH 7.2; 0.15 M chloride; 0.01 M phosphate) was used to perform the dilutions. As for the negative control, only 1x PBS was used to estimate the maximum amount of PGE₂ and LTB₄ produced in the inflammatory process. The samples, inducers, and blood were plated on sterile 96-well plates (86 × 128 mm) in a laminar flow hood. The plating sequence consisted of 35 μL of the Dec, fractions, isolated substances from the Dec, and reference drugs, 280 μL of heparinized blood in all wells, and 35 μL of LPS solution at 1 μg/mL and calcium ionophore at 2 μmol/L (final well concentration) in all wells, resulting in a total volume of 350 μL per well. The 96-well plates were then incubated for 24 h in an atmosphere of 5% CO₂ at 37 °C. After the incubation period, the plates were centrifuged for 5 min at 157 rcf and 4 °C. Subsequently, 180 μL of plasma were removed from the blood in each well, and the plasmas were frozen, freeze-dried, and stored until the protein precipitation process. The 280 μL of the precipitating agent (ACN/MeOH, 1:1, v/v) containing the internal standard limaprost (23 ng/mL) was added to the dried plasma. The controls and samples were vortexed, subjected to ultrasound for 2 min, and centrifuged (4025 rcf, 4 °C) for 10 min. The supernatant (200 μL) was transferred to a polypropylene tube containing 1.8 mL of ultrapure water. The chemical mediators of inflammation were extracted from the samples on an LC-18 solid-phase cartridge. The cartridges were conditioned with 2 mL of MeOH, and 2 mL of ultrapure water acidified with 0.1% AcOH. Consecutively, 2 mL of the samples were eluted into the cartridges, which were then washed with 2 mL of an aqueous solution containing 0.1% AcOH. The eluates were transferred to polypropylene tubes using 1.0 mL of metabolic solution containing 0.1% AcOH, dried in a speed vacuum sample concentrator for about 4 h, and stored in a freezer at –20 °C until the quantification analyses of PGE₂ and LTB₄.

2.7.1. Prostaglandin E2 and Leukotriene B4 quantification by UHPLC-MS/MS

Quantification was performed using UHPLC-MS/MS on an ultra-performance liquid chromatography coupled with a triple quadrupole mass analyzer operating in negative mode. Controls and samples were resuspended in 50 μL of ACN and injected (30 μL) into the chromatographic system containing an Agilent® Poroshell 120 EC-C18 column (2.7 μm, 3.0 × 100 mm) and a matching guard column EC-C18 (2.7 μm, 3.0 × 5 mm) maintained at 30 °C. The mobile phase consisted of (solvent A) ultrapure water acidified with 0.1% formic acid and (solvent B) 100% ACN at a constant flow rate of 0.3 mL/min. The elution gradient was established as flows: 40% A and 60% B to 90% B over 4.5 min (0–4.5 min), 90%–100% B in 1 min (4.5–5.5 min), 100% B for 1 min (5.5–6.5 min), finally returning to the initial mobile phase of 60% B over 1 min,

followed by 3.5 min of re-equilibration. The mass spectrometer settings were as follows: nitrogen gas nebulization at a flow rate of 2 L/min, nitrogen gas drying at a flow rate of 15 L/min at 400 °C, interface voltage at 3.5 kV, DL temperature at 250 °C, oven temperature at 35 °C, detector voltage at 2.16 kV, and argon collision gas at 230 kPa. The multiple reaction monitoring transitions in (*m/z*) in negative mode [M-H]⁻ for the chemical mediators and internal standards are shown in Table S2, as validated by Rosa et al. (2021). Data acquisitions were performed using LabSolutions® software.

The linear equation of the calibration curve for PGE2 ($y = 0.01307x + 0.1424$) and LTB4 ($y = 0.009332x + 0.01324$) yielded a coefficient of correlation (R^2) = 0.9933 for PGE2 and LTB4 (R^2) = 0.9925, where *y* is the relative area of PGE2/limaprost or LTB4/limaprost and *x* is the concentration of PGE2 and LTB4 measured in ng/mL, respectively. The concentration of PGE2 and LTB4 in ng/mL and the percentage of inhibition were calculated for the isolated substances from *C. gonyglodes* and the Dec, the fractions, and subfractions were evaluated by the ratio of the signal area of PGE2 to the internal standard. All determinations were performed in triplicates, and the results of the concentration of PGE2 and LTB4 were expressed as mean ± standard error (SD). The data statistics were conducted by one-way analysis of variance (ANOVA) ($p \leq 0.05$) followed by Dunnett's multiple comparison tests in GraphPad Prism® 9.1.2 (GraphPad© software, La Jolla, CA, USA). The IC₅₀ value and statistical analyses were calculated using the GraphPad Prism® software.

2.8. Evaluation of CaOx in human urine

The effects of calcium oxalate (CaOx) crystal dissolution were conducted following modified protocols as per Barros et al. (2003), Moreno et al. (2021), and Salem et al. (2020). The experiment was approved by the Research Ethics Committee of the Federal University of Alfenas (registration number 67746523.7.0000.5142, approved on June 1, 2023), and first-morning urine samples were obtained from individuals with a history of crystalluria. All individuals voluntarily agreed to participate in the study and signed informed consent forms. Urine samples were collected in sterile containers, stored in Falcon tubes, and centrifuged at 239 rcf for 10 min at room temperature (25 °C). To induce CaOx crystal formation, 400 µL of 0.1 mol/L sodium oxalate solution per milliliter of centrifuged urine was added, which was kept at 100 rpm agitation for 24 h at room temperature. After incubation, an aliquot of urine was evaluated to observe CaOx crystal formation under a binocular microscope. The urine containing crystals was diluted with crystal-free urine to a concentration of 200 CaOx crystals/µL and 10% (v/v) of the penicillin and streptomycin antibiotic solution at 10.000 IU/mL.

The anti-urolithiatic activity of the FoAc, FoCl, and FoHi fractions was screened at doses of 1, 2, and 3 mg/mL based on the studies of Barros et al. (2003) and Moreno et al. (2021). The Dec and active fractions FoAc and FoHi were evaluated at 0.25, 0.5, and 1.0 mg/mL doses. These doses were defined based on the screening evaluation to allow assessment of the dose-response curve. Screening of the subfractions Ac-1 to Ac-10 and Hi-1 to Hi-10 was assessed at a dose of 2 mg/mL, while the isolated substances P1 and P2 were tested at doses of 0.25, 0.5, and 1.0 mg/mL, and substance P3 was tested at a dose of 1.0 mg/mL. Only the subfractions H-8 and H-9 could not be evaluated due to their low yield. The positive controls sodium citrate and Cystone® were evaluated according to the respective doses of the tested samples, and the negative control consisted of urine with crystals only. The samples were resuspended in water, filtered through a 25 mm × 0.22 µm cellulose ester filter, diluted in urine with crystals, and incubated in 96-well plates (86 × 128 mm), totaling a volume of 200 µL per well. The samples were then incubated for 24 h to evaluate crystal dissolution compared to positive and negative controls. After incubation, aliquots of 20 µL samples were assessed in a Neubauer counting chamber enhanced by the Nikon Eclipse E100 binocular microscope. Crystal images were captured

using the Axio Scope A1 microscope, Carl Zeiss™, Germany, and the area and quantity of CaOx crystals were analyzed using Carl Zeiss AxioVision Rel. 4.8 software. The results were analyzed by one-way analysis of variance (ANOVA) ($p \leq 0.05$), followed by Dunnett's multiple comparison test. The IC₅₀ value and statistical analyses were calculated using the GraphPad Prism® software.

3. Results and discussion

3.1. Anti-inflammatory activity of the fractions from the Dec of *C. gonyglodes*

The Dec and the fractions FoAc and FoHi exhibited dual anti-inflammatory activity by inhibiting the inflammatory mediators PGE2 and LTB4, synthesized through the COX and LOX pathways, respectively. These effects were significantly different from the negative control in both pathways: COX ($p \leq 0.0001$) and LOX ($p \leq 0.0001$), at the tested dose of 10 µg/mL (Fig. 1). The Dec showed 58% inhibition in the production of PGE2 and 52% inhibition in the production of LTB4. The inhibitory values for the FoAc and FoHi fractions were 51% and 54% for the COX pathway and 43% and 37% for the LOX pathway, respectively. Only the FoCl fraction did not exhibit satisfactory anti-inflammatory activity in either pathway compared to the negative control group ($p \leq 0.0001$). Dexamethasone and indomethacin respectively displayed 71% and 80% inhibition of PGE2 production, while dexamethasone demonstrated 70% inhibition of LTB4 release.

Dexamethasone belongs to the class of anti-inflammatory SAIDs, which inhibit the production of arachidonic acid by and consequently the COX and LOX pathways. On the other hand, indomethacin acts only on the inhibition of the COX pathway and belongs to the class of NSAIDs (Chagas-Paula et al., 2011). The substances present in the Dec, as well as in the FoAc and FoHi fractions demonstrated the ability to inhibit the production of PGE2 and LTB4, exhibiting a dual action in inhibiting the COX and LOX pathways. Based on these results, the FoAc and FoHi fractions appeared promising, inhibiting both two main pathways of the inflammatory process. Consequently, they were selected for further fractionation and investigation.

The FoAc and FoHi fractions underwent fractionation using SPE, resulting in the subfractions Ac-1 to Ac-10 and Hi-1 to Hi-10, respectively. Similar to the FoAc and FoHi fractions, their subfractions Ac-1, Ac-2, Ac-3, Ac-4, Ac-5, Ac-6, Ac-8, Ac-10, Hi-1, Hi-2, Hi-5, and Hi-6 exhibited dual activity by inhibiting both inflammation pathways ($p \leq 0.0001$). In contrast, the subfractions Ac-7, Ac-9, Hi-4, and Hi-10 inhibited the synthesis of PGE2 ($p \leq 0.0001$) (Figs. 2 and 3). Only the Hi-3 subfraction did not show any anti-inflammatory activity compared to the negative control group ($p \leq 0.05$). Indomethacin, the reference medication group, demonstrated a significant difference from the negative control in inhibiting PGE2 ($p \leq 0.0001$). However, it was inactive in the LOX pathway, similar to the negative control, according to its known function as a nonsteroidal anti-inflammatory drug acting through the COX pathway (Alcantara et al., 2023). Subfractions Ac-5 and Hi-5 through Hi-7 exhibited PGE2 inhibition percentages greater than 50%, whereas the drug indomethacin showed 88% inhibition via COX and inhibition percentages of LTB4 ranging from 31% to 54%. The Ac-5 and Hi-5 to Hi-7 subfractions demonstrated PGE2 inhibition rates exceeding 50%, while the drug indomethacin showed 88% inhibition via the COX pathway. Therefore, these subfractions were selected for chemical isolation to identify compounds with anti-inflammatory potential that can act on both the COX and LOX pathways.

3.2. Anti-urolithiatic activity of *C. gonyglodes* Dec fractions

Positive controls, the polyherbal formulation Cystone® and sodium citrate, along with the FoAc and FoHi fractions, showed significant ability to dissolve COM and COD crystals in human urine compared to the negative control group at the tested doses ($p \leq 0.0001$) (Fig. 4). The

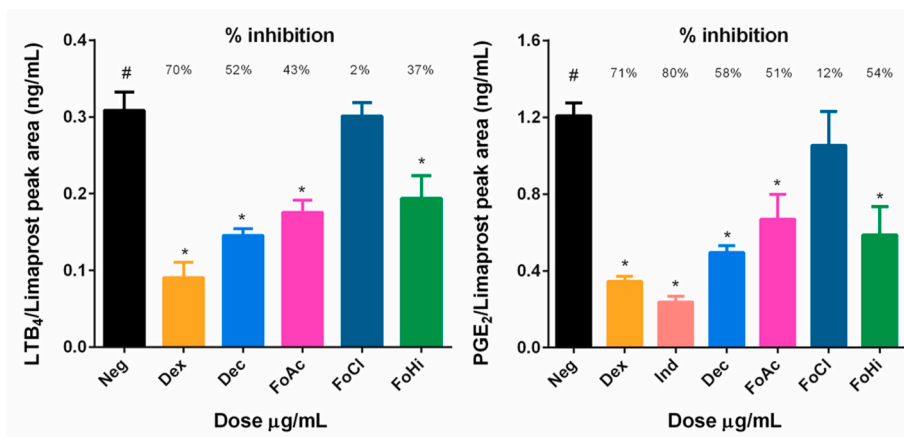


Fig. 1. Anti-inflammatory evaluation of PGE2 and LTB4 quantification of the decoction (Dec) and its fractions, acetate fraction (FoAc), chloroform fraction (FoCl), and hydroalcoholic Fraction (FoHi) derived from the leaves of *C. gongyloides*. The peak area values of PGE2/limaprost and LTB4/limaprost are expressed as mean ± SD (n = 4). *p ≤ 0.0001 compared to the negative control group (Neg), #p ≤ 0.0001 compared to the positive control group, dexamethasone (Dex). Results were analyzed using *one-way* analysis of variance (ANOVA) and subsequent Dunnett’s multiple comparison test.

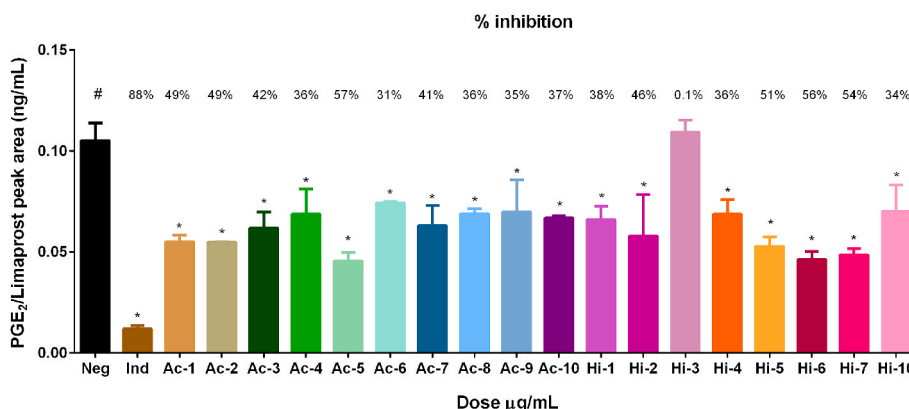


Fig. 2. Comparison of anti-inflammatory activity in the COX pathway of the subfractions from the acetate fraction (Ac-1 to Ac-10) and subfractions from the hydroalcoholic fraction (Hi-1 to Hi-7 and Hi-10) fractionated using SPE-C18. The peak area values of PGE2/limaprost are expressed as mean ± SD (n = 4) for the negative control group (Neg) and the positive control group indomethacin (Ind), and the subfractions as mean ± SD (n = 3). *p ≤ 0.0001 compared to the negative control group (Neg), and #p ≤ 0.0001 compared to the positive control group indomethacin (Ind). Results were analyzed using *one-way* analysis of variance (ANOVA) and subsequent Dunnett’s multiple comparison test.

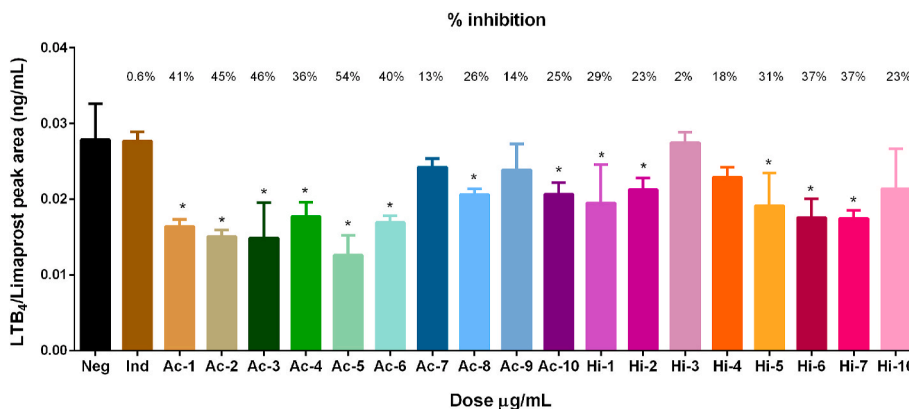


Fig. 3. Comparison of anti-inflammatory activity in the LOX pathway of the subfractions from the acetate fraction (Ac-1 to Ac-10) and subfractions from the hydroalcoholic fraction (Hi-1 to Hi-7 and Hi-10) fractionated using SPE-C18. The peak area values of LTB4/limaprost were expressed as mean ± SD (n = 4) for the negative control group (Neg) and the positive control group indomethacin (Ind), and the subfractions as mean ± SD (n = 3). The reference control and negative control groups were not significantly different, and neither inhibited the quantification of LTB4. *p ≤ 0.0001 compared to control groups. Results were analyzed using *one-way* analysis of variance (ANOVA) and subsequent Dunnett’s multiple comparison test.

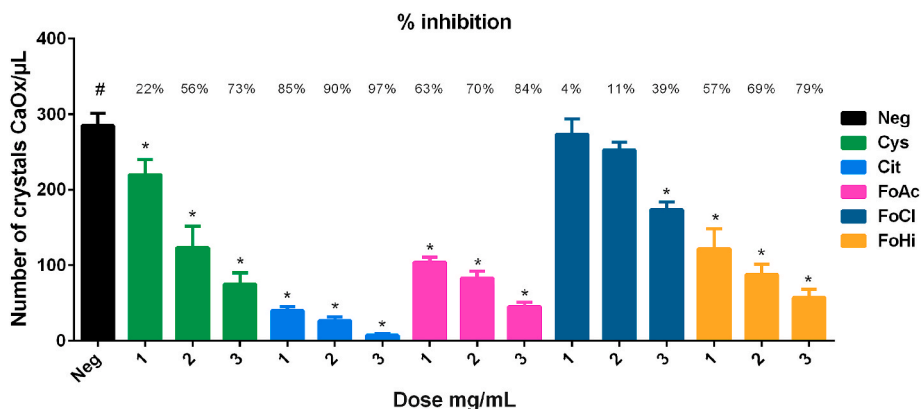


Fig. 4. The percentage inhibition of CaOx crystal at concentrations of 1, 2, and 3 mg/mL of the acetate fraction (FoAc), chloroform fraction (FoCl), and hydroalcoholic fraction (FoHi). * $p \leq 0.0001$ compared to the negative control group (neg). # $p \leq 0.0001$ compared to the positive controls sodium citrate (Cit) and Cystone® (Cys) at all three tested doses. Data are expressed as mean \pm SD (n = 3). Results were analyzed using one-way analysis of variance (ANOVA) and subsequent Dunnett’s multiple comparison test.

FoAc and FoHi fractions exhibited substantial anti-urolithiatic activity ($p \leq 0.0001$), with inhibition percentages of 63%, 70%, and 84%, and 57%, 69%, and 79%, respectively, at the evaluated concentrations. The positive control, Cystone®, demonstrated inhibition percentages of 22%, 56% and 73%, while sodium citrate showed 85%, 90%, and 97%. The FoCl fraction exhibited significant dissolution of CaOx only at the highest dose of 3 mg/mL, with an inhibition percentage of 39%.

At all doses (1–3 mg/mL), the active fractions FoAc and FoHi, were further evaluated to understand the dissolution of CaOx crystals, observing a reduction in crystal area at concentrations of 0.25, 0.5, and 1 mg/mL (Fig. 5). The Dec and its active fractions significantly reduced the area of CaOx crystals compared to the negative control group ($p \leq 0.05$), demonstrating inhibition percentages exceeding 50%. No significant differences were observed between the active fractions and the Dec ($p \leq 0.05$), indicating dose-dependent, anti-urolithiatic action. The inhibitory concentrations (IC_{50}) of the Dec and active fractions were 0.22, 0.21, and 0.16 mg/mL, while the positive controls, poly-herbal Cystone® and sodium citrate, each exhibited an IC_{50} of 0.23 mg/mL (Table S3). Microscopic observations showed that increasing doses of the Dec and fractions FoAc and FoHi reduced the quantity and size of CaOx crystals, completely dissolving them. These results indicate the presence of substances in the *C. gongyloides* Dec and its fractions that confer anti-urolithiatic activity. Based on this, subfractions (Ac-1 to Ac-10 and Hi-1 to Hi-7 and Hi-10) of the active fractions FoAc and FoHi

were also subjected to anti-urolithiatic evaluation.

Screening for CaOx crystal dissolution allowed the selection of the most active subfractions for bioguided isolation of substances responsible for anti-urolithiatic activity. All subfractions Ac-1 to Ac-10, Hi-1 to Hi-7, and Hi-10 showed significant differences compared to the negative control group and the positive controls Cystone® and sodium citrate ($p \leq 0.05$) (Fig. 6). The percentage inhibition of CaOx crystal area ranged from 39% to 82% compared to the positive controls Cystone® and sodium citrate, which achieved 80% and 95%, respectively. Among all the subfractions, Ac-5, Hi-5, Hi-6, and Hi-7 stood out with the highest inhibition rates, showing 78%, 76%, 76%, and 82%, respectively. Therefore, these subfractions were also chosen for chemical isolation.

