UNIVERSIDADE FEDERAL DA BAHIA

INSTITUTO DE QUÍMICA

PROGRAMA DE PÓS-GRADUAÇÃO EM QUÍMICA

ANDRÉ BARRETO CUNHA

OCORRÊNCIA, DETECÇÃO E QUANTIFICAÇÃO DE ÁCIDOS TRITERPÊNICOS EM ESPÉCIES DE DILLENIACEAE E LAMIACEAE, ATIVIDADES BIOLÓGICAS DE DERIVADOS SEMISSINTÉTICOS E COMPOSIÇÃO, BIOATIVIDADE, USOS E QUIMIOTAXONOMIA DA FAMÍLIA ANACARDIACEAE

Salvador

2023

ANDRÉ BARRETO CUNHA

OCORRÊNCIA, DETECÇÃO E QUANTIFICAÇÃO DE ÁCIDOS TRITERPÊNICOS EM ESPÉCIES DE DILLENIACEAE E LAMIACEAE, ATIVIDADES BIOLÓGICAS DE DERIVADOS SEMISSINTÉTICOS E COMPOSIÇÃO, BIOATIVIDADE, USOS E QUIMIOTAXONOMIA DA FAMÍLIA ANACARDIACEAE

Tese de Doutorado apresentada ao Programa de Pós-graduação em Química da Universidade Federal da Bahia como requisito parcial para obtenção do título de doutor em Química.

Orientador: Prof. Dr. Jorge Maurício David

Salvador

2023

TERMO DE APROVAÇÃO

ANDRÉ BARRETO CUNHA

"OCORRÊNCIA, DETECÇÃO E QUANTIFICAÇÃO DE ÁCIDOS TRITERPÊNICOS EM ESPÉCIES DE DILLENIACEAE E LAMIACEAE, ATIVIDADES BIOLÓGICAS DE DERIVADOS SEMISSINTÉTICOS E COMPOSIÇÃO, BIOATIVIDADE, USOS E QUIMIOTAXONOMIA DA FAMÍLIA ANACARDIACEAE"

Tese aprovada como requisito parcial para obtenção do grau de Doutor em Química, Universidade Federal da Bahia, pela seguinte banca examinadora:

Prof. Dr. Jorge Mauricio David
Doutorado em Ciências, Universidade de São Paulo (USP)
Universidade Federal da Bahia

Profa. Dra. Valéria Belli Riatto
Doutorado em Química, Universidade Estadual de Campinas (UNICAMP)
Universidade Federal da Bahia

Profa. Dra. Eliane de Oliveira Silva
Doutorado em Ciências, Universidade de São Paulo (USP)
Universidade Federal da Bahia

Prof. Dr. Clayton Queiroz Alves
Doutorado em Química, Universidade Federal da Bahia (UFBA)
Universidade Estadual de Feira de Santana

Prof. Dr. Hugo Neves Brandão
Doutorado em Química, Universidade Federal da Bahia (UFBA)
Universidade Estadual de Feira de Santana

Salvador, 22 de junho de 2023.

Rua Barão de Jeremoabo, 147 – Campus Universitário de Ondina 40170-115 Salvador, BA
Tel: (71) 3283-6805
E-mail: secpgquim@ufba.br

C972 Cunha, André Barreto

Ocorrência, detecção e quantificação de ácidos triterpênicos em espécies de Dilleniaceae e Lamiaceae, atividades biológicas de derivados semissintéticos e composição, bioatividade, usos e quimiotaxonomia da família Anacardiaceae/ André Barreto Cunha. – Salvador, 2023.

167 f.

Orientador: Prof. Dr. Jorge Maurício David

Tese (Doutorado) – Universidade Federal da Bahia. Instituto de Química, 2023.

1. Química. 2. Produtos Naturais - Obtenção. 3. Síntese Orgânica. I. David, Jorge Maurício. II. Universidade Federal da Bahia. III. Título.

CDU 547

DEDICATÓRIA / DEDICATION

Ao meu Deus pela força, sabedoria e perseverança concedidas a cada dia (a quem seja a glória somente e para sempre!), à minha família pelo apoio constante – sobretudo ao meu padrasto Alberto Pereira (*in memoriam*), cujo bom exemplo sempre guardarei –, aos meus colegas de academia pela amizade, conhecimento e aprendizado mútuo bem como aos demais amigos por todas as alegrias e superações compartilhadas.

AGRADECIMENTOS / ACKNOWLEDGEMENTS

À minha mãe Solange por estar presente em todos os momentos – e não apenas durante minha caminhada acadêmica. Minha gratidão mais especial!

Aos demais membros de minha família (minha avó, tios, primos etc.) por dividirem comigo alegrias e desafios durante essa fase, pelo amor sincero e pelo apoio constante. Obrigado!

Ao professor Dr. Jorge Maurício David pela orientação e colaboração para minha formação científica, bem como pela sincera amizade e paciência constante. Minha sincera gratidão!

A todos os parceiros (estudantes de iniciação científica, mestres e doutores) do Grupo de Pesquisa de Produtos Naturais (GPPN) pela amizade, bons momentos e conhecimentos compartilhados. Obrigado pessoal!

Aos professores Rauldenis Fonseca (IFBA, Porto Seguro), Ronan Batista e Sílvio Cunha (UFBA) e María Ángeles Castro (Universidad de Salamanca, Espanha) pela significativa colaboração em diferentes partes deste trabalho. Muito obrigado! ¡Muchísimas gracias!

A CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) pelo apoio financeiro concedido durante a realização deste trabalho, sem o qual o mesmo não seria possível. Obrigado!

À Cru Campus, movimento pelo qual sou grato e do qual levarei valores e lembranças para toda a vida! Muito obrigado, “amigos irmãos”!

A toda a comunidade do Instituto de Química da UFBA (professores, pesquisadores e funcionários) que direta ou indiretamente são parte de mais esse degrau em minha formação acadêmica e profissional.

SUMÁRIO / SUMMARY

LISTA DE FIGURAS / LIST OF FIGURES

LISTA DE TABELAS / LIST OF TABLES

LISTA DE ESQUEMAS / LIST OF SCHEMES

ABREVIATURAS E SIGLAS / ABBREVIATIONS AND ACRONYMS

AB/BA: ácido betulínico/betulinic acid

AO/OA: ácido oleanólico/oleanolic acid

AU/UA: ácido ursólico/ursolic acid

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ANVISA: Brazilian Health Regulatory Agency (Brazil)

AZT: azidothymidine

BHA: butylated hydroxyanisole

Caco-2: cell lineage related to human colorectal epithelial adenocarcinoma

CC: column chromatography

COX-1 and COX-2: cyclooxygenases 1 and 2

DCC: *N*,*N*'-dicyclohexylcarbodiimide

D-GalN: D-(+)-Galactosamine

DHP: 3,4-dihydropyran

DIB: diacetoxy-iodobenzene

DMAP: dimethylaminopyridine

EOs: essential oils

EPP: ethyl phenylpropiolate

FRAP: Fe^{3+} reducing power assay

FTC: ferric thiocyanate method

HPLC: high-performance liquid chromatography

HPLC-DAD-UV: high-performance liquid chromatography with UV-diode array detection

HepG2: cell lineage related to human liver cancer

LAAO: L-amino-acid oxidase

IC_{50}: half-maximal inhibitory concentration

INMETRO: National Institute of Metrology, Standardization and Industrial Quality (Brazil)

LPO: lactoperoxidase

LD: limt of detection

LQ: limit of quantification

MAE: microwave-assisted extraction

MBC: minimum bactericidal concentration

MCF-7: cell lineage related to human breast adenocarcinoma

MFC: minimum fungicidal concentration

MIC: minimum inhibitory concentration

MRSA: methicillin-resistant *Staphylococcus aureus*

OCCL: ovarian cancer cell lines

PGG: penta-*O*-galloyl-D-glucose

PKSs: polyketide biosynthetic enzymes

PLA_2: phospholipase A2

PPTS: pyridinium p-toluenesulfonate

ρ: relative yield

R_t: retention time

SAM: *S*-adenosyl-l-methionine

SDIs: succinate dehydrogenase inhibitors

SERCA: sarcoendoplasmic reticulum calcium ATPase

SFME: serum-free mouse embryonic cells

TEA: triethylamine

TEMPO: 2,2,6,6-tetramethylpiperidine 1-oxyl

TFA: trifluoroacetic acid

TLC: thin layer chromatography

TPA: 12-*O*-tetradecanoylphorbol acetate

VOCs: Volatile Organic Compounds

CUNHA, André Barreto. Ocorrência, detecção e quantificação de ácidos triterpênicos em espécies de Dilleniaceae e Lamiaceae, atividades biológicas de derivados semissintéticos e composição, bioatividade, usos e quimiotaxonomia da família Anacardiaceae. 167 f. il. 2023. Tese (Doutorado em Química) – Instituto de Química, Universidade Federal da Bahia, Salvador, 2023.

RESUMO

O presente trabalho apresenta um estudo referente à importância dos produtos naturais nos diversos âmbitos da pesquisa científica (em particular a Química, a Farmacologia, a Biotecnologia e áreas afins), tais como os ácidos triterpênicos, os alquilfenóis, os flavonoides e biflavonoides. Dentre as diferentes subclasses de metabólitos especializados, os ácidos triterpênicos são de significativa relevância devido às suas propriedades biológicas, aspectos quimiotaxonômicos e determinados usos comerciais e tecnológicos. Com base em sua ampla ocorrência em plantas, novas investigações com o objetivo de desenvolver metodologias de obtenção que sejam posteriormente validadas se mostram mais promissoras e necessárias. Desse modo, este trabalho apresenta o desenvolvimento de métodos de obtenção, detecção e quantificação dos ácidos betulínico (AB), oleanólico (AO) e ursólico (AU) em extratos de *Davilla rugosa* Poir. et St. Hill (Dilleniaceae) e *Eriope blanchetii* (Benth.) Harley (Lamiaceae), com subsequente validação do método desenvolvido no estudo com *E. blanchetii*. Dentre os principais resultados, o AB foi quantificado no caule de *D. rugosa* (2.4×10^{-2} % do peso seco) assim como nas folhas de *E. blanchetii* (2.5×10^{-2} % do peso seco). Além disso, AO foi determinado nos pecíolos das folhas (1.1×10^{-2} % do peso seco) de *E. blanchetii* e AU foi identificado em nível de traços em todas as partes da planta (folhas, pecíolos, caule e raiz). Finalmente, a extração assistida por micro-ondas (MAE) se mostrou mais eficiente em relação à maceração com aquecimento (em termos de rendimento relativo, ρ) sob as mesmas condições analíticas. No tocante à validação analítica, o método cromatográfico (HPLC-DAD-UV) utilizado na quantificação simultânea desses compostos foi devidamente seletivo e preciso, apresentando linearidade, robustez e valores de recuperação com limites satisfatórios (LD = 4.1096 and LQ = 12.4535; µg/mL) nas faixas de concentração experimentais. Por outro lado, diversas estratégias químicas para a síntese do AB (e de análogos bioativos) a partir da betulina foram revisadas (e aplicadas), de modo que diferentes derivados exibiram atividade antiplasmodial (acil-compostos com modificações na posição C-3, incluindo derivados diméricos), anti *Leishmania* spp. (heterociclos fundidos e derivados de amidas em C-2/C-3) ou antitripanossômica (derivados de amidas na posição C-28) superiores àquelas correspondentes ao ácido betulínico. De maneira complementar, foi realizada uma revisão detalhada concernente à família Anacardiaceae, destacando sua composição química, suas propriedades biológicas, diversos usos tecnológicos e medicinais e as descobertas mais recentes com relação à biossíntese de alquilfenóis (o principal marcador quimiotaxonômico na família). Desse modo, novos metabólitos bioativos (óleos essenciais, terpenoides, flavonoides e biflavonoides, chalconas etc.) têm sido

encontrados em diferentes gêneros, diferentes composições cosméticas e farmacológicas vêm sendo patenteadas e novos mecanismos para a biossíntese dos lipídios fenólicos têm sido propostos, cujos resultados ratificam a importância desta família para a Química de Produtos Naturais e áreas correlatas.

Palavras-chave: ácidos triterpênicos; quantificação; validação; derivados bioativos; Anacardiaceae.

CUNHA, André Barreto. Occurrence, detection and quantification of triterpenic acids in species of Dilleniaceae and Lamiaceae, biological activities of semisynthetic derivatives and composition, bioactivity, uses and chemotaxonomy of Anacardiaceae family. 167 pp. ill. 2023. Thesis (Doctor's degree in Chemistry) – Instituto de Química, Universidade Federal da Bahia, Salvador, 2023.

ABSTRACT

The present work reports a study regarding to the importance of natural products in various fields of scientific research (particularly Chemistry, Pharmacology, Biotechnology and related areas), such as triterpenic acids, alkyl phenols, flavonoids and biflavonoids. Amongst the different subclasses of specialized metabolites, triterpenic acids are of significant relevance due to their biological properties, chemotaxonomic aspects and certain commercial and technological uses. Based on their wide occurrence in plants, new investigations aiming the development of novel methodologies of obtention that are later validated are more promising and necessary. Therefore, this work presents the development of methods for obtaining, identifying and quantifying betulinic (BA), oleanolic (OA) and ursolic (UA) acids in extracts of *Davilla rugosa* Poir. et St. Hill (Dilleniaceae) and *Eriope blanchetii* (Benth.) Harley (Lamiaceae) with subsequent validation of the method developed in the study with *E. blanchetii*. Among the main results, BA was quantified in the stems of *D. rugosa* (2.4 x 10^{-2} % of dry weight) as well as in the leaves of *E. blanchetii* (2.5 x 10^{-2} % of dry weight). Furthermore, OA was determined in leaf petioles of *E. blanchetii* (1.1 x 10^{-2} % of dry weight) and AU was identified in traits in all parts of the plant (leaves, petioles, stem and root). Lastly, microwave-assisted extraction (MAE) was more efficient than heated maceration (in terms of relative yield, ρ) under the same analytical conditions. Concerning to the analytical validation, the chromatographic method (HPLC-DAD-UV) used in the simultaneous quantification of the triterpenic acids was properly selective and precise, showing satisfactory linearity, robustness and recovery values with acceptable limits (LD = 4.1096 and LQ = 12.4535; µg/mL) in the experimental concentration ranges. On the other hand, several chemical strategies for the synthesis of AB (and bioactive analogues) from betulin were reviewed and applied. Thus, different derivatives exhibited antiplasmodial activity (acylated compounds with modifications at C-3 position, including dimeric derivatives), anti *Leishmania* spp. (fused heterocycles to C-2/C-3 and amide derivatives) or antitrypanosomal (amide derivatives at C-28 position), which were superior to those corresponding to betulinic acid. Additionally, a detailed review concerning to Anacardiaceae family was carried out, highlighting the chemical composition, the biological properties, the several technological and medicinal uses and the most recent discoveries regarding the biosynthesis of alkyl phenols (the main chemotaxonomic marker in the family). Thereby, new bioactive metabolites (essential oils, terpenoids, flavonoids and biflavonoids, chalcones, etc.) have been found in different genera, different cosmetic and pharmacological compositions have been patented and new mechanisms for the biosynthesis of phenolic lipids have been

proposed, whose results confirm the importance of this family for the Chemistry of Natural Products and other scientific areas.

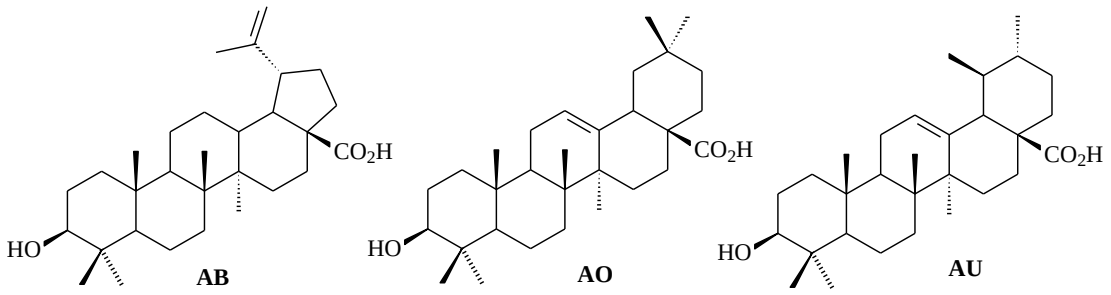
Keywords: triterpenic acids; quantification; validation; bioactive derivatives; Anacardiaceae.

INTRODUÇÃO GERAL

Os produtos naturais são divididos em diferentes classes e em suas respectivas subclasses de acordo com suas características estruturais e aspectos biossintéticos. Dentre as classes principais, destacam-se os compostos fenólicos, tais como flavonoides, lipídios fenólicos e cumarinas, além dos alcaloides, lignanas e ligninas, terpenos e terpenoides, policetídeos e outros de ocorrência menos significativa. Nesse sentido, os terpenoides são dignos de destaque, uma vez que estes metabólitos são os mais abundantemente encontrados nos vegetais, bem como apresentam propriedades de particular importância nos âmbitos medicinal, químico e biotecnológico (Patočka, 2003; Dewick, 2009).

Considerando-se os diferentes tipos de terpenoides, os triterpenoides constituem um grupo de metabólitos especializados (i.e., especiais ou secundários) de ocorrência frequente em vegetais, de forma que os ácidos betulínico (AB), oleanólico (AO) e ursólico (AU) são os mais conhecidos (Figura 1). Tais compostos, assim como seus respectivos derivados, possuem um amplo espectro de atividades biológicas (Tarvainen, 2010; Mandal, 2010) e usos comerciais, o que pode ser ratificado pelo crescente desenvolvimento de novas patentes envolvendo estas substâncias nas últimas décadas (Xu, 2007; Cho, 1996; Lee, 1997; Scheffler, 2010). Além disso, novas metodologias de isolamento e purificação desses metabólitos têm sido estudadas e avaliadas, tendo em vista a sua obtenção com maior eficiência e rendimentos razoáveis (Frighetto, 2005; Razboršek, 2008; Silvestre, 2006; Domingues, 2011).

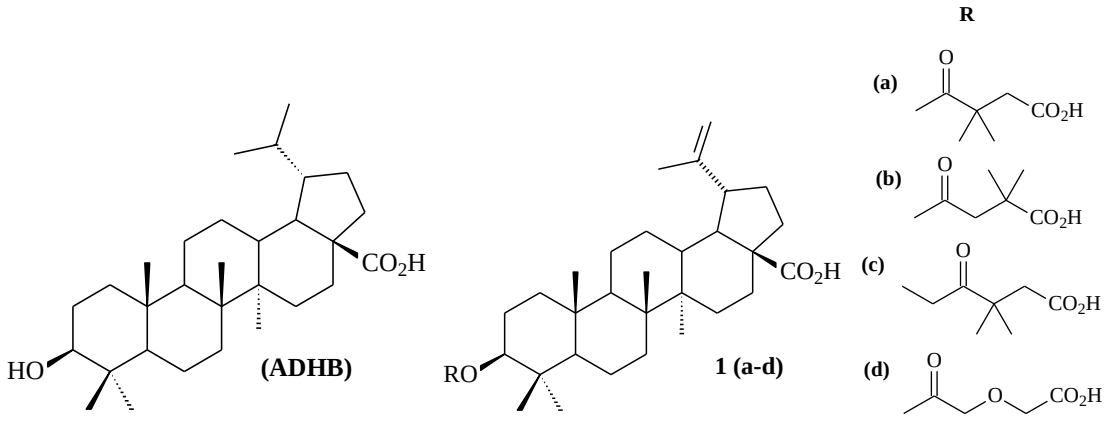
Figura 1. Estruturas dos ácidos betulínico (AB), oleanólico (AO) e ursólico (AU)

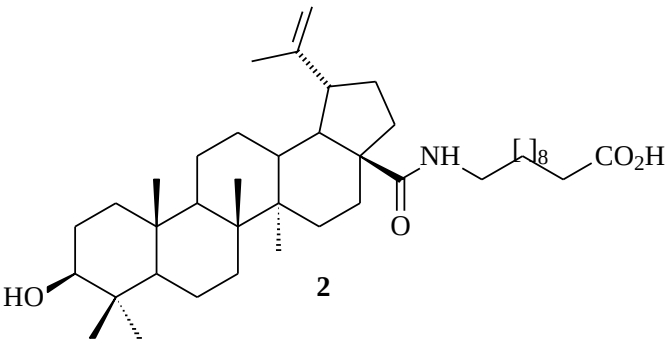


Com respeito à quimiotaxonomia, esses compostos ocorrem comumente em espécies de betuláceas (Betulaceae) do gênero *Betula*, lamiáceas (Lamiaceae) dos gêneros *Eriope*, *Salvia* e *Rosmarinus*, mirtáceas (Myrtaceae) dos gêneros *Eugenia* e *Eucalyptus* e dileniáceas (Dilleniaceae) dos gêneros *Davilla*, *Dillenia*, *Wormia* e *Acrotrema*, de modo que a sua significativa ocorrência em espécies dos últimos três gêneros mencionados permite considerar o AB como marcador quimiotaxonômico em Dilleniaceae spp. (Pavanasasivam, 1974). Por outro lado, a distribuição de cada um destes ácidos é seletiva ao longo das famílias botânicas em que estão presentes, de modo que estes podem ser encontrados concomitantemente (p.ex., em Adoxaceae, Apocynaceae, Oleaceae, Rosaceae, Rubiaceae spp. etc.) ou separadamente (i.e., quando apenas um ou até dois compostos são produzidos) (David, 2001; David, 2006; Frighetto, 2005; Patočka, 2003; Fai, 2009), o que pode justificar a obtenção preferencial de algum dos ácidos em detrimento de outros em determinados estudos analíticos.

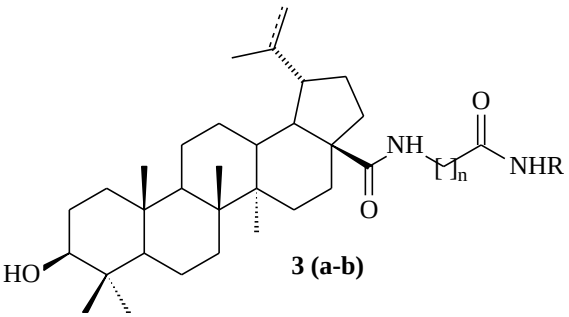
No que se refere às propriedades biológicas, pode-se destacar a atividade anti-HIV-1 *in vitro* (provavelmente relacionada à inibição de enzimas como a HIV-protease e a transcriptase reversa) do AB e de alguns de seus análogos semissintéticos (Figura 2), de tal modo que modificações na molécula do AB para a obtenção de novos derivados podem estar associadas ao aumento ou à perda dessa atividade (Yogeeswari, 2005).

Figura 2. Derivados semissintéticos do AB com atividade antiviral (anti-HIV-1). ADHB (ácido di-hidrobetulínico); compostos 1a-d: ácidos 3-*O*-(3',3'-dimetilsuccinil betulínico) (a), 3-*O*-(2',2'-dimetilsuccinil betulínico) (b) e acil-derivados obtidos após tratamento com anidrido 3,3-dimetilglutárico (c) e anidrido diglicólico (d); derivado C-17 alquilamido (2) e derivados ω-aminoalcanóicos (3a-b)





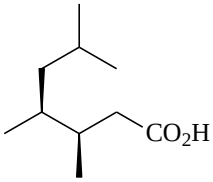
2



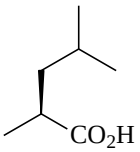
3 (a-b)

R:

a)



b)



Além disso, o AB tem apresentado ação seletiva contra células do neuroblastoma e inibição do crescimento de linhagens celulares associadas ao melanoma humano na ausência de efeitos colaterais (como a perda de peso) assim como ação antitumoral *in vitro* e/ou *in vivo* fortemente seletiva contra o melanoma humano, cujos resultados tornam o AB único em comparação aos compostos correntemente utilizados na terapia do câncer (e.g., taxol/paclitaxel, camptotecina, elipticina, etoposídeo, vimblastina, vincristina e outros análogos), cuja citotoxicidade e efeito inibitório não são seletivos (Schimidt, 1997; Fulda, 1998; Selzer, 2000; Patočka, 2003). Em estudos mais recentes, tem sido atribuída ao AB a atividade anticâncer alta e/ou moderada contra diferentes linhagens neoplásicas relacionadas à leucemia (U937, HL-60, K562), ao carcinoma de cólon hepático altamente metastático (26-L5), ao câncer de fígado humano (VA-13 e HepG2) e a fibroblastos do tipo WI-38 *in vitro*, além de efeito inibitório frente a determinadas enzimas como a DNA topoisomerase II, tirosina fosfatase 1B e enzimas da via do ácido araquidônico (Kumar, 2010; Tezuka, 2000; Wada e Tanaka, 2005; Fu, 2005; Seung, 2009; Patočka, 2003).