Kidney stones can form from various crystalline structures, with calcium oxalate monohydrate (COM) crystals being the most prevalent (Sorensen et al., 2022). COM crystals exhibit a more stable structure compared to calcium oxalate dihydrate (COD) crystals. Morphological differences include that COM has a hexagonal structure, making it less soluble and more prone to adhesion in the kidneys, playing a central role in the development of nephrolithiasis (Dinnimath et al., 2017; Rez, 2017). In contrast, COD has a bipyramidal shape, which is thermodynamically less stable and dissolves more easily. Considering this prevalence and affinity, it is essential to develop therapies that target the dissolution of COM and COD crystals for more effective treatment of urolithiasis (Ammar et al., 2022). In light of the results, Dec fractions

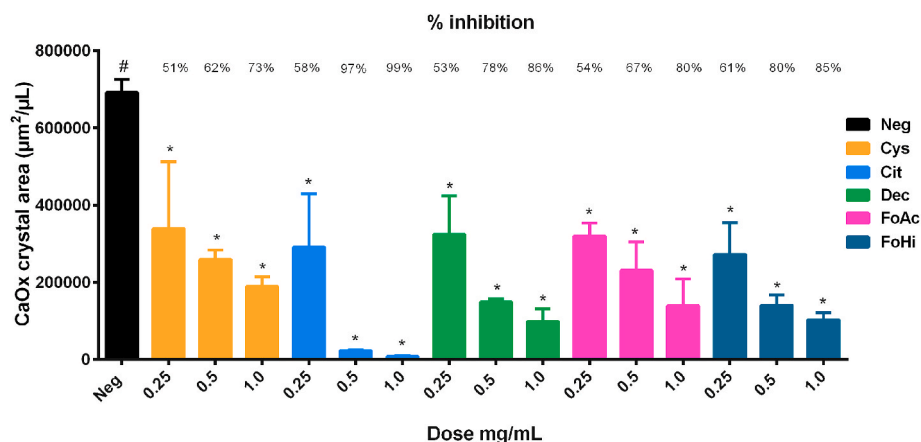


Fig. 5. Anti-urolithiatic activity of the decoction (Dec) and its active fractions, ethyl acetate fraction (FoAc), and hydroalcoholic fraction (FoHi) at doses of 0.25, 0.5, and 1 mg/mL # $p \leq 0.05$ compared to the positive controls sodium citrate (Cit) and Cystone® (Cys) at all three tested doses. * $p \leq 0.05$ compared to the negative control group (Neg). Values are expressed as mean \pm SD (n = 3). Results were analyzed using one-way analysis of variance (ANOVA) and subsequent Dunnett’s multiple comparison test.

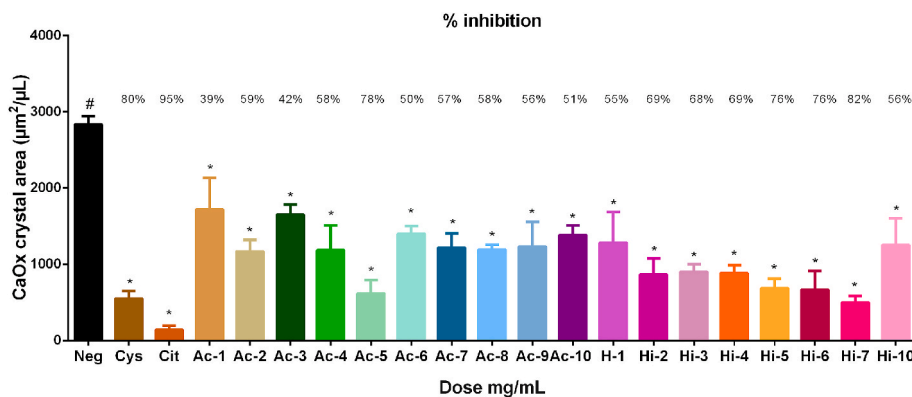


Fig. 6. Comparison of the anti-urolithiatic activity of subfractions from the ethyl acetate fraction (Ac-1 to Ac-10) and subfractions from the hydroalcoholic fraction (Hi-1 to Hi-7 and Hi-10) isolated by SPE-C18 from the active fractions at a dose of 2 mg/mL # $p \leq 0.0001$ compared to the positive controls sodium citrate (Cit) and Cystone® (Cys). * $p \leq 0.05$ compared to the negative control group (Neg). Values are expressed as mean \pm SD (n = 3). Results were analyzed using one-way analysis of variance (ANOVA) and subsequent Dunnett's multiple comparison test.

from *C. gonylodes* leaves have shown promising activity in dissolving both types of crystals, indicating significant potential as anti-urolithiatic agents. Therefore, these extracts may be valuable for developing new therapies for urolithiasis treatment.

3.3. Bioguided isolation of anti-inflammatory anti-urolithiatic substances

The Dec of *C. gonylodes* leaf powder was fractionated and the FoAc, FoCl and HoHi fractions were obtained. FoAc and FoHi demonstrated dual activity, dose-dependently dissolving CaOx crystals and inhibiting the production of key inflammatory mediators PGE2 and LTB4. The fraction FoCl showed significant CaOx dissolution only at the highest dose (3 mg/mL) and did not exhibit anti-inflammatory activity. Therefore, only FoAc and FoHi were selected for bioguided phytochemical fractionation by SPE, resulting in twenty subfractions Ac-1 to Ac-10 and H-1 to H-10. Due to low yield, subfractions Hi-8 and Hi-9 were not evaluated for biological activities. The chemical profiles of the subfractions were analyzed using HPLC-UV-DAD at 254 nm. Subfractions Hi-5 to Hi-7 exhibited similar chemical composition with few differences on the peak area/intensity, and thus they were grouped as fraction Hi-5a. Subfractions Hi-5a from FoHi and Ac-5 from FoAc exhibited significant multi-target activity, both anti-urolithiatic and anti-inflammatory, with lower chemical complexity, and were selected for isolation and purification by HPLC-UV-DAD. The active fractions underwent isolation, resulting in the isolation of rutin (P1) from subfraction Hi-5a, and the compounds eriodictyol 4'-O-glucoside (P2) and

isoquercetin (P3) from subfraction Ac-5.

3.4. Anti-inflammatory activity of bioactive compounds

The three glycosylated flavonoids rutin (P1), eriodictyol 4'-O-glucoside (P2), and isoquercetin (P3) isolated from the active fractions of the Dec of the *C. gonylodes* demonstrated significant anti-inflammatory activity compared to the negative control group ($p \leq 0.05$). Both rutin and isoquercetin exhibited dose-dependent dual action, inhibiting the production of inflammatory mediators PGE2 and LTB4 across various tested concentrations (0.5, 1, 2, 4, 10 μ g/mL) (Figs. 7 and 8). The eriodictyol 4'-O-glucoside also showed dual anti-inflammatory activity through COX and LOX pathways at higher concentrations (2, 4, and 10 μ g/mL) and inhibited LTB4 synthesis at all tested doses. However, eriodictyol 4'-O-glucoside displayed selectivity for dose-dependent reduction of LTB4 levels, with no significant effect on PGE2 levels at lower doses (0.5 and 1 μ g/mL).

The rutin and eriodictyol 4'-O-glucoside demonstrated over 50% inhibition in the COX pathway at doses of 1–10 μ g/mL, whereas isoquercetin showed this level of inhibition across all tested concentrations (0.5–10 μ g/mL) (Table S4). For the LOX pathway, rutin exhibited over 50% inhibition at doses of 4–10 μ g/mL, eriodictyol 4'-O-glucoside only at the highest dose (10 μ g/mL), and isoquercetin at concentrations of 2–10 μ g/mL. Additionally, the IC₅₀ values for PGE2 inhibition were 1.33 μ g/mL for isoquercetin, 2.27 μ g/mL for rutin, and 7.99 μ g/mL for eriodictyol 4'-O-glucoside. By comparison, dexamethasone and

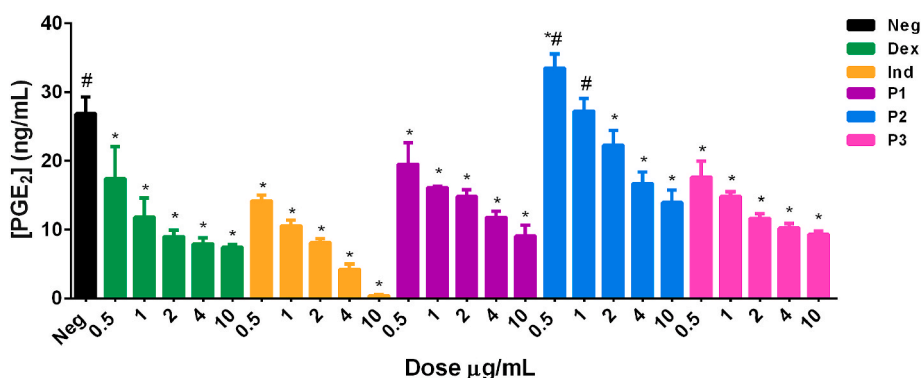


Fig. 7. Quantification of PGE2 from the compounds rutin (P1), eriodictyol 4'-O-glucoside (P2), and isoquercetin (P3) isolated from *C. gonylodes* in the ex-vivo assay with human blood at doses of 0.5, 1, 2, 4, and 10 μ g/mL. Values are expressed as mean \pm SD (n = 4) for the negative control group (Neg) (n = 3), positive control groups dexamethasone (Dex) and indomethacin (Ind), and the isolated compounds (n = 4). # $p \leq 0.05$ compared to the reference indomethacin (Ind). * $p \leq 0.05$ compared to the negative control group (Neg). *# $p \leq 0.05$ compared to the negative control group (Neg) and the positive control groups dexamethasone (Dex) and indomethacin (Ind). Results were analyzed using one-way analysis of variance (ANOVA) and subsequent Dunnett's multiple comparison test.

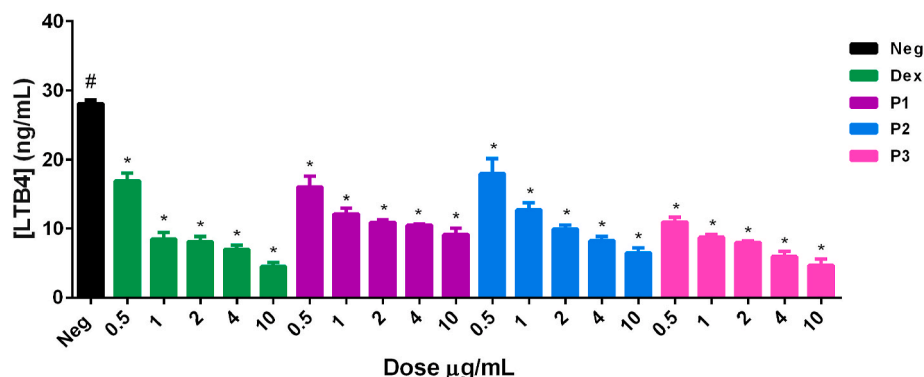


Fig. 8. Quantification of LTB₄ from substances rutin (P1), eriodictyol 4'-O-glucoside (P2), and isoquercetin (P3) isolated from *C. gongyloides* in the *ex-vivo* assay with human blood at doses of 0.5, 1, 2, 4, and 10 µg/mL. Values are expressed as mean ± SD (n = 4) for the negative control group (Neg) (n = 3), positive control group dexamethasone (Dex), and the isolated substances (n = 4). #p ≤ 0.0001 compared to the reference drug dexamethasone. *p ≤ 0.0001 compared to the negative control group. Results were analyzed using *one-way* analysis of variance (ANOVA) and subsequent Dunnett's multiple comparison test.

indomethacin exhibited IC₅₀ values of 0.78 µg/mL and 0.56 µg/mL, respectively. Regarding LTB₄, the IC₅₀ values were 0.68 µg/mL for rutin, 0.92 µg/mL for eriodictyol 4'-O-glucoside, and 0.15 µg/mL for isoquercetin, while dexamethasone showed an IC₅₀ of 0.57 µg/mL (Table S5).

The results of our studies corroborate previous research, which demonstrated the anti-inflammatory effect of rutin by inhibiting the COX and LOX pathways, resulting in reduced prostaglandin E2 (PGE2) and leukotriene B4 (LTB₄) levels in LPS-stimulated RAW 264.7 cells (Morikawa et al., 2003; Muvhulawa et al., 2022). Isoquercetin has also been shown to decrease PGE2 levels in carrageenan-treated rats (Choi et al., 2021). Furthermore, for the first time, we investigated the ability of isoquercetin to inhibit the LOX pathway, as well as the anti-inflammatory activity of eriodictyol 4'-O-glucoside, by evaluating the reduction of PGE2 and LTB₄ levels induced by LPS in human blood samples.

Flavonoids are widely recognized for their therapeutic potential against pain and inflammation, and they are considered promising candidates for the development of new drugs due to their wide range of biological activities, attributed to their great chemical diversity (Arulselvan et al., 2016; Newman and Cragg, 2020). The structural diversity of flavonoids results from variations in levels of oxidation and reactions such as alkylation and glycosylation. These modifications in the central C ring give rise to different classes of flavonoids, which present variations in the inhibition of COX and LOX enzymes (Comalada et al., 2006).

The isolated compounds belong to the classes of flavanones and flavonols. The presence of hydroxyl groups at positions C5 and C7 of ring A, C3' and C4' of ring B (3',4'-dihydroxy), the carbonyl group at C4 in ring C, the C2-C3 double bond in ring C, and the planarity of the molecule contribute significantly to the anti-inflammatory activity, favoring interaction with the active sites of the COX and LOX enzymes (Shamsudin et al., 2022; Wang et al., 2022). Planarity, promoted by the C2-C3 double bond, increases the affinity for the enzyme receptors, while the delocalization of the π electron, generated by the conjugated system between the carbonyl group at C4 and the C2-C3 double bond, stabilizes intermediates formed during chemical reactions with flavonoids (Es-Safi et al., 2007; Niquini et al., 2020). On the other hand, glycosylation reduces the anti-inflammatory activity by decreasing lipophilicity, weakening the interaction with the active groups of the enzymes. The presence of a hydroxyl group at C3 in the C ring decreases enzyme inhibition (Chen et al., 2018). In contrast, methylation of flavonoids increases lipophilicity, allowing a more efficient interaction with hydrophobic regions of proteins involved in inflammation. Thus, the combination of hydroxyls in strategic positions, methylation, and specific double bonds is essential to optimize the anti-inflammatory action of flavonoids (Md Idris et al., 2022; Ribeiro et al., 2014).

Among the isolated compounds, the flavonol class, represented by rutin and isoquercetin, showed greater inhibition of the COX and LOX pathways compared to eriodictyol 4'-O-glucoside, a compound of the flavanone class. The presence of a glycosidic group at the C3 position of the C ring contributed to the greater anti-inflammatory activity of rutin and isoquercetin. In contrast, the absence of the double bond between the C2 and C3 carbons in the B ring resulted in less inhibition by eriodictyol 4'-O-glucoside on the COX and LOX pathways (Table S5) (Du et al., 2023; Oniga et al., 2017). These discoveries highlight the importance of specific structural characteristics of flavonoids in modulating inflammatory pathways. Therefore, continued structure-activity relationship studies are essential to improving the understanding of molecular interactions and identifying new flavonoid-based therapeutic strategies for the treatment of inflammatory processes.

Compounds isolated from *C. gongyloides* Dec showed a dual anti-inflammatory action, offering more effective control of inflammation by reducing multiple inflammatory mediators. This effect may decrease the typical side effects of NSAIDs and SAIDs (Januario et al., 2018; Santos et al., 2019). These findings are relevant for future research, highlighting the importance of identifying compounds that simultaneously inhibit the COX and LOX pathways and cause fewer adverse effects (Maiolini et al., 2023).

3.5. Anti-uroliathatic activity of bioactive compounds

The substances rutin (P1), eriodictyol 4'-O-glucoside (P2), and isoquercetin (P3), isolated from the most active fractions, demonstrated significant action in dissolving both COM and COD crystals in human urine samples. In the study, both the polyherbal formulation Cystone® (Cys) and sodium citrate (Cit), used as positive controls, showed significant efficacy compared to the negative control group, with a statistically relevant difference (p ≤ 0.0001) (Fig. 9). Dissolution of CaOx stones was dose-dependent for rutin, eriodictyol 4'-O-glucoside, and isoquercetin at tested concentrations of 0.25, 0.5, and 1 mg/mL. Specifically, at the highest tested dose of 1 mg/mL, rutin and eriodictyol 4'-O-glucoside achieved 86% dissolution, and isoquercetin achieved 88% dissolution. In comparison, the positive controls Cystone® and sodium citrate showed dissolution percentages rates of 70% and 95%, respectively. The observed inhibition percentages (IC₅₀) for the tested doses were: 0.55 mg/mL for Cystone®, 0.22 mg/mL for sodium citrate, 0.37 mg/mL for rutin, and 0.18 mg/mL for eriodictyol 4'-O-glucoside (Table S6).

The constituents present in Dec and active fractions, as well as isolated compounds such as rutin, eriodictyol 4'-O-glucoside, and isoquercetin, which have hydrophilic and hydrogen bond-forming groups, can form complexes with CaOx crystals. The nature of these phytochemical complexes can affect their efficacy, resulting in greater

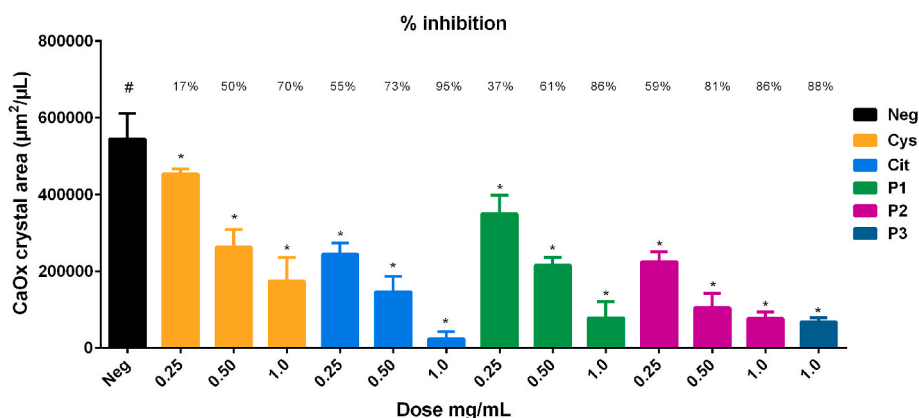


Fig. 9. Anti-uro lithiatic activity of the substances rutin (P1), eriodictyol 4'-O-glucoside (P2), and isoquercetin (P3) isolated from *C. gongyloides* evaluated at doses of 0.25, 0.5, and 1 mg/mL #p ≤ 0.0001 compared to the positive controls sodium citrate (Cit) and Cystone® (Cys). *p ≤ 0.0001 compared to the negative control group (Neg). Values are expressed as mean ± SD (n = 3). Results were analyzed using one-way analysis of variance (ANOVA) and subsequent Dunnett's multiple comparison test.

solubility of CaOx crystals (Liu et al., 2022). Furthermore, the concentrations and sizes of the crystals directly influence dissolution. Smaller crystals, with a larger surface area, facilitate more efficient interactions with the compounds in the fractions, accelerating dissolution through hydrogen bonds and ionic interactions between Ca²⁺ and C₂O₄²⁻ ions on the crystal surface (Polat and Eral, 2021). In contrast, larger crystals with a smaller contact area slow down this process. Higher crystal concentrations may also reduce the efficacy of the fractions due to competition for interactions with the active compounds (Li et al., 2022; Sheng et al., 2005).

Decoctions, infusions, and plant extracts have been the focus of intensive research due to their potential anti-uro lithiatic, antioxidant, and anti-inflammatory properties (Zeng et al., 2019). Biological assays highlight these extracts as promising alternatives for urolithiasis treatment, showing fewer side effects than conventional therapies (Al-Khayri et al., 2022). Studies suggest that flavonoids and extracts rich in flavonoids play a crucial role in preventing and treating kidney stones. These substances are likely the primary agents responsible for the observed pharmacological activity (Ahmed et al., 2018; Bawari et al., 2023; Sharma et al., 2017; Zhang et al., 2014; Zhou et al., 2018). However, only a small fraction of flavonoids present in plant extracts have been identified, isolated, and evaluated for their anti-uro lithiatic action. A detailed understanding of these substances is essential for elucidating their mechanisms of action in nephrolithiasis (Sorokin et al., 2017).

Additionally, among the substances investigated for anti-uro lithiatic activity (rutin, hyperoside, quercetin, catechin, and diosmin), rutin has shown significant efficacy in inhibiting kidney stone formation by reducing the concentration of CaOx stone-forming constituents such as calcium and oxalate (Chaiyarit et al., 2024; Chen et al., 2001). This effect resulted in minimal damage to kidney tissues in animals induced with ethylene glycol and ammonium chloride (Ghodasara et al., 2010). Rutin acts as a potent agent in treating nephrolithiasis by inhibiting oxidative stress, autophagy, and apoptosis while also promoting the recovery of the structural and functional integrity of kidneys compromised by gentamicin (Kandemir et al., 2015). It also reduces oxidative stress and the expression of inflammation and cell death markers such as TNF-α, NF-kB, and caspase-3 proteins, protecting against inflammation and cell death in rats with nephrotoxicity (Arjumand and Sultana, 2011). Its combination with vitamin C has demonstrated protective effects against kidney injuries induced by high-cholesterol diets (Al-Rejaie et al., 2012). Furthermore, the novel insights into the mechanisms of action of isoquercetin and eriodictyol 4'-O-glucoside significantly enhance our understanding of the processes involved in renal stone dissolution by flavonoids. This evidence confirms the anti-uro lithiatic effects of rutin and extends previous research, supporting flavonoids'

effectiveness in eliminating CaOx stones.

The Dec of *C. gongyloides* leaves harbors a high concentration of flavonoids, potentially elucidating their observed anti-uro lithiatic action. These substances are crucial in justifying their therapeutic use in kidney stone removal, demonstrating their effectiveness in treating this condition among the population. It is essential to evaluate the Dec of *C. gongyloides* in different experimental models to deepen the understanding of its mechanisms of action in the formation and dissolution of kidney stones in urolithiasis, especially considering its medicinal use for managing this disease.

3.6. Identification of the structural compounds isolated via NMR and MS

The bioactive subfractions Hi-5a and Ac-5, selected for phytochemical isolation, produced three glycosylated flavonoids (Fig. 10). The three isolated compounds, P1, P2, and P3, were identified as flavonoids of the flavonol (P1 and P3) and flavanone (P2) types. Their chemical structures were identified from NMR (1D and 2D) and UHPLC/ESI-QTOF-MS in positive mode and comparison with data described in the literature. The compounds were identified as:

Rutin (Quercetin 3-O-rutinoside), (P1), was confirmed by QTOF-MS in positive mode, where the *m/z* of the ion adduct [M+H]⁺ = 611.1614 Da (calc. *m/z* 611.1607 Da, error of 1.14 ppm) was attributed to the molecular formula C₂₇H₃₀O₁₆. The MS/MS spectrum (Fig. S1) with retention time of 2.25 min showed the fragment of *m/z* 303.0499 (error of 0.00 ppm) and 465.1033 (error of 1.07 ppm) corresponding to the aglycone and the loss of the glycoside, respectively - ¹H NMR (300 MHz, MeOD) δ: 7.66 (d, 1H, J_m = 1.6 Hz, H-2'), 6.86 (d, 1H, J_o = 8.5 Hz, H-5'), 7.63 (dd, 1H, J_o = 8.5 Hz; J_m = 1.6 Hz; H-6'), 6.21 (d, 1H, J_m = 1.7 Hz, H-6), 6.40 (d, 1H, J_m = 1.5 Hz, H-8), 5.11 (d, 1H, J_{aa} = 7.35 Hz, H-1''), 3.27 (m, H-2''), 3.42 (dd, 1H, J_{ee} = 2.2 Hz; J_{aa} = 8.4 Hz, H-3''), 3.38 (m, H-4''), 3.30 (m, H-5''), 3.36 (m; Ha-6''), 3.79 (d, 1H, J_{aa} = 10.4 Hz, Hb-6''); 4.51 (brs, 1H, H-1'''), 3.27 (m, 1H, H-2'''), 3.62 (d, 1H, J_{ee} = 2.3 Hz, H-3'''), 3.30 (m, H-4'''), 3.53 (dd, 1H, J_{aa} = 9.4 Hz; J_{ae} = 3.2 Hz, H-5'''), 1.11 (d, 3H, J = 6.1 Hz, H-6'''). ¹³C (75 MHz, MeOD) δ: 121.9 (C-1'), 118.1 (C-2'), 144.5 (C-3'), 148.3 (C-4'), 116.5 (C-5'), 124.0 (C-6'), 157.7 (C-2), 134.1 (C-3), 177.2 (C-4), 161.8 (C-5), 100.5 (C-6), 164.7 (C-7), 95.1 (C-8), 157.1 (C-9), 104.1 (C-10), 105.1 (C-1''), 74.4 (C-2''), 76.3 (C-3''), 68.9 (C-4''), 77.6 (C-5''), 69.0 (C-6''), 102.8 (C-1'''), 71.9 (C-2'''), 72.6 (C-3'''), 74.4 (C-4'''), 72.7 (C-5'''), 18.4 (C-6'''). The compared experimental data and literature are presented in Table S7 and additional information relying on spectral correlations is presented at the 2D spectra (Figs. S6–8) and Table S8 (Srinivasan et al., 2015).

Eriodictyol 4'-O-glucoside, (P2), confirmed by QTOF-MS in positive mode, presented *m/z* of the ion adduct [M+H]⁺ = 451.1248 Da (calc. *m/z*

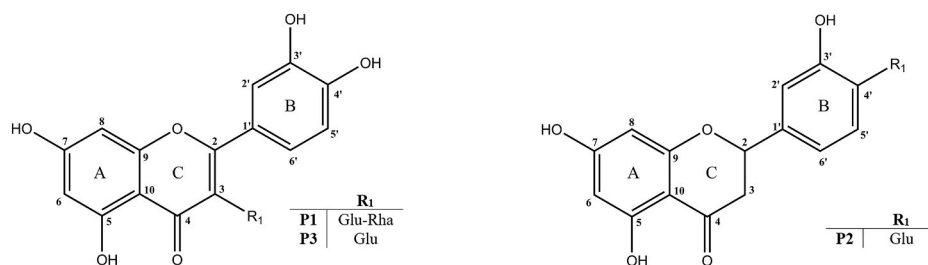


Fig. 10. Representation of the compounds identified through phytochemical analysis of the decoction of *C. gongylodes* (Dec). Legend: Rha = rhamnose; Glu-Rha = glucose-rhamnose.

z 451.1235, error of 2.88 ppm), corresponding to the molecular formula $C_{21}H_{22}O_{11}$ and the MS/MS spectrum (Fig. S2) of the ion with the retention time 2.40 min ^{-1}H NMR (300 MHz, MeOD) δ : 7.67 (d, 1H, $J_m = 2.16$ Hz, H-2'), 6.89 (d, 1H, $J_o = 8.5$ Hz, H-5'), 7.61 (dd, 1H, $J_o = 8.5$ Hz; $J_m = 2.16$ Hz; H-6'), 4.39 (dd, 1H, $J = 4.65$ Hz, $J = 7.26$ Hz, H-2), 2.51 (dd, 1H, $J = 7.26$ Hz; $J = 15.57$ Hz, Ha-3), 2.57 (dd, 1H, $J = 4.65$ Hz; $J = 15.57$ Hz, Hb-3), 6.24 (d, 1H, $J_m = 1.98$ Hz, H-6), 6.44 (d, 1H, $J_m = 1.98$ Hz, H-8), 5.19 (d, 1H, $J_{aa} = 7.65$ Hz, H-1''), 3.41 (m, H-2''), 3.42 (m, H-3''), 3.42 (m, H-4''), 3.44 (m, H-5''), 3.63 (brs, 1H, Ha-6''), 4.22 (brs, 1H, Hb-6''). ^{13}C (75 MHz, MeOD) δ : 122.0 (C-1'), 118.1 (C-2'), 145.0 (C-3'), 149.3 (C-4'), 123.8 (C-5'), 116.5 (C-6'), 69.4 (C-2), 41.7 (C-3), 175.4 (C-4), 159.7 (C-5), 100.5 (C-6), 164.8 (C-7), 95.4 (C-8), 157.5 (C-9), 104.5 (C-10), 104.8 (C-1''), 76.0 (C-2''), 78.3 (C-3''), 71.5 (C-4''), 78.4 (C-5''), 64.8 (C-6''). The compared experimental data and literature is presented in Table S9, and additional information relying on spectral correlations is presented in the 2D spectra (Figs. S12–14) and Table S10 (Liu et al., 2014).

Isoquercetin (Quercetin-3-O-glucoside), (P3), was also confirmed by QTOF-MS in positive mode, with m/z of the ion adduct $[M+H]^+ = 465.1027$ Da (calc. m/z 465.1028, error of 0.21 ppm) and corresponding molecular formula $C_{21}H_{20}O_{12}$. The MS/MS spectrum (Fig. S3) of the ion with retention time of 2.44 min showed the fragment of m/z 303.0496 (error of 0.99 ppm) of the aglycone corresponding to the loss of the glycoside - 1H NMR (300 MHz, MeOD) δ : 7.70 (d, 1H, $J_m = 2.0$ Hz, H-2'), 6.86 (d, 1H, $J_o = 8.4$ Hz, H-5'), 7.58 (dd, 1H, $J_o = 8.4$ Hz; $J_m = 2.0$ Hz; H-6'), 6.39 (d, 1H, $J_m = 1.8$ Hz, H-6), 6.19 (d, 1H, $J_m = 1.8$ Hz, H-8), 5.26 (d, 1H, $J_{aa} = 7.29$ Hz, H-1''), 3.45 (brs, 1H, H-2''), 3.41 (brs, 1H, H-3''), 3.47 (brs, 1H, H-4''), 3.38 (brd, 1H, $J = 5.10$ Hz, H-5''), 3.58 (dd, 1H, $J = 5.10$ Hz; Ha-6''), 3.71 (dd, 1H, $J = 11.7$ Hz; $J = 2.43$ Hz, Hb-6''). ^{13}C (75 MHz, MeOD) δ : 121.7 (C-1'), 116.2 (C-2'), 144.7 (C-3'), 148.4 (C-4'), 114.6 (C-5'), 121.8 (C-6'), 157.3 (C-2), 134.2 (C-3), 178.2 (C-4), 165.5 (C-5), 93.2 (C-6), 164.8 (C-7), 98.5 (C-8), 157.0 (C-9), 104.4 (C-10), 102.9 (C-1''), 74.4 (C-2''), 76.9 (C-3''), 74.5 (C-4''), 76.7 (C-5''), 61.2 (C-6''). The compared experimental data and literature is presented in Table S11 and additional information relying on spectral correlations are presented at the 2D spectra (Figs. S18–20) and Table S12 (Fitrah et al., 2024).

The compounds P1 and P3, identified as rutin and isoquercetin, respectively, were determined to be flavonoids of the flavonol 3-O-glycosylated type. This subclass of flavonoids is characterized by the absence of aliphatic hydrogens at positions 2 and 3 of the C ring in the 1H NMR spectrum, which indicates the presence of an α,β -unsaturated flavonol system (Wawer and Zielinska, 2001).

The NMR spectra for compound P1 (Figs. S4–S9) were identical to those reported in the literature (Table S7), with the 1H -NMR spectrum exhibited signals corresponding to aromatic hydrogens from five sp^2 carbon protons (δ_H 6.21–7.66) (Srinivasan et al., 2015). Among these, two proton signals at δ_{H-6} 6.21 and δ_{H-8} 6.40, doublets with the same coupling constant ($J_m = 1.7$ Hz), were attributed to the A ring of the aglycone. The other three protons, at $\delta_{H-2'}$ 7.66 (d, 1H, $J_m = 1.6$ Hz), $\delta_{H-5'}$ 6.86 (d, 1H, $J_o = 8.5$ Hz), and $\delta_{H-6'}$ 7.63 (dd, 1H, $J_o = 8.5$ Hz; $J_m = 1.6$ Hz), were assigned to the B ring of quercetin. These data

unequivocally supported the conclusion that the aglycone fraction of P1 is quercetin. The glycosidic hydrogens were detected with chemical shifts ranging from δ_H 3.08 to δ_H 5.30, indicating the presence of a glucose unit, confirmed by the oxymethylene carbon at $\delta_{C-6''}$ 69.0. Additionally, the connection to a rhamnose unit was confirmed by the methyl group at $\delta_{C-6''}$ 18.4, observed in the ^{13}C NMR spectrum. The main correlations observed in the two-dimensional spectra, such as COSY, HSQC, and HMBC, are presented in Table S8 and Fig. S9.

Compound P2 was identified as eriodictyol 4'-O-glucoside, which belongs to the flavanone class. Its NMR spectra are detailed in Table S9 and Figs. S10–S14 (Liu et al., 2014). The 1H NMR spectrum of compound P2 showed doublets at δ_{H-6} 6.24 and δ_{H-8} 6.44 (each 1H, d, $J_m = 1.95$ Hz) due to meta coupling of two protons from ring A. The other three aromatic protons at $\delta_{H-2'}$ 7.67 (d, 1H, $J_m = 2.16$ Hz), $\delta_{H-5'}$ 6.89 (d, 1H, $J_o = 8.5$ Hz), and $\delta_{H-6'}$ 7.61 (dd, 1H, $J_o = 8.5$ Hz; $J_m = 2.16$ Hz) exhibited a trisubstituted coupling pattern at positions 1, 3, and 4. The flavanone aglycone was confirmed by signals in the 1H NMR spectrum of H-2, Ha-3, and Hb-3 protons, coupled at δ_{H-2} 4.39 (dd, 1H, $J = 4.65$ Hz; $J = 7.26$ Hz), δ_{Ha-3} 2.51 (dd, 1H, $J = 7.26$ Hz; $J = 15.57$ Hz), and δ_{Hb-3} 2.57 (dd, 1H, $J = 4.65$ Hz; $J = 15.57$ Hz), respectively. The primary correlations observed in the two-dimensional spectra, including COSY, HSQC, and HMBC, are detailed in Table S10 and Fig. S15.

The NMR spectra for substance P3 identify it as isoquercetin, as detailed in Table S11 and Figs. S16–S20 (Fitrah et al., 2024). This substance is very similar to rutin, differing only in the type of glycoside. In the ^{13}C NMR spectrum, an anomeric carbon was observed at $\delta_{C-1''}$ 104.8, along with four oxygenated methine carbons at $\delta_{C-2''}$ 76.0, $\delta_{C-3''}$ 78.3, $\delta_{C-4''}$ 71.6, and $\delta_{C-5''}$ 78.4 and an oxygenated methylene carbon at $\delta_{C-6''}$ 64.8, all attributed to glucose. The primary correlations observed in the two-dimensional spectra are detailed in Table S12 and Fig. S21.

In all spectra, the sugar linkage can be distinguished as O-glycosidic, indicated by the ^{13}C NMR (δ_C 98–112), in contrast to C-glycosides (δ_C 60–82) (Silva et al., 2023). Furthermore, the J values of the anomeric proton observed in all spectra (7.29–7.65 Hz) suggest a β -configuration of D-glucose, consistent with findings reported in the literature (Seo et al., 2017).

Rutin has been isolated from various species of the genus *Cissus*, including the leaves of *Cissus ibuensis* (Ahmadu et al., 2010), the fruits of *Cissus aovata* (Hazarika et al., 2023), the aerial parts of *Cissus quadrangularis* (Ruangsuriya et al., 2020), and the leaves of *Cissus sicyoides* Xu et al. (2009). Isoquercetin has been reported in the leaves of *C. sicyoides* (Xu et al., 2009) and *Cissus rotundifolia* (Said et al., 2018). Rutin and isoquercetin have been reported for the first time in the species *C. gongylodes*, while eriodictyol 4'-O-glucoside has been reported in the genus *Cissus*.

3.7. Characterization of the Dec and fractions of *C. gongylodes* by UHPLC-ESI-HRMS

Data-independent acquisition (DIA) was employed to enhance spectral coverage compared to data-dependent acquisition (DDA), providing a more comprehensive view of the metabolic profile of Dec

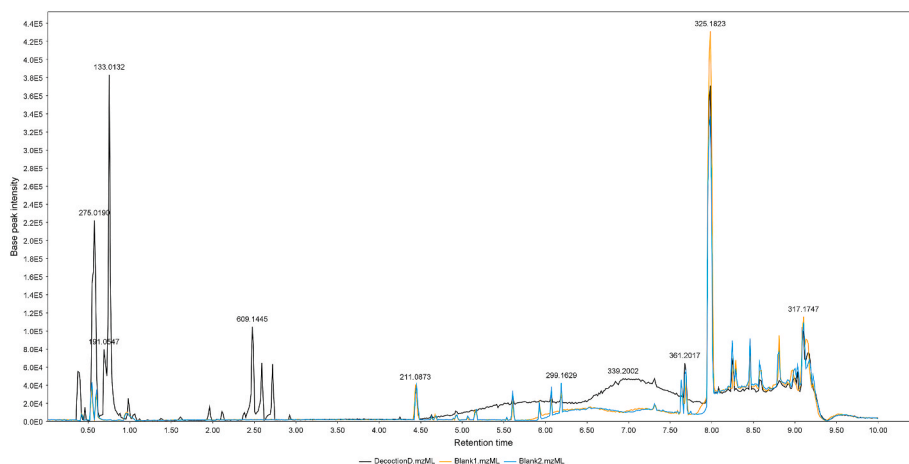


Fig. 11. Base peak chromatograms obtained in negative ion mode of *C. gongyloides* (Dec) and blanks.

(Schultz et al., 2013; Zhang et al., 2012). The Dec of *C. gongyloides* were subjected to metabolic profiling using UHPLC-ESI-QTOF/MS (Fig. 11).

Features were confidence annotated at level 2 (12 compounds) and level 0 for the identified compounds (Sumner et al., 2007). The integration of in-house databases containing compounds identified from the *Cissus* genus enhances the reliability of annotations in metabolomic studies (Katchborian-Neto et al., 2024). In the positive ion mode, 60 features were detected, and 7 substances were annotated (Table S13). In the negative ion mode, 67 features were detected, and 12 substances were annotated (Table S14). The main classes of annotated metabolites were flavonoids and organic acids. Compounds 1, 2, 3, 5, 6, 8, and 12 were identified as flavonols, with compounds 1, 2, 5, and 8 being

glycosylated. Compounds 4 and 14 were classified as flavones, while compound 7 was identified as a glycosylated flavone. Compounds 9, 10, 11, and 13 were categorized as organic acids (Fig. 12).

Among the annotated compounds, 1, 2, 3, 5, 6, 8, and 12 were identified as flavonols, with compounds 1, 2, 5, and 8 showing glycosylation. Compounds 4 and 14 were classified as flavones, while compound 7 was identified as a glycosylated flavone. Finally, compounds 9, 10, 11, and 13 were categorized as organic acids. Compound 1 exhibited a pseudomolecular ion $[M-H]^-$ at m/z 609.1445 and $[M+H]^+$ at m/z 611.1612, indicating a molecular formula of $C_{27}H_{30}O_{16}$. Fragmentation produced product ions at m/z 463.0867 ($[M-146-H]^-$) and m/z 300.0278 ($[M-146-163-H]^-$), corresponding to the loss of rhamnose (146 Da) and

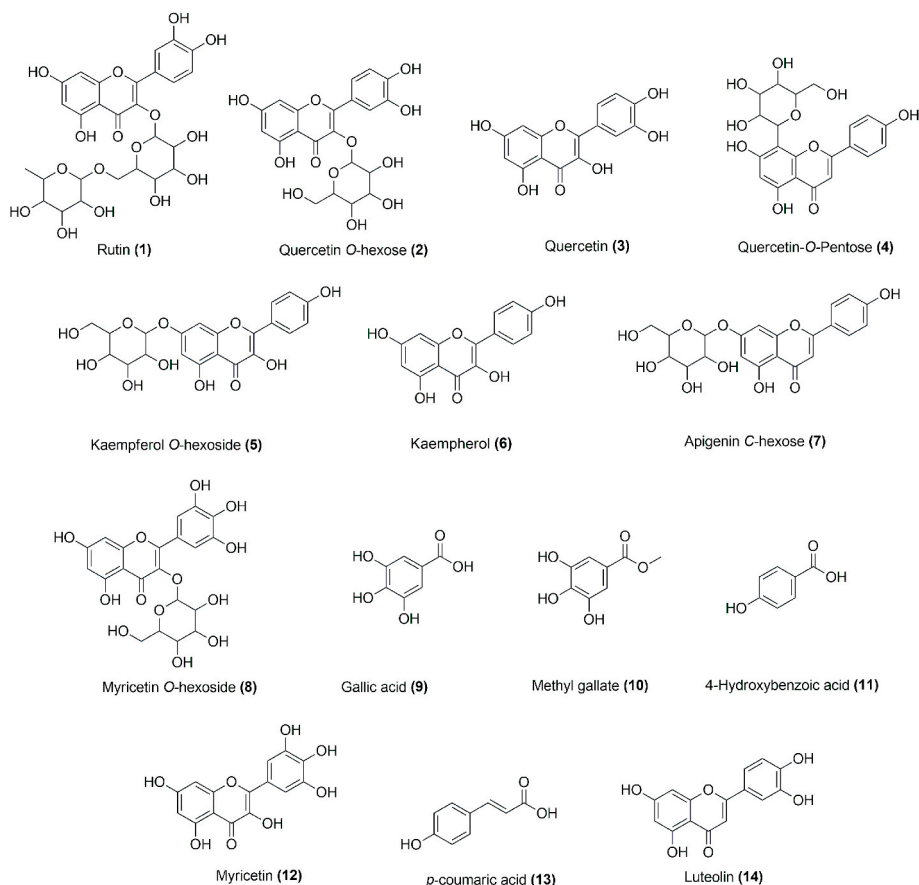


Fig. 12. Chemical structure of the annotated compounds.

glucose (163 Da), respectively, which are indicative of rutin.