O ácido betulínico também possui comprovada atividade antibacteriana, conforme mostrado num estudo realizado com *Syncarpia glomulifera* (Myrtaceae), cujo

extrato clorofórmico bruto das cascas apresentou atividades antibacteriana e citotóxica. Nesse caso, com base na considerável abundância relativa deste composto nesta espécie – cerca de 10% do extrato $CHCl_3$ bruto –, pode-se inferir que o AB está relacionado ao efeito correspondente (Setzer, 2000). Similarmente, num estudo químico desenvolvido com as cascas do caule de *Zizyphus joazeiro* (Rhamnaceae, planta medicinal típica do Brasil), o AB foi obtido a partir do extrato CH_2Cl_2 e apresentou considerável atividade contra bactérias Gram-positivas (Schühly, 1999). Finalmente, o AB também possui atividades antimalárica/antiplasmodial *in vitro*, anti-helmíntica (superior àquela da piperazina em 100 e 500 ppm), anti-inflamatória (contra edema de pata – induzido por carragenina e serotonina – bem como em relação ao edema de orelha – induzido por TPA e EPP), antinociceptiva, antitripanossômica *in vitro*, dentre outras (Bringmann, 1997; Ahmad, 2012).

De modo análogo, o ácido oleanólico também apresenta diversas propriedades biológicas, a exemplo da atividade hepatoprotetora *in vivo* contra ferimentos no fígado (induzidos por CCl_4, acetaminofeno e outros agentes citotóxicos) em ratos, da ação anti-inflamatória relativa à inibição de edema de pata induzido por carragenina em ratos e de artrite induzida por formaldeído, do efeito antimutagênico *in vitro* produzido por benzo-*α*-pireno em bactérias e do decréscimo da incidência e da multiplicação de tumores intestinais induzido por azoximetano em ratos (Ma, 1982; Gupta, 1969; Niikawa, 1993 e Yoshimi, 1992). Além disso, o AO tem sido recomendado para a terapia do câncer de pele, de modo que o Japão possui patentes da indústria cosmética que vêm sendo utilizadas para sua prevenção desde os anos 90 (Ishida, 1990; Liu, 1987). Finalmente, deve-se também mencionar suas atividades anti-hiperlipidêmica, antibacteriana *in vitro* (frente a *Mycobacterium tuberculosis*), antioxidante, anticariogênica e inibitória da DNA polimerase tipo-*β*, as quais podem ser igualmente associadas a alguns de seus derivados semissintéticos (Hada, 1990; Horiuchi, 2007; Vasilenko, 1982; Kingston, 2003; Bremner, 2008; Liu, 1995).

Com relação ao AU, sabe-se que este composto possui atividades hepatoprotetora e anti-inflamatória (relacionada à prevenção de edema de pata induzido por carragenina em ratos e de contorções induzidas por ácido acético), assim como atua na inibição de mutagenicidade produzida por aflatoxina B-1 (em *Salmonella typhimurium*) ou por benzo-α-pireno (em outras bactérias). De maneira adicional, pode-se citar as atividades anti-hiperlipidêmica e antimicobacteriana *in vitro* (expressa pelo

AU e por alguns de seus derivados esterificados), o efeito inibitório da enzima COX-2, a ação antitumoral, a redução do estresse oxidativo de células nervosas induzido pela proteína amilóide tipo-β (associado ao Mal de Alzheimer), além das atividades adaptogênica, antimicrobiana e estimuladora da produção de citocinas como a interferon gama (IFN-γ) (Liu, 1995; Bremner, 2008; Tarvainen, 2010; Ringbom, 1998; Janicsák, 2003).

Nesse ínterim, o AU tem sido considerado como um potente agente antitumoral, conforme mostrado num estudo realizado com *Malus pumila* (espécie de maçã nativa do Japão), no qual o AU foi isolado a partir das cascas dos frutos (Yamaguchi, 2008). Este estudo possibilitou comprovar o efeito antiproliferativo desse composto contra células HepG2 (causadoras de câncer de fígado), frente a células MCF-7 (relacionadas ao câncer de mama) e contra linhagens de células Caco-2 (associadas ao câncer de cólon).

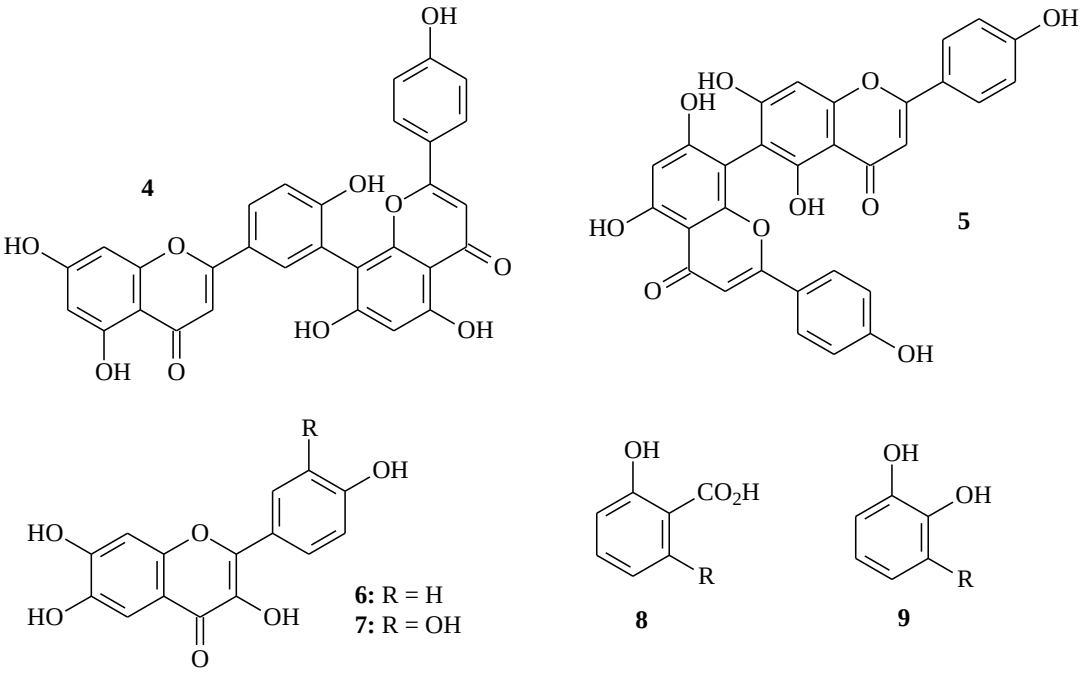
Nesse sentido, a busca por estratégias químicas para a síntese de novos derivados de produtos naturais bioativos tem contribuído significativamente para a elucidação dos fatores determinantes para a bioatividade (seja dos metabólitos, seja dos respectivos derivados) bem como para a sua potencialização, além das contribuições referentes às pesquisas em síntese química (especialmente a síntese orgânica). Portanto, considerando-se que os ácidos triterpênicos possuem diversas propriedades biológicas amplamente estudadas, deve-se ressaltar que o AB tem sido objeto de particular interesse, de modo que sua síntese (bem como de seus derivados ativos) tem sido realizada através de diferentes estratégias, sobretudo envolvendo a betulina como principal precursor (Wickholm, 2018; Qi-He, 2009; Ressmann, 2017; Wu, 2017; Csuk, 2006; Murzin, 2016; Moghaddam, 2010). Dentre tais derivados, podem ser destacados alguns compostos que têm exibido considerável atividade antiparasitária (particularmente contra *Plasmodium* spp., *Leishmania* spp. e *Trypanosoma* spp.), cujos resultados se mostram promissores para o tratamento de diversas doenças tropicais como a malária, a leishmaniose e as tripanossomíases.

De maneira complementar, é pertinente salientar que esses compostos (assim como outros triterpenoides bioativos) têm sido encontrados em espécies de Anacardiaceae – como a *Rhus tripartitum* (Ucria) (Nasri, 2014) –, família de grande importância em virtude de sua relação com várias dermatites de contato (*Toxicodendron* spp. e/ou *Rhus* spp.), com a alimentação (gêneros *Anacardium*, *Mangifera* e *Pistacia*) e outros usos (p.ex., na medicina popular, *Spondias* spp. e *Schinus* spp.). Além disso,

sabendo-se que a família Anacardiaceae é uma fonte de metabólitos bioativos de outras subclasses (especialmente flavonoides, biflavonoides, alquil e alquenilfenóis, Figura 3), novos estudos podem possibilitar a obtenção de compostos bioativos inéditos assim como contribuir para um conhecimento mais detalhado do perfil químico dos diversos gêneros da família (inclusos os menos estudados), das propriedades biológicas das diferentes espécies de cada gênero investigado e de suas aplicações industriais e/ou tecnologias correspondentes (Correia, 2006; Baer, 1979).

Desse modo, este trabalho está organizado em três capítulos nos quais são apresentados 1) novos métodos de obtenção e quantificação de ácidos triterpênicos em *Davilla rugosa* (Dilleniaceae) e *Eriope blanchetii* (Lamiaceae), 2) diferentes estratégias de síntese de derivados do ácido betulínico com atividade antiparasitária e 3) uma revisão detalhada dos aspectos fundamentais (composição, bioatividade, usos e quimiotaxonomia) de todos os gêneros da família Anacardiaceae.

Figura 3. Alguns biflavonoides, flavonoides e alquilfenóis comuns em Anacardiaceae. (**4**): amentoflavona; (**5**): agathisflavona; (**6**) e (**7**): kaempferol e quercetina; (**8**): esqueleto estrutural dos ácidos anacárdicos; (**9**): urushiol e derivados



REFERÊNCIAS

[1] Patočka, J., 2003. **Biologically active pentacyclic triterpenes and their current medicine signification.** *J. Appl. Biomed.*, 1, p. *7*.

[2] Dewick, P. M., 2009. **Medicinal Natural Products: a biosynthetic approach.** 3rd edition, John Wiley & Sons, p. *187-241*.

[3] Tarvainen, M.; Jukka-Pekka, S.; Kallio, H.; Yang, B., 2010. **Triterpene acids in *Plantago major*: Identification, quantification, and comparison of different extraction methods.** *Chromatographia*, 71, p. *279*.

[4] Mandal, V.; Mandal, S. C., 2010. **Design and performance evaluation of a microwave based low carbon yielding extraction technique for naturally occurring bioactive triterpenoid: oleanolic acid.** *Biochem. Eng. J.*, 50, p. *63*.

[5] Xu, Ze-Qi.; Koohang, A.; Mar, A. A.; Majewski, N. D.; Eiznhamer, D. A.; Flavin, M. T., 2007. **Synthetic pentacyclic triterpenoids and derivatives of betulinic acid and betulin**. *Patent Application Publication*, US 2007/0232577 A1.

[6] Cho, S. H.; Gottlieb, K.; Santhanam, U., 1996. **Cosmetic compositions containing betulinic acid**. 14 pp., *Patent Application Publication*, US 005529769 A, EP 717983 A1.

[7] Lee. K-H; Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Manak, M., 1997. **Betulinic acid and dihydrobetulinic acid derivatives and uses therefor**. 10 pp., *Patent Application Publication*, US 005679828 A.

[8] Scheffler, A.; Jäeger, S.; Beffert, M.; Hoppe, K., 2010. **Method for preparing an aqueous solution containing triterpenic acid (aqueous solution containing triterpenic acid) and use thereof.** US *Patent Appl. Publication*, nº US 2010/0104669 A1.

[9] Friguetto, N.; Welendorf, R. M.; Da Silva, A. M. P.; Nakamura, M. J.; Siani, A. C., 2005. **Purification of betulinic acid from *Eugenia florida* (Myrtaceae) by High-Speed Counter-Current Chromatography.** *Phytochem. Anal.*, 16, p. *411*.

[10] Razboršek, M. I.; Voncina, D. B.; Dolecek, V.; Voncina, E., 2008. **Determination of oleanolic, betulinic and ursolic acid in Lamiaceae and Mass Spectral Fragmentation of their trimethylsilylated derivatives**. *Chromatographia*, 67(*5-6*), p. *433*.

[11] Silvestre, A. J. D.; Sousa, A. F.; Pinto, P. C. R. O.; Neto, C. P., 2006. **Triterpenic and other lipophilic components from industrial cork byproducts.** *J. Agric. Food Chem.*, 54, p. *6888*.

[12] Domingues, R. M. A.; Sousa, G. D. A.; Silva, C. M.; Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P., 2011. **High value triterpenic compounds from the outer barks of several *Eucalyptus* species cultivated in Brazil and in Portugal.** *Ind. Crops Prod.*, 33, p. *158*.

[13] Pavanasasivam, G.; Sultanbawa, M. U. S., 1974. **Chemical investigation of ceylonese plants. IX. Betulinic acid in the Dilleniaceae and a review of its natural distribution**. *Phytochemistry* (Elsevier), 13(*9*), p. *2002*.

[14] David, J. P. L.; Da Silva, E. F.; de Moura, D. L.; Guedes, M. L. S.; Assunção, R. J.; David, J. M., 2001. **Lignanas e triterpenos do extrato citotóxico de *Eriope blanchetii***. *Quim. Nova*, (24)*6*, p. *730*.

[15] David, J. M.; Souza, J. C.; Guedes, M. L. S.; David, J. P. L., 2006. **Estudo fitoquímico de *Davilla rugosa*: flavonóides e terpenóides**. *Rev. Bras. Farmacogn.*, 16(*1*), p. *105*.

[16] Fai, Y. M.; Tao, C. C., 2009 **A review of presence of oleanolic acid in natural products.** *Nat. Prod. Med*., University of Hong Kong, Hong Kong.

[17] Yogeeswari, P.; Sriram, D., 2005. **Betulinic acid and its derivatives: a review on their biological properties.** *Curr. Med. Chem*., 12, p. *657*.

[18] Schmidt, M. L.; Kuzmanoff, K. L.; Ling-Indeck, L.; Pezzuto, J. M., 1997. **Betulinic acid induces apoptosis in human neuroblastoma cell lines**. *Eur J. Cancer* 33, p. *2007*.

[19] Fulda S.; Jeremias, I.; Steiner, H. H.; Pietsch, T.; Debatin, K. M., 1998. **Betulinic acid: a new cytotoxic agent against malignant brain-tumor cells**. *Int J. Cancer* 82, p. *435*.

[20] Selzer E.; Pimentel, E.; Wacheck, V.; Schlegel, W.; Pehamberger, H.; Jansen, B.; Kodym, R., 2000. **Effects of betulinic acid alone and in combination with irradiation in human melanoma cells**. *J. Invest. Dermatol*. 114, p. *935*.

[21] Kumar, D.; Mallick, S.; Vedasiromoni, J. R.; Pal, B. C., 2010. **Anti-Leukemicactivity of *Dillenia indica* L. Fruit Extract and Quantification of Betulinic Acid by HPLC**. *Phytomedicine*, (17)*6*, p. *431*.

[22] Tezuka, Y.; Stampoulis, P.; Banskota, A. H.; Awale, S.; Tran, K. Q.; Saiki, I.; Kadota, S., 2000. **Constituents of the Vietnamese Medicinal Plant *Orthosiphon stamineus***. *Chem. Pharm. Bull.*, (48)*11*, p. *1711*.

[23] Wada, S.; Tanaka, R., 2005. **Betulinic Acid and Its Derivatives - potent DNA Topoisomerase II Inhibitors from the Bark of *Bischofia javanica***. *Chem. Biodiversity*, (2)*5*, p. *689*.

[24] Fu, L.; Zhang, S.; Li, N.; Wang, J.; Zhao, M.; Sakai, J. et al., 2005. **Three New Triterpenes from *Nerium oleander* and Biological Activity of the Isolated Compounds**. *J. Nat. Prod.*, (68)*2*, p. *198*.

[25] Seung, H. L.; Choi, J. Y.; MinKyun, N.; Hwang, I. H.; Bae, E. Y.; Kim, B. Y.; Ahn, J. S., 2009. **Isolation of betulinic acid, its methyl ester and guaiane sesquiterpenoids with protein Tyrosine Phosphatase 1B inhibitory activity from the roots of *Saussurea lappa* C.B.Clarke.** *Molecules*, 14, p. *266*.

[26] Setzer, W. N.; Setzer, M. C.; Bates, R. B.; Jackes, B. R., 2000. **Biologically Active Triterpenoids of *Syncarpia glomulifera* Bark Extract from Paluma, North Queensland, Australia**. *Planta Med.*, (66)*2*, p. *176*.

[27] Schühly, W.; Heilmann, J.; Callis, I.; Sticher, O., 1999. **New Triterpenoids with Antibacterial Activity from *Zizyphus joazeiro***. *Planta Med.*, (65)*8*, p. *740*.

[28] Bringmann, G.; Saeb, W.; Assi, L.; A.; Francois, G.; Narayanan, A. S. S.; Peters, K.; Peters, E. M., 1997. **Betulinic Acid: Isolation from *Triphyophyllum peltatum* and *Ancistrocladus heyneanus*, Antimalarial Activitv and Crystal Structure of the Benzyl Ester**. *Planta Med.*, (63)*3*, p. *255*.

[29] Ahmad, F. B. H.; Moghaddam, M. G; Samzadeh-Kermani, A., 2012. **Biological Activity of Betulinic Acid: A Review**. *Pharmacol. Pharm.* 3, p. *119*.

[30] Ma, X. H.; Zhao, Y. C.; Yin, L.; Han, D. W.; Ji, C. X., 1982. **Studies on the effect of oleanolic acid on experimental liver injury.** *Acta Pharm. Sin*. B 17, p. *93*.

[31] Gupta, M. B.; Bhalla, T. N.; Gupta, G. P.; Mitra, C. R.; Bhargava, K. P., 1969. **Anti-inflammatory activity of natural products (I) triterpenoids.** *Eur. J. Pharm*. 6, p. *67*.

[32] Niikawa, M.; Hayashi, H.; Sato, T.; Nagase, H.; Kito, H., 1993. **Isolation of substances from glossy privet (*Ligustrum lucidum* Ait) inhibiting the mutagenicity of benzo(a)pyrene in bacteria.** *Mut. Res.*, 319(*1*), p. *1-9*.

[33] Yoshimi, N.; Wang, A.; Morishita, Y.; Tanaka, T.; Sugie, S.; Kawai, K.; Yamahara, J.; Mori, H., 1992. **Modifying effects of fungal and herb metabolites on azoxymethane-induced intestinal carcinogenesis in rats.** *Jpn. J. Cancer Res*., 83, p. *1273*.

[34] Ishida, M.; Okubo, T.; Koshimizu, K.; Daito, H.; Tokuda, H.; Kin, T.; Yamamoto, T.; Yamazaki, N., 1990. **Topical preparations containing ursolic acid and/or oleanolic acid for prevention of skin cancer**. Chemical Abstracts 113, *12173*y.

[35] Liu, J. K.; Zuo, C. X., 1987. **Studies on the chemical constituents of *Pyrus communis*.** *Acta Bot. Sin.* 29, **p.** *84*.

[36] Hada, S.; Hattori, T.; Namba T., 1990. **Dental caries prevention by traditional medicines: effect of components of *Ganoderma lucidum* on glucosyltransferase from *Streptococcus mutans.*** Chemical Abstracts 113, *91423*f.

[37] Horiuchi, K.; Shiota, S.; Hatano, T.; Yoshida, T.; Kuroda, T.; Tsuchiya, T., 2007. **Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on Vancomycin-Resistant Enterococci (VRE).** *Biol. Pharm. Bull*., 30(*6*), p. *1147*.

[38] Vasilenko, L.; Lisevitskaia, L. I.; Frolova, L. M. et al., 1982. **Hypolipidemic properties of triterpenoids.** *Farmakol. Toksikol*., 45, p. *66*.

[39] Kingston, D. G. I.; Chaturvedula, V. S. P.; Gao, Z.; Hecht, S. M.; Jones, S. H., 2003. **A new acylated oleanane triterpenoid from *Couepia polyandra* that inhibits the lyase activity of DNA Polymerase *β*.** *J. Nat. Prod*., 66, p. *1463*.

[40] Bremner, J. B; Tanachatchairatana, T.; Chokchaisiri, R.; Suksamrarn, A., 2008. **Antimycobacterial activity of cinnamate-based esters of the triterpenes betulinic, oleanolic and ursolic acids.** *Chem. Pharm. Bull*., 56(*2*), p. *194*.

[41] Liu, J., 1995. **Pharmacology of oleanolic acid and ursolic acid.** *J. Ethnopharmacol*. 49, p. *57*.

[42] Ringbom, T.; Segura, L.; Noreen, Y.; Perera, P.; Bohlin, L., 1998. **Ursolic acid from *Plantago major,* a Selective Inhibitor of Cyclooxygenase-2 Catalyzed Prostaglandin Biosynthesis.** *J. Nat. Prod*., 61, p. *1212*.

[43] Janicsák, G.; Veres, K.; Kállai, M.; Máthé, I., 2003. **Gas Chromatographic Method for Routine Determination of Oleanolic and Ursolic Acids in Medicinal Plants.** *Chromatographia*, 58, p. *295*.

[44] Yamaguchi, H.; Noshita, T.; Kidachi, Y.; Umetsu, H.; Hayashi, M.; Komiyama, K.; Funayama, S.; Ryoyama, K., 2008. **Isolation of ursolic acid from apple peels and its specific efficacy as a potent antitumor agent.** *J. Health Sci*., 54(*6*), p. *654*.

[45] Wickholm. N., Alakurtti, S., Yli-Kauhaluoma, J.; Koskimies, S., 2018. **Method for preparation of betulinic acid**. *European Patent Specification,* EP 2 755 989 B1.

[46] Qi-He, C., Jing, L., Hai-Feng, Z., Guo-Qing, H.; Ming-Liang, F., 2009. **The betulinic acid production from betulin through biotransformation by fungi**. *Enzyme Microb. Technol*., 45, p. *175*.

[47] Ressmann, A. K., Kremsmayr, T., Gaertner, P., Zirbs, R.; Bica, K., 2017. **Toward a benign strategy for the manufacturing of betulinic acid**. *Green Chem.*, 19, p. *1014*.

[48] Wu, J.; Niu, Y.;, B, Abdelmoneim.; Li, H.; Chen, Q., 2017. **Cell-free production of pentacyclic triterpenoid compound betulinic acid from betulin by the engineered *Saccharomyces cerevisiae***. *Molecules*, 22(*7*), p. *1075*.

[49] Csuk, R., Schmuck, K.; Schäfer, R., 2006. **A practical synthesis of betulinic acid**. *Tetrahedron Lett.*, 47, p. *8769*.

[50] Murzin, D. Y., Mäki-Arvela, P., Barsukova, M. et al., 2016. **Unprecedented Selective Heterogeneously Catalysed "Green" Oxidation of Betulin to Biologically Active Compounds using Synthetic Air and Supported Ru Catalysts**. *ChemSelect.* 1, p. *3866*.

[51] Moghaddam, M. G.; Ahmad, F. B. H.; Basri, M.; Abdul Rahman, M. B., 2010. **Lipase-catalyzed esterification of betulinic acid using phthalic anhydride in organic solvent media: study of reaction parameters**. *J. Appl. Sci.*, (10)*4*, p. *337*.

[52] Nasri, N.; Tlili, N.; Mejri, H.; Yahia, Y.; Saadaoui, E.; Rejeb, S.; Khaldi, A., 2014. **Phytochemicals and antioxidant activities of *Rhus tripartitum* (Ucria) fruits depending on locality and different stages of maturity.** *Food Chem*. 160, p. *98*.

[53] Correia, S. de J.; David, J. P.; David, J. M., 2006. **Metabólitos secundários de espécies de Anacardiaceae**. *Quim. Nova*, 29, p. *1287*.

[54] Baer, H. **The poisonous Anacardiaceae**. Edited by Kinghorn, A. Douglas. *Toxic Plants* [Symp.], 1979, p. *161-170*.

OBJETIVOS

Esse estudo está subdividido em três seções, cujos objetivos são:

a) Apresentar o desenvolvimento de diferentes métodos de obtenção e quantificação de AB, AO e AU, incluindo a comparação das metodologias de extração aplicadas;

b) Desenvolver a validação analítica do método aplicado na separação e determinação simultâneas dos três ácidos triterpênicos;

c) Fazer um levantamento de ocorrência de AB e subsequente aplicação de diferentes estratégias para a semissíntese de análogos bioativos do AB com elevadas atividades antiplasmodial, anti *Leishmania* e antitripanossômica;

d) Desenvolver uma revisão minuciosa dos aspectos fundamentais referentes à família Anacardiaceae (a qual se constitui uma investigação pioneira), tendo em vista uma contribuição altamente significativa para o estudo desta família nos âmbitos químico, farmacológico e tecnológico, bem como em relação à biossíntese de marcadores quimiotaxonômicos dessa família.