In flavonoid fragmentation, the loss of CO and CO₂ is quite common. This process generates pseudomolecular ions [M-H]⁻ with *m/z* 271.0229 (C₁₄H₇O₆) and 245.0270 (C₁₃H₅O₅), resulting from the consecutive loss of CO from the ion at *m/z* 300.0278. The loss of CO₂ from *m/z* 301.0278 can lead to the formation of ions at *m/z* 256.0229 (C₁₄H₉O₅) and 227.0362 (C₁₃H₇O₄). Ions at *m/z* 119.0503 (C₇H₃O₂) and 178.9980 (C₈H₃O₅) may form through the cleavage of the C1-C2 and C2-C3 bonds in the ion, respectively. The ion at *m/z* 119.0503 contains the B ring, while the ion at *m/z* 178.9980 contains the A ring. The ion at *m/z* 178.9980 can lose CO and CO₂, generating ions at *m/z* 151.0013 (C₇H₃O₄) and 107.01395 (C₆H₃O₂). The Retro-Diels–Alder reaction can occur in flavonoids, forming an intense ion at *m/z* 151.0013 (C₇H₃O₄). Compounds 1–4 were annotated in both ionization modes: product ions of quercetin-type flavonoids exhibit *m/z* values of 301.03, 271.03, 178.99, and 151.00, as observed in compounds 1–4 in negative mode, and positive mode, the product ions of flavonoids showed intense signals at *m/z* 303.04, 273.03, 181.06, and 153.01 (Fu et al., 2020).

Compounds 5 and 6 exhibited a structure similar to quercetin. Due to the absence of the -OH group at C-5, the product ions of kaempferol are all 16 Da smaller than those of quercetin, showing intense ions at *m/z* 285.03, 257.05, and 163.01 (Vinayavekhin et al., 2010). Compound 7 displayed a pseudomolecular ion [M+H]⁺ at *m/z* 433.1115, indicating a molecular formula of C₂₁H₂₀O₁₀. Fragmentation produced signals at *m/z* 287.0569 ([M-146-H]⁻), indicating O-hexose loss. Diagnostic ions of apigenin O-hexose-type flavonoids are typically seen at *m/z* 433.11, 271.03, and 153.02 (Ivanova and Spiteller, 2021).

Compound 8 exhibited a pseudomolecular ion [M+H]⁻ at *m/z* 479.0835, with a retention time of 0.59 min, corresponding to a molecular formula of C₂₁H₂₀O₁₃. Its fragmentation (MS/MS) generated representative fragment ions at *m/z* 316.0289 ([M-162-H]⁻), indicating the loss of an O-hexoside. Compound 12, with ion [M-H]⁻ at *m/z* 317.0298, a retention time of 2.27 min, and a molecular formula of C₁₅H₁₀O₈, showed product ions characteristic of myricetin-type flavonoids at *m/z* 479.08, 316.02, and 271.02, as seen in compounds 8 and 12 (Candela et al., 2020). Compound 15 exhibited a structure similar to quercetin. Due to the absence of the -OH group at C-3, the product ions of luteolin-type flavonoids are all 16 Da smaller than those of quercetin-type flavonoids, showing characteristic intense ions at *m/z* 285.03, 151.05, and 133.01 (Śliwka-Kaszyńska et al., 2022).

4. Conclusion

The Dec and its fractions obtained from *C. gongylodes* leaves exhibited multi-target activity, demonstrating dual anti-inflammatory effects through the key inflammatory pathways COX and LOX and promoting the dissolution of CaOx crystals. The characterization of the Dec using UHPLC-ESI-HRMS revealed a high content of flavonoids, primarily glycosylated flavonoids. Bioguided isolation led to the identification of flavonoid compounds: rutin, eriodictyol 4'-O-glucoside, and isoquercetin, which were isolated for the first time from *C. gongylodes*. These isolated compounds confirmed their multi-action, acting as anti-inflammatory agents through COX and LOX pathways by inhibiting the synthesis of inflammatory mediators PGE2 and LTB4 and exhibiting anti-urolithiatic effects by dissolving CaOx stones. Both biological activities are highly desirable for the treatment of urolithiasis, a condition associated with kidney stone formation and inflammation. The isolated compounds support ethnopharmacological use against urolithiasis and inflammation, and identifying compounds with dual biological activity may present fewer side effects compared to conventional therapies. In this sense, the Dec of *C. gongylodes* leaves appears promising as a candidate for the development of new treatments for urolithiasis. Therefore, the extract of *C. gongylodes* deserve further studies to confirm *in vivo* anti-urolithiatic activity and the absence of toxicity. Additionally, standardized, seasonal metabolomic and chemometric studies of the extracts should also be conducted to assess the stability of the chemical

composition before clinical trials.

CRediT authorship contribution statement

Paula P.O. Salem: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Daniele O. Silva:** Writing – review & editing, Methodology, Investigation. **Paulo R.S. Silva:** Writing – review & editing, Methodology, Investigation. **Lara P. D.M. Costa:** Writing – review & editing, Methodology, Investigation. **Karen J. Nicácio:** Writing – review & editing, Methodology, Investigation. **Michael Murgu:** Writing – review & editing, Methodology, Investigation. **Ivo S. Caldas:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **Fernanda B. Leite:** Writing – review & editing, Methodology, Investigation. **Ana C.C. de Paula:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Danielle F. Dias:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Marisi G. Soares:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Daniela A. Chagas-Paula:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2024.118950>.

Data availability

Data will be made available on request.

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3 CONSIDERAÇÕES FINAIS

O Dec e suas frações obtidas das folhas de *Cissus gongylodes* apresentaram atividade multialvo, com efeitos anti-inflamatórios duais através das vias COX e LOX, além de promoverem a dissolução de cristais de CaOx. A caracterização do Dec por UHPLC-HRMS revelou alto teor de flavonoides, principalmente flavonoides glicosilados. O isolamento bioguiado permitiu identificar substâncias pertencente a classe dos flavonoides como rutina, eriodictiol 4'-O-glicosídeo e isoquercetina isolados pela primeira vez na espécie *C. gongylodes*. Essas substâncias demonstraram atividade anti-inflamatória por meio da inibição de mediadores inflamatórios (PGE2 e LTB4) e atividade antiurolítica, dissolvendo cálculos prevalentes de CaOx. Ambas as atividades são desejáveis para o tratamento da urolitíase, condição associada à formação de cálculos renais e a inflamação. A ação dual das substâncias isoladas reforça o uso etnofarmacológico contra a urolitíase e inflamação e sugerem menor risco de efeitos colaterais em comparação às terapias convencionais. Dessa forma, o Dec de *C. gongylodes* surge como um candidato promissor para novos tratamentos de urolitíase. No entanto, estudos adicionais são necessários para confirmar sua atividade antiurolítica *in vivo* e ausência de toxicidade, além de investigações metabolômicas e quimiométricas sazonais para avaliar a estabilidade da composição química antes de ensaios clínicos.

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APÊNDICE A – Colaborações científicas

As colaborações científicas realizadas pelo nosso grupo de pesquisa e com colaboradores, publicadas na literatura durante o período deste doutorado. O conhecimento, as técnicas e o trabalho em equipe desenvolvidos ao longo do doutorado também foram aplicados nesses artigos. Em resumo, a Tabela 1 apresenta o periódico acadêmico, ano de publicação, tema principal e título de cada pesquisa, além do DOI correspondente.

Tabela 1 – Colaborações científicas

Nº	Jornal Acadêmico	Ano	Colaboração	Título (DOI Hiperlink)
1	Photochemistry And Photobiology	2019	Isolamento de Tag C e a conversão em Tag F	<u>Fast and efficient method to obtain tagitinin f by photocyclization of tagitinin C. photochemistry and photobiology</u>
2	Phytochemical Analysis	2021	Preparo de amostras e LC-MS/MS	<u>In vivo anti-inflammatory activity of Fabaceae species extracts screened by a new ex vivo assay using human whole blood.</u>
3	Phytochemistry Letters	2021	Ensaio anti-inflamatório	<u>Chemistry of leaves, bark, and essential oils from <i>Ocotea diospyrifolia</i> and anti-inflammatory activity - Dual inhibition of edema and neutrophil recruitment.</u>
4	Bioorganic & Medicinal Chemistry Letters	2023	Purificação de substâncias	<u>Structure-activity relationship study of antitrypanosomal analogues of gibbilimbol B using multivariate analysis and computation-aided drug design</u>
5	Natural Product Research	2023	Preparo de amostras e ensaio anti-inflamatório	<u>Chemical annotation of the infusion of <i>Jungia floribunda</i> Less and its inhibitory potential on the elastase enzyme</u>
6	Patente	2023	Ensaio anti-inflamatório	Inflamatórios, os referidos compostos, composição

farmacêutica e uso dos referidos compostos e da composição farmacêutica

7	Fitoterapia	2024	Preparo de amostras e isolamento de substâncias	<u>Biological and metabolomics-guided isolation of tetrahydrofurofuran lignan from <i>Croton</i> spp. with antiproliferative activity against human melanoma cell line</u>
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Fonte: Autor (2024).

Nota: Cada publicação pode ser acessada clicando com o botão direito do mouse no hiperlink DOI em cada título.

As publicações científicas listadas na Tabela 1 refletem as colaborações realizadas em cada trabalho científico. Essas colaborações foram selecionadas para posterior discriminação, pois estão relacionadas à formação científica obtida durante o doutorado, contribuindo para o amadurecimento e desenvolvimento da presente tese. Assim, serão apresentados um breve resumo e as contribuições específicas em cada publicação.

Nº 1– Artigo de pesquisa – (2019): Fast and efficient method to obtain tagitinin F by photocyclization of tagitinin C photochemistry and photobiology

Há evidências na literatura de que a Tagitinina C (1) pode sofrer fotociclização para formar Tagitinina F (2). O composto 2 possui elevado potencial farmacológico, mas sua obtenção é dificultada, enquanto o composto 1 é facilmente extraído de *Tithonia diversifolia*, uma planta amplamente disseminada. Após avaliar diversas condições reacionais, foi identificada uma condição fotodependente que promove a ciclização de 1 em 2 em menos de 15 minutos. A ampliação da escala da reação de fotociclização de 1 para 2 demonstrou um rendimento de 100%. Além disso, a quantidade de 2 isolada de um extrato irradiado com UV foi oito vezes maior do que a isolada de um extrato não irradiado. Este estudo permitiu um melhor entendimento do processo de fotoconversão, além de estabelecer métodos eficazes para o isolamento e quantificação dessas substâncias, conhecidos por suas significativas atividades antitumorais, entre outras propriedades farmacológicas relevantes.

Principais contribuições: Auxílio na elaboração do manuscrito, extração do material vegetal e isolamento por HPLC-UV-DAD, e a fotoconversão da Tag C em F. Elaboração da versão final do manuscrito.

N° 2 – Artigo de pesquisa – (2021): *In vivo* anti-inflammatory activity of Fabaceae species extracts screened by a new *ex vivo* assay using human whole blood.

As plantas são uma fonte promissora na busca por novos candidatos a fármacos. A família Fabaceae inclui diversas espécies com potencial terapêutico, como atividade anti-inflamatória. Devido às limitações dos anti-inflamatórios atuais, há uma demanda por estruturas mais eficazes e métodos éticos e confiáveis para triagem de múltiplas amostras. Um método *ex vivo* para quantificação de prostaglandina E2 (PGE2) foi validado para rastrear a atividade anti-inflamatória em extratos de Fabaceae. O ensaio demonstrou que cinco de 47 espécies inibiram a produção de PGE2. Ensaio *in vivo* e perfis metabólicos de quatro extratos confirmaram seu potencial anti-inflamatório. O método se mostrou rápido, sensível e preciso, oferecendo uma abordagem confiável para triagem inicial antes de ensaios *in vivo* e contribuindo para o desenvolvimento de anti-inflamatórios mais eficazes.

Principais contribuições: Auxílio no preparo de amostras, execução do ensaio anti-inflamatório e a operação do LC-MS/MS. Elaboração da versão final do manuscrito.

N° 3 – Artigo de pesquisa – (2021): Chemistry of leaves, bark, and essential oils from *Ocotea diospyrifolia* and anti-inflammatory activity - Dual inhibition of edema and neutrophil recruitment.

Espécies do gênero *Ocotea* demonstraram atividade anti-inflamatória por meio de um mecanismo de ação promissor. Este estudo apresenta a primeira avaliação *in vivo* do potencial anti-inflamatório e do mecanismo de ação dos extratos e óleos essenciais das folhas e cascas de *Ocotea diospyrifolia*. Substâncias foram isoladas e identificadas nos extratos das folhas e cascas por UPLC-HRMS, enquanto os óleos essenciais foram caracterizados por GC-MS. Entre as nove substâncias isoladas, destaca-se a descoberta da diospirifolina, um alcaloide

aporfínico inédito no gênero *Ocotea*. Outras substâncias isoladas pela primeira vez neste gênero incluem mururina A e salsolina, e os alcaloides coclaurina, reticulina e isoboldina apresentaram-se como isômeros 6aR(-), diferindo dos comumente encontrados em outras espécies do gênero. A análise por UPLC-HRMS identificou classes dos flavonolignanas, flavonoides, ácidos clorogênicos e alcaloides, enquanto a análise por GC-MS revelou o α -felandreno como composto majoritário nos óleos essenciais, sem a presença do safrol, marcador químico típico da família Lauraceae. Algumas substâncias, como o ácido 5-cafeoilquínico, quercitrina, α -humuleno e (E)-cariofileno, possuem atividades anti-inflamatórias conhecidas. Tanto o extrato das folhas e sua fração acetato de etila quanto os óleos essenciais demonstraram atividade anti-inflamatória in vivo, inibindo edema e recrutamento de neutrófilos, o que sugere atuação em vias inflamatórias principais e potencial maior eficácia e segurança em relação aos anti-inflamatórios convencionais. A alta diversidade química e o mecanismo anti-inflamatório promissor de *O. diospyrifolia* reforçam seu potencial para investigações futuras.

Principais contribuições: Auxílio na elaboração do manuscrito, realização da extração do material vegetal, preparo de amostras, isolamento em HPLC-UV-DAD, execução dos ensaios anti-inflamatórios e análise estatística dos resultados. Elaboração da versão final do manuscrito.

N° 4 – Artigo de Pesquisa – (2023): Structure-activity relationship study of antitrypanosomal analogues of gibbilimbol B using multivariate analysis and computation-aided drug design

Gibbilimbol B e análogos, isolados da planta brasileira *Piper malacophyllum*, mostraram atividade contra formas tripomastigotas de *Trypanosoma cruzi*, com baixa toxicidade em células NCTC. Esses resultados motivaram a síntese de 24 análogos relacionados para investigar o potencial antitripanossômico e explorar relações estrutura-atividade. Inicialmente, 12 substâncias foram planejadas mantendo a cadeia lateral linear de gibbilimbol B e insaturação em C-4, mas com variação de grupos éster e amida e substituintes na posição *para* do anel aromático. Outras 12 substâncias foram sintetizadas com uma cadeia lateral ramificada contendo um grupo etil em C-2. Esses análogos demonstraram atividade promissora contra tripomastigotas ($EC_{50} < 20 \mu M$) e ausência de citotoxicidade em fibroblastos de

mamíferos (CC50 > 200 µM). Análises de estatística multivariada e aprendizado de máquina indicaram que a atividade biológica está relacionada à estrutura tridimensional e aos substituintes aromáticos, especialmente com grupos *t*-butil ou nitro na posição *para*. Esses grupos afetam propriedades como o estado topológico eletrônico, volumes de Van der Waals, área superficial e polarizabilidade, essenciais para a atividade contra *T. cruzi*.

Principais contribuições: Auxílio no isolamento e purificação de substâncias em placa cromatográfica de camada delgada preparativa. Elaboração da versão final do manuscrito.

Nº 5 – Artigo de pesquisa – (2023): Chemical annotation of the infusion of *Jungia floribunda* Less and its inhibitory potential on the elastase enzyme

A *Jungia floribunda* Less., um arbusto da família Asteraceae, tem suas folhas tradicionalmente utilizadas na medicina popular de diversos países da América do Sul como agente anti-inflamatório e hipoglicemiante. No presente estudo, uma infusão das folhas de *J. floribunda* foi preparada e sua composição química foi analisada por UHPLC-MS, combinada com análise de rede molecular, permitindo a anotação de flavonoides, lactonas sesquiterpênicas, cumarinas e derivados do ácido clorogênico. Adicionalmente, realizou-se um ensaio de atividade *in vitro* para inibição da elastase com a infusão, onde se observou inibição enzimática em concentrações entre 15 e 240 µg/mL, alcançando até 71% de inibição na concentração máxima avaliada. Considerando que plantas representam fontes promissoras para o desenvolvimento de novos fármacos, esses resultados corroboram o potencial da infusão de *J. floribunda* como uma fonte de substâncias bioativas com capacidade inibitória da elastase, reforçando também seus aspectos etnofarmacológicos.

Principais contribuições: Auxílio na elaboração do manuscrito, preparo de amostras, execução dos ensaios anti-inflamatórios. Elaboração da versão final do manuscrito.

Nº 6 – Patente – (2023): Processo de Obtenção de compostos anti-inflamatórios, os referidos compostos, composição farmacêutica e uso dos referidos compostos e da composição farmacêutica

A patente refere-se a um processo de produção de compostos anti-inflamatórios derivados do espilantol, uma N-alquilamida presente no jambu, planta comum no Brasil e conhecida por seu efeito anestésico. O derivado desenvolvido demonstrou maior eficácia e menor risco de efeitos colaterais em comparação aos corticosteroides, amplamente utilizados para reduzir inflamações, mas com sérias reações adversas quando usados cronicamente. A pesquisa identificou compostos com alta potência anti-inflamatória e sua composição farmacêutica. Esses compostos têm potencial aplicação na indústria farmacêutica para tratar doenças inflamatórias, como enxaqueca, reumatismo, osteoartrite, infecções e lesões ortopédicas. Além disso, seu mecanismo de ação difere dos fármacos convencionais, sugerindo um perfil de segurança aprimorado.

Principais contribuições: Auxílio na elaboração do manuscrito, execução dos ensaios anti-inflamatórios e na análise estatística dos resultados. Elaboração da versão final do manuscrito.

Nº 7 – Artigo de pesquisa – (2024): Biological and metabolomics-guided isolation of tetrahydrofurofuran lignan from *Croton* spp. with antiproliferative activity against human melanoma cell line

O gênero *Croton* (Euphorbiaceae) é reconhecido como fonte promissora de substâncias bioativas com atividade antiproliferativa, embora o conhecimento sobre a composição e atividade de *Croton floribundus*, *Croton echinocarpus* e *Croton zehntneri* seja ainda limitado. Este estudo visou investigar a atividade antiproliferativa dessas espécies em células tumorais de mama, pulmão e melanoma, além de fibroblastos humanos. Estratégias metabolômicas foram aplicadas usando cromatografia líquida de ultra-desempenho acoplada à espectrometria de massas de alta resolução e análise estatística multivariada para identificar o principal composto ativo. O extrato das folhas de *C. floribundus* apresentou a maior atividade antiproliferativa, com um valor de IC₅₀ inferior ao do fármaco temozolomida na linhagem de melanoma SK-MEL-147 e em outras linhagens de melanoma testadas. Quatro lignanas tetrahidrofurofurânicas foram isoladas pela primeira vez da fração mais promissora desse extrato, sendo a epi-yangambina identificada como o principal composto antiproliferativo contra SK-MEL-147. A epi-yangambina mostrou-se seletiva para essa linhagem celular (IC₅₀ =

13,09 $\mu\text{g/mL}$; índice de seletividade = 3,82), superando a temozolomida (IC_{50} = 121,50 $\mu\text{g/mL}$) ao inibir a progressão do ciclo celular em G2/M. Essa descoberta é relevante devido à alta resistência das células de melanoma a tratamentos convencionais, posicionando a epi-yangambina como um potencial protótipo para futuras pesquisas antiproliferativas.

Principais contribuições: Auxílio na elaboração do manuscrito, preparo de amostras e isolamento em HPLC-UV-DAD. Elaboração da versão final do manuscrito

A colaboração em artigos científicos durante o doutorado foi fundamental para o desenvolvimento da formação científica, proporcionando experiência prática e técnica em diversas etapas do processo de pesquisa. Contribuições como a extração do material vegetal, preparo de amostras, isolamento por HPLC-UV-DAD, e realização de ensaios anti-inflamatórios reforçaram habilidades laboratoriais essenciais, enquanto a análise estatística dos resultados aprimorou a capacidade de interpretação de dados. Participar da elaboração e finalização de manuscritos também foi crucial para o aprimoramento de competências na redação científica, essenciais para a comunicação de resultados complexos de forma clara e precisa. Esses aspectos não só enriqueceram a formação, como também ampliaram o conhecimento técnico e a capacidade de colaboração em equipe, essenciais para o desenvolvimento da tese e para futuras pesquisas na área.

APÊNDICE B – Material suplementar

Supplementary material

Bioguided isolation of anti-inflammatory and anti-urolithiatic active compounds from the decoction of *Cissus gongylodes* leaves

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Table S1. Mobile phases used in solid-phase extraction and the corresponding aliquots obtained.

Mobile Phase	(v/v)	FoAc aliquots	FoHi aliquots
H ₂ O	Only	Ac-1	Hi-1
H ₂ O/MeOH	9:1	Ac-2	Hi-2
H ₂ O/MeOH	8:2	Ac-3	Hi-3
H ₂ O/MeOH	7:3	Ac-4	Hi-4
H ₂ O/MeOH	6:4	Ac-5	Hi-5
H ₂ O/MeOH	1:1	Ac-6	Hi-6
H ₂ O/MeOH	3:7	Ac-7	Hi-7
H ₂ O/MeOH	2:8	Ac-8	Hi-8
H ₂ O/MeOH	1:9	Ac-9	Hi-9
MeOH	Only	Ac-10	Hi-10

Table S2. MRM Transitions of mediators and internal standards.