CAPÍTULO 1

DEVELOPMENT OF METHODS EMPLOYING MAE FOR OBTENTION AND QUANTIFICATION OF TRITERPENE ACIDS FROM *Davilla rugosa* AND *Eriope blanchetii*

1. INTRODUCTION

Triterpenes are a group of specialized metabolites of common occurrence in plants, and the most known examples of triterpene acids are ursolic, oleanolic and betulinic acids (UA, OA and BA). These compounds and their derivatives present a broad spectrum of biological activities (Tarvainen, 2010; Mandal and Mandal, 2010) and commercial applicability, once in the last decades a growing development of new patents involving these metabolites can be observed (Xu, 2007; Cho, 1996; Lee, 1997; Scheffler, 2010). Besides, the search for more efficient isolation and purification methods (Frighetto, 2005; Razborsek, 2008; Silvestre, 2006; Domingues, 2011) has also been studied.

Regarding these compounds' occurrence, some triterpene acids have already been found in species of the families Dilleniaceae (e.g., in *Davilla rugosa*) and Lamiaceae such as *Rosmarinus*, *Salvia*, and *Eriope* spp. (Pavanasasivam and Sultanbawa, 1974; David, 2001; David, 2006). *Davilla rugosa* Poir. et St. Hill is a woody bindweed of variable size and popularly known as “cipó-caboclo”, which is widely used in Brazilian folk medicine for various purposes (Corrêa, 1984). On the other hand, the genus *Eriope* is typical of the tropical and subtropical regions of South America and gathers about 20 species (18 of them endemic in Brazil), whose geographic distribution includes areas of rocky fields and vegetation of coastal regions. Considering the genus above, the species *Eriope blanchetii* (Benth.) Harley is an endemic shrub of the Brazilian Northeastern Atlantic Forest (David, 2001; Harley, 2012; Viana, 2002), and it is known to present lignans (α-peltatin, diacetyl-α-peltatin and *β*-peltatin), besides the 3*β*-glycosyl sitosterol and the ursolic and oleanolic acids (Brandão, 2017; David, 2001).

These three triterpene acids exhibit unique and notable biological and pharmacological activities, including anti-inflammatory, antimicrobial, antiviral, cytotoxic and cardiovascular effects (Connolly and Hill, 2008; Huang, 1994; Vechia, 2009, Maia, 2006). The birch cream is employed commercially as a cosmetic that helps delay the signs of aging of the skin, improves the shape of the face, the elasticity and has anti-cellulite properties. These activities are probably due to the anti-elastase

activity of triterpenes such as betulinic and oleanolic acids (Kim, 2009; https://eliveragroup.com/collections/cosmetics-online-beauty-products/products/birch-betulinic-cream-50ml). Thus, given the broad importance of these compounds and the contribution coming from new studies involving the highlighted species, this work presents the development of methods for obtaining and quantifying betulinic acid by HPLC in chloroform extracts from *D. rugosa* stems as well as quantification of oleanolic, ursolic and betulinic acids from $CHCl_3$ extracts from different parts of *E. blanchetii* (leaves, stem, root and leaf petioles). The methodologies developed included the comparison of different techniques of extraction – maceration under heating vs. microwave-assisted extraction (MAE) – under different conditions, with subsequent validation of the chromatographic method used in the study with *E. blanchetii*.

2. MATERIALS AND METHODS

2.1. General procedures

The plant samples (root, stem, leaves and petioles of the leaves from *Eriope blanchetii*, as well as the stems of *Davilla rugosa*) were collected in Abaeté Metropolitan Park region, Salvador, BA, Brazil (12°56'38.6"S 38°21'32.5"W and 12°55'48.5"S 38°20'55.9"W, respectively). The species' vouchers are deposited in the Herbarium Alexandre Leal Costa (Institute of Biology, Federal University of Bahia) under # 045599. Then, all plant materials – *D. rugosa* twigs and root, stem, leaves and petioles of *E. blanchetii* – were dried using a forced ventilation oven (Model 320-SE, FANEM®) and, afterward, powered with the Willye TE-650 Tecnal® mill.

The analyses by thin layer chromatography (TLC) were carried out using silica gel (SiO_2) plates supported on aluminum foil (silica gel 60 F254 sheet, 0.2 mm thick, 2,5 x 7.5 cm, Riedel-deHäen®). After each elution, all the chromatographic plates were exposed to UV radiation in a Spectroline Model CM-10 booth (with a Spectroline Model Enf-260C lamp, at wavelengths of 254 and 365 nm) as well as to Liebermann-

Bürchard reagent for subsequent revelation. This reagent was prepared by mixing 10 mL of concentrated sulfuric acid and 10 mL of acetic anhydride, adding 50 mL of ice bath-cooled ethanol. After the reagent was sprayed onto the chromatographic plates, they were heated in a plate between 100 and 150°C.

In column chromatography (CC), Acros® silica gel 60 (63-200 μm) was used as the stationary phase, so the choice of length of column was made based on the amount of sample that would be used in the preparation of the tablet (1,0 g of chloroform extract on flash silica) with subsequent separation. All collected fractions (75 mL each) were monitored by TLC, gathered according to their respective chromatographic profiles (e.g., R_f similarity), concentrated under reduced pressure in a rotary evaporator and transferred to glass flasks pre-weighed and labeled.

Samples obtained by CC were concentrated on IKA® RV10 Digital (40-50°C, 100-120 rpm) and Buchi Rotavapor RII (50°C, minimum pressure 25 mbar) rotary evaporators. Finally, the solvents (MeOH, $CHCl_3$, CH_2Cl_2, Hex, EtOH, and EtOAc) used in the bench activities (maceration, extraction, TLC, and CC procedures) were analytical grade (Vetec® or QHEMIS®).

The analyses by HPLC-DAD-UV were carried out on 1) the semiUPLC Dionex with a UV-Vis diode array detector (DAD) Mod. Ultimate 3000 and on 2) the Shimadzu mod. 10ADVP LC with SPD-M20A (DAD) UV-Vis detector mod. Prominence. Both studies used the stationary phase of the Dionex C18 Acclaim® 120 (120Å, 1 x 100 mm, particle size 5 μm) column. Furthermore, MeCN: H_2O (9:1) was used as a mobile phase in all Dionex semiUPLC analyses and, likewise, the MeCN: orthophosphoric acid (85%, 0.3% v/v aqueous solution) in all Shimadzu LC chromatographic experiments. In these procedures, the solvents used were MeCN (TEDIA®) and Milli-Q purified water in the Millipore system (resistivity 18.2 $\mu\Omega\ cm^{-1}$), in addition to MeOH (TEDIA® or JT Baker®) for the preparation of corresponding solutions. The triterpene acids (BA, UA, and OA) standards were from Sigma Aldrich® and isolated from the plants. Lastly, all microwave-assisted extraction (MAE) procedures were made using the equipment CEM model Discover®-SP, W/Activent (SN: DC6562) at the adequate frequency between 50-60 Hz.

2.2. Obtention of the betulinic acid-enriched fraction from *D. rugosa* stems and chloroform soluble fractions from different parts of *E. blanchetii*

Firstly, hexane and $CHCl_3$ extracts were prepared from the dried stems/twigs of *D. rugosa*, so that the $CHCl_3$ soluble extract was used to obtain the BA enriched fraction by CC separation, which included the preparation of a silica pellet with 1.0 g of the extract. The CC was eluted with Hex: EtOAc (6:4) mixture and 43 fractions (50 mL each) were collected. TLC accomplished the collected fractions, what has permitted the grouping of similar ones. The 2^{nd} to 9^{th} fractions were grouped (87.4 mg, after drying) due to the presence of BA when compared with the standard.

All dried parts of *E. blanchetii* were properly powdered, obtaining 157.09 g from leaves, 435.56 g from stems, 43.38 g from petioles and about 30 g from roots. Afterward, subsequent extractions were performed with each related material. In particular, the extractions of *E. blanchetti* leaves were performed in triplicate (20 g of leaves, 10 g of petioles, 50 g of stems, and 5 g of roots in each extraction) and extracted with 150 mL of a MeOH:H_2O mixture (8:2) in a system under magnetic stirring at 50°C for 1h. After this step, the hydromethanolic extracts were submitted to simple filtration and the filtrates were concentrated to obtain three extracts. Sequentially, these extracts were individually treated with 25 mL of a MeOH: H_2O (6:4) mixture and, later, partitioned between $CHCl_3$ (3x, 30 mL for each extract) and hydroalcoholic solution. The hydromethanolic phases were discarded, and the $CHCl_3$ soluble fractions were finally stored. After drying, the fractions EBF01 (278.6 mg), EBF02 (244.8 mg) and EBF03 (249.7 mg) were obtained from leaves, EBPA01 (86.0 mg), EBPA02 (77.4 mg) and EBPA03 (74.8 mg) from petioles, EBCC3.1 (144.8 mg), EBCC3.2 (144.1 mg) and EBCC3.3 (144.0 mg) from stems and EBRC01 (58.1 mg), EBRC02 (27.4 mg) and EBRC03 (36.3 mg) from roots.

2.3. Quantification of betulinic acid-enriched fraction from *D. rugosa* stems

The betulinic acid standard was analyzed under the conditions established in the external standardization method. Firstly, five solutions of different concentrations (0.7,

0.35, 0.175, 0.0875 and 0.0438 mg mL^{-1}) of the standard were prepared by successive dilutions and analyzed in triplicate on the DIONEX semiUPLC equipment during 6.82-10.0 min, with sequential autoinjection and isocratic elution with MeCN:H_2O (9:1) at 0.300 mL/min, monitoring at 205 nm and 5.0 µL injection volume. After the analyses of the standard solutions, a solution of the enriched fraction (1.0 mg mL^{-1}) was prepared to be analyzed in triplicate under the same chromatographic conditions.

2.4. HPLC-DAD-UV analyses of BA, UA and OA of all parts of *E. blanchetii*

Three standard stock solutions (BA 0.13 mg mL^{-1}, OA 0.12 mg $mL^{-1,}$ and UA 0.11 mg mL^{-1}) were prepared in 10 mL volumetric flasks. In sequence, 1.0 mL of each stock solution was transferred into another 10 mL volumetric flask and the volume was made up with MeOH to prepare a solution of the acid mixture (solution M, at the approximate concentration was 10.0 µg mL^{-1}), whose analysis was performed to verify the chromatographic profile of the respective standards.

Similarly, solutions of the $CHCl_3$ soluble fractions from the extracts of leaves, petioles, stem and roots of *E. blanchetii* (each one at 50.0 µg mL^{-1}) were prepared (after dilution of the respective stock solutions at 0.2 mg mL^{-1}) and analyzed together with solution M by HPLC. The chromatographic method used included automatic injection followed by elution with MeCN:aqueous solution of 85% orthophosphoric acid (0.3% (v/v)), in gradient mode (30-100% MeCN), injection volume 5.0 µL, mobile phase flow 0.500 mL/min, monitoring at 210 nm and analyses of 36 min.

2.5. Analyses of triterpene acids' standards: calibration curves

Initially, the solution concentrations of each of the standards were determined from the results of the previous analyses, whose values were 2.0, 0.5, 0.2, 0.1 and 0.05 µg mL^{-1} (concerning ursolic acid), 50.0, 24.8, 4.8, 3.2 and 1.6 µg mL^{-1} (relatively to oleanolic acid) and 50.0, 24.0, 12.0, 1,0 and 0.5 µg mL^{-1} (for betulinic acid). Subsequently, the standard solutions were adequately prepared with HPLC grade methanol in volumetric flasks (10 mL) and transferred (1.0-1.3 mL of solution) with the

aid of a syringe and a 0.25 mm Sartorius® cartridge for previously identified vials, so that all solutions were analyzed via HPLC-DAD-UV under the same conditions aforementioned.

2.6. Comparison between microwave-assisted extraction (MAE) and maceration with heating extraction

In order to compare the efficiency of extraction, three different extraction methodologies were elaborated and later evaluated. Thus, two different strategies involving MAE and an alternative extraction method by maceration under heating were compared. Microwave extractions were performed in a 10 mL Pyrex pressure vial for closed vessel experiments under the indicated power automatically to reach and maintain the temperature set specified in each case, with IR temperature control and medium stirring speed using cylindrical stir bars (10 x 3 mm) and 10 min default ramp time.

The first methodology was based on MAE using the MeOH:H_2O (8:2) system starting from 1.0 g of *E. blanchetii* leaves in each extraction in triplicate. Three portions of plant material were transferred to three glass tubes (35 mL), to which 14.5 mL (3x) of the MeOH:H_2O (8:2) mixture was added in a microwave system. The extractions were completed in 30 min at specific temperatures (T_1 = 50ºC, T_2 = 100ºC, and T_3 = 125°C) to obtain EBF.MWM1 (87.2 mg), EBF.MWM2 (130.2 mg) and EBF.MWM3 (216.4 mg) extracts, respectively. These extracts were submitted to simple filtration, treated with 14.5 mL (3x) of a MeOH: H_2O (6:4) mixture and partitioned between $CHCl_3$ (3x, 10 mL for each extract). Then, the $CHCl_3$ soluble fractions were concentrated and transferred to properly weighed glass flasks furnishing the EBFCMO1 (42.2 mg), EBFCMO2 (44.6 mg) and EBFCMO3 (46.0 mg) samples.

Similarly, another triplicate extraction via MAE was carried out from three 1.0 g portions of plant material, but with 14.5 mL (3x) of a MeOH:acetone (1:1) mixture and a constant temperature of 50ºC in all extractions. The crude extracts obtained – EBF.MAM1 (64.6 mg), EBF.MAM2 (73.6 mg) and EBF.MAM3 (57.8 mg) – were filtered and concentrated, being subsequently treated with 14.5 mL (3x) MeOH:H_2O (6:4) and partitioned with $CHCl_3$ as in the preceding example. After the concentration

of the $CHCl_3$ phases, the samples EBFCMO4 (13.2 mg), EBFCMO5 (62.8 mg) and EBFCMO6 (32.0 mg) were obtained.

The extraction by maceration under heating was achieved from 1.0 g of *E. blanchetii* leaves, whose material was extracted 14.5 mL (3x) of the MeOH:acetone (1:1) mixture were added in a system under heating (50°C) and magnetic stirring for 30 min (in triplicate). The EBF.MA01 (47.5 mg), EBF.MA02 (43.9 mg) and EBF.MA03 (43.6 mg) extracts were filtered and concentrated, treated with MeOH:H_2O (6:4, 14.5 mL, 3x) and partitioned with $CHCl_3$ (3x, 10 mL to each extract). The chloroform phases were concentrated and transferred to pre-weighed glass flasks. Thus, the samples EBFCMA1 (26.3 mg), EBFCMA2 (19.1 mg) and EBFCMA3 (28.7 mg) were obtained. In all extraction sets, hydromethanolic phases were discarded after $CHCl_3$ partition procedures.

2.7 HPLC-DAD-UV analyses of chloroform soluble fractions obtained from MAE and heated maceration.

After obtaining the chloroform phases mentioned in the previous item, solutions of each sample were prepared (50.0 µg mL^{-1}). These solutions were transferred to identified flasks with a syringe and a PVDF Whatman Uniflo® cartridge (0.45 mm) and then analyzed in HPLC/DAD under the same conditions as sub-item 2.4.

2.8. Validation parameters

The validation of the HPLC method developed with *E. blanchetii* was performed using manual injection mode (volume at 10-15 µL), with mobile phase flow at 0.150 mL/min. Besides, a new chromatographic column (Dionex C-18 Acclaim® 300, 300Å, 2.1 x 50 mm, 3 µm p.s) was used as stationary phase, whose features were slightly different from those in the column mentioned in topic 2.1. In this work, the resolution RE nº 899/2003 (from ANVISA/Brazil) was followed (Ribani, 2004).

2.8.1. Linearity and Selectivity

Linearity was determined by constructing two analytical curves using the external standardization method, so that at least five distinct BA standard concentrations were used for each curve. According to topic 2.6, the first curve (curve A) was prepared at concentrations of 0.5, 1.0, 12.0, 24.0 and 50.0 μg mL^{-1}. Moreover, the curve B was constructed after the abovementioned modifications (Figure 4) at the concentrations of 4.0, 12.0, 24.0, 50.0 and 100.0 μg mL^{-1}. All solutions were prepared in HPLC methanol (TEDIA® or J. T. Baker®). On the other hand, the selectivity of the method was evaluated by comparing retention times (R_t) and UV spectra (homogeneity in the ascending, apical and descending regions) of the BA standard and EBF01 (20.0 μg mL^{-1} solution).

2.8.2. Limit of Detection (LD) and Limit of Quantification (LQ)

The curve B parameters were considered together with the equations (1) and (2) to determine the respective figures of merit:

(1) LD = 3.3 x (SDe/Ca) and (2) LQ = 10 x (SDe/Ca)

wherein SDe is the estimated standard deviation of the regression line equation and Ca is the angular coefficient of the analytical curve.

2.8.3. Accuracy

The accuracy of the method was verified via recovery assays, in which three EBF01 solutions (20.0 μg mL^{-1}) were strengthened with three different BA standard concentrations – 2.0 μg mL^{-1} (low), 10.0 μg mL^{-1} (intermediate) and 20.0 μg mL^{-1} (high). Afterward, all these samples were analyzed (at least) in triplicate – nine times. Then, the recovery rates were determined considering the results obtained for the standard of interest.

2.8.4. Precision

The precision of the chromatographic method was evaluated by “intra-run” and “inter-run” experiments using BA standard solutions at concentrations of 10.0, 50.0, and 200.0 μg mL^{-1} (low, medium and high, respectively).

Intraday precision was assessed through a series of triplicate analyses of each standard solution (nine analyses in total) on the same day, by the same experimenter and under the same methodological and instrumental conditions. Likewise, interday precision was assessed by three-day non-consecutive analysis in triplicate of the standard solution (50.0 μg mL^{-1}, nine runs in total).

2.8.5. Robustness

The robustness assay was made to evaluate the analytical system's behavior in the face of small changes in optimal operating conditions. In this experiment, a betulinic acid solution (100.0 μg.mL^{-1}) was used in all experiments and analyzed under the optimized chromatographic conditions after the former methodological modifications.

The robustness assessment performed deliberate changes only in the mobile phase flow rate (from 0.150 mL/min to 0.100 mL/min). Thus, it is noteworthy that this evaluation was simplified without involving a more detailed statistical treatment or a more comprehensive strategy (e.g., the Youden Test recommended by INMETRO, Brazil) (Ribani, 2004), so that the chromatograms obtained from the corresponding R_t values and UV spectra were compared.

3. RESULTS AND DISCUSSION

3.1. Validation of the analytical method

The analytical validation (validation "in house") was made through the evaluation of the parameters of selectivity and linearity, detection and quantification limits (LD and LQ), accuracy (by recovery assays), precision intra and interday and robustness (see Table 1). The validation was performed using betulinic acid as a standard due to its relative abundance in *E. blanchetii* and, since it has accumulated preferably in the leaves, the corresponding $CHCl_3$ soluble fractions of the extracts were used. Verification of the performance efficiency of the analytical method requires the assessment of repeatability. In this case, from the coefficient of variation data (or relative standard deviation, CV (%)) referring to the area and Rt values present in Table 1 – which should be less than 15%, so that smaller values indicate good selectivity –, it can be confirmed that the method presents satisfactory performance in terms of repeatability, as well as being selective for the substance of interest. However, the CV value (%) concerning the peak area is reasonably high.

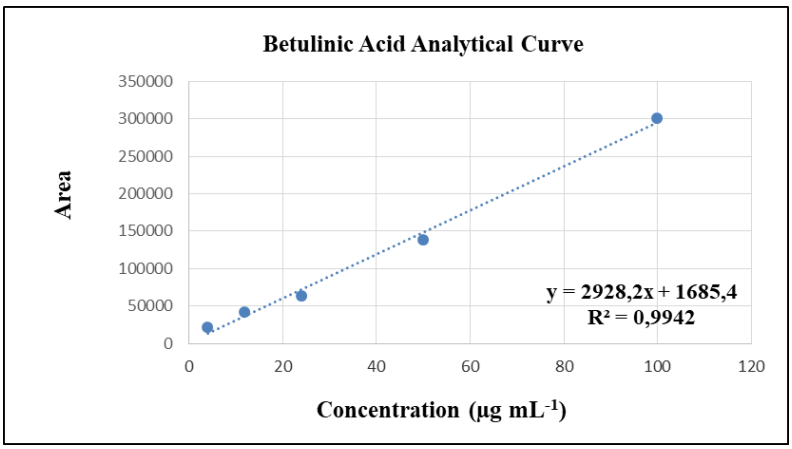
Table 1. Validation parameters of the chromatographic method used to quantify BA in the $CHCl_3$ extracts of *E. blanchetii* leaves. (SD: standard deviation; CV: coefficient of variation; % Rec: recovery percentage; CVm: medial CV)

Selectivity	**Major peak (EBF01 20.0 µg mL^{-1})**	**Average value (n = 3) ± SD**	**CV (%)**	-	-
	Area	6.2274 ± 0.5879	9.44	-	-
	R_t	6.4283 ± 0.0564	0.88	-	-
LD (µg mL^{-1})	4.1096	-	-	-	-
LQ (µg mL^{-1})	12.4535	-	-	-	-
Accuracy (recovery assays)	**Sample conc. (µg mL^{-1})**	**Added conc. (µg mL^{-1})**	**Detected conc. (µg mL^{-1})**	**% Rec**	**CV (%)**
	0.2276	2.0	1.8675	81.99	12.43
	0.2276	10.0	10.2812	100.54	0.37
	0.2276	20.0	20.3211	100.47	0.33
Intraday precision	**Concentration (µg mL^{-1})**	**CV (%)**	-	-	-
	10.0	2.09	-	-	-

	50.0	0.85	-	-	-
	200.0	0.99	-	-	-
Interday precision	**Concentration (µg mL^{-1})**	**CV (%) 1st day**	**CV (%) 2nd day**	**CV (%) 3rd day**	**CV$_m$ (%)**
	50.0	0.85	0.66	1.47	0.99
Robustness	**Concentration (µg mL^{-1})**	**CV (%)**	**-**	**-**	**-**
	100.0	3.67	-	-	-

Although the robustness assessment usually involves changing upon more than one analytical parameter to obtain a more reliable result as well as a subsequent more statistically sophisticated data processing, the results obtained might indicate that the method is satisfactorily robust, since the chromatographic profiles were similar to each other. Thus, based on Table 1, it is also inferred that the method is robust since the CV (%) is less than 5% for the concentration tested (which indicates that the change in mobile phase flow rate did not meaningfully interfere with the method precision), as well as the fact that the chromatographic profiles and their UV spectra are analogous or corresponding.

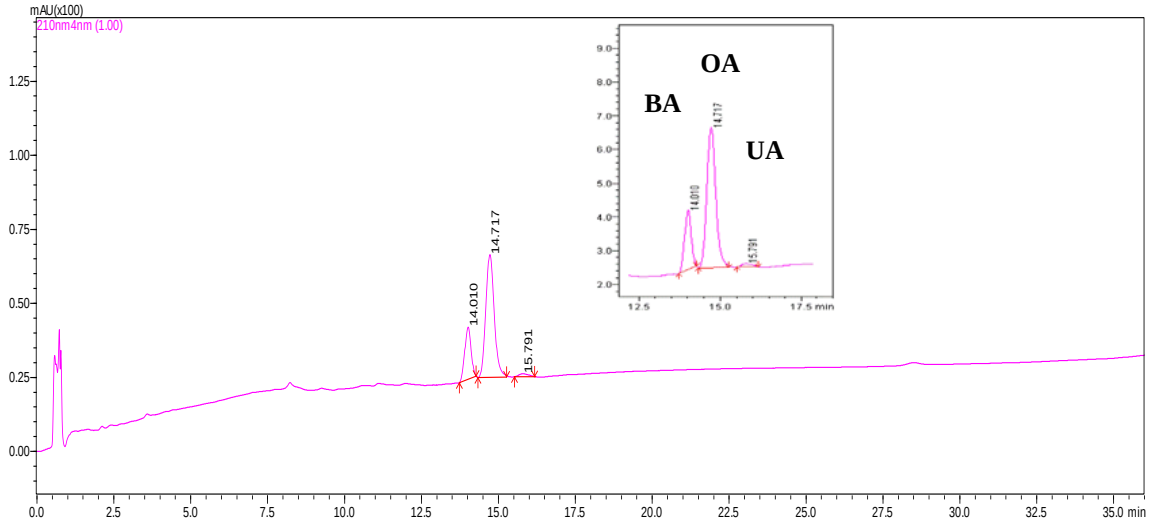
Figure 4. Analytical curve of BA regarding to the linearity assays (4.0, 12.0, 24.0, 50.0 and 100.0 µg mL^{-1})



3.2. Development of the method for simultaneous separation of triterpene acids

The simultaneous separation of BA, OA, and UA has been performed by triplicate analysis of samples according to the previously developed and described method, whose chromatographic profile is shown in Figure 5. The peaks for each substance showed an adequate resolution (although the respective R_t values were relatively close), confirming that the developed method was suitable for the separation of the analytes in the concentration range applied.