Compound	Transition (<i>m/z</i>)	Q1	CE	Q3
Limaprost	379.20 → 299.30	14	21	21
PGE2	351.20 → 315,25	14	15	21
LTB4	335.20 → 195,20	11	18	19

Table S3. IC₅₀ inhibitory concentration of the anti-urolithiatic activity of the active fractions obtained from the leaves of Dec.

Samples IDs*	IC ₅₀ (mg/mL)
Cys	0.2351
Cit	0.2323
Dec	0.2200
FoAc	0.2145
FoHi	0.1582

Legend: *Cystone® (Cys), sodium citrate (Cit), *C. gongyloides* decoction (Dec), ethyl acetate fraction (FoAc), and hydroalcoholic fraction (FoHi).

Table S4. Percentage inhibition of PGE2 and LTB4 concentrations by compounds isolated from *C. gongylodes*.

Samples IDs	Doses μg/mL	% PGE2 inhibition	[PGE2] ng/mL	% LTB4 inhibition	[LTB4] ng/mL
Neg		-	27.92 ± 1.52	-	28.19 ± 0.56
Dex	0.5	37.64	17.41 ± 4.61	39.92	16.93 ± 1.07
	1	57.78	11.78 ± 2.80	69.88	8.49 ± 0.91
	2	67.82	8.98 ± 0.96	71.19	8.12 ± 0.74
	4	71.69	7.90 ± 0.88	75.25	6.98 ± 0.62
	10	73.45	7.41 ± 0.42	83.98	4.51 ± 0.56
Ind	0.5	49.06	14.22 ± 0.75	ND	ND
	1	62.19	10.55 ± 0.77	ND	ND
	2	71.02	8.09 ± 0.57	ND	ND
	4	90.22	4.19 ± 0.79	ND	ND
	10	93.56	0.33 ± 0.19	ND	ND
P1	0.5	30.13	19.51 ± 3.11	43.04	16.06 ± 1.51
	1	42.42	16.08 ± 0.24	57.03	12.11 ± 0.86
	2	46.97	14.80 ± 0.99	61.26	10.92 ± 0.36
	4	57.87	11.76 ± 0.91	62.83	10.48 ± 0.20
	10	67.58	9.05 ± 1.63	67.67	9.11 ± 0.95
P2	0.5	-17.83	32.90 ± 2.11	36.34	17.95 ± 2.18

	1	2.45	27.24 ± 1.82	54.89	12.71 ± 1.03
	2	20.27	22.26 ± 2.16	64.73	9.94 ± 0.53
	4	40.17	16.71 ± 1.63	70.65	8.27 ± 0.60
	10	50.03	13.95 ± 1.76	77.00	6.48 ± 0.71
P3	0.5	36.83	17.64 ± 2.29	61.05	10.98 ± 0.67
	1	46.92	14.82 ± 0.71	69.01	8.73 ± 0.39
	2	58.47	11.59 ± 0.75	71.68	7.98 ± 0.23
	4	63.25	10.26 ± 0.65	78.84	5.96 ± 0.75
	10	66.94	9.23 ± 0.58	83.41	4.68 ± 0.91

Legend: Dex: Corticosteroid (Steroidal Anti-Inflammatory Drug), Ind: Non-Steroidal Anti-Inflammatory Drug; P1: Rutin; P2: Eriodictyol 4'-*O*-glucoside, P3: Isoquercetin; ND: Not determined due to lack of activity in the LOX pathway.

Table S5. IC₅₀ inhibitory concentration of the anti-inflammatory activity against PGE2 and LTB4 of the isolated substances from *C. gongyloides*.

Samples IDs *	IC ₅₀ (µg/mL) PGE2	IC ₅₀ (µg/mL) LTB4
Dex	0.7769	0.5705
Ind	0.5600	-
P1	2.263	0.6854
P2	7.988	0.9801
P3	1.331	0.1482

Legend: *Reference drugs, dexamethasone (Dex) and indomethacin (Ind), the isolated substances from the active fractions of the decoction of *C. gongyloides* rutin (P1), eriodictyol 4'-*O*-glucoside (P2) and isoquercetin (P3).

Table S6. IC₅₀ inhibitory concentration of the anti-urolithiatic activity of isolated substances from *C. gongylodes*.

Samples IDs*	IC ₅₀ (mg/mL)
Cys	0.5475
Cit	0.2245
P1	0.3601
P2	0.1759

Legend: *Polyherbal Cystone® (Cys), sodium citrate (Cit), and the isolated substances from the active fractions of the decoction of *C. gongylodes*, rutin (P1), and eriodictyol 4'-*O*-glucoside (P2).

Table S7. NMR spectroscopic data for rutin (P1).

Position	Experimental data ^a		Literature data ^b	
	¹ H	¹³ C	¹ H	¹³ C
1'	–	121.9	–	122.05
2'	7.66 (d, 1H, J _m = 1.6 Hz)	118.1	7.540 (brs, 1H)	116.73
3'	–	144.5	–	145.20
4'	–	148.3	–	148.86
5'	6.86 (d, 1H, J _o = 8.5 Hz)	116.5	6.841 – 6.858 (d, 1H, J _o = 8.5 Hz)	115.69
6'	7.63 (dd, 1H, J _o = 8.5 Hz; J _m = 1.6 Hz)	124.0	7.555 – 7.559 (d, 1H, J _m = 2.4 Hz)	121.64
2	–	157.7	–	156.88
3	–	134.1	–	133.76
4	–	178.1	–	177.83
5	–	161.9	–	161.68
6	6.21 (d, 1H, J _m = 1.7 Hz)	100.5	6.20 – 6.204 (d, 1H, J _m = 2 Hz)	99.13
7	–	164.7	–	164.52
8	6.40 (d, 1H, J _m = 1.7 Hz)	95.1	6.392 – 6.396 (d, 1H, J _m = 2 Hz)	94.04
9	–	157.1	–	157.07
10	–	104.1	–	104.43
1''	5.11 (d, 1H, J _{aa} = 7.35 Hz)	105.1	5.352 – 5.359 (d, 1H, J _{aa} = 7.5 Hz)	101.64
2''	3.27 (m)	74.4	3.221 – 3.303 (m, 4H)	74.53
3''	3.42 (dd, 1H, J _{ee} = 2.2 Hz; J _{aa} = 8.4 Hz)	76.3	n.o.	76.36

4''	3.38 (m)	68.9	n.o.	68.70
5''	3.30 (m)	77.6	n.o.	76.90
6''	3.36 (m); 3.79 (d, 1H, J _{aa} = 10.4 Hz)	69.0	3.221 – 3.303 (m, 1H); 3.703 – 3.724 (d, 1H, J _{aa} = 10.5 Hz)	67.45
1'''	4.51 (brs, 1H)	102.8	4.353 (brs, 1H)	101.20
2'''	3.27 (m, 1H)	71.9	3.221 – 3.303 (m, 1H)	71.02
3'''	3.62 (d, 1H, J _{ee} = 2.3 Hz)	72.6	3.060 – 3.078	70.46
4'''	3.30 (m)	74.4	3.221 – 3.203	70.83
5'''	3.53 (dd, 1H, J _{aa} = 9.4 Hz; J _{ae} = 3.2 Hz)	72.7	3.060 – 3.078	72.30
6'''	1.11 (d, 3H, J = 6.1 Hz)	18.4	0.992 – 1.004 (d, 3H, J = 6 Hz)	18.19

^a Compound P1 (300 MHz to ¹H e 75 MHz to ¹³C em MeOD);
^b Srinivasan et al., 2015 (500 MHz, DMSO).

Table S8. Spectral correlations of rutin (P1).

Position	¹³ C	HSQC	HMBC	COSY
1'	121.9	–	6.86; 7.66	–
2'	118.1	7.66	–	–
3'	144.5	–	6.86; 7.66	–
4'	148.3	–	6.86; 7.63; 7.66	–
5'	116.5	6.86	7.63	–
6'	124.0	7.63	–	6.86
2	157.7	–	–	–
3	134.1	–	5.11	–
4	178.1	–	–	–
5	161.9	–	6.21	–
6	100.5	6.21	6.40	6.40
7	164.7	–	6.21; 6.40	–
8	95.1	6.40	6.21	6.21
9	157.1	–	6.40	–
10	104.1	–	6.21; 6.40	–
1''	105.1	5.11	–	–
2''	74.4	3.27	3.38	–

3''	76.3	3.42	–	–
4''	68.9	3.38	–	–
5''	77.6	3.30	–	–
6''	69.0	3.36; 3.79	–	3.42
1'''	102.8	4.51	69.0	–
2'''	71.9	3.27	–	–
3'''	72.6	3.62	–	3.53
4'''	74.4	3.30	–	3.53
5'''	72.7	3.53	–	3.30
6'''	18.4	1.11	–	3.53

Table S9. NMR spectroscopic data for eriodictyol 4'-*O*-glucoside (P2).

Position	Experimental data ^a		Literature data ^b	
	¹ H	¹³ C	¹ H	¹³ C
1'	–	122.0	–	133.2
2'	7.67 (d, 1H, J _m = 2.16 Hz)	118.1	6.95 (d, 1H, J _m = 2.04 Hz)	114.4
3'	–	145.0	–	146.7
4'	–	149.3	–	145.4
5'	6.89 (d, 1H, J _o = 8.46 Hz)	123.8	7.12 (d, 1H, J _o = 8.22 Hz)	116.6
6'	7.61 (dd, 1H, J _o = 8.55 Hz; J _m = 2.28 Hz)	116.5	6.85 (dd, 1H, J _m = 2.4 Hz; J _o = 8.22 Hz)	117.7
2	4.39 (dd, 1H, J = 4.62 Hz; J = 7.26 Hz)	69.4	5.44 (dd, 1H, J = 3.42 Hz; J = 12.36 Hz)	78.1
3	2.57 (dd, 1H, J = 4.65 Hz; J = 15.57 Hz); 2.51 (dd, 1H, J = 7.23 Hz; J = 15.48 Hz)	41.7	2.71 (dd, 1H, J = 3.42 Hz; J = 17.16); 3.21 (dd, 1H, J = 12.36 Hz; J = 17.16 Hz)	42.0
4	–	175.4	–	196.1
5	–	159.7	–	163.4
6	6.24 (d, 1H, J _m = 1.95 Hz)	100.5	5.80 (d, 1H, J _m = 2.10 Hz)	95.8
7	–	164.8	–	166.6
8	6.44 (d, 1H, J _m = 1.95 Hz)	95.4	5.89 (d, 1H, J _m = 2.10 Hz)	95.0
9	–	157.5	–	162.7

10	–	104.5	–	101.8
1''	5.19 (d, 1H, J _{aa} = 7.65Hz)	104.8	4.69 (d, 1H, J _{aa} = 7.56 Hz)	102.2
2''	3.41 (m)	76.0	3.46 (m)	73.3
3''	3.42 (m)	78.3	3.46 (m)	75.8
4''	3.42 (m)	71.5	3.40 (m)	69.8
5''	3.44 (m)	78.4	3.40 (m)	77.2
6''	3.63 (brs, 1H); 4.22 (brs, 1H)	64.8	3.71 (dd, 2H, J _{ae} = 3.42; J _{aa} = 11.7 Hz)	60.7

^a 300 MHz to ¹H e 75 MHz to ¹³C em MeOD.

^b Liu et al., 2014 (600 MHz, DMSO-d₆).

Table S10. Spectral correlations for eriodictyol 4'-O-glucoside (P2)

Position	¹³ C	HSQC	HMBC	COSY
1'	122.0	–	6.89	–
2'	118.1	7.67	–	–
3'	145.0	–	6.89	–
4'	149.3	–	6.89	–
5'	123.8	6.89	–	–
6'	116.5	7.61	–	6.89
2	69.4	4.39	–	2.51; 2.57
3	41.7	2.57; 2.51	4.39	–
4	175.4	–	2.51; 2.57; 4.39	–
5	159.7	–	6.24	–
6	100.5	6.24	–	6.44
7	164.8	–	6.24; 6.44	–
8	95.4	6.44	–	6.24
9	157.5	–	6.44	–
10	104.5	–	–	–

1''	104.8	5.19	–	–
2''	76.0	3.41	–	–
3''	78.3	3.42	–	–
4''	71.5	3.42	–	–
5''	78.4	3.44	–	–
6''	64.8	3.63; 4.22	–	–

Table S11. NMR spectroscopic data for isoquercetin (P3).

Position	Experimental data ^a		Literature data ^b	
	¹ H	¹³ C	¹ H	¹³ C
1'	–	121.7	–	123.2
2'	7.70 (d, 1H, J _m = 2.0 Hz)	116.2	7.71 (1H, br s)	116.2
3'	–	144.7	–	146.1
4'	–	148.4	–	150.0
5'	6.86 (d, 1H, J _o = 8.4 Hz)	114.6	6.88 (d, 1H, J = 8.3 Hz)	117.7
6'	7.58 (dd, 1H, J _o = 8.4 Hz; J _m = 2.0 Hz)	121.8	7.59 (dd, 1H, J = 8.3 Hz, 1.7 Hz)	123.3
2	–	157.3	–	158.6
3	–	134.2	–	135.8
4	–	178.2	–	179.6
5	–	165.5	–	163.2
6	6.39 (d, 1H, J _m = 1.8 Hz)	93.2	6.21 (1H, br s)	100.0
7	–	164.8	–	166.2
8	6.19 (d, 1H, J _m = 1.8 Hz)	98.5	6.40 (1H, brs)	94.9
9	–	157.0	–	159.2
10	–	104.4	–	105.8
1''	5.26 (d, 1H, J _{aa} = 7.29 Hz)	102.9	5.25 (d, 1H, J = 7.7 Hz)	104.4
2''	3.45 (1H, br s)	74.4	3.49 (brt, 1H, J = 8.4 Hz)	75.9
3''	3.41 (1H, br s)	76.9	3.43 (br t, 1H, J = 8.9 Hz)	78.2

4''	3.47 (1H, brs)	74.5	3.35 (br t, 1H, J = 9.2 Hz)	71.4
5''	3.38 (brd, 1H, J = 5.10 Hz)	76.7	3.22 (ddd, 1H, J = 9.2 Hz, 5.1 Hz, 2.0 Hz)	78.5
6''	3.58 (dd, 1H, J = 11.7 Hz; 5.10 Hz); 3.71 (dd, 1H, J = 11.7 Hz; 2.43 Hz)	61.2	3.58 (dd, 1H, J = 11.8 Hz, J = 5.1 Hz); 3.71 (dd, 1H, J = 11.8 Hz, J = 2.0 Hz)	62.7

^a 300 MHz to ¹H e 75 MHz to ¹³C em MeOD.

^b Fitrah et al., 2024 (500 MHz, MeOD).

Table S12. Spectral correlations of isoquercetin (P3).

Position	¹³ C	HSQC	HMBC	COSY
1'	121.7	–	6.86; 7.70	–
2'	116.2	7.70	–	–
3'	144.7	–	6.86; 7.70	–
4'	148.4	–	6.86; 7.58; 7.70	–
5'	114.6	6.86	–	–
6'	121.8	7.58	7.70	6.86
2	157.3	–	7.70	–
3	134.2	–	5.26	–
4	178.2	–	–	–
5	165.5	–	6.19	–
6	93.2	6.39	6.19	6.19
7	164.8	–	6.19; 6.39	–
8	98.5	6.19	6.39	6.39
9	157.0	–	6.39	–
10	104.4	–	6.19	–
1''	102.9	5.26	–	3.45
2''	74.4	3.45	3.41	–
3''	76.9	3.41	3.47	–

4"	74.5	3.47	3.41	—
5"	76.7	3.38	—	—
6"	61.2	3.58; 3.71	—	—

Table S13. Annotation of *C. gongylodes* leaf Dec by UPLC/ESI-QTOF-MS in positive mode.

Dec	Rt	Compounds	MF	Exact Mass	Adduct	<i>m/z</i>	Error (ppm)	MS/MS MassBank PubChem	MS/MS fragments	Database
8.7E ³	2.47	Rutin (1)	C ₂₇ H ₃₀ O ₁₆	610.1533	[M+H] ⁺	611.1612	-0.1391	303.049; 127.037; 097.029;	303.0483; 127.0339; 097.0288;	<i>Cissus</i> genus
4.7E ³	2.59	Isoquercetin (2)	C ₂₁ H ₂₀ O ₁₂	464.0954	[M+H] ⁺	465.1025	-1.8876	303.048; 305.031; 229.048; 257.007; 273.031;	303.0498; 305.0559; 229.0490; 257.0447; 273.0395;	<i>Cissus</i> genus
1.7E ³	2.72	Quercetin (3)	C ₁₅ H ₁₀ O ₇	302.0426	[M+H] ⁺	303.0486	-3.1541	153.017; 137.022; 229.050; 201.053;	153.0172; 137.0229; 229.0482; 155.0480;	<i>Cissus</i> genus

7.8E ¹	2.75	Quercetin- <i>O</i> - Pentose (4)	C ₂₀ H ₁₈ O ₁₁	434.0849	[M+H] ⁺	435.0910	-2.7906	303.048; 356.965; 229.048; 153.017;	303.0385; 229.0427; 153.0172; 356.9632;	<i>Cissus</i> genus
3.5E ²	2.81	Kaempferol <i>O</i> -hexoside (5)	C ₂₁ H ₂₀ O ₁₁	448.1005	[M+H] ⁺	449.1068	-2.2316	287.056; 258.059; 289.060; 127.039;	287.0535; 258.0593; 289.0634; 127.0524;	<i>Cissus</i> genus
6.9E ²	2.81	Kaempferol (6)	C ₁₅ H ₁₀ O ₆	286.0477	[M+H] ⁺	287.0534	-5.7264	287.055; 241.049; 213.054; 165.018;	287.0535; 241.0480; 213.0490; 165.0164;	<i>Cissus</i> genus
8.9E ¹	2.83	Apigenin C- hexose (7)	C ₂₁ H ₂₀ O ₁₀	432.1056	[M+H] ⁺	433.1115	-4.0425	271.060; 433.113; 153.018; 187.056;	271.0577; 433.1139; 153.0147; 187.0569;	<i>Cissus</i> genus

Table S14. Annotation of *C. gongylodes* leaf Dec by UPLC/ESI-QTOF-MS in negative mode

Dec	Rt	Compounds	MF	Exact Mass	Adduct	<i>m/z</i>	Error (ppm)	MS/MS MassBank PubChem	MS/MS fragments	Database
1.5E ³	0.59	Myricetin <i>O</i> -hexoside (8)	C ₂₁ H ₂₀ O ₁₃	480.0903	[M-H] ⁻	479.0835	2.1023	316.022; 479.082; 271.025; 287.020; 317.029;	316.0233; 479.0836; 271,0229; 287.0189; 317.0289;	<i>Cissus</i> genus
2.7E ²	0.80	Gallic acid (9)	C ₇ H ₆ O ₅	170.0215	[M-H] ⁻	169.0125	-3.0777	125.023; 169.013; 170.016; 124.014;	125.0221; 169.0125; 170.0069; 124.0144;	<i>Cissus</i> genus
9.2E ²	1.43	Methyl gallate (10)	C ₈ H ₈ O ₅	184.0371	[M-H] ⁻	183.0287	-3.1154	124.020; 183.035; 123.009;	124.0166; 183.0355; 123.0010;	<i>Cissus</i> genus
3.2E ²	1.61	4-Hydroxybenzoic acid (11)	C ₇ H ₆ O ₃	138.0316	[M-H] ⁻	137.0235	-2.1303	137.023; 094.054; 093.035;	137.0232; 094.0364; 093.0331;	<i>Cissus</i> genus

1.8E ¹	2.27	Myricetin (12)	C ₁₅ H ₁₀ O ₈	318.0375	[M-H] ⁻	317.0298	-1.1548	315.013; 316.016; 317.027; 287.018; 178.999;	315.0121; 316.0233; 317.0289; 287.0195; 178.9990;	<i>Cissus</i> genus
4.0E ³	2.47	Rutin (1)	C ₂₇ H ₃₀ O ₁₆	610.1533	[M-H] ⁻	609.1445	-1.6141	301.033; 271.023; 255.022; 609.144; 178.998;	301.0324; 271.0229; 255.0303; 609.1445; 178.9980;	<i>Cissus</i> genus
1.8E ²	2.48	<i>p</i> -coumaric acid (13)	C ₉ H ₈ O ₃	164.0579	[M-H] ⁻	163.0389	-3.3168	178.998; 106.347; 133.034; 134;036; 160.842;	178.0198; 106.0396; 133.0282; 134.0377; 160.8415;	<i>Cissus</i> genus
2.5E ³	2.59	Isoquercetin (2)	C ₂₁ H ₂₀ O ₁₂	464.0954	[M-H] ⁻	463.0879	0.6979	301.038; 255.032; 151.004; 463.097; 178.998;	301.0324; 255.0303; 151.0013; 463.0888; 178.9981;	<i>Cissus</i> genus
3.9E ²	2.72	Quercetin (3)	C ₁₅ H ₁₀ O ₇	302.0426	[M-H] ⁻	301.0351	1.1500	121.029; 107.013; 151.003; 109.028; 178.998;	121.0269; 107.0023; 151.0013; 109.0284; 178.9981;	<i>Cissus</i> genus

2.6E ¹	2.75	Quercetin <i>O</i> - pentose (4)	C ₂₀ H ₁₈ O ₁₁	434.0849	[M-H] ⁻	433.0783	2.9685	300.027; 301.034; 433.077; 271.024; 151.002; 178.998;	300.0278; 301.0324; 433.0783; 271.0229; 151.0038; 178.9980;	<i>Cissus</i> genus
7.2E ¹	2.81	Kaempferol <i>O</i> -hexoside (5)	C ₂₁ H ₂₀ O ₁₁	448.1005	[M-H] ⁻	447.0937	2.3176	447.093; 285.039; 284.032; 257.055; 163.012;	447.0911; 285.0385; 284.0333; 257.0554; 163.0121;	<i>Cissus</i> genus
1.2E ¹	3.51	Luteolin (14)	C ₁₅ H ₁₀ O ₆	286.0477	[M-H] ⁻	285.0404	1.9716	133.027; 132.019; 151.001;	133.0190; 132.0182; 151.0013;	<i>Cissus</i> genus

Fig. S1. The molecular mass of rutin was confirmed by QTOF-MS in positive mode, yielding $[M+H]^+ = 611.1614$ Da, with an error of 1.14 ppm and a molecular formula of $C_{27}H_{30}O_{16}$.

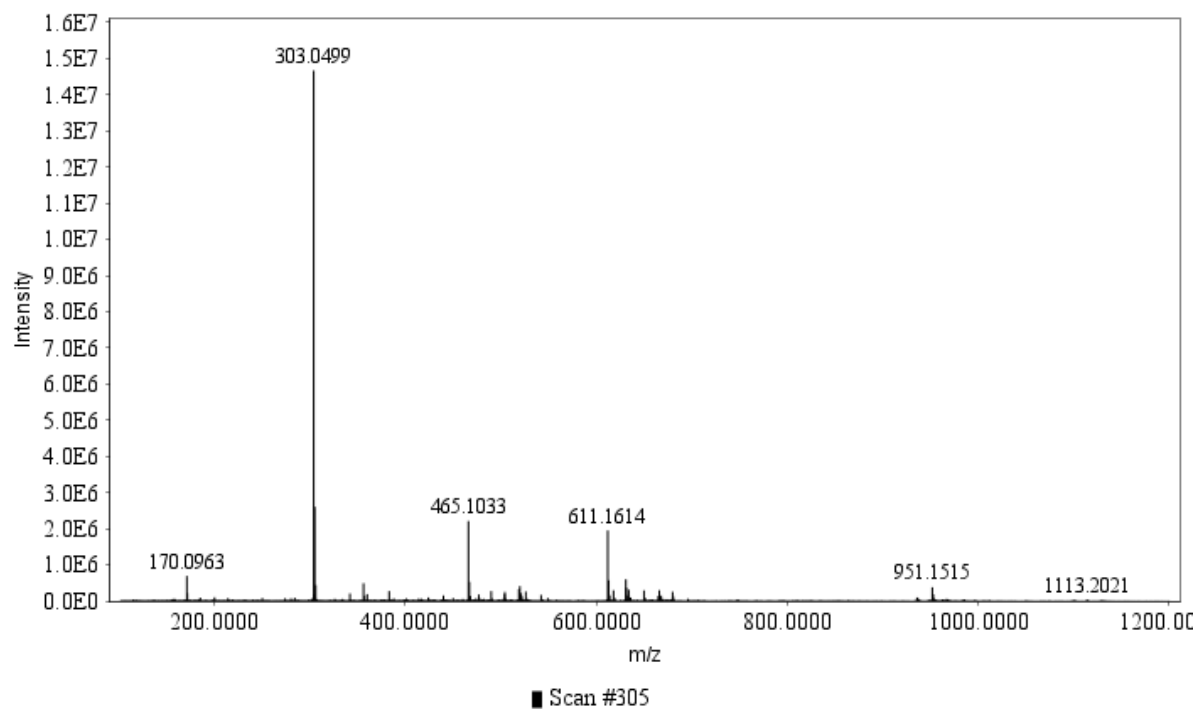


Fig. S2. The molecular mass of eriodictyol 4'-*O*-glucoside was confirmed by QTOF-MS in positive mode, yielding $[M+H]^+ = 451.1248$ Da, with an error of 2.88 ppm and a molecular formula of $C_{21}H_{22}O_{11}$.

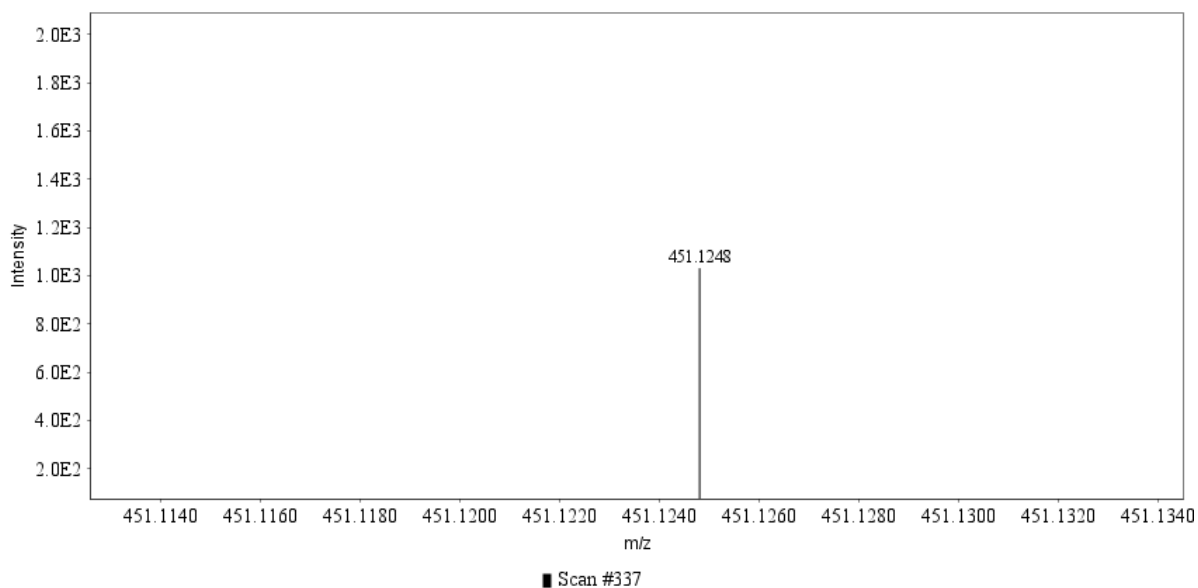


Fig. S3. The molecular mass of isoquercetin was confirmed by QTOF-MS in positive mode, yielding $[M+H]^+ = 465.1027$ Da, with an error of 0.21 ppm and a molecular formula of $C_{21}H_{20}O_{12}$.

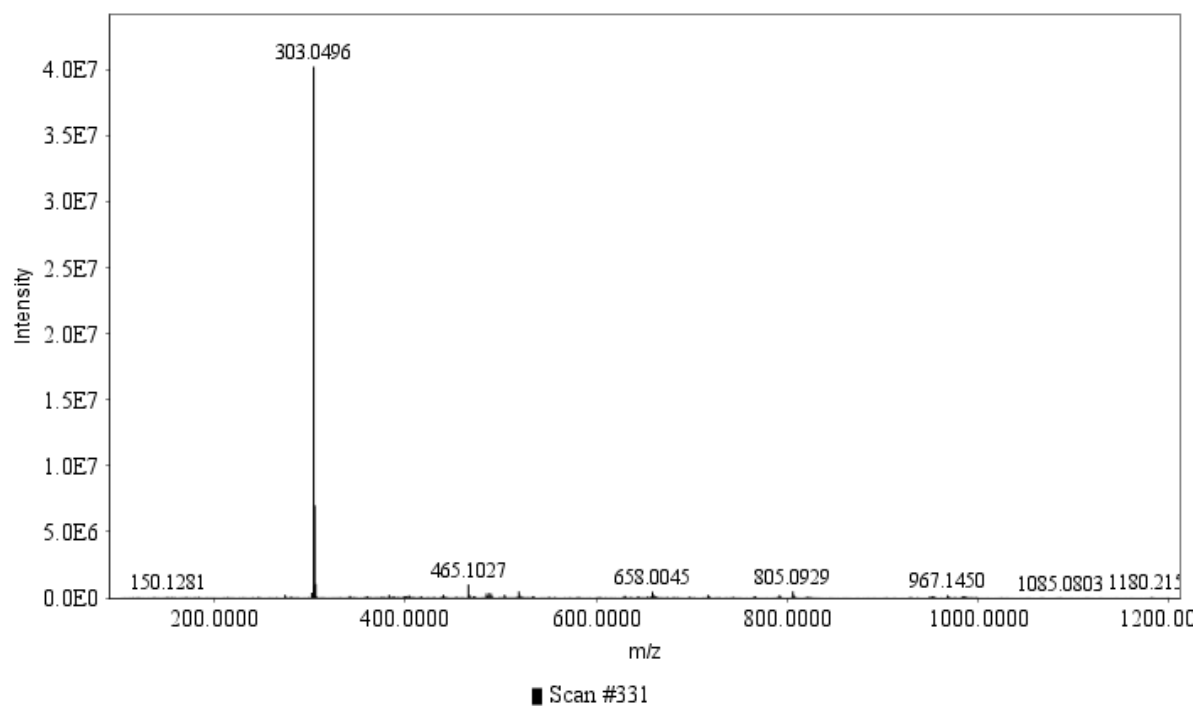


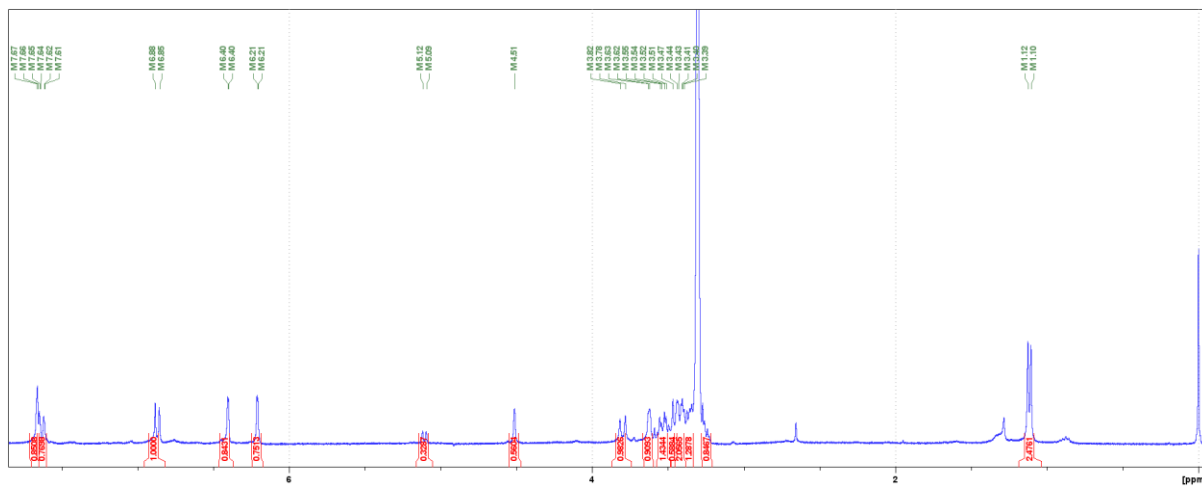
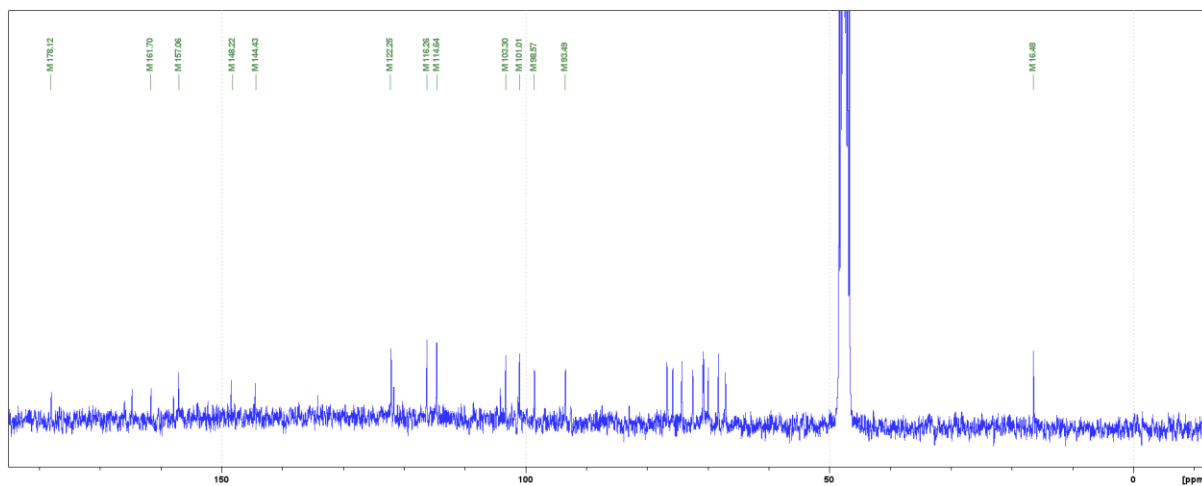
Fig. S4. ^1H NMR spectrum of rutin (Compound P1) recorded at 300 MHz in MeOD.Fig. S5. ^{13}C NMR spectrum of rutin (Compound P1) recorded at 300 MHz in MeOD.

Fig. S6. COSY spectrum of rutin (Compound P1) recorded at 300 MHz in MeOD.

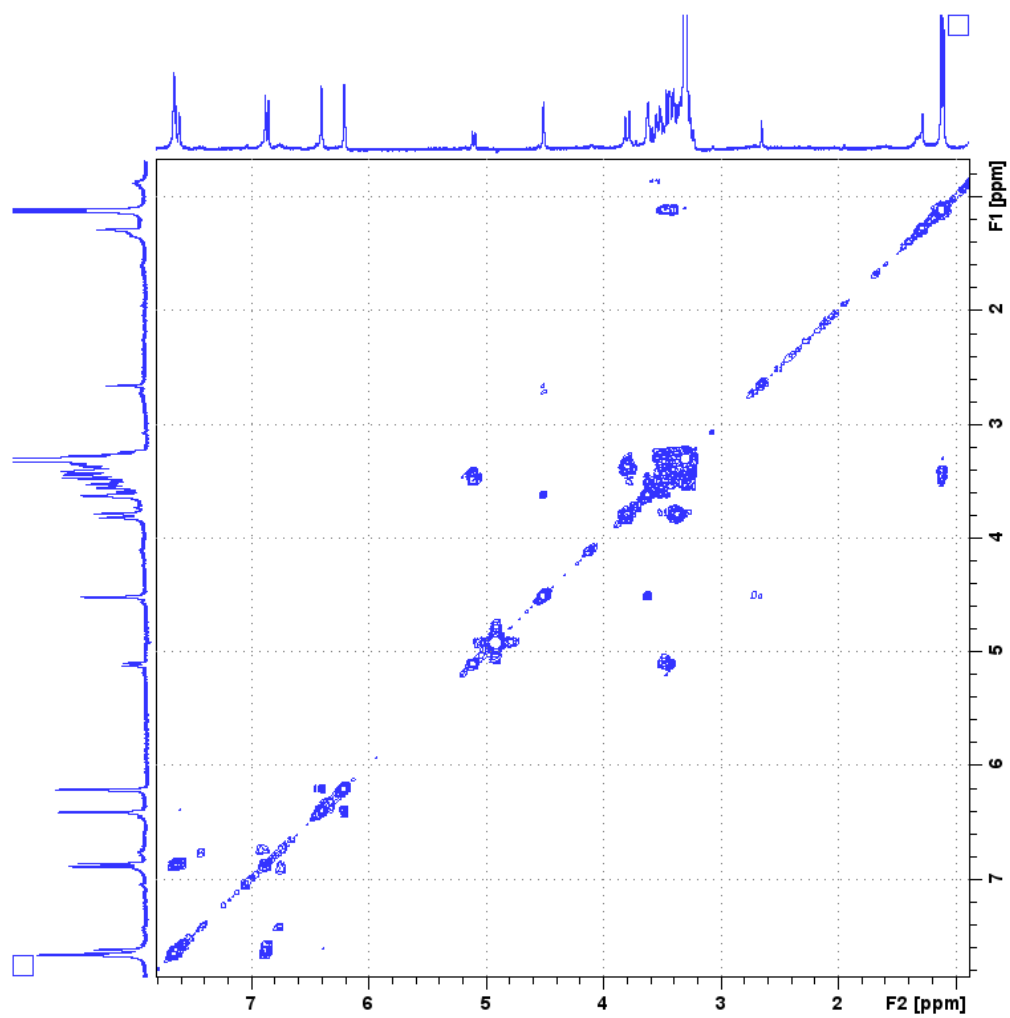


Fig. S7. HSQC spectrum of rutin (Compound P1) recorded at 300 MHz in MeOD.

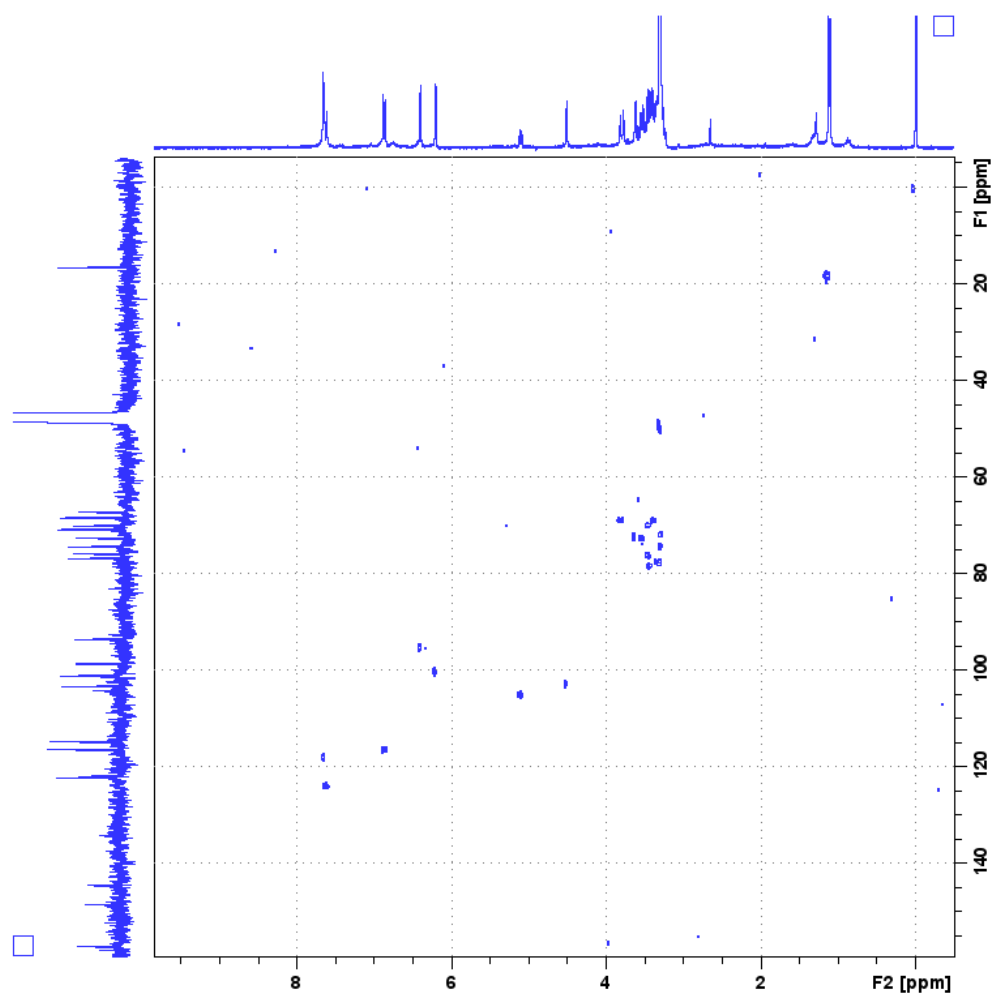


Fig. S8. HMBC spectrum of rutin (Compound P1) recorded at 300 MHz in MeOD.