Figure 5. Chromatogram for the mixture of BA (R_t = 14.010), OA (R_t = 14.717), and UA (R_t = 15.791)



3.3. Quantification of the betulinic acid obtained from *D. rugosa* stems

The TLC analysis of the fractions obtained by CC (including their respective groupings) indicated the fractions containing BA when the plates were compared with the standard. After the BA standard solutions analysis, different chromatograms were obtained, in such a way that their respective UV profiles and Rt were analogous in all experiment series. Then, the corresponding standard curve was constructed from the mean peak area and concentration values (y = 142.41x + 3.6002, R^2 0.993, Figure 6). Similarly, chromatograms and UV spectra for the standard and the enriched fraction (DR2-9) containing BA showed similar profiles (Figure 7). These fractions are constituted mainly by betulinic acid, whose matrix effect conditions can often be

reflected in slight variations in R_t values. Thus, the concentration and amount of BA determined in DR2-9 were approximately 0.56 mg mL^{-1} and 48.53 mg, respectively. Subsequently, the percentage of AB was determined concerning the $CHCl_3$ soluble fraction (0.92 g), the crude methanolic extract (21.66 g), and the weight of dry plant material (201.42 g), whose respective values were 5.275, 0.224 and 0.024%. Therefore, the methodology employed proved to be simple and provided quick analyses, whose results reiterate the possibility of using *D. rugosa* as a natural source of BA in reasonable yields.

Figure 6. Analytical curve for BA (concentration range of 0.0438-0.7000 mg mL^{-1})

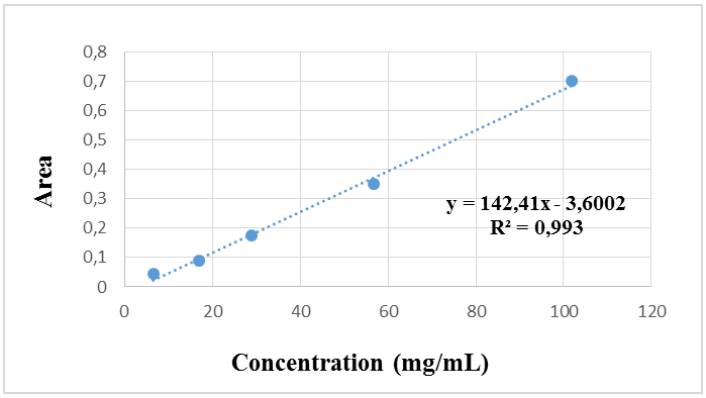
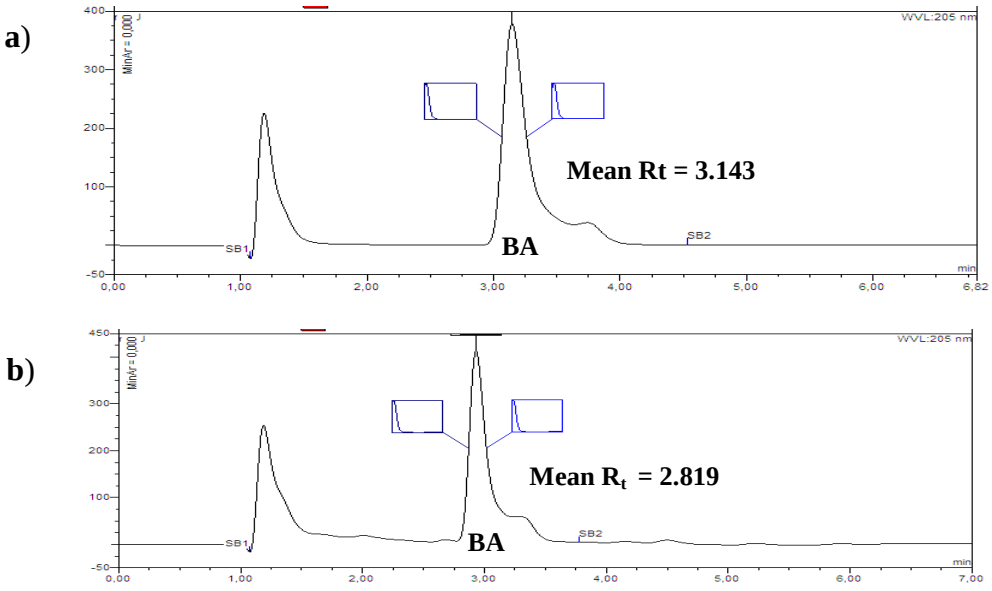
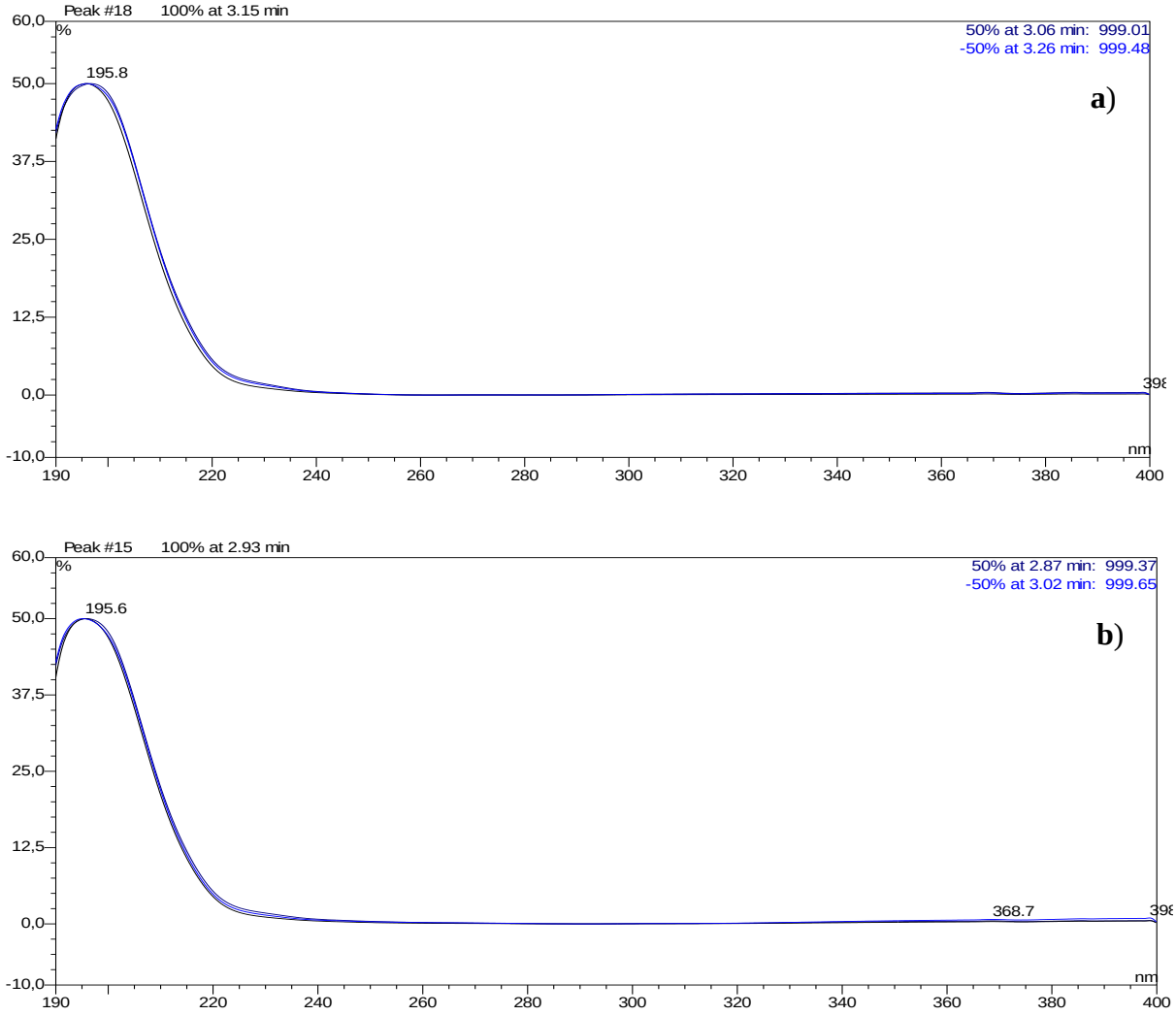


Figure 7. HPLC-DAD-UV data for betulinic acid standard (a, 0.7 mg mL^{-1}) and DR2-9 (b, enriched fraction, 1.0 mg mL^{-1})





3.4. Detection and quantification of triterpene acids in fractions from all parts of *E. blanchetii*

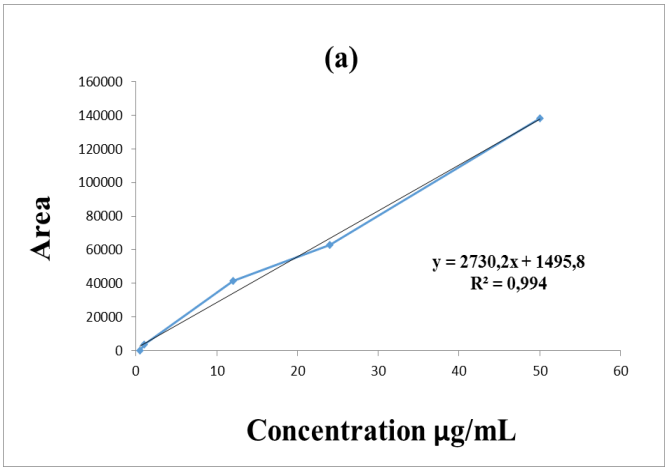
The analytical curves corresponding to BA, OA and UA (Figure 8) – were constructed, and since betulinic acid was identified in both species, this compound was quantified in all extracts from all parts of *E. blanchetii*. The relation of the respective values of concentrations and quantities of BA, OA and UA regarding to the $CHCl_3$ soluble fractions from all studied parts of *E. blanchetii* are described in Table 2.

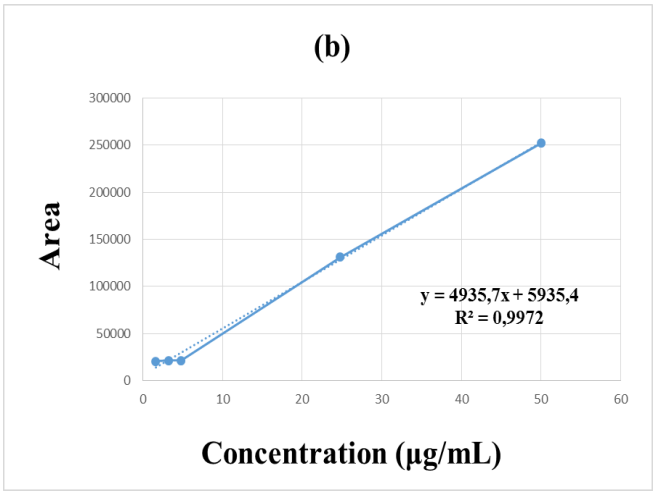
Table 2. BA, OA and UA average concentrations (µg mL^{-1}) and average amounts (mg/g dry plant material) from the $CHCl_3$ soluble fractions of all studied parts of *E. blanchetii*

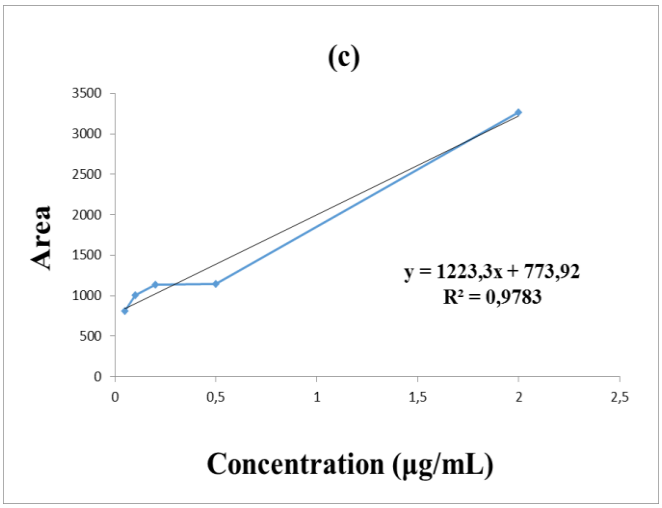
Sample / Triterpene acid amounts	Leaves	Petioles	Stem	Roots
BA concentration ± SDc	29.020 ± 0.645	14.7055*	1.6251 ± 0.0985	n.d.
BA quantity ± SDq	14.893 ± 1.940	2.5294*	0.4690 ± 0.0284	n.d.
OA concentration ± SDc	n.d.	20.3597 ± 1.5103	3.3514*	n.d.
OA quantity ± SDq	n.d.	3.3764 ± 0.2686	0.9706*	n.d.
UA concentration ± SDc	0.4959 ± 0.0419	0.3933 ± 0.0823	0.4526 ± 0.0502	0.4245 ± 0.0321
UA quantity ± SDq	0.2469 ± 0.0172	0.0642 ± 0.0139	0.1310 ± 0.0146	0.0310 ± 0.0023

n.d.: not detected; (SDc: standard deviation of concentration; SDq: standard deviation of quantity); *: data without SD

Figure 8. Analytical curves for BA (a, 0.5-50.0 µg mL^{-1}), OA (b, 1.6-50.0 µg mL^{-1}) and UA (c, 0.05-2.00 µg mL^{-1})

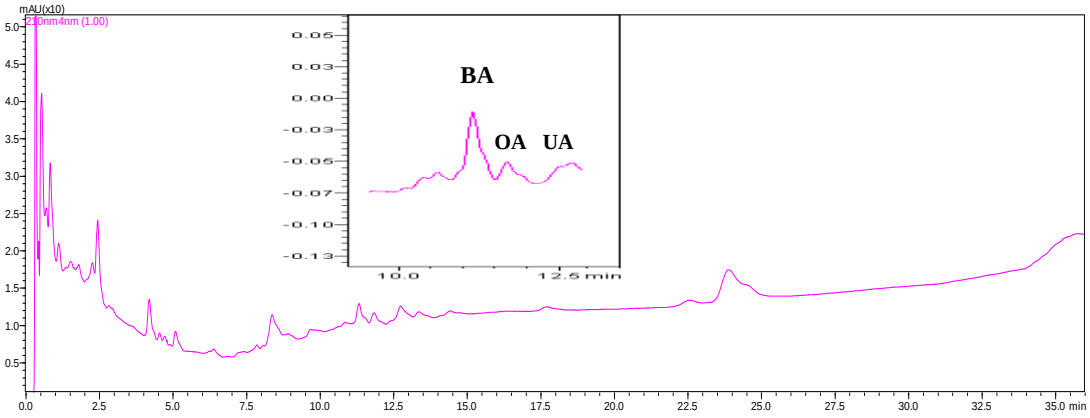






To date, Figure 9 presents the chromatographic profile of $CHCl_3$ extracts of *E. blanchetii* stems (EBCC3.1, EBCC.3.2 e EBCC3.3).

Figure 9. Chromatographic profile of $CHCl_3$ soluble fractions of *E. blanchetii* stems.



According to the Table 2 data, the average amount of BA determined in the $CHCl_3$ soluble fractions (EBF01, EBF02, and EBF03) of *E. blanchetii* leaves was about 5.78% and its relative amount in plant material was approximately 0.025%, so that BA was the most predominant compound amongst the acids in the plant. Besides, BA was detected in petioles and stems in lower quantities, but was absent in root extracts. Moreover, oleanolic acid was predominantly identified in leaf petiole samples and in EBCC3.1, but not in leaf and root extracts. In this case, OA was obtained as the major constituent in the petioles of *E. blanchetii*, so that its relative amount (%) in $CHCl_3$ fractions as well as in the dry material was, respectively, 4.250 and 0.011. However, although the OA represents 0.67% of the mass of the EBCC3.1 sample, its occurrence in the stems was less significant than the AB's, since it was not detected in all stem $CHCl_3$ extracts. Lastly, based on the obtained data, UA was the only compound identified in all parts of *E. blanchetii*, although it appears as the minor constituent amongst the triterpene acids in most samples obtained. On the other hand, the Figure 9 ilustrates the efficiency of the HPLC-DAD-UV method applied therein, since BA, OA and UA were simultaneously detected and appropriately separated.

3.5. Comparison of the efficiency between MAE and maceration extraction methodologies

The efficiency of each methodology was evaluated according to the extraction yields (y, %) of the plant material used (1.0 g) in each extraction (Table 3). Moreover, the relative yield (ρ) values between the following pairs of crude extracts obtained under the same conditions (EBF.MAM1 and EBF.MA01; EBF.MAM2 and EBF.MA02; EBF.MAM3 and EBF.MA03) are shown in Table 4.

Table 3**.** Percentage values of yield (w/w) relating to the MAE with MeOH:H_2O (8:2) and MeOH:acetone (1:1) and the maceration under heating with MeOH:acetone (1:1)

Condition/System	Temperature (°C)	Extract	Yield (%)
	50	EBF.MWM1	8.72

MAE MeOH:H_2O (8:2)	100	EBF.MWM2	13.02
	125	EBF.MWM3	21.64
MAE MeOH:acetone (1:1)	50	EBF.MAM1	6.46
		EBF.MAM2	7.36
		EBF.MAM3	5.78
Heated maceration	50	EBF.MA01	4.75
		EBF.MA02	4.39
		EBF.MA03	4.36

Table 4. Percentage values of extraction yield with respective SD concerning the pairs of extracts obtained with MeOH:acetone (1:1). (SD: standard deviation)

Extract pair	**EBF.MAM1/EBF.MA01**	**EBF.MAM2/EBF.MA02**	**EBF.MAM3/EBF.MA03**
Relative yield (ρ, %)	(1.36 ± 0.04)	(1.68 ± 0.04)	(1.33 ± 0.04)

From the aforementioned data, it was confirmed that, under certain standard conditions (type and volume of solvent used, temperature and extraction time, as well as the amount of starting material), MAE was subtly more efficient (for a factor of almost 1.5) compared to maceration under heating, which can be justified from factors related to extraction mechanisms.

In MAE, fast and localized sample heating (whose process predominantly includes molecular rotation and/or ion migration due to the establishment of permanent/induced dipoles in the molecules/ions of the material components) may lead to an increased pressure within the extraction container, so that its constituents are transferred more quickly to the extracting solvent. However, in a conventional heating process, the extraction usually involves conduction and convection processes followed by heating through sample surface, so that the removing of plant material components by extracting solvent works out less effectively. In this case, MAE using MeOH:H_2O (8:2) was the most efficient extraction by the data obtained.

On the other hand, concerning the MeOH:H_2O (8:2) MAE, it is observed that the yield values (r,%) of the extracts were higher as the extraction temperature increased (see Table 3). Knowing that the gradual increase in temperature raises the average kinetic energy of the molecules in the medium – which implies stronger interactions between the solvent and the corresponding constituents –, it is confirmed that the extractions performed at higher temperatures are usually more efficient. In conclusion, it must be highlighted that the microwave-assisted extractions with MeOH:acetone (1:1) were carried out at 50°C due to operational limitations, which have prevented extraction at temperatures above 60ºC under the corresponding conditions.

3.6. Quantification of BA in $CHCl_3$ soluble fractions of extracts obtained by MAE and by maceration with heating from *E. blanchetii*

Although the analytical curves for all three acids were obtained, only the quantification of BA in these respective fractions was performed (as it is shown in Table 5):

Table 5. Relation of BA concentration values and its respective quantities contained in the $CHCl_3$ soluble fractions obtained by MAE and by maceration of *E. blanchetii* leaves

Sample (50 µg/mL)	Concentration (µg/mL)	Mass (mg)
EBF.CMO1	0.1979	0.1670
EBF.CMO2	0.0396	0.0353
EBF.CMO3	0.0609	0.0560
EBF.CMO4	0.5165	0.1364
EBF.CMO5	0.4004	0.5029
EBF.CMO6	0.0155	0.0099
EBF.CMA1	0.0970	0.0510
EBF.CMA2	0.1142	0.0436
EBF.CMA3	0.1556	0.0893

The results allowed to infer that the most efficient extraction methodology, in terms of concentration of BA, was the MAE using MeOH:acetone (1:1). However, although MAE with MeOH:H_2O (8:2) at 125°C showed the best total yield (w/w), BA was determined in slightly higher amounts in the $CHCl_3$ soluble fractions derived from the extracts obtained by MAE with MeOH:acetone (1:1) at 50°C. This fact probably accrues from the effect of the temperature or the polarity difference of the solvent mixtures used, so that BA could be preferably extracted at lower temperatures and/or by using medium polar solvents. In these cases, we can avoid the possibility of degradation of these metabolites or extractions under harsher conditions and, since these acids have an intermediate or low polarity, their extraction can be more efficient. Table 6 shows the contents of BA in the samples wherein it was obtained.

Table 6. Relation of the percentage contents of BA and some of their respective $CHCl_3$ soluble fractions

Sample	**EBF.CMO1**	**EBF.CMO4**	**EBF.CMO5**
Yield (r, %)	0.396	1.033	0.801

Thus, the data presented in Table 6 underscore the inference that MAE with MeOH: acetone (1:1) resulted in richer extracts of triterpene acids (particularly BA), so that EBF.CMO4 sample contains 1.03% of BA (w/w). Therefore, it can be supposed that MAE at 50°C was the most suitable alternative among the evaluated methodologies.

CONCLUSIONS

The chromatographic methods developed were efficient in the identification, separation, and quantification of oleanolic (OA), ursolic (UA), and betulinic (BA) acids in $CHCl_3$ extracts from different parts of *Eriope blanchetii* and from *Davilla rugosa*

stems. In addition, the method used in *E. blanchetii* investigation has been satisfactorily validated on a laboratory scale according to the parameters required for this purpose, so that it can later be applied in similar studies. On the other hand, from the comparison between MAE and maceration under heating, it was concluded that MAE was the most efficient methodology, inasmuch as the extraction with MeOH:H_2O (8:2) showed a higher yield than that with MeOH:acetone (1:1) under similar conditions, being more favored at higher temperatures (125°C). However, concerning the quantification, it was found that BA was determined in higher quantities in the extracts obtained by MAE with MeOH: acetone (1:1) under milder conditions (50ºC).

REFERENCES

[1] Tarvainen, M.; Jukka-Pekka, S.; Kallio, H.; Yang, B., 2010. **Triterpene acids in *Plantago major*: Identification, quantification, and comparison of different extraction methods**. *Chromatographia* 71, p. *279*. http://dx.doi.org/10.1365/s10337-009-1439-7

[2] Mandal, V.; Mandal, S. C., 2010. **Design and performance evaluation of a microwave based low carbon yielding extraction technique for naturally occurring bioactive triterpenoid: oleanolic acid**. *Biochem. Eng. J.*, 50, p. *63*. https://doi.org/10.1016/j.bej.2010.03.005

[3] Xu, Ze-Qi.; Koohang, A.; Mar, A. A.; Majewski, N. D.; Eiznhamer, D. A.; Flavin, M. T., 2007. **Synthetic pentacyclic triterpenoids and derivatives of betulinic acid and betulin**. *Patent Application Publication*, US 2007/0232577 A1, 83 pp..

[4] Cho, S. H.; Gottlieb, K.; Santhanam, U., 1996. **Cosmetic compositions containing betulinic acid**. *Patent Application Publication*, US 005529769 A, EP 717983 A1, 14 pp..

[5] Lee, K-H.; Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Manak, M., 1997. **Betulinic acid and dihydrobetulinic acid derivatives and uses therefor**. *Patent Application Publication*, US 005679828 A, 10 pp..