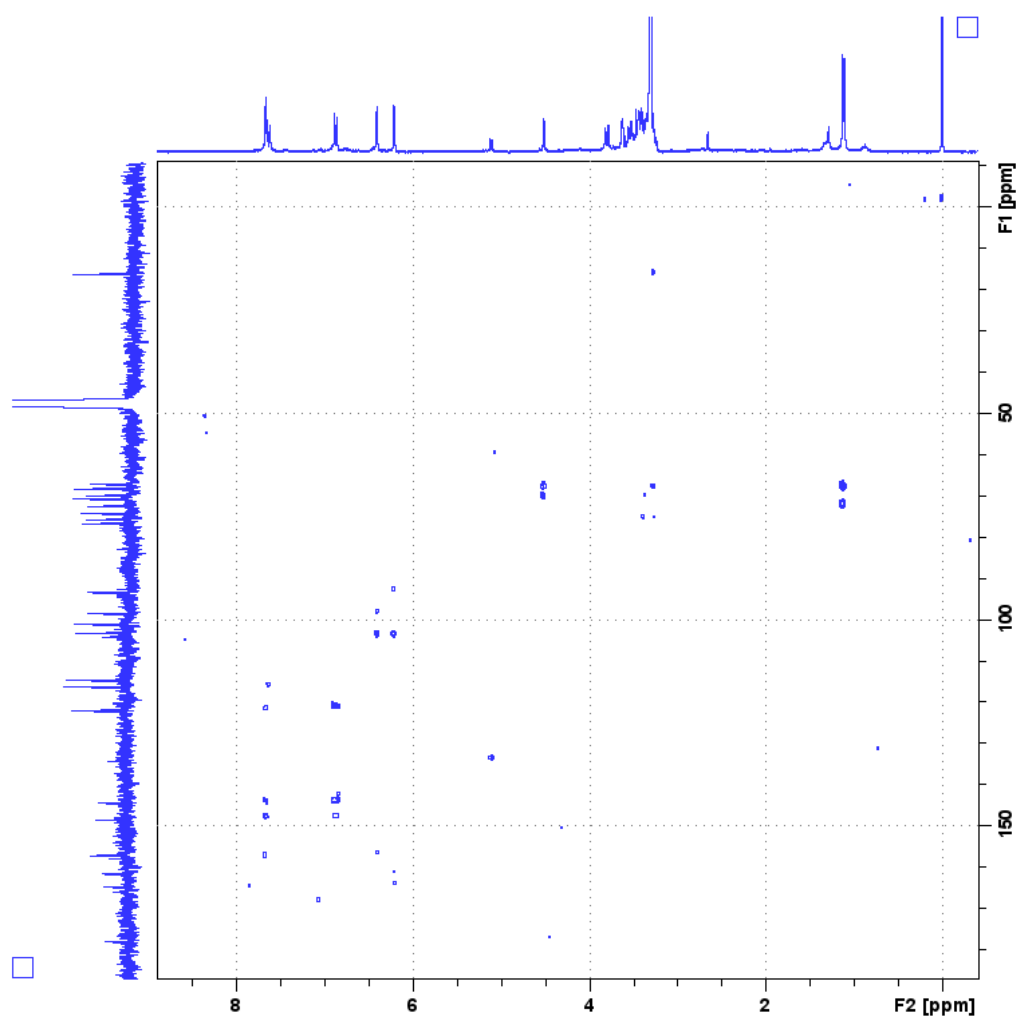


Fig. S9. Main HMBC correlations for rutin (Compound P1).

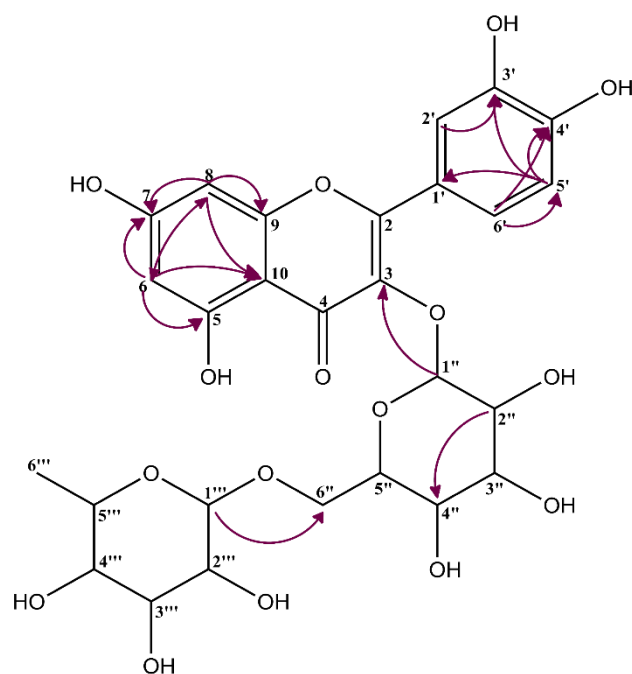


Fig. S10. ^1H NMR spectrum of eriodictyol 4'-*O*-glucoside (Compound P2) recorded at 300 MHz in MeOD.

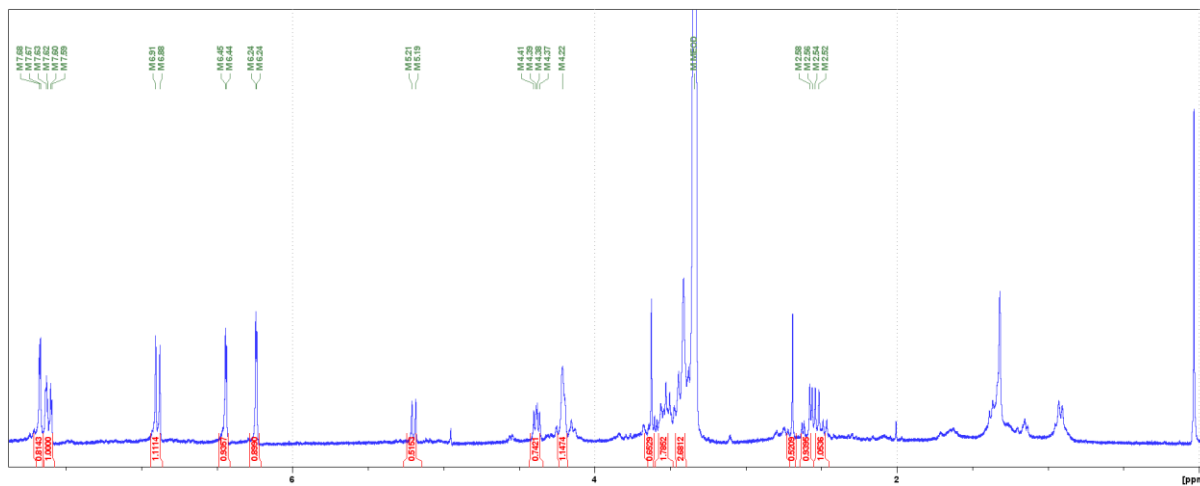


Fig. S11. ^{13}C NMR spectrum of eriodictyol 4'-*O*-glucoside (Compound P2) recorded at 300 MHz in MeOD.

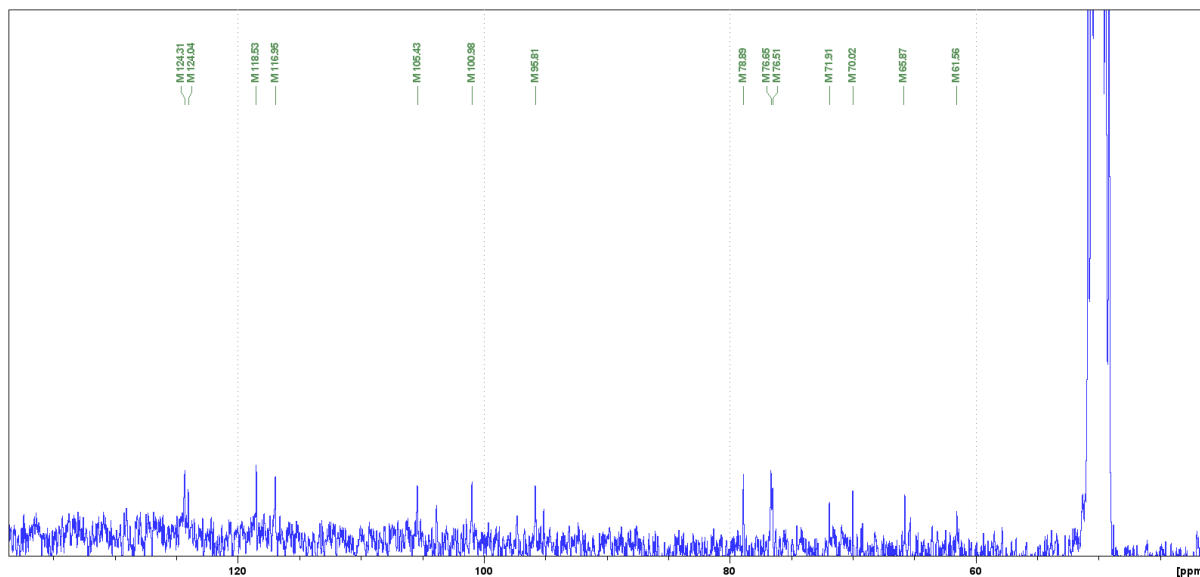


Fig. S12. COSY spectrum of eriodictyol 4'-*O*-glucoside (Compound P2) recorded at 300 MHz in MeOD.

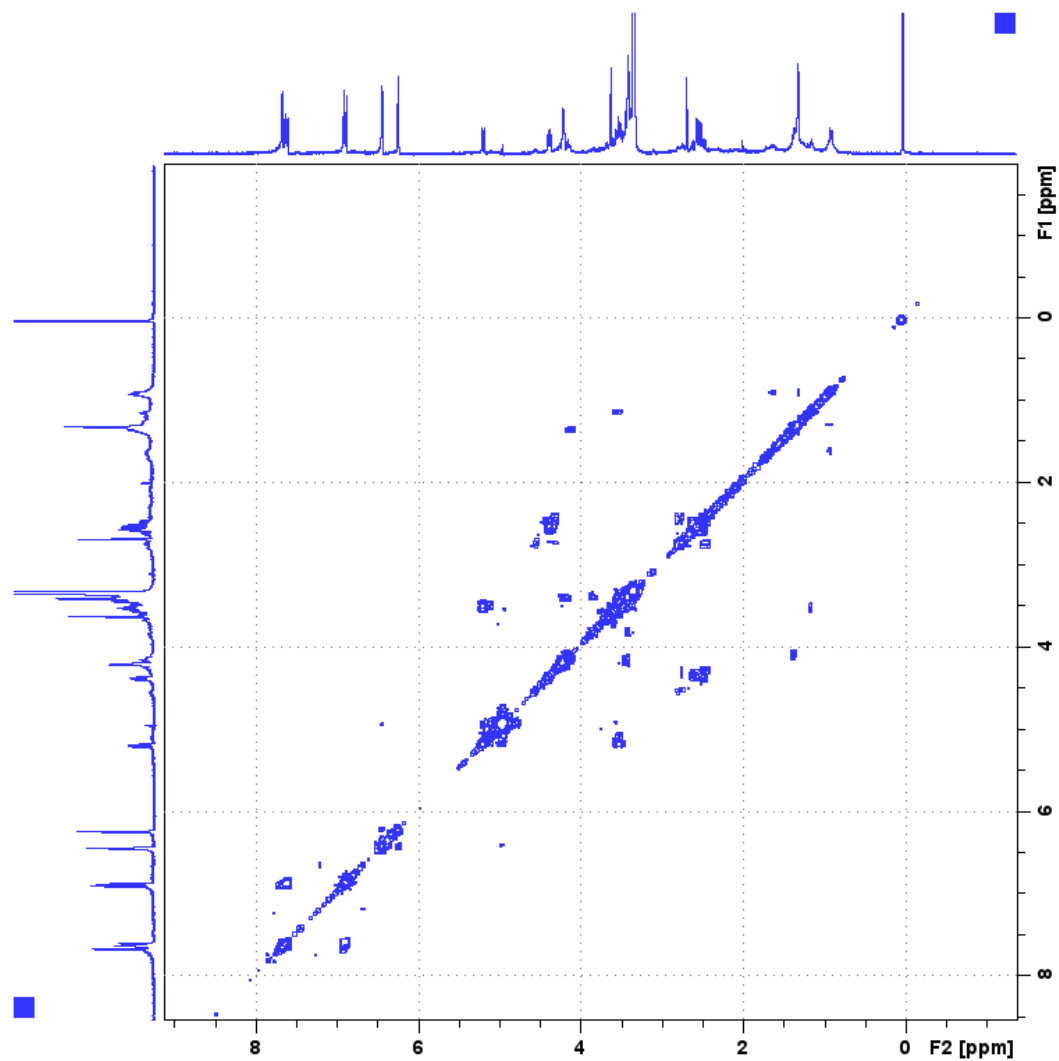


Fig. S13. HSQC spectrum of eriodictyol 4'-*O*-glucoside (Compound P2) recorded at 300 MHz in MeOD.

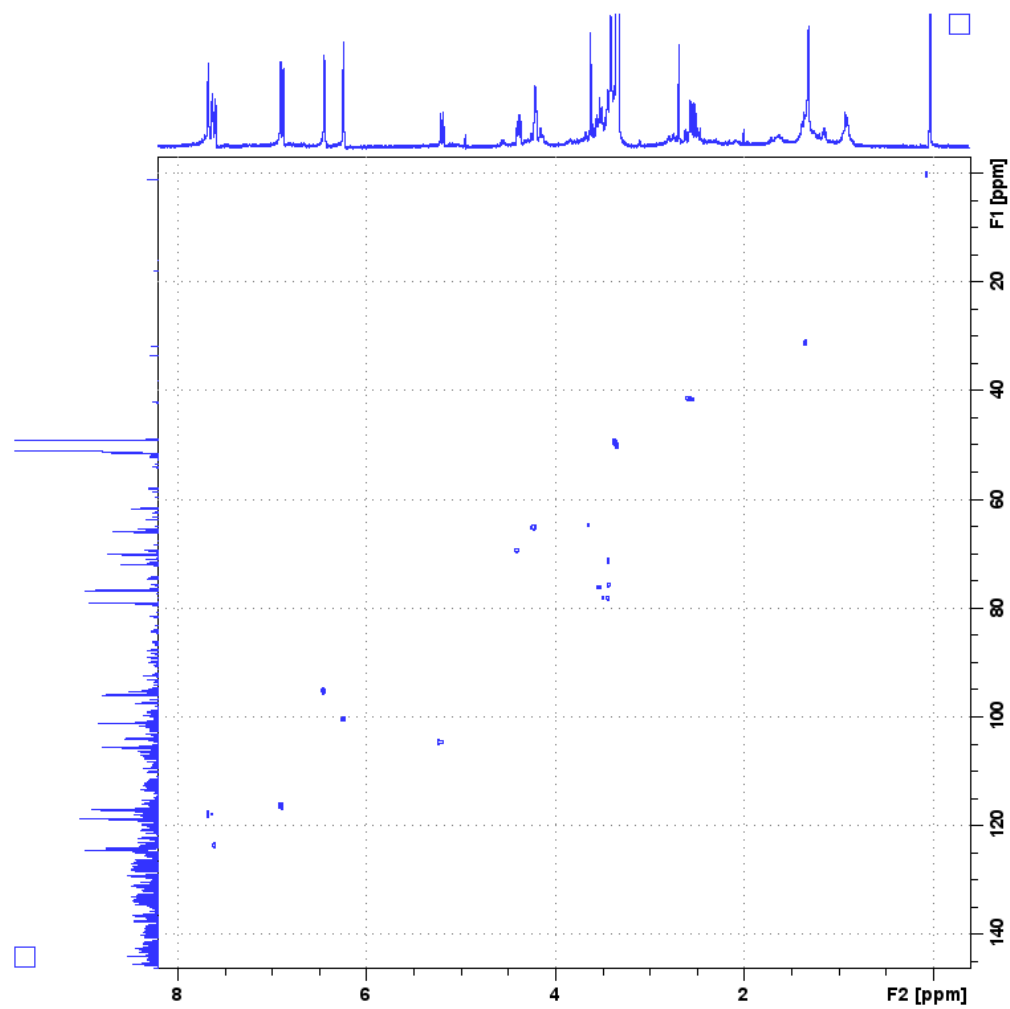


Fig. S14. HMBC spectrum of eriodictyol 4'-*O*-glucoside (Compound P2) recorded at 300 MHz in MeOD.

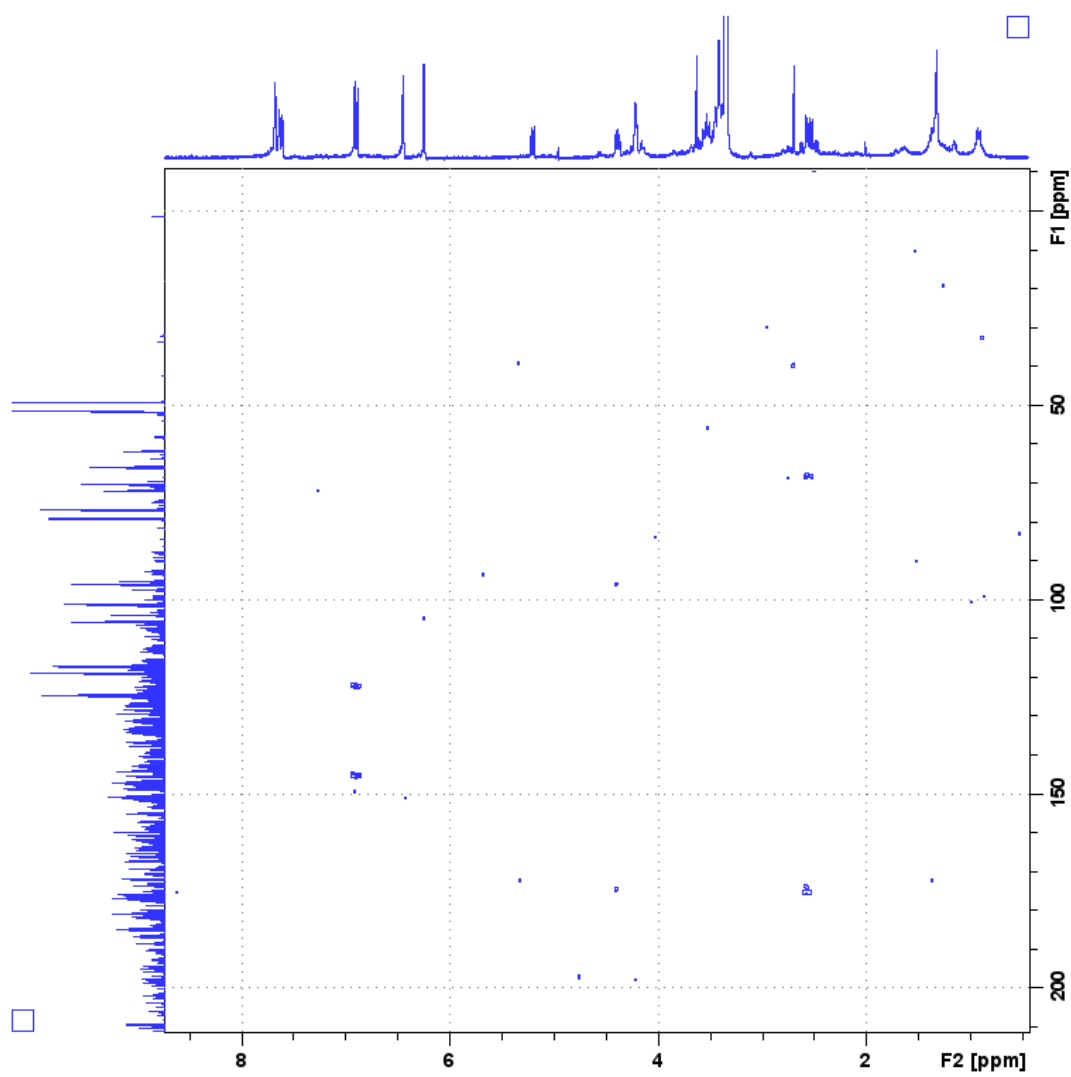


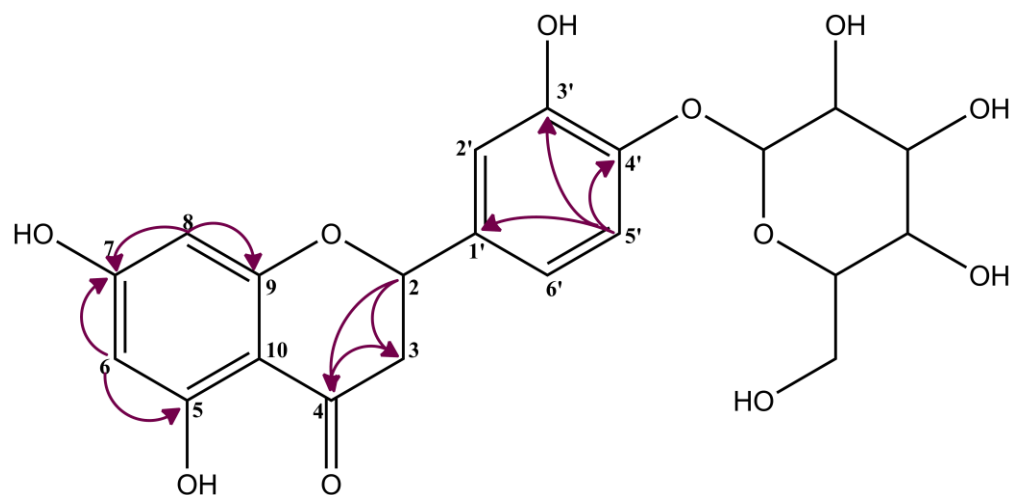
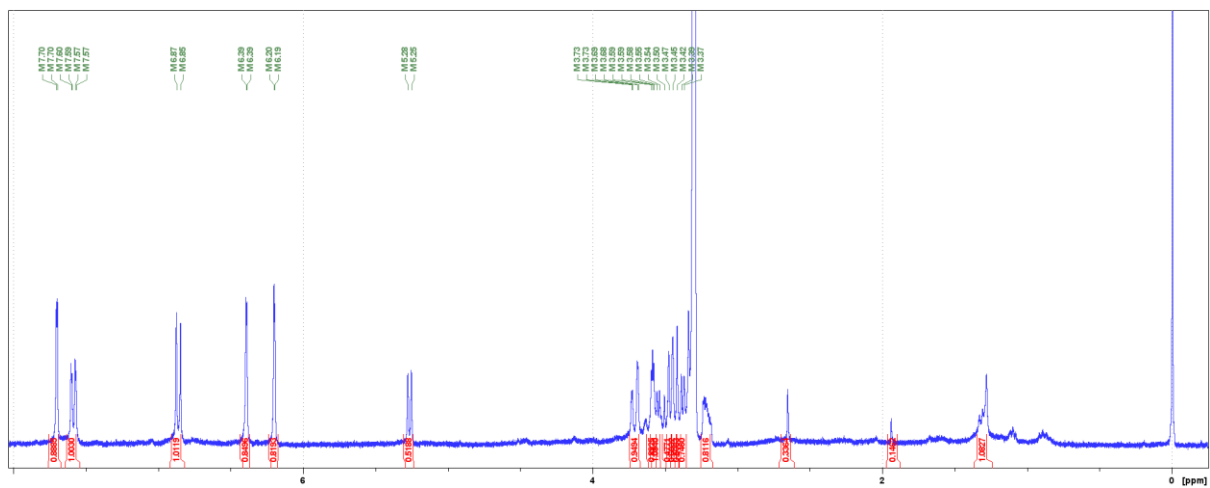
Fig. S15. Main HMBC correlations for eriodictyol 4'-*O*-glucoside (Compound P2).Fig. S16. ^1H NMR spectrum of isoquercetin (Compound P3) recorded at 300 MHz in MeOD.