[6] Scheffler, A.; Jäeger, S.; Beffert, M.; Hoppe, K., 2010. **Method for preparing an aqueous solution containing triterpenic acid (aqueous solution containing triterpenic acid) and use thereof.** US *Patent Appl. Publication*, US 2010/0104669 A1, 6 pp., 2010.

[7] Friguetto, N.; Welendorf, R. M.; Da Silva, A. M. P.; Nakamura, M. J.; Siani, A. C., 2005. **Purification of betulinic acid from *Eugenia florida* (Myrtaceae) by High-Speed Counter-Current Chromatography.** *Phytochem. Anal*. 16, p. *411*. https://doi.org/10.1016/10.1002/pca.860

[8] Razboršek, M. I.; Voncina, D. B.; Dolecek, V.; Voncina, E., 2008. **Determination of oleanolic, betulinic and ursolic acid in Lamiaceae and Mass Spectral Fragmentation of their trimethylsilylated derivatives**. *Chromatographia* 67, p. *433*. https://doi.org/10.1365/s10337-008-0533-6

[9] Silvestre, A. J. D.; Sousa, A. F.; Pinto, P. C. R. O.; Pacoal-Neto, C., 2006. **Triterpenic and other lipophilic components from industrial cork byproducts**. *J. Agric. Food Chem*. 54, p. *6888*. https://doi.org/10.1021/jf060987

[10] Domingues, R. M. A.; Sousa, G. D. A.; Sousa, C. M.; Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P., 2011. **High value triterpenic compounds from the outer barks of several *Eucalyptus* species cultivated in Brazil and in Portugal**. *Ind. Crops Prod*. 33, p. *158*. https://doi.org/10.1016/j.indcrop.2010.10.006

[11] Pavanasasivam, G.; Sultanbawa, M. U. S., 1974. **Chemical investigation of ceylonese plants. IX. Betulinic acid in the Dilleniaceae and a review of its natural distribution**. *Phytochemistry* 13, p. *2002*. https://doi.org/10.1016/0031-9422(74)85143-5

[12] David, J. P. L.; Da Silva, E. F.; De Moura, D. L.; Guedes, M. L. S.; Assunção, R. J.; David, J. M., 2001. **Lignanas e triterpenos do extrato citotóxico de Eriope blanchetii**. *Quim*. *Nova* 24, p. *730*. https://doi.org/10.1590/S0100-40422001000600004

[13] David, J. M.; Souza, J. C.; Guedes, M. L. S.; David, J. P, 2006. **Estudo fitoquímico de *Davilla rugosa*: flavonoides e terpenoides**. *Rev*. *Bras*. Farmacogn. 16, p. *105*. https://doi.org/10.1590/S0102-695X2006000100019

[14] Corrêa, M. P., 1984. **Dicionário das Plantas Úteis do Brasil**. Ed. Ministério da Agricultura, *Instituto Brasileiro de Desenvolvimento Florestal*, Brasília – DF, vol. 2, p. *266*.

[15] Harley, R.; França, F.; Santos, E. P.; Santos, J. S. **Lamiaceae in Lista de Espécies da Flora do Brasil, Jardim Botânico do Rio de Janeiro**. *Jardim Botânico do Rio de Janeiro*, http://floradobrasil.jbrj.gov.br (accessed 11 April 2023).

[16] Viana, B. F.; Kleinert, A. D. M. P.; Silva, F. O. D., 2002. **Ecologia de Xylocopa (Neoxylocopa) cearensis (Hymenoptera, Anthophoridae) nas Dunas Litorâneas de Abaeté, Salvador, Bahia**. *Iheringia – Série Zoológica* 92, p. *47*. https://doi.org/10.1590/S0073-47212002000400007

[17] Brandão, H. N.; Medrado, H. H. S.; David, J. P.; David, J. M.; Pastore, J. F. B.; Meira, M., 2017. **Determination of podophyllotoxin and related aryltetralin lignans by HPLC/DAD/MS from Lamiaceae species**. *Microchem*. *J*. 130, p. *179***.** https://doi.org/10.1016/j.microc.2016.09.002

[18] Connolly, J.; Hill, R., 2008. **Triterpenoids**. *Nat*. *Prod*. *Rep.* 25, p. *794*. https://doi.org/10.1039/B718038C

[19] Huang, M-T.; Ho, C-H.; Wang, Z. Y.; Ferraro, T.; Lou, Y-R.; Stauber, K.; Ma, W.; Georgiadis, C.; Laskin, J. D.; Conney, A. H., 1994. **Inhibition of Skin Tumorigenesis by Rosemary and Its Constituents Carnosol and Ursolic Acid**. *Cancer Res*. 54, p. *701*.

[20] Vechia, L. D.; Gnoatto, S. C. B.; Gosmann, G., 2009. **Oleanane and ursane derivatives and their importance on the discovery of potential antitumour, anti-inflammatory and antioxidant drugs**. *Quim*. *Nova* 32, p. *1245*. https://doi.org/10.1590/S0100-40422009000500031

[21] Maia, J. L.; Lima-Junior, R. C. P.; David, J. P.; David, J. M.; Santos, F. A.; Rao, V. S., 2006. **Oleanolic Acid, a Pentacyclic Triterpene Attenuates the Mustard Oil-Induced Colonic Nociception in Mice**. *Biol*. *Pharm*. *Bull*. 29, p. *82*. https://doi.org/10.1248/bpb.29.82

[22] Kim, J. H.; Byun, J. C.; Bandi, A. K.; Hyun, C. G.; Lee, N. H., 2009. **Compounds with elastase inhibition and free radical scavenging activities from *Callistemon lanceolatus***. *J*. *Med*. *Plant*. *Res*. 3, p. *914*.

[23] Elivera Group. https://eliveragroup.com/search?q=betulinic+cream (accessed 18 April 2023).

[24] Ribani, M.; Bottoli, C. B. G.; Collins, C. H.; Jardim, I. C. S. F.; Melo, L. F. C., 2004. **Validação em métodos cromatográficos e eletroforéticos**. *Quim*. *Nova*, 27(*5*), *771*. DOI: https://dx.doi.org/10.1590/S0100-40422004000500017

CAPÍTULO 2

CHEMICAL STRATEGIES TOWARDS THE SYNTHESIS OF BETULINIC ACID AND ITS MORE POTENT ANTIPROTOZOAL ANALOGUES

1. INTRODUCTION

Betulinic acid (BA), namely 3-hydroxy-lup-20(29)-en-28-oic acid, is a pentacyclic lupane-type triterpene which was allegedly isolated for the first time from the bark of *Cornus florida* L. (Cornaceae) in 1939. At that time, its structural elucidation was based on data obtained by comparison with a series of synthetic derivatives (Robertson, 1939). Subsequently, BA was also found in the seeds of *Zizyphus vulgaris* Lam. (var. Spinosus Bunge, Rhamnaceae) (Kawaguiti, 1940), in the bark of *Platanus acerifolia* (Bruckner, 1948) and in the fibrous outer bark of *Syncarpia laurifolia* (currently var. *glomulifera*, Myrtaceae) (Ralph, 1949). For the proper identification of the isolates, the preparation of known chemical derivatives was also necessary. Curiously, it is assumed that BA was found even earlier in other species, but under different trivial names, such as gratiolone (isolated from *Gratiola officinalis*, Schropulariaceae) (Retzlaff, 1902), platanol and/or platanine (from *Platanus* spp.) and an unidentified compound obtained from *Cornus sanguinea* L. and named as "platanoic acid" (Bruckner, 1948; Retzlaff, 1902; Zellner, 1925; Stabursvik, 1953).

The name "betulinic acid" has been given to this compound because of its prevalence in birch trees, which belong to the genus *Betula* (family Betulaceae), especially *Betula alba*, *B. pubescens*, *B. platyphylla*, *B. maximowicziana* and *B. mandshurica*. Additionally, although this family is the main natural source of BA, this triterpene is spread across many plant species belonging to the families Amaranthaceae, Ancistrocladaceae, Apocynaceae, Asteraceae, Chrysobalanaceae, Convolvulaceae, Dichapetalaceae, Dilleniaceae, Dioncophyllaceae, Ebenaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Loganiaceae, Melastomataceae, Moraceae, Myrtaceae, Onagraceae, Ramnaceae, Ranunculaceae, Rhamnaceae, Rosaceae, Rubiaceae, Trochodendraceae, Verbenaceae and Viscaceae (Pai, 2014). Interestingly, this triterpene can be extracted in large quantities from *Akania lucens* Hook f. (Akaniaceae), *Nerium oleander* L. (Apocynaceae), *Avicennia marina* L. (Verbenaceae), *Lemaireocereus* spp. (Cactaceae), *Arbutus menziesii* Pursh., *Arctostaphylos uva-ursi* (Ericaceae), *Lavandula angustifolia* var. *officinalis* (Lamiaceae), *Nuytsia floribunda* R. Br. (Loranthaceae), *Tectona grandis* L. f. (Verbenaceae), *Davilla rugosa* and other species of Dilleniaceae

family (*Dillenia*,*Wormia* and *Acrotrema*). Thus, BA may be considered as a chemotaxonomic marker for these families/genera (Pavanasasivam, 1974; David, 2006; Moghaddam, 2012). This compound seems to be biosynthesized from lupeol (LU) by the cytochrome CYP716A12, which has been characterized as a multifunctional enzyme showing lupeol 28-oxidase activities. The same cytochrome is responsible for the methyl C-28 oxidation of β- and α-amyrins, providing, in addition to BA, the other common triterpenes olenanolic (OA) and ursolic (UA) acids (Fukushima, 2011; Zhou, 2016).

Only in 1976 the compound's pharmacological importance begin to be evidenced. A study employing plant extracts containing BA exhibited high cytotoxicity and selectivity against lymphocytic leukemia P388 cells (Trumbull, 1976). Sequentially, BA was identified as a melanoma-specific cytotoxic compound given that *in vivo* studies showed tumor growth inhibition without toxicity (Pisha, 1995). Since then, various studies with BA and derivatives as antitumor agents have been published (Zhang, 2008; Zhang, 2016). Recent investigations have shown that the presence of the carboxyl function at C-28 is required for the cytotoxicity. This conclusion is supported in the fact of BA derivatives are usually significantly more potent than those derived from betulin (BE) (Hoenke, 2020). BA also shows potential as an anti-HIV agent and many of its derivatives, especially hybrids compounds, are highly effective against HIV (Huang, 2018; Wu, 2020). Compared to azidothymidine (AZT), specific hybrid derivatives presented more potent or equipotent anti-HIV activities but displayed less cytotoxicity (Wang, 2020). Additionally, numerous studies have been published describing a broad spectrum of other remarkable pharmacological properties for BA against fungi, bacteria, protozoa, diabetes and inflammatory disorders (Ghaffari Moghaddam, 2012; Rios, 2018; Hordyjewska, 2019). Among all these potential applications for BA and its analogues, we have focused this review on their antiprotozoal activity and, particularly, on their application to tropical diseases transmitted by vectors.

Vector-borne tropical diseases are disorders that are prevalent in tropical and subtropical regions since the cold season in temperate climates often controls the insect populations (vectors) by forcing their hibernation. Among the most impactful tropical diseases, malaria, leishmaniases and trypanosomiases are globally widespread, with potentially harmful consequences if left untreated (Saccoliti, 2020; Sarma, 2019).

Malaria is a life-threatening protozoan disease caused by five *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. knowlesi*, *P. ovale* and *P. malariae*) that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. *P. falciparum* and *P. vivax* account for more than 95% of all human malaria infections and therefore represent a great threat and serious challenge to public health. In 2019, with an estimated 229 million cases and 409.000 deaths, nearly half of the world's population was at risk of malaria. Most cases and deaths occur in sub-Saharan Africa and more severely affect children under 5 years old (WHO Malaria: https://www.who.int/health-topics/malaria#tab=tab_1).

In turn, the leishmaniases are a group of diseases caused by protozoan parasites from more than 20 *Leishmania* species. These parasites are transmitted to humans by the bite of an infected female phlebotomine sandfly, so that three main forms of the disease may arise: cutaneous leishmaniasis (CL), the most common form; visceral leishmaniasis (VL), also known as kala-azar, the most severe form; and mucocutaneous leishmaniasis (MCL), the most disabling form of the disease. More than 1 billion people live in areas endemic for leishmaniasis and are at risk of infection. An estimated 30.000 new cases of VL and more than 1 million new cases of CL occur annually (WHO Leishmaniasis: https://www.who.int/health-topics/leishmaniasis#tab=tab_1; WHO Human African Trypanosomiasis/Sleeping Sickness: https://www.who.int/health-topics/human-african-trypanosomiasis#tab=tab_1).

Finally, human trypanosomiasis comprises African trypanosomiasis and Chagas disease, which are caused by protozoan parasites of the genus *Trypanosoma*. African trypanosomiasis is caused by either *T. brucei gambiense* or *T. brucei rhodesiense* and threatens some 65 million people in sub-Saharan Africa, especially in rural areas and populations disrupted by war or poverty. Alternatively, Chagas disease is caused by *T. cruzi* and is responsible for 21.000 deaths per year, occurring mainly in Latin America (WHO Human African Trypanosomiasis/Sleeping Sickness: https://www.who.int/health-topics/human-african-trypanosomiasis#tab=tab_1; WHO Chagas Disease/American Trypanosomiasis: https://www.who.int/health-topics/chagas-disease#tab=tab_1).

Since tropical diseases are not very attractive to pharmaceutical companies for the development of novel drugs (Manner, 2019), few options are available on the market to treat these protozoan disorders. Currently, the most effective antimalarial

drugs available are chloroquine and artemisinin derivatives, whereas amphotericin B, paromomycin, miltefosine, pentamidine and sodium stibogluconate are the most important antileishmanial agents. Additionally, pentamidine, benznidazole and nifurtimox are the drugs of choice for the treatment of human trypanosomiasis. The problems related to the high economic and social costs of these drugs, along with their toxic effects and the emergence of drug resistance, point to the urgent need for novel antiprotozoal drugs (Bernal, 2020). This review aims to cover all chemical strategies published so far towards the synthesis of BA and its analogues with potent antiprotozoal activities against *Plasmodium*, *Leishmania* and *Trypanosoma* parasites.

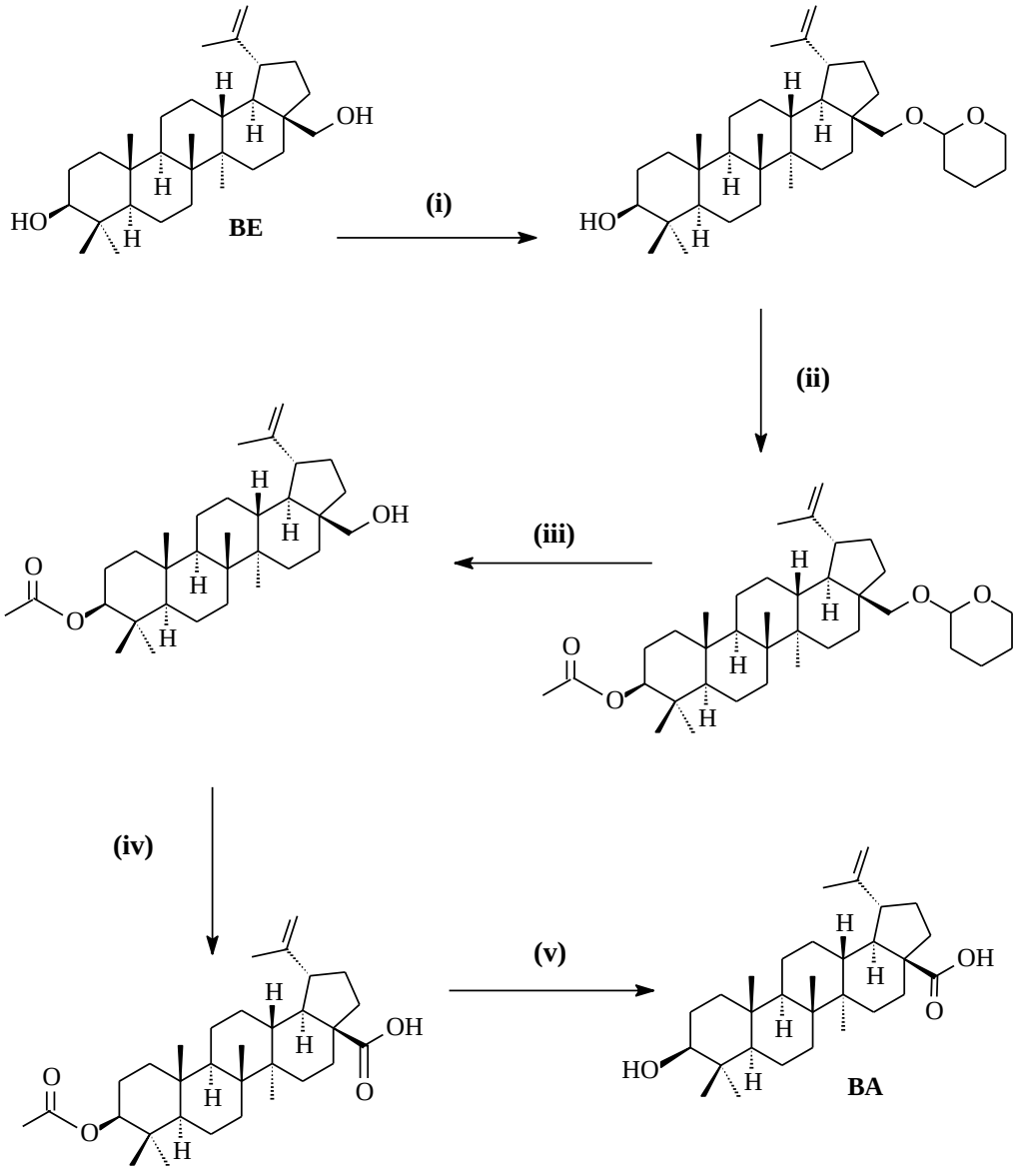
2. SYNTHESIS OF BA FROM BETULIN

In view of the highly valuable potential of BA for the development of novel drugs to treat protozoal and other impacting diseases, it is necessary to establish viable sources of this triterpene to allow its use as a starting material for the preparation of new and improved bioactive analogues. Within this scenario, it is worth mentioning that promising results regarding the production of BA and derivatives by biocatalysis and other biotechnological strategies have been widely reported in literature (Chen, 2009; Liu, 2011; An, 2020), which will not be covered in this review.

To the best of our knowledge, no total synthesis of BA has been reported yet. Although LU, its lupane C-28 methyl co-related triterpene, has been totally synthesized since the 1970s (Stork, 1971; Surendra, 2009). Currently, commercial production of BA substantially depends on traditional phytochemical extraction from birch bark (An, 2020), that yields around 0.025% w/w from dry material (Kim, 1997). The structure related to BA is the triterpene alcohol BE, which is much more abundant in birch trees (up to 34% of the dry barks) (Laszczyk, 2009), but it is biologically less active (Kim, 1997; Csuk, 2006) than BA. Therefore, BE could be employed as a starting material to easily prepare BA through semisynthesis, by oxidation of the primary hydroxyl group present at C-28.

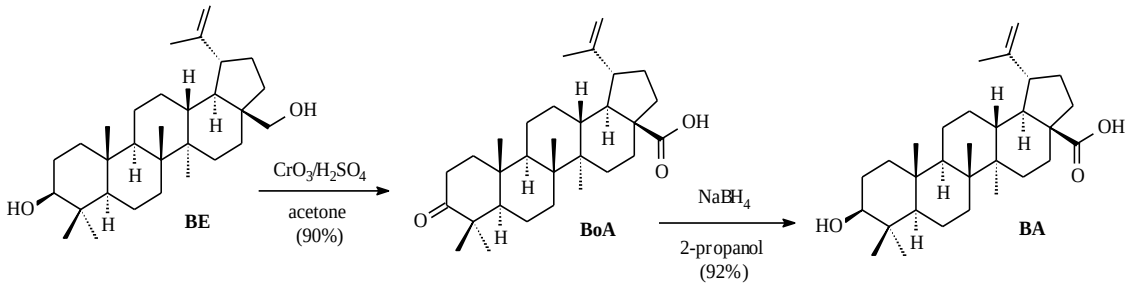
The first report of the synthesis of BA from BE were published in the late 1930s (Robertson 1939; Ruzicka, 1938). In this study, the authors used routes with a large number of steps and, consequently, the overall yield was low. Decades later, a classical method was reported using protecting groups to selectively mask the C-3 OH group (secondary alcohol) to avoid its isomerization (Scheme 1). In this study, although this classical route involves five steps and uses chemical agents that are harmful to both health and the environment (e.g., CrO_3), BA could be advantageously produced with high stereoselectivity and a good overall yield (Kim, 1997).

Scheme 1. Synthesis of betulinic acid (BA) from betulin (BE) using protecting groups. Reagents and conditions: (**i**) DHP/CH_2C1_2/PPTS (95%); (**ii**) Ac_2O/pyridine (87%); (**iii**) EtOH/PPTS (95%); (**iv**) CrO_3/H_2SO_4/acetone (80%); (**v**) K_2CO_3/MeOH/H_2O (88%)



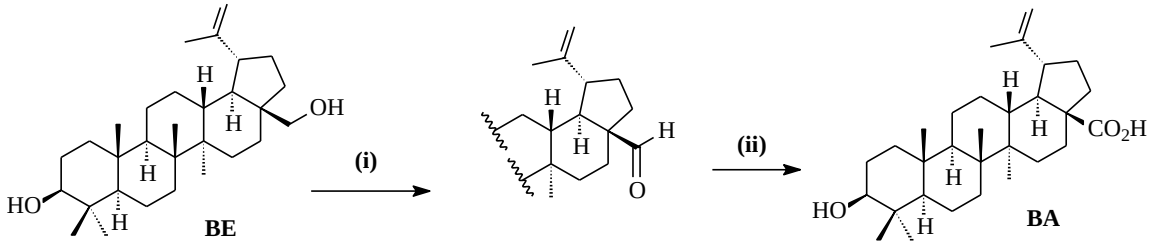
However, the most common methodology employed to synthesize BA from BE involves a direct Jones oxidation step (CrO_3/H_2SO_4/acetone) to form first the intermediate betulonic acid (BoA), with a yield of 90%. Then, BoA is reduced with $NaBH_4$/2-propanol to obtain, after recrystallization in hot methanol, pure BA. This way, a 3*β*-isomer yield of 92% is obtained (Scheme 2) (Baltina, 2003).

Scheme 2. Synthesis of BA from BE through direct Jones oxidation



Recently, a two-step route using solid-supported chromium oxide and potassium permanganate has been suggested to improve BA synthesis (Pichette, 2004). The main step of this approach is the selective oxidation of the primary alcohol function of BE, accomplished with chromic oxide adsorbed on silica gel to obtain BeAL (betulinal) with a reasonable yield (64%). Afterwards, the aldehyde derivative is oxidized to BA by potassium permanganate, resulting in a yield of 85% (Scheme 3).

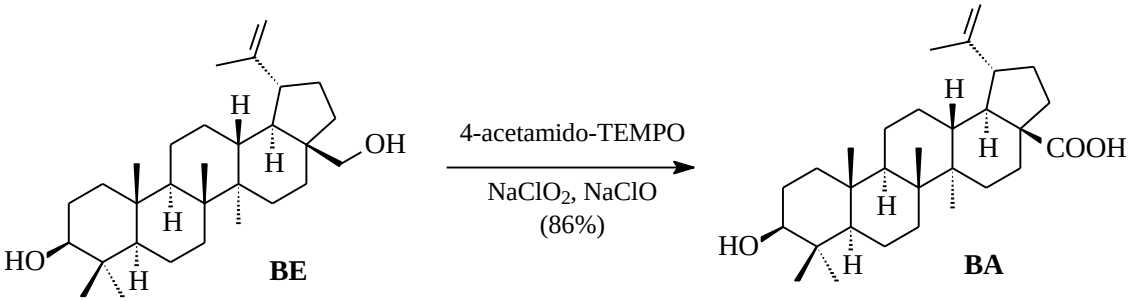
Scheme 3. Synthesis of BA from BE via solid-supported CrO_3 oxidation. Reagents and conditions: (**i**) 2 eq. CrO_3/SiO_2 (1:10), toluene, 60 min (84%); (**ii**) 2 eq. $KMnO_4$, acetone, 0ºC, 30 min (~100%)



BeAL + secondary products

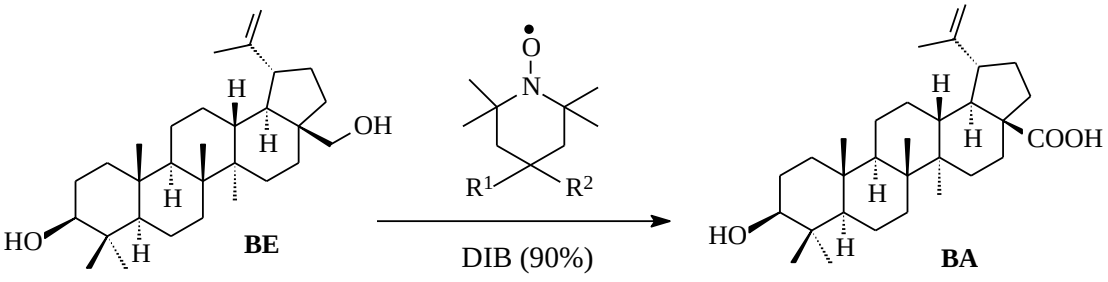
All of the above-mentioned approaches were designed to be small-scale preparations, employing more than one synthetic step with overall moderate to good yields. To overcome these limitations, Csuk and collaborators developed the short one-step route represented in Scheme 4 (Csuk, 2006). The method was based on the catalytic conversion of BE into BA, mediated by 4-acetamide-2,2,6,6-tetramethylpiperidine-1-oxyl (4-acetamide-TEMPO) in a reaction medium containing a mixture of NaClO and $NaClO_2$ at 35ºC. It is worth mentioning that the simplicity of the synthetic route is the main advantage resulting from this study, in addition to the use of a cheap starting material and, especially, the possibility of obtaining BA on a larger scale, with a yield of 86% in just one step.

Scheme 4. One-step synthesis of BA from BE via 4-acetamido-TEMPO/NaClO/$NaClO_2$



In a slightly different one-step method, a patented route also employing TEMPO-type catalysts, the hypervalent iodine reagent diacetoxy-iodobenzene (DIB) was used as the oxidizer (Wickholm, 2013). The reaction occurs under mild conditions and is both economically and environmentally friendly, with a yield of up to 90% (Scheme 5).

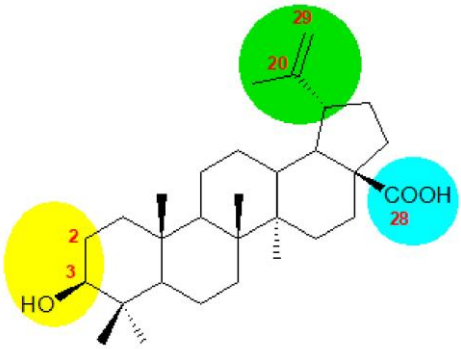
Scheme 5. Synthesis of BA from BE via TEMPO-type catalysts/DIB



3. SYNTHESIS OF ANTIPROTOZOAL BA ANALOGUES

Previous studies have shown a vast range of antimicrobial (including antiprotozoal) properties displayed by triterpenes with lupane skeletons (Hordyjewska, 2019; Domínguez-Carmona, 2010). These findings are indicative that the BA could be a promising scaffold for several modifications leading to BA derivatives with more potent antiprotozoal activities. Figure 10 offers an overview of the molecular structure of BA, highlighting positions C-2/C-3, C-20/C-29 and C-28 as those susceptible to chemical derivatization. Thus, in recent years several BA analogues have been designed, synthesized and evaluated against parasites *Plasmodium* sp., *Leishmania* sp. and *Trypanosoma* sp., which are described below.

Figure 10. The structural formula of BA and C-2/C-3, C-20/C-29 and C-28 positions amenable to chemical derivatization



3.1. Antiplasmodial Analogues

The *in vitro* antiplasmodial properties of BA were reported for the first time by Bringmann (1997). This study described that BA exhibited moderate to good antimalarial activity *in vitro* against asexual erythrocytic stages of the human malaria parasite *P. falciparum*. In a subsequent investigation, the IC_{50} values for BA against chloroquine-resistant (K1) and -sensitive (T9-96) strains of *P. falciparum* were calculated and ranged from 42.9 to 56.7 μM, respectively (Steele, 1999). In addition,

this study also revealed that BA was ineffective at reducing parasitaemia when tested *in vivo* in a murine malaria model (*P. berghei*), even when the highest dosage of 250 mg/kg/day was used intraperitoneally.

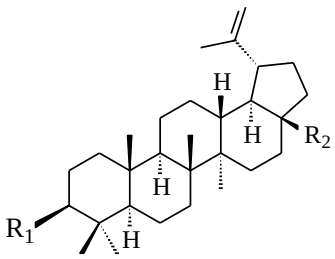
Another interesting study showed that erythrocytes preloaded either with BA or its simple analogues BeAL, LU (**1**), BE, BA methyl ester (**2**) and BA amide (**3**) (Figure 10) did not serve as hosts for *P. falciparum* parasites (Ziegler, 2004). All the above compounds inhibited *P. falciparum* growth with IC_{50} values in the range of 7–28 μM, and this inhibition correlates well with the extent of membrane curvature changes towards stomatocytes or echinocytes in a concentration-dependent approach. The authors concluded that the antiplasmodial activities of BA and its analogues are clearly related to the incorporation of such compounds into the lipid bilayer of erythrocytes, causing modifications of cholesterol-rich membrane rafts that play an important role in parasite vacuolization. The authors also concluded that this established link between erythrocyte membrane modifications and antiplasmodial activity might suggest a novel target for the development of novel antimalarial drugs (Ziegler, 2004).

The *in vitro* evaluation of BA and its simple derivatives BoA, betulinic acid acetate (**4**), BA methyl ester (**2**) and BA methyl ester acetate (**5**) (Figure 11) against chloroquine-resistant *P. falciparum* parasites showed IC_{50} values of 9.89, 10.01, 5.99, 51.58 and 45.79 μM, respectively (De Sá, 2009). In addition, mice infected with *P. berghei* and treated with **4** showed a dose-dependent reduction in parasitemia. These results are in agreement with those later obtained by Domínguez-Carmona (2010), who showed that simple esterification of the C-3 hydroxyl group results in an improvement of the antiplasmodial activity.

Based on studies that reported that piperazine derivatives possess antimalarial action, new piperazinyl derivatives at the C-28 position of **4** (Innocente, 2012) were designed and synthesized as prototypes for new antimalarial compounds against the *P. falciparum* chloroquine-sensitive 3D7 strain (Scheme 6). Among them, compounds **4** (IC_{50} = 4 μM) and **6** (IC_{50} = 5 μM) were found to show good antiplasmodial activity, while **7** was shown to be much more potent (IC_{50} = 220 nM), also presenting a selectivity index = 18. However, subsequent *in vivo* studies (Diedrich, 2018) revealed that **7** showed toxicity to mice, and docking studies indicated the putative mechanism of action of **7** in inhibiting PfATP6 (a SERCA-type Ca+2-ATPase present in *P.*

falciparum) as well as the mammalian SERCA protein with greater affinity, compromising the selectivity expected towards the parasite.

Figure 11. Simple antiplasmodial BA analogues



BeAL: R_1 = OH; R_2 = CHO

BoA: R_1 = O; R_2 = COOH

BE: R_1 = OH; R_2 = CH_2OH

1: R_1 = OH; R_2 = CH_3

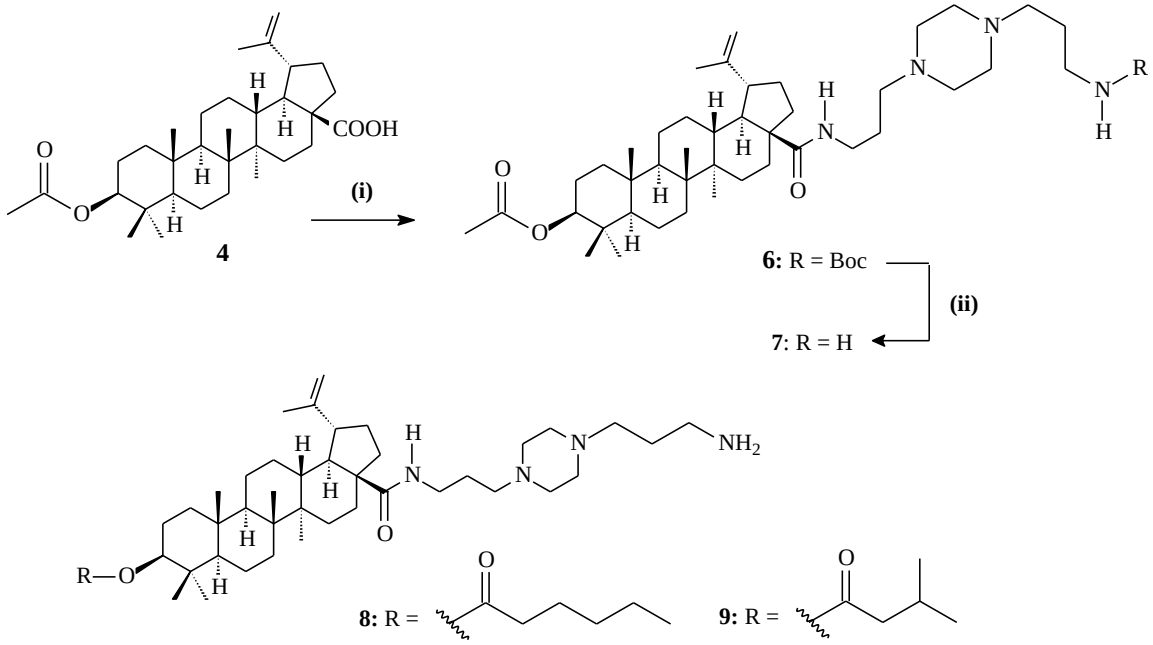
2: R_1 = OH; R_2 = $COOCH_3$

3: R_1 = OH; R_2 = $CONH_2$

4: R_1 = OAc; R_2: COOH

5: R_1 = OAc; R_2 = $COOCH_3$

Scheme 6. Synthesis of new piperazinyl derivatives of acetyl betulinic acid. Reagents and conditions: (**i**) ClCOCOCl, 0ºC, 3 h; TEA, *N*-tert-butyloxycarbonyl-1,4-bis(3-aminopropyl)piperazine, RT, 24 h; (**ii**) TFA 10%/CH_2Cl_2, RT, 6 h

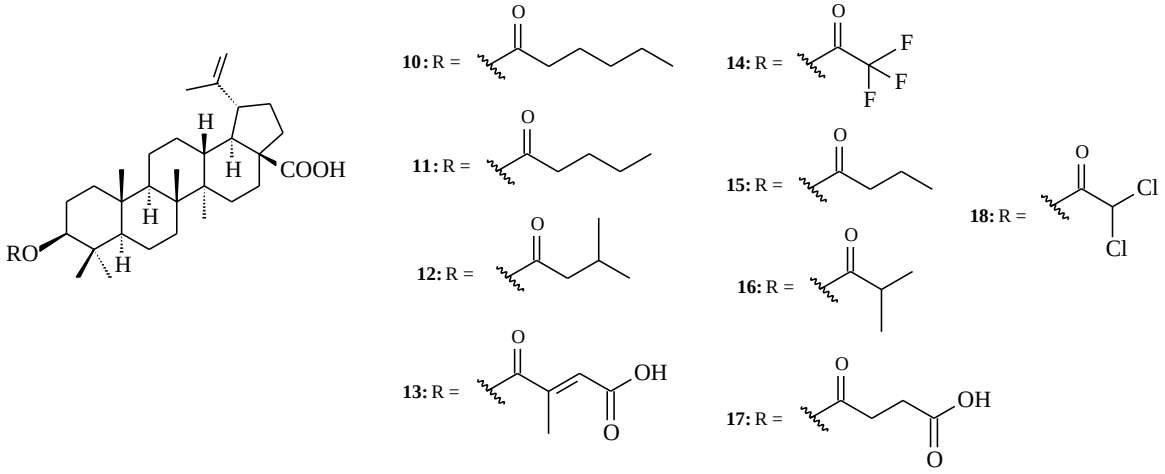


The study performed by Innocente (2012) was useful to inspire the synthesis and antiplasmodial assessment of novel BA piperazinyl analogues, whose results were published some years later (Silva, 2015). Analogues **8** (IC_{50} = 1 μM) and **9** (IC_{50} = 4 μM) (Scheme 6) displayed good antiplasmodial potency against the *P. falciparum*

chloroquine-sensitive 3D7 strain. Moreover, the authors investigated the mechanism of action of **8** and noted that this derivative led to an increase in cytosolic Ca^{2+}, as well as causing a lower inhibition of *β*-haematin formation than that observed for chloroquine.

A new series of BA derivatives **10**–**18** obtained by carboxylic acid esterification at the C-3 OH group was synthesized in an attempt to improve the antimalarial properties of the BA skeleton. Among the nine derivatives prepared (Figure 12), two presenting smaller side chains (**15** and **16**, IC_{50} = 5–8 μM) were found to be two to three times more potent than BA (IC_{50} = 18 μM) against chloroquine-sensitive *P. falciparum* 3D7. Additionally, such analogues were non-cytotoxic towards the mammalian cell line HEK293T (Da Silva, 2013).

Figure 12. Antiplasmodial BA analogues from esterification at the C-3 OH group

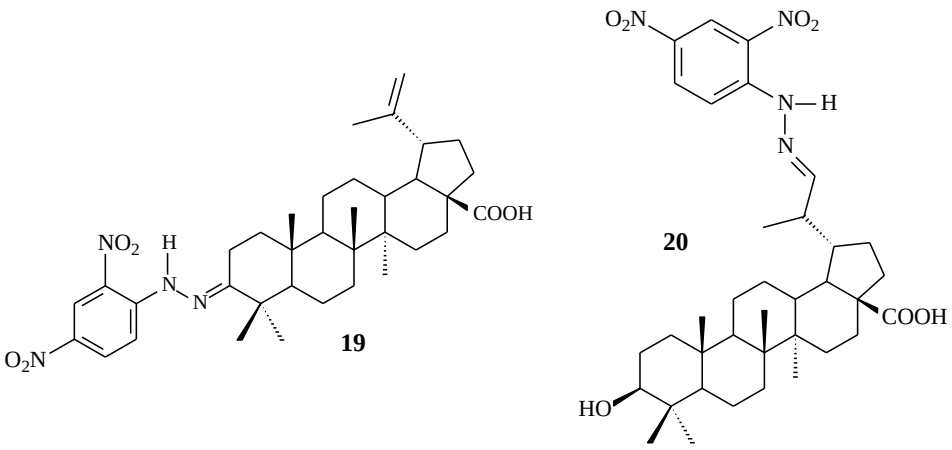


In another study, the synthesis of a novel series of BA derivatives was performed by acetic, butyric and isobutyric esterification at the C-3 OH group, as well as by incorporating methoxy and imidazole moieties at the carboxyl at the C-28 position (Cargnin, 2018). The antiplasmodial activities of all analogues were assessed against the chloroquine-resistant *P. falciparum* W2 strain. The results permitted the conclusion that structural modifications at C-3 were more promising for increasing the antiplasmodial properties of the BA skeleton rather than simultaneous modifications at the C-3 and C-28 positions. From all the prepared compounds, the ester derivative 3*β*-butanoyl betulinic acid (**15**) was shown to be the most potent (IC_{50} = 3.4 μM) and also did not present cytotoxicity against VERO nor HepG2 cells (CC_{50} > 400 μM), thus displaying a

selectivity index higher than 117. Docking studies indicated a possible interaction of **15** with the *Plasmodium* protease PfSUB1. Moreover, additional data suggest that the main target of **15** on *Plasmodium* might be related to other molecules and processes on the ring stage.

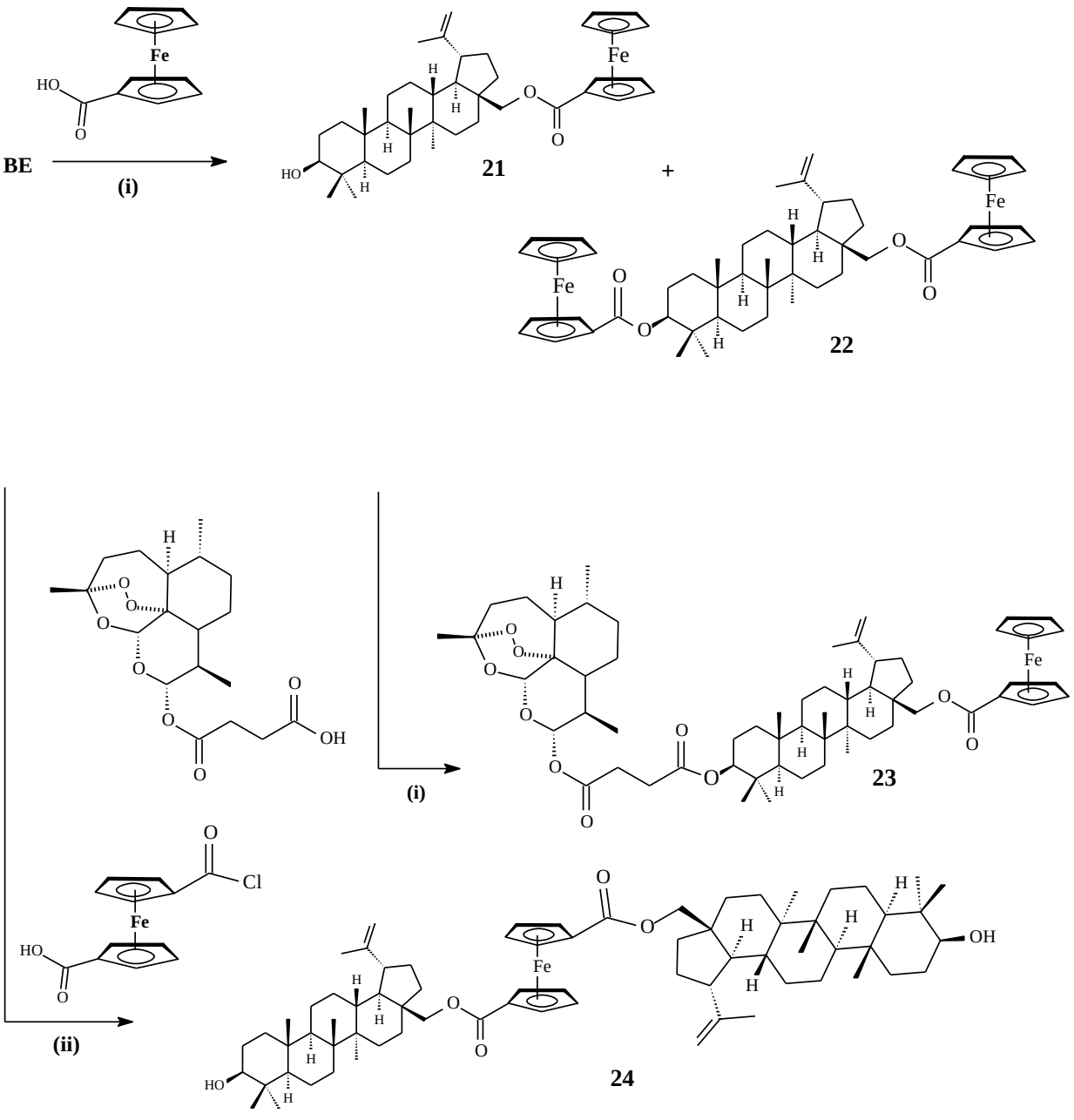
In a different chemical approach, nine 2,4-dinitrophenyl-hydrazono-BA analogues were prepared (Ullah, 2016), but only compounds **19** (IC_{50} = 15.3 μM) and **20** (IC_{50} = 10.2 μM) (Figure 13) showed better antiplasmodial activity than BA (IC_{50} = 38.8 μM) against the chloroquine-resistant *P. falciparum* W2 strain.

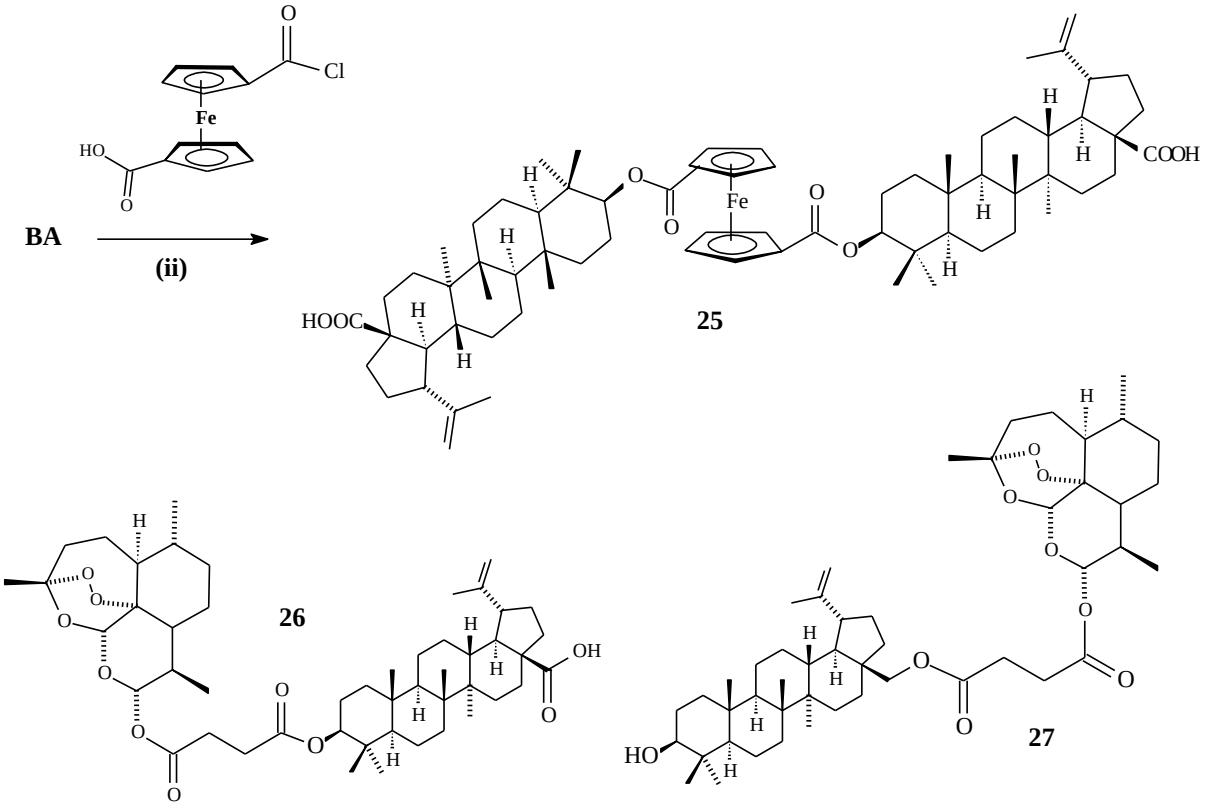
Figure 13. 2,4-dinitrophenyl-hydrazone-BA analogues with moderate antiplasmodial activity



Inspired by the highly active antimalarial compound ferroquine and by the previously prepared artemisinin–ferrocene hybrid, a series of BA/BE based dimer and hybrid compounds carrying ferrocene and/or artesunic acid moieties were designed and synthesized (Scheme 7), to include the ferrocene moiety into the new molecules as a linker or a subunit (Karagöz, 2019). The antiplasmodial activity of all newly synthesized compounds **21–25** as well as the previously synthesized hybrids **26** and **27,** were evaluated against *P. falciparum* 3D7 strain. Compounds **23** (IC_{50} = 1.49 μM), **26** (IC_{50} = 0.26 μM) and **27** (IC_{50} = 0.09 μM), which are hybrids of BA and artesunic acid, were found to be the most active antiplasmodial analogues.

Scheme 7. Synthetic routes to BA/BE-derived dimers and hybrids **21**–**25**. Reagents and conditions: (**i**) DCC, DMAP, CH_2Cl_2, 0ºC to rt; (**ii**) DMAP, CH_2Cl_2, 0ºC to rt





All the above described results indicated that BA is a weak antimalarial agent and, consequently, it is not suitable to be employed as a drug for this disease. However, the improvement in the antiplasmodial activity that was found for some of its derivatives makes BA a good starting material to design and prepare new analogues that are able to reach and even improve the potency of the commercially available antimalarial drugs. In this sense, new chemical approaches may explore other functionalities at the C-3 and/or C-28 positions in order to synthesize more potent antiplasmodial analogues. Within this scenario, hybridization with other antimalarial drugs seems to be a promising synthetic strategy.