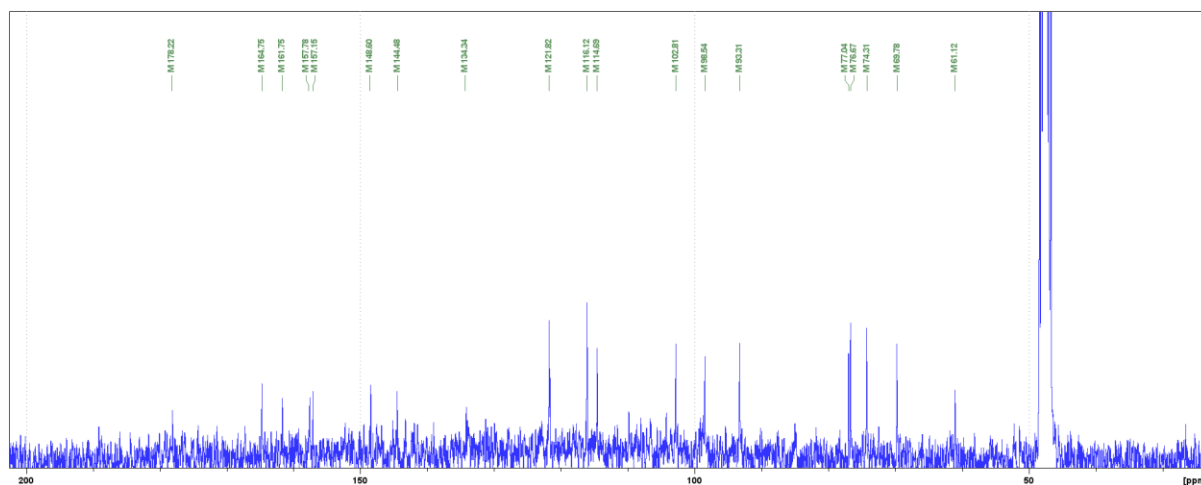
Fig. S17. ^{13}C NMR spectrum of isoquercetin (Compound P3) recorded at 300 MHz in MeOD.

Fig. S18. COSY spectrum of isoquercetin (Compound P3) recorded at 300 MHz in MeOD.

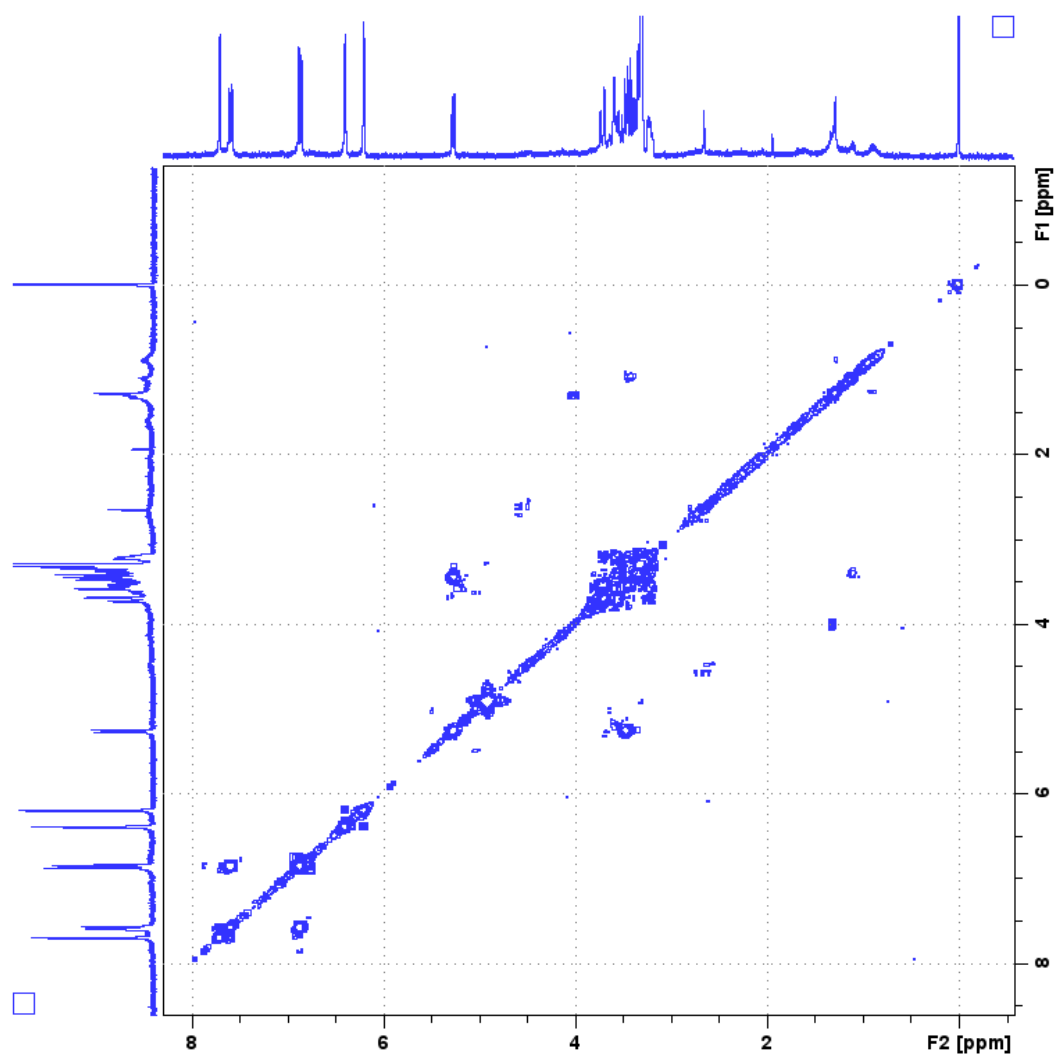


Fig. S19. HSQC spectrum of isoquercetin (Compound P3) recorded at 300 MHz in MeOD.

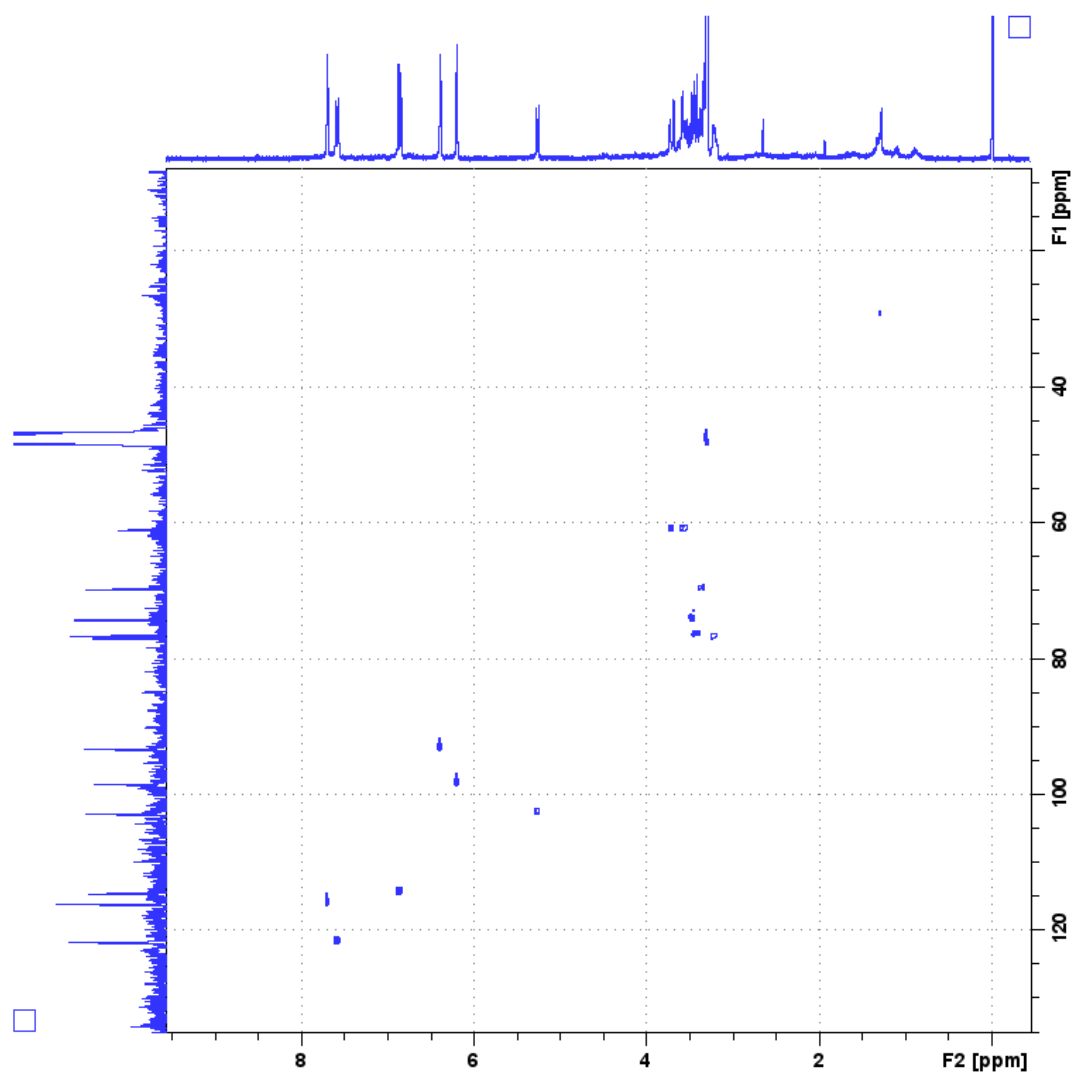


Fig. S20. HMBC spectrum of isoquercetin (Compound P3) (recorded at 300 MHz in MeOD).

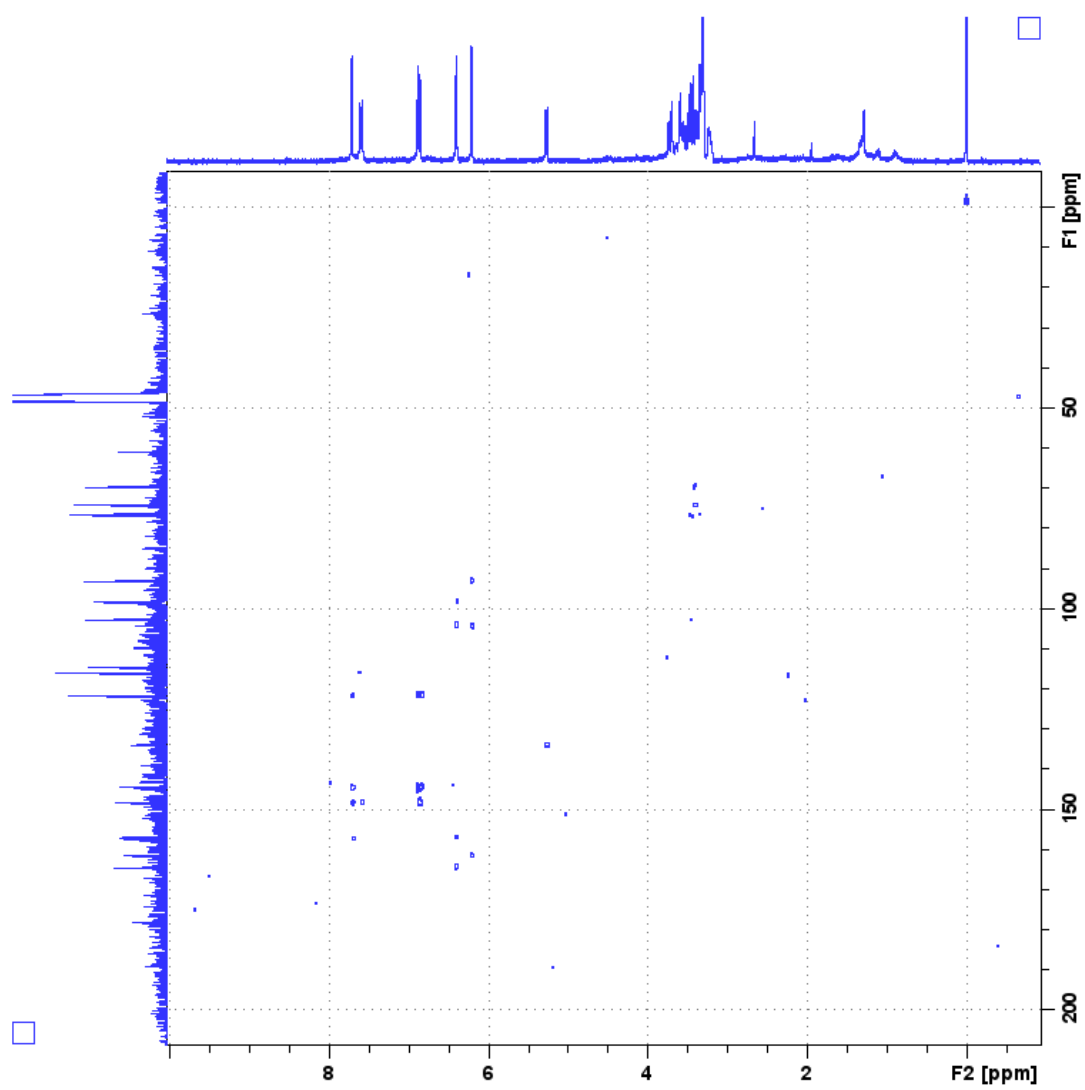
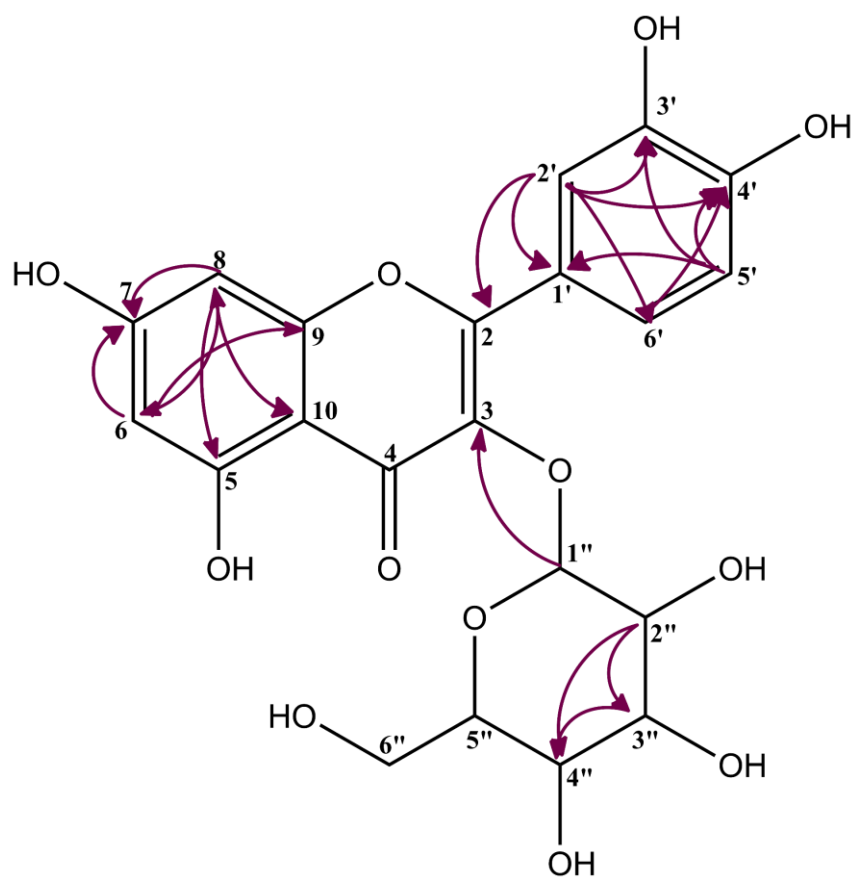


Fig. S21. Main HMBC correlations for isoquercetin (Compound P3).



References

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<https://doi.org/10.1007/s11418-023-01771-1>

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Srinivasan, R., Natarajan, D., Shivakumar, M., S. 2015. Antioxidant Compound Quercetin-3-*O*- α -L-rhamnoside (1 \rightarrow 6)- β -D-glucose (Rutin) isolated from ethyl acetate leaf extracts of *Memecylon edule* Roxb (Melastamataceae). *Free Radicals Antioxid.* 5, 35– 42.

<https://doi.org/10.5530/fra.2015>

ANEXO A – Parecer consubstanciado do Comitê de Ética

UNIVERSIDADE FEDERAL DE
ALFENAS

**PARECER CONSUBSTANCIADO DO CEP****DADOS DO PROJETO DE PESQUISA**

Título da Pesquisa: METABOLÔMICA, FITOQUÍMICA E AVALIAÇÃO ANTI-INFLAMATÓRIA EX VIVO DE PRODUTOS NATURAIS, SUBSTÂNCIAS ISOLADAS E SINTÉTICAS.

Pesquisador: Daniela Aparecida Chagas de Paula

Área Temática:

Versão: 1

CAAE: 60344622.1.0000.5142

Instituição Proponente: UNIVERSIDADE FEDERAL DE ALFENAS - UNIFAL-MG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.582.522

Apresentação do Projeto:

Trata-se de um estudo de extratos de diferentes espécies de famílias com reconhecido potencial para atividade anti-inflamatória, por meio de estratégias metabolômicas, fitoquímicas, de síntese e o uso de ensaios ex vivo em sangue humano para triagem da atividade anti-inflamatória para descoberta de extratos e substâncias com propriedades anti-inflamatórias com mecanismo de ação inovador.

Serão realizadas coletas de amostra de sangue de voluntários que serão utilizadas para os testes ex vivo.

O Pesquisador não indica o tipo de projeto de (IC, mestrado ou doutorado).

Não apresenta indícios de conflito de interesse.

Financiamento Próprio.

Objetivo da Pesquisa:

Estudar os extratos de diferentes espécies de famílias com reconhecido potencial para atividade anti-inflamatória, por meio de estratégias metabolômicas, fitoquímicas, de síntese e o uso de ensaios ex vivo em sangue humano para triagem da atividade anti-inflamatória para descoberta

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ANEXO B – Parecer consubstanciado do Comitê de Ética

UNIVERSIDADE FEDERAL DE
ALFENAS - UNIFAL

**PARECER CONSUBSTANCIADO DO CEP****DADOS DO PROJETO DE PESQUISA**

Título da Pesquisa: Isolamento bioguiado de substâncias antiurolíticas e anti-inflamatórias de *Cissus gongylodes* (VITACEAE)

Pesquisador: PAULA PIO DE OLIVEIRA SALEM

Área Temática:

Versão: 2

CAAE: 67746523.7.0000.5142

Instituição Proponente: UNIVERSIDADE FEDERAL DE ALFENAS - UNIFAL-MG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.095.634

Apresentação do Projeto:

O projeto trata de proposta de tese de doutorado, com financiamento próprio, que aborda pesquisa bioguiada da possível ação de frações isoladas da planta *Cissus gongylodes*, conhecida como cupá, cujo o histórico etnofarmacológico descreve o uso do seu decocto para ação antiurolítica e estudos in vitro apontam resultados promissores. O decocto e as frações isoladas cromatograficamente (acetato de etila), clorofórmica e hidroalcoólica serão avaliadas em ensaios in vitro para verificar a capacidade urolítica sobre cristais de oxalato de cálcio obtidos por cristalização da urina humana.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar o decocto das folhas de cupá e suas frações quanto à sua atividade antiurolítica.

Objetivo Secundário:

- Coleta do material vegetal das folhas de cupá;
- Obtenção do decocto e suas frações acetato de etila, clorofórmica e hidroalcoólica;
- Estruturação de bando de dados das substâncias isoladas do gênero *Cissus*;
- Caracterização do perfil cromatográfico do decocto e de suas frações por HPLC-UV

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