3.2. Antileishmanial Analogues

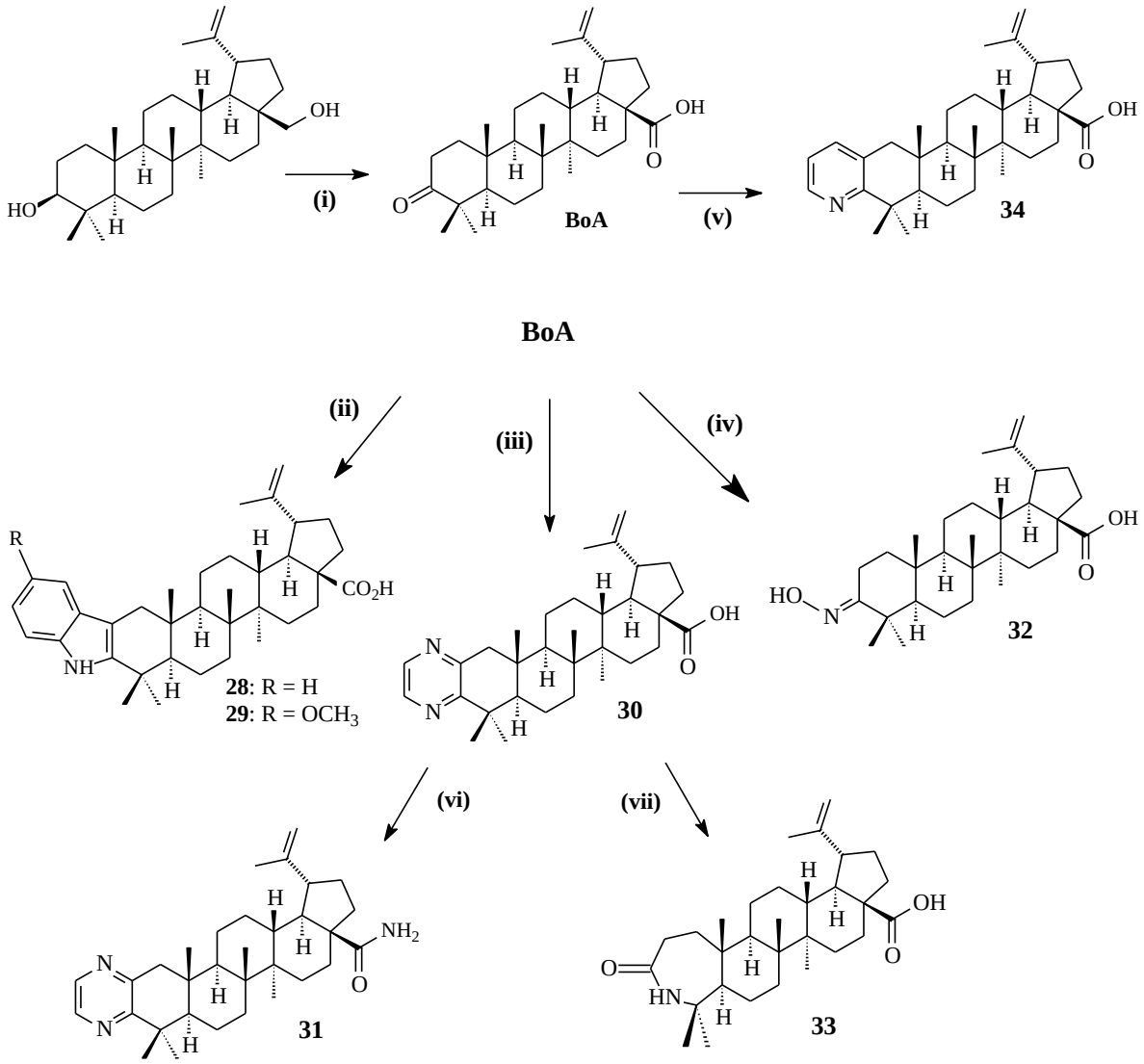
Betulinal (BeAL) was the first naturally occurring BA-structurally related compound with antileishmanial properties to be reported in literature. It showed a weak activity *in vitro*, reducing *Leishmania amazonensis* amastigotes infection by 88% at 136

μM, but this high dose was also significantly toxic to peritoneal macrophages (Sauvain, 1996). However, the analogue 20(29)-dihydrobetulinic acid (DHBA) was described as being capable of strongly inhibiting the growth of *L. donovani* promastigotes (IC_{50} = 2.6 μM) and amastigotes (IC_{50} = 4.1 μM) at lower concentrations (Chowdhuri, 2003). Furthermore, DHBA was also shown to be a dual inhibitor of DNA topoisomerases I and II and acts by preventing the formation of enzyme-DNA binary complex, in such a way as to induce apoptosis.

Later, in a screening study aiming to investigate the *in vitro* leishmanicidal activity of 46 scarce natural products, the weak antileishmanial effect of BA (IC_{50} = 87.5 μM) was then first evidenced against *L. major* promastigotes (Takahashi, 2004). Similarly, betulinic acid acetate (**4**, IC_{50} = 44.9 μM) and BoA (IC_{50} = 51.2 μM) derivatives were also both weakly active against *L. amazonensis* (Domínguez-Carmona, 2010).

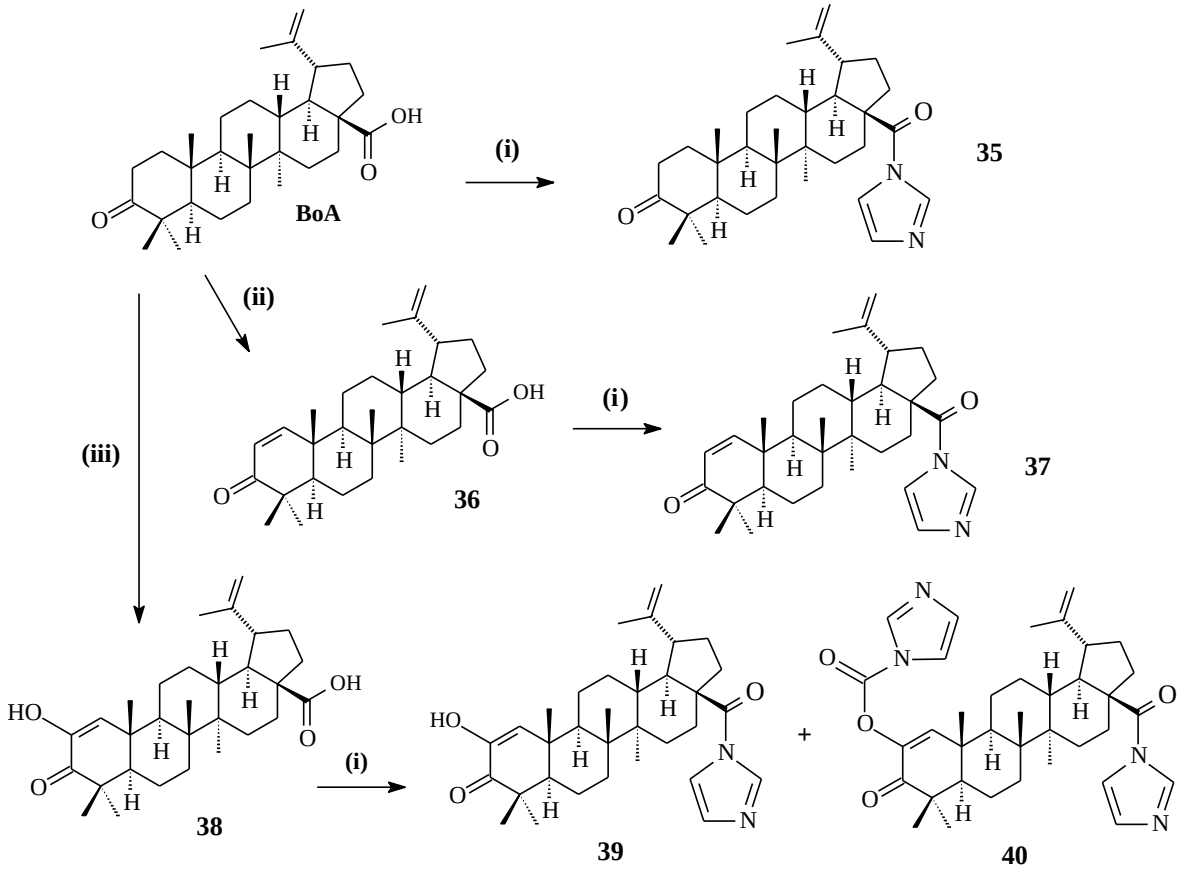
Heterocyclic analogues **28**–**34** were designed and synthesized from BE, employing BoA as a versatile intermediate. The employment of the cheap BE instead of BA is an advantage of this route. Different heterocycles were fused to the 2,3-position of the lupane skeleton, including isoxazole, pyrazine, pyridine, indole and pyrazole groups, while the carboxyl group at the C-28 position was also transformed into an amide derivative (Scheme 8). Among all compounds tested, the analogues **30** (IC_{50} = 13.2 μM) and **33** (IC_{50} = 4.3 μM) were found to display the best antileishmanial activity against axenic amastigotes of *L. donovani*, with derivative **33** being both the most potent and selective (SI = 12.9) antileishmanial analogue of all those reported in this study (Haavikko, 2014).

Scheme 8. Synthesis of heterocyclic BA analogues. Reagents and conditions: (**i**) Jones oxidation, $Na_2Cr_2O_7$, H_2SO_4, H_2O, acetone, rt, 21 h (44%); (**ii**) appropriate phenylhydrazine hydrochloride, HOAc, reflux, 3 h, (21–42%); (**iii**) ethylenediamine, sulfur, morpholine, reflux, 21 h (68%); (**iv**) NH_2OH/HCl, pyridine, MeOH, reflux, 16 h (84%); (**v**) propargylamine, Cu(I)Cl, EtOH, reflux, 17 h (11%); (**vi**) 1. oxalyl chloride, DCM, rt, 3 h; 2. aqueous ammonia, DCM, rt, 1 h, (~100%); (**vii**) TFAA, DCM, rt, 20 h, (33%); DCM 1/4 dichloromethane, TFAA 1/4 trifluoroacetic anhydride



The new imidazole carbamate and *N*-acylimidazole derivatives **35**–**40** were synthesized from BoA (Scheme 9) and firstly, they were screened for *in vitro* cytotoxic activity against human cancer cell lines (Santos, 2009). Later, these imidazole analogues were also assessed against *L. infantum* promastigotes (Sousa, 2014). Compound **40** (IC_{50} = 25.8 μM), which has an additional imidazole carbamate at the C-2 position, was shown to be the most active analogue, and its antileishmanial activity was synergistically increased when **40** was associated with miltefosine (the medication used to treat leishmaniasis).

Scheme 9. Synthesis of imidazole BA analogues. Reagents and conditions: (**i**) CDI, dry THF, N_2, reflux, 8–9 h; (**ii**) DDQ, dioxane, N_2, reflux, 15 h; (**iii**) O_2, t-BuOK, t-BuOH, 40ºC, 2 h



Antileishmanial studies employing BA derivatives are still in early stages. Even though only a few results have been obtained up to the present time, they suggest that other known BA analogues may display interesting antileishmanial profiles and, thus, they also deserve to be assayed *in vitro* and *in vivo* against *Leishmania* sp. parasites, including in combination with traditional antileishmanial drugs. Furthermore, taking into account that heterocyclic betulin derivatives displayed antileishmanial activities with GI_{50} values between 8.9–30.0 μM (Alakurtti, 2010), novel heterocyclic BA analogues are worthy of attention for synthesis and antileishmanial investigation.

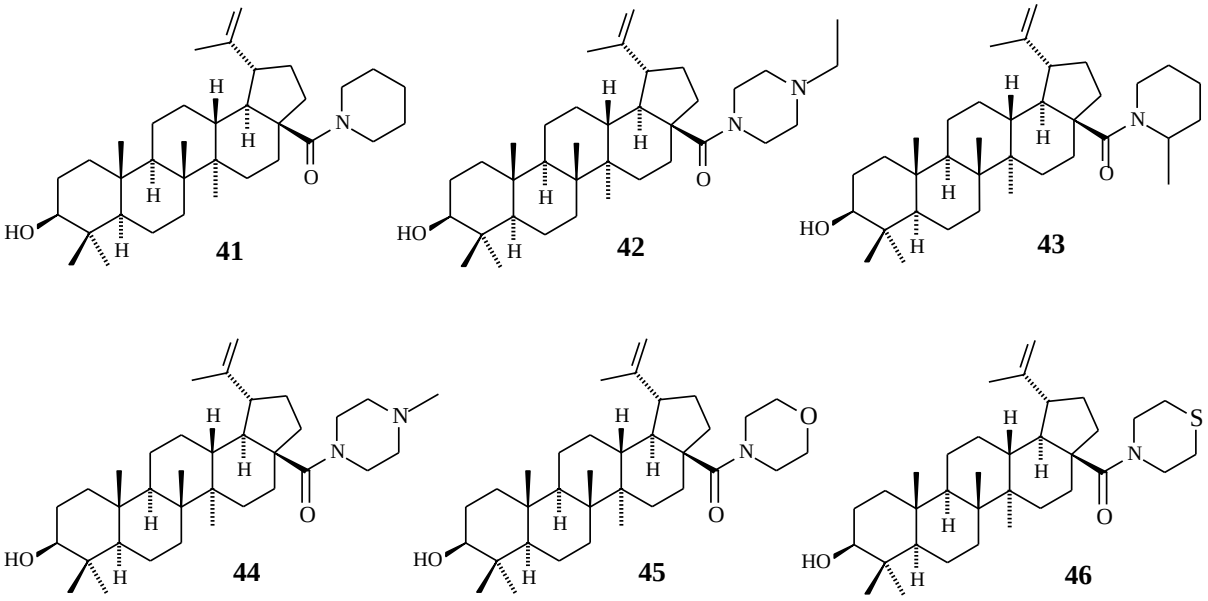
3.3. Antitrypanosomal Analogues

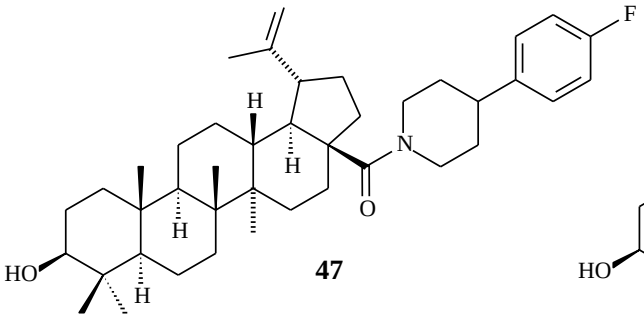
BA shows weak antitrypanosomal properties, first revealed when this triterpene was isolated from a *Strychnos spinose* Lam. lipophilic leaf extract showing antitrypanosomal activity. This plant of the Loganiaceae family is traditionally used to treat African trypanosomiasis. The BA isolated was assayed against bloodstream forms

of *Trypanosoma brucei* and showed IC_{50} = 32.6 μM (SI = 1.3) (Hoet, 2007). Subsequently, BA was also assayed against epimastigote forms of *T. cruzi* Tulahuen strain and again BA was weakly active (IC_{50} = 50.0 μM) (Domínguez-Carmona, 2010).

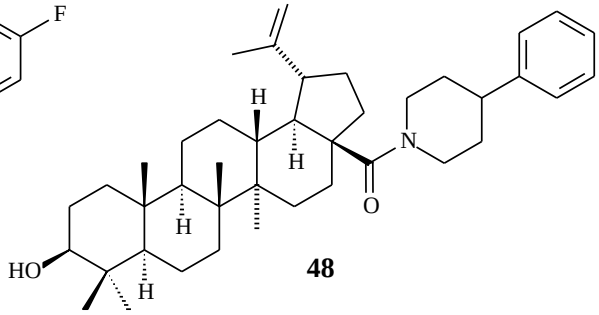
These findings indicated that BA could be considered a good lead for the design and synthesis of more potent and selective antitrypanosomal analogues (Meira, 2016). Previous studies have suggested that changes at the C-28 carboxyl group could lead to analogues with enhanced antiprotozoal activities when compared to BA. Thus, new amide analogues **41**–**48** (Figure 14) were prepared and assayed against trypomastigotes forms of *T. cruzi*. The most potent antitrypanosomal effects were observed for **45** (IC_{50} = 1.8 μM; SI = 17.3), **46** (IC_{50} = 5.4 μM; SI = 5.3) and **48** (IC_{50} = 5.0 μM; SI = 10.7), while BA displayed an IC_{50} value of 19.5 μM and SI = 1. In this study, the authors identified **45** as a selective anti-*T. cruzi* agent, and additional experimental data showed this analogue destroys parasite cells by necrotic death as well as exhibits synergistic activity when used in combination with benznidazole, the antiparasitic drug commonly employed in the treatment of Chagas disease (Meira, 2016).

Figure 14. Amide BA analogues with antitrypanosomal activity





47



48

These initial results reveal promising antitrypanosomal properties of BA analogues and open the door to unexplored opportunities for assaying numerous other BA derivatives that have already been synthesized (Borkova, 2018; Hodon, 2019) against *Trypanosoma* sp. parasites. Moreover, new chemical approaches aiming at incorporating triphenylphosphonium groups into the BA skeleton (Grymel, 2019) may improve not only physical properties, but also the mechanism of action of BA analogues, leading to more potent antitrypanosomal compounds.

4. CONCLUDING REMARKS AND PERSPECTIVES

BA is a compound that is easily obtained from natural sources or by chemical transformation of biosynthetically related triterpenes. Its total synthesis is still not commercially possible, but semisynthesis from BE has yielded good results. In addition, besides the already reported chemical procedures used to obtain BA and its derivatives from natural precursors, some promising biotechnological developments have also been achieved in the same direction by employing biocatalysis of BE and/or BA by microorganisms (Zhou, 2016; Liu, 2011; Feng, 2013; Chatterjee, 2000; Wu, 2017) and plant cells (Hakkinen, 2018).

BA and its derivatives present a range of biological activities, especially anticancer and anti-HIV properties. However, the antiprotozoal activity is also very interesting, particularly when dealing with vector-borne tropical diseases such as

malaria, leishmaniases and trypanosomiases, which have a high prevalence in tropical and subtropical regions. The antiplasmodial activity described for BA was moderate, nevertheless some analogues that were acylated at the C-3 position showed an improvement of this activity and the hybrid models with artesunic acid showed the most interesting properties. Similarly, several analogues also presented more intense antileishmanial activities than BA, and heterocycles fused to the C-2/C-3 positions and amide derivatives were the most promising analogues. Regarding the antitrypanosomal activity and besides the weak activity of BA, some interesting antitrypanosomal analogues were also prepared by amide formation at the C-28 carboxylic group of the lupane skeleton.

To date, most of the chemical transformations performed on the BA structure deal with esterification of the C-3 hydroxyl group and derivatization of the C-28 carboxylic group, however, another functional group present in BA that is susceptible to transformation is the double bond at C-20/C-29. This has not been well explored, and no robust studies aimed at synthesizing BA antiparasitic analogs from structural modifications at these positions have been found in literature yet. Recently, just one published study suggested that BA bromination at the C-20/C-29 positions might generate more potent antileishmanial analogues (Cargnin, 2017). Thus, further studies would be of interest to obtain new derivatives by transformation not only of the double bond itself (but also of the allylic positions), whose evaluation as an antiprotozoal would allow for gaining more in-depth knowledge regarding the structure–activity relationships of BA.

Besides, it is well known that molecular conjugation or hybridization can yield compounds with improved pharmacokinetic and pharmacological properties, and even overcome resistance in comparison with their precursors (Tietze, 2003; Ojima, 2008; Bansal, 2014). In this sense, taking into account that hybridization of BA with artesunic acid gave good results, it will be worth exploring the application of this strategy to other known antimalarial, antileishmanial and antitrypanosomal agents.

Lastly, BA can be produced either by isolation of different plant extracts or by the chemical transformation of betulin easily obtained from *Betula* spp. Consequently, it can be said that BA could be a lead molecule of great interest for further research into obtaining new commercial products with the potential to be used as antiprotozoal agents, including for the treatment of neglected tropical diseases.

REFERENCES

[1] Robertson, A.; Soliman, G.; Owen, E. C., 1939. **Polyterpenoid compounds. Part, I. Betulic acid from *Cornus florida* L**. *J. Chem. Soc*., p. *1267*.

[2] Kawaguti, R.; Kim, K. W., 1940. **The constituents of *Zyzyphus vulgaris* Lamark var. *spinosus* Bunge. II. Betulinic acid**. *Yakugaku Zasshi*, 60, p. *595*.

[3] Bruckner, G. J.; Kovacs, J.; Koczka, I., 1948. **Occurrence of betulinic acid in the bark of the plane tree**. *J. Chem. Soc*., p. *948*.

[4] Ralph, C. S.; White, D. E., 1949. **Betulic acid from *Syncarpia laurifolia***. *J. Chem. Soc*., p. *3433*.

[5] Retzlaff, F. Ueber., 1902. **Herba gratiolae**. *Arch. Pharm*. (Weinh.)., 240, p. *561*.

[6] Zellner, J.; Fajner, R., 1925. **Wachsbestandteile in Hartriegelrinden (Cornaceae)**. *Monatshefte*, 46, p. *611*.

[7] Stabursvik, A., 1953. **Occurrence of betulinic acid in *Menyanthes trifoliata* L**. *Acta Chem. Scand*., 7, p. *446*.

[8] Pai, S. R.; Joshi, R. K., 2014. **Distribution of betulinic acid in plant kingdom**. *Plant. Sci. Today*, 1, p. *103*.

[9] Pavanasasivam, G.; Sultanbawa, M.U.S., 1974. **Betulinic acid in the dilleniaceae and a review of its natural distribution**. *Phytochemistry*, 13, p. *2002*.

[10] David, J. M.; Souza, J. C.; Guedes, M. L. S.; David, J. P., 2006. **Estudo fitoquimico de *Davilla rugosa*: flavonóides e terpenóides**. *Braz. J. Pharmacogn*., 16, p. *105*.

[11] Moghaddam, M. G.; Ahmad, F. B. H., 2012. **Various botanical sources of betulinic acid: A review**. *Asian J. Chem*. 24, p. *4843*.

[12] Fukushima, E. O.; Seki, H.; Ohyama, K.; Ono, E.; Umemoto, N.; Mizutani, M.; Saito, K.; Muranaka, T., 2011. **CYP716A subfamily members are multifunctional oxidases in triterpenoid biosynthesis**. *Plant. Cell Physiol*. 52, p. *2050*.

[13] Zhou, C.; Li, J.; Li, C.; Zhang, Y., 2016. **Improvement of betulinic acid biosynthesis in yeast employing multiple strategies**. *BMC Biotechnol*., 16, p. *1–9*.

[14] Trumbull, E. R.; Bianchi, E.; Eckert, D. J.; Wiedhopf, R. M.; Cole, J. R., 1976. **Tumor inhibitory agents from *Vauquelinia corymbosa* (Rosaceae)**. *J. Pharm. Sci*., 65, p. *1407*.

[15] Pisha, E.; Chai, H.; Lee, I.-S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; *et al*., 1995. **Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis**. *Nat. Med*., 1, p. *1046*.

[16] Zhang, D.; Xu, H.; Wang, L.; Li, Y.; Sun, P.; Wu, X.; Wang, G.; Chen, W.; Ye, W., 2008. **Betulinic Acid and its Derivatives as Potential Antitumor Agents**. *Harv. Bus. Rev*., 86, p. *84*.

[17] Zhang, X.; Hu, J.; Chen, Y., 2016. **Betulinic acid and the pharmacological effects of tumor suppression (Review)**. *Mol. Med. Rep*., 14, p. *4489*.

[18] Hoenke, S.; Heise, N. V.; Kahnt, M.; Deigner, H. P.; Csuk, R., 2020. **Betulinic acid derived amides are highly cytotoxic, apoptotic and selective**. *Eur. J. Med. Chem.*, 207, p. *112815*.

[19] Huang, Q.; Chen, H.; Luo, X.; Zhang, Y.; Yao, X.; Zheng, X., 2018. **Structure and Anti-HIV Activity of Betulinic Acid Analogues**. *Curr. Med. Sci.*, 38, p. *387*.

[20] Wu, H. F.; Morris-Natschke, S. L.; Xu, X. D.; Yang, M. H.; Cheng, Y. Y.; Yu, S. S.; Lee, K. H., 2020. **Recent advances in natural anti-HIV triterpenoids and analogs**. *Med. Res. Rev.*, 40, p. *2339*.

[21] Wang, Q.; Li, Y.; Zheng, L.; Huang, X.; Wang, Y.; Chen, C. H.; Cheng, Y. Y.; Morris-Natschke, S. L.; Lee, K. H., 2020. **Novel Betulinic Acid-Nucleoside Hybrids with Potent Anti-HIV Activity**. *Acs Med. Chem. Lett.*, 11, p. *2290*.

[22] Ghaffari Moghaddam, M.; Bin, H.; Ahmad, F.; Samzadeh-Kermani, A., 2012. **Biological Activity of Betulinic Acid: A Review**. *Pharmacol. Pharm.*, 03, p. *119*.

[23] Ríos, J. L.; Máñez, S., 2018. **New Pharmacological Opportunities for Betulinic Acid**. *Planta Med.*, 84, p. *8*.

[24] Hordyjewska, A.; Ostapiuk, A.; Horecka, A.; Kurzepa, J., 2019. **Betulin and betulinic acid: triterpenoids derivatives with a powerful biological potential**. *Phytochem. Rev.*, 18, p. *929*.

[25] Saccoliti, F.; Di Santo, R.; Costi, R., 2020. **Recent Advancement in the Search of Innovative Antiprotozoal Agents Targeting Trypanothione Metabolism**. *Chem. Med. Chem.*, p. *1–17*.

[26] Sarma, N.; Patouillard, E.; Cibulskis, R. E.; Arcand, J. L., 2019. **The economic burden of Malaria: Revisiting the evidence**. *Am. J. Trop. Med. Hyg.*, 101, p. *1405*.

[27] World Health Organization. Malaria. Available online: https://www.who.int/health-topics/malaria#tab=tab_1 (accessed on May 2023).

[28] World Health Organization. Leishmaniasis. Available online: https://www.who.int/healthtopics/leishmaniasis#tab=tab_1 (accessed on May 2023).

[29] World Health Organization. Human African Trypanosomiasis (Sleeping Sickness). Available online: https://www.who.int/health-topics/human-african-trypanosomiasis#tab=tab_1 (accessed on 05 May 2023).

[30] World Health Organization. Chagas Disease (American Trypanosomiasis). Available online: https://www.who.int/healthtopics/chagas-disease#tab=tab_1 (accessed on May 2023).

[31] Manner, C. K.; Graef, K. M.; Dent, J., 2019. WIPO Re:Search: **Catalyzing public-private partnerships to accelerate tropical disease drug discovery and development**. *Trop. Med. Infect. Dis.*, 4, p. *53*.

[32] Bernal, F. A.; Kaiser, M.; Wünsch, B.; Schmidt, T. J., 2020. **Structure-Activity Relationships of Cinnamate Ester Analogues as Potent Antiprotozoal Agents**. *Chem. Med. Chem.*, 15, p. *68*.

[33] Chen, Q.-h.; Liu, J.; Zhang, H.-f.; He, G.-q.; Fu, M.-l., 2009. **The betulinic acid production from betulin through biotransformation by fungi**. *Enzym. Microb. Technol.*, 45, p. *175*.

[34] Liu, J.; Fu, M. L.; Chen, Q.H., 2011. **Biotransformation optimization of betulin into betulinic acid production catalysed by cultured *Armillaria luteo-virens* Sacc ZJUQH100-6 cells**. *J. Appl. Microbiol.*, 110, p. *90*.

[35] An, T.; Zha, W.; Zi, J., 2020. **Biotechnological production of betulinic acid and derivatives and their applications**. *Appl. Microbiol. Biotechnol.*, 104, p. *3339*.

[36] Stork, G.; Uyeo, S.; Wakamatsu, T.; Grieco, P.; Labovitz, J., 1971. **The Total Synthesis of Lupeol**. *J. Am. Chem. Soc.*, 93, p. *4945*.

[37] Surendra, K.; Corey, E. J., 2009. **A short enantioselective total synthesis of the fundamental pentacyclic triterpene lupeol**. *J. Am. Chem. Soc.*, 131, p. *13928*.

[38] Kim, D. S. H. L.; Chen, Z.; Van Nguyen, T.; Pezzuto, J. M.; Qiu, S.; Lu, Z. Z., 1997. **A concise semi-synthetic approach to betulinic acid from betulin**. *Synth. Commun.*, 27, p. *1607*.

[39] Laszczyk, M. N., 2009. **Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy**. *Planta Med.*, 75, p. *1549*.

[40] Csuk, R.; Schmuck, K.; Schäfer, R., 2006. **A practical synthesis of betulinic acid**. *Tetrahedron Lett.*, 47, p. *8769*.

[41] Ruzicka, L.; Lamberton, A. H.; Christie, E. W., 1938. Zur Kenntnis der Triterpene. **41. Mitteilung. Oxydation des Betulin-mono-acetats mit Chromtrioxyd zu sauren Produkten**. *Helv. Chim. Acta*, p. *1706*.

[42] Baltina, L. A.; Flekhter, O. B.; Nigmatullina, L.R.; Boreko, E.I.; Pavlova, N.I.; Nikolaeva, S. N.; Savinova, O. V.; Tolstikov, G. A., 2003. **Lupane triterpenes and derivatives with antiviral activity**. *Bioorganic Med. Chem. Lett.*, 13, p. *3549*.

[43] Pichette, A.; Liu, H.; Roy, C.; Tanguay, S.; Simard, F.; Lavoie, S., 2004. **Selective oxidation of betulin for the preparation of betulinic acid, an antitumoral compound**. *Synth. Commun.*, 34, p. *3925*.

[44] Wickholm, N.; Alakurtti, S.; Yli-Kauhaluoma, J.; Koskimies, J. **Method for preparation of betulinic acid.** WO2013038316A1, 21 March 2013.

[45] Domínguez-Carmona, D. B.; Escalante-Erosa, F.; García-Sosa, K.; Ruiz-Pinell, G.; Gutierrez-Yapu, D.; Chan-Bacab, M. J.; Giménez-Turba, A.; Peña-Rodríguez, L. M., 2010. **Antiprotozoal activity of betulinic acid derivatives**. *Phytomedicine*, 17, p. *379*.

[46] Bringmann, G.; Saeb, W.; Assi, L. A.; François, G.; Narayanan, A. S. S.; Peters, K.; Peters, E.-M., 1997. **Betulinic acid: Isolation from *Triphyophyllum peltatum* and *Ancistrocladus heyneanus*, antimalarial activity, and crystal structure of the benzyl ester**. *Planta Med.*, 63, p. *255*.

[47] Steele, J.; Warhurst, D.; Kirby, G.; Simmonds, M., 1999. ***In vitro* and *in vivo* evaluation of betulinic acid as an antimalarial**. *Phyther. Res.*, 13, p. *115*.

[48] Ziegler, H. L.; Franzyk, H.; Sairafianpour, M.; Tabatabai, M.; Tehrani, M. D.; Bagherzadeh, K.; Hägerstrand, H.; Stærk, D.; Jaroszewski, J. W., 2004. **Erythrocyte membrane modifying agents and the inhibition of *Plasmodium falciparum* growth: Structure-activity relationships for betulinic acid analogues**. *Bioorganic Med. Chem.*, 12, p. *119*.

[49] De Sá, M. S.; Costa, J. F. O.; Krettli, A. U.; Zalis, M. G.; De Azevedo Maia, G. L.; Sette, I. M. F.; De Amorim Câmara, C.; Filho, J. M. B.; Giulietti-Harley, A. M.; Ribeiro

Dos Santos, R.; *et al*., 2009. **Antimalarial activity of betulinic acid and derivatives *in vitro* against *Plasmodium falciparum* and *in vivo* in *P. berghei*-infected mice**. *Parasitol*. *Res*., 105, p. *275*.

[50] Innocente, A. M.; Silva, G. N. S.; Cruz, L. N.; Moraes, M. S.; Nakabashi, M.; Sonnet, P.; Gosmann, G.; Garcia, C. R. S.; Gnoatto, S. C. B., 2012. **Synthesis and antiplasmodial activity of betulinic acid and ursolic acid analogues**. *Molecules*, 17, p. 12003.

[51] Diedrich, D.; Wildner, A. C.; Silveira, T. F.; Silva, G. N. S.; dos Santos, F.; da Silva, E. F.; do Canto, V. P.; Visioli, F.; Gosmann, G.; Bergold, A. M.; *et al*., 2018. **SERCA plays a crucial role in the toxicity of a betulinic acid derivative with potential antimalarial activity**. *Chem*. *Biol*. *Interact*., 287, p. *70*.

[52] Silva, G. N. S.; Schuck, D. C.; Cruz, L. N.; Moraes, M. S.; Nakabashi, M.; Gosmann, G.; Garcia, C. R. S.; Gnoatto, S. C. B., 2015**. Investigation of antimalarial activity, cytotoxicity and action mechanism of piperazine derivatives of betulinic acid**. *Trop*. *Med*. *Int*. *Heal*., 20, p. *29*.

[53] Da Silva, G. N. S.; Maria, N. R. G.; Schuck, D. C.; Cruz, L. N.; De Moraes, M. S.; Nakabashi, M.; Graebin, C.; Gosmann, G.; Garcia, C. R. S.; Gnoatto, S. C. B., 2013. **Two series of new semisynthetic triterpene derivatives: Differences in anti-malarial activity, cytotoxicity and mechanism of action**. *Malar*. *J*., 12, p. *1–7*.

[54] Cargnin, S. T.; Staudt, A. F.; Medeiros, P.; de Medeiros Sol S., D.; de Azevedo dos Santos, A. P.; Zanchi, F. B.; Gosmann, G.; Puyet, A.; Garcia Teles, C. B.; Gnoatto, S. B., 2018. **Semisynthesis, cytotoxicity, antimalarial evaluation and structure-activity relationship of two series of triterpene derivatives**. *Bioorganic Med*. *Chem*. *Lett*., 28, p. *265*.

[55] Ullah, A.; Baratto, L. C.; Paula, R. C.; Silva, L. H. V.; Soares, M. J.; Oliveira, B. H., 2016. **Preparation of derivatives of betulinic acid, steviol and isosteviol and evaluation of antitrypanosomal and antimalarial activities**. *J*. *Braz*. *Chem*. *Soc*., 27, p. *1245*.

[56] Karagöz, A. Ç.; Leidenberger, M.; Hahn, F.; Hampel, F.; Friedrich, O.; Marschall, M.; Kappes, B.; Tsogoeva, S. B., 2019. **Synthesis of new betulinic acid/betulin-derived dimers and hybrids with potent antimalarial and antiviral activities**. *Bioorganic Med*. *Chem*., 27, p. *110*.

[57] Sauvain, M.; Kunesch, N.; Poisson, J.; Gantier, J. C.; Gayral, P.; Dedet, J. P., **1996. Isolation of leishmanicidal triterpenes and lignans from the amazonian liana *Doliocarpus dentatus* (Dilleniaceae)**. *Phyther*. *Res*., 10, p. *1–4*.

[58] Chowdhury, A. R.; Mandal, S.; Goswami, A.; Ghosh, M.; Mandal, L.; Chakraborty, D.; Ganguly, A.; Tripathi, G.; Mukhopadhyay, S.; Bandyopadhyay, S.; *et al*., 2003. **Dihydrobetulinic acid induces apoptosis in *Leishmania donovani* by targeting DNA topoisomerase I and II: implications in antileishmanial therapy**. *Mol*. *Med*., 9, p. *26*.

[59] Takahashi, M.; Fuchino, H.; Sekita, S.; Satake, M., 2004. ***In vitro* leishmanicidal activity of some scarce natural products**. *Phyther*. *Res*., 18, p. *573*.

[60] Haavikko, R.; Nasereddin, A.; Sacerdoti-Sierra, N.; Kopelyanskiy, D.; Alakurtti, S.; Tikka, M.; Jaffe, C. L.; Yli-Kauhaluoma, J., 2014. **Heterocycle-fused lupane triterpenoids inhibit *Leishmania donovani* amastigotes**. *Med. Chem. Comm.*, 5, p. *445*.

[61] Santos, R. C.; Salvador, J. A. R.; Marín, S.; Cascante, M., 2009. **Novel semisynthetic derivatives of betulin and betulinic acid with cytotoxic activity**. *Bioorganic Med. Chem*., 17, p. *6241*.

[62] Sousa, M. C.; Varandas, R.; Santos, R. C.; Santos-Rosa, M.; Alves, V.; Salvador, J. A. R., 2014. **Antileishmanial activity of semisynthetic lupane triterpenoids betulin and betulinic acid derivatives: synergistic effects with miltefosine**. *PLoS ONE*, 9, e89939.

[63] Alakurtti, S.; Heiska, T.; Kiriazis, A.; Sacerdoti-Sierra, N.; Jaffe, C. L.; Yli-Kauhaluoma, J., 2010. **Synthesis and anti-leishmanial activity of heterocyclic betulin derivatives**. *Bioorganic Med. Chem*., 18, p. *1573*.

[64] Hoet, S.; Pieters, L.; Muccioli, G. G.; Habib-Jiwan, J. L.; Opperdoes, F. R.; Quetin-Leclercq, J., 2007. **Antitrypanosomal activity of triterpenoids and sterols from the leaves of *Strychnos spinosa* and related compounds**. *J. Nat. Prod*., 70, p. *1360*.

[65] Meira, C. S.; Barbosa-Filho, J. M.; Lanfredi-Rangel, A.; Guimarães, E. T.; Moreira, D. R. M.; Soares, M. B. P., 2016. **Antiparasitic evaluation of betulinic acid derivatives reveals effective and selective anti-*Trypanosoma cruzi* inhibitors**. *Exp. Parasitol*., 166, p. *108*.

[66] Borkova, L.; Hodon, J.; Urban, M., 2018**. Synthesis of Betulinic Acid Derivatives with Modified A-Rings and their Application as Potential Drug Candidates**. *Asian J. Org. Chem*., 7, p. *1542*.

[67] Hodon, J.; Borkova, L.; Pokorny, J.; Kazakova, A.; Urban, M., 2019. **Design and synthesis of pentacyclic triterpene conjugates and their use in medicinal research**. *Eur. J. Med. Chem*., 182, p. *1–25*.

[68] Grymel, M.; Zawojak, M.; Adamek, J., 2019. **Triphenylphosphonium Analogues of Betulin and Betulinic Acid with Biological Activity: A Comprehensive Review**. *J. Nat. Prod*., 82, p. *1719*.

[69] Feng, Y.; Li, M.; Liu, J.; Xu, T. Y.; Fang, R. S.; Chen, Q. H.; He, G. Q., 2013. **A novel one-step microbial transformation of betulin to betulinic acid catalysed by *Cunninghamella blakesleeana***. *Food Chem*., 136, p. *73*.

[70] Chatterjee, P.; Kouzi, S. A.; Pezzuto, J. M.; Hamann, M. T., 2000**. Biotransformation of the antimelanoma agent betulinic acid by *Bacillus megaterium* ATCC 13368**. *Appl. Environ. Microbiol*., 66, p. *3850*.

[71] Wu, J.; Niu, Y.; Bakur, A.; Li, H.; Chen, Q., 2017. **Cell-free production of pentacyclic triterpenoid compound betulinic acid from betulin by the engineered *Saccharomyces cerevisiae***. *Molecules*, 22, p. *1075*.

[72] Häkkinen, S. T.; Nygren, H.; Maiorova, N.; Haavikko, R.; Alakurtti, S.; Yli-Kauhaluoma, J.; Rischer, H.; Oksman-Caldentey, K. M., 2018. **Biotransformation of Cyclodextrine-Complexed Semisynthetic Betulin Derivatives by Plant Cells**. *Planta Med*., 84, p. *743*.

[73] Cargnin, S. T.; Staudt, A. F.; Paula de Azevedo dos Santos, A.; Gosmann, G.; Bioni, C.; Teles, G.; Gnoatto, S. B., 2017. **Effective approach to semi-synthesis of lupane and ursane brominated derivatives and its effects on viability of *Leishmania amazonensis***. *Ann. Med. Chem. Res*., 3, p. *1–5*.

[74] Tietze, L. F.; Bell, H. P.; Chandrasekhar, S., 2003. **Natural product hybrids as new leads for drug discovery**. *Angew. Chem. Int. Ed.*, 42, p. *3996*.

[75] Ojima, I., 2008. **Modern Molecular Approaches to Drug Design and Discovery**. *Acc. Chem. Res.*, 41, p. *2*.

[76] Bansal, Y.; Silakari, O., 2014. **Multifunctional compounds: smart molecules for multifactorial diseases**. *Eur. J. Med. Chem.*, 76, p. *31*.

CAPÍTULO 3

CHEMICAL COMPOSITION, BIOLOGICAL ACTIVITIES AND USES OF ANACARDIACEAE SPECIES: AN UPDATED REVIEW

1. INTRODUCTION

Anacardiaceae is a family consisting of about 600 species distributed in 76 genera. The genera are subdivided into five tribes (Anacardieae, Dobineae, Rhoeae, Semecarpeae and Spondiadeae). The plants of this family are known as sources of edible fruits and condiments such as mango, cashew, pistachios (*Pistacia* spp.), sumac (*Rhus coriaria*) and pink peppercorns (*Schinus terebinthifolius*). Approximately 25% of genera present toxic phenolics, which are compounds responsible for several contact dermatitis. In general, the poisonous species of this family are restricted to the tribes Anacardieae, Rhoeae and Semecarpeae (Correia, 2006; Baer, 1979).

Phenolic and catecholic lipids are usual compounds present in these plants, and these compounds are usually responsible for their toxic properties, whether alone or in mixtures of different saturated or unsaturated aliphatic chains. These compounds are present in different plant parts and frequently occur in *Rhus* species. Then, species of this family have been frequently studied from a chemical and biological point of view due to their potential as sources of new bioactive compounds. The most studied genera are *Mangifera*, *Rhus* (*Toxicodendron*), *Spondias*, *Schinus*, *Lannea*, *Semecarpus*, *Pistacia*, *Lithraea*, *Tapirira*, *Melanorrhoea* and *Anacardium*. However, most of Anacardiaceae species remain unknown about their chemical composition, pharmacological and other biological activities. Alongside some recent reviews about some class or specific compound in Anacardiaceae (Schulze-Kaysers, 2015; Singh, 2009) and genus *Schinus* (El-Nashar, 2022), there are just two reviews of all genera, the last one dated from 2006 (Correia, 2006; Baer, 1979).

The current review with approximately 170 references is an update of the chemical composition, biological activities in extracts and pure compounds isolated from different Anacardiaceae plant species since 2006. Moreover, some processes, technological applications and new insights about the biosynthesis of phenolic lipids were also reviewed.

2. LITERATURE SEARCH STRATEGY

A bibliographic survey of scientific articles published in indexed journals and deposited process patents was performed to develop this review. For this purpose, the databases SciFinder, Web of Science, Science Direct, PubMed, Scielo and Google Scholar were utilized. All articles published from 2006 to March 2023 were considered, including papers not considered in a previous review. In the quest "Advanced Search" feature combined with the keywords "Anacardiaceae", "chemical composition", "bioactivity", "biosynthesis" and all the genera described in specialized literature were used (https://www.mobot.org/mobot/research/apweb/genera/anacardiaceaegen.html, accessed on November 2022). The titles of articles and patents found were scanned and organized in a file when considered meaningful. Afterward, duplicates were removed and, thus, the abstracts of the articles obtained were checked for relevant information as part of the inclusion strategy. Finally, all articles and patents were carefully read and, after reviewing, 149 papers and 21 patents were selected to compose the present work.

3. CHEMICAL COMPOSITION OF ANACARDIACEAE spp.

3.1. Essential Oils (EOs) and Volatile Other Compounds (VOCs)

Studies concerning to essential oils (EOs) and Volatile Organic Compounds (VOCs) of Anacardiaceae family are frequently performed, especially for edible species (such as fruits and seeds). These EOs are usually obtained from plants' leaves, flowers and other aerial parts. The most recent works will be highlighted herein, including the compositions of these metabolites in different species and genera, the identification of new substances and other relevant information.

The investigation of the EOs' chemical composition of *Mangifera indica* (var. "coquinho"; plant leaves) (Simionatto, 2010) indicated that the sesquiterpene hydrocarbons are the leading representative compounds (66.4%) against the oxygenated ones (8.7%), which have presented anticancer, antimicrobial and antioxidant activities. On the other hand, in barks of *M. indica* L. was reported (Nyegue, 2018) that the sesquiterpene hydrocarbons reached 97.0% in an analogue study (Table 7). The other components of these EOs are monoterpenes (≤ 2%).

Table 7. Relative composition (in %) of the more abundant compounds in the essential oils from some *Mangifera indica* varieties

Compounds	**(var. coquinho) - leaves**		**barks**
Sesquiterpene hydrocarbons	α-gurjunene	24.0	-
	β-selinene	24.0	-
	β-caryophyllene	11.2	60.3
	α-humulene	7.2	36.7
Oxygenated sesquiterpenes	caryophyllene oxide	5.5	-
	humulene epoxide	2.4	-

Rhus cotinus L. (syn. *Cotinus coggygria* Scop.) is a European tree commonly grown as an ornamental plant, presenting different cultivars due to the different purple foliage and flowers. The wood of this species presented economic importance because it was formerly used to make the yellow dye called young fustic (fisetin), now replaced by synthetic dyes. The profile of EOs obtained from the same species varies according to the biome, the part of the plant (fresh aerial parts, leaves or flowering aerial parts) and seasonally, even between regions that are close in latitude (Joshi, 2014; Ulukanli, 2014; Fraternale, 2018). Thus, the total composition of monoterpenes was not similar in both cases (Table 8).

Table 8. Relative composition (in %) of the most abundant essential oils from *Rhus cotinus*

Subgroup	Compounds	Fresh aerial parts[9] (%)	Leaves[10] (%)	Flowering aerial parts[11] (%)
	α-pinene	5.2	43.1	8.8
	β-pinene	30.6	3.4	n.d.
	limonene	12.4	21.3	49.2
Monoterpene	camphene	13.6	n.d.	n.d.
hydrocarbons	*p*-cymene	4.6	n.d.	n.d.
[66.4–79.6]	*β*-myrcene	n.d.	8.5	n.d.
	α-terpinene	n.d.	3.3	n.d.
	(*Z*)-*β*-ocimene	n.d.	n.d.	13.6
	(*E*)-*β*-ocimene	n.d.	n.d.	5.9
Sesquiterpene	*β*-caryophyllene	4.4	2.4	n.d.
hydrocarbons	bicyclogermacrene	12.6	n.d.	n.d.
[0-21.4]	germacrene D	2.0	n.d.	n.d.
Oxygenated				
Monoterpenes	α-terpinolene	n.d.	5.0	n.d.
[0-5.0]				

n.d.: not detected

Pistacia spp. (such as *P. lentiscus* L., *P. vera*, *P. terebinthus* L. and *P. khinjuk* Stocks) are employed in Europe and the Mediterranean region as food, for cooking and other purposes (e.g., the oleoresin). Consequently, this is the most probable reason for the expressive number of studies dealing with the EOs composition of these species.

The EOs profile of *P. lentiscus is* similar to species from different habitats (Negro, 2015) (Southern Italy and Morocco, Tunisia, Greece or France) but distinct from other EOs profiles obtained from specimens from Egypt, Sardinia Island/Italy and Spain, probably due to the different climate and seasonal changes, besides insect presence, physicochemical soil properties, extraction methods and others. Furthermore, mastic gum essential oils (MGEOs) of wild plants of *P. lentiscus* (Tabanca, 2020) are quantitatively different compared to the cultivated plants, so that the tree age could also

affect this chemical composition. Other subsequent studies (Abed, 2017; Komaitis, 2008) present several data that confirm the exact behavior of the EOs profile.

Similarly, the EOs content in *P. terebinthus* (Hadjari, 2016; Ulukanli, 2014) is also related to the plant organ and population origins. In these studies, the variability of the composition was carefully analyzed by the Principal Component Analysis (PCA), and the conclusion is that abiotic (climatic, edaphic, chemical, among others) and biotic (genotypic diversity and nutritional variations) factors may be related to these variations. At last, since no previous published data deals with the *P. khinjuk* EOs leaf profile, it was impossible to compare the current study with the EOs composition of other *P. khinjuk* (Abolghasemi, 2018) trees from other regions (particularly from Iran).

Studies employing PCA (Principal Component Analysis) and HCA (Hierarchical Cluster Analysis) permitted us to evaluate if EOs constituents could reflect the chemotaxonomic relationships in *Pistacia* species (Ismail, 2013). Based on the most abundant compounds present in the EOs (contents ≥ 3.5%), the groups were classified as chemotypes 1) Group A (α-pinene, *β*-pinene, limonene and terpinen-4-ol, *P. lentiscus*) and 2) Group B (B1, α-terpinene, *P. terebinthus*; B2, limonene, *P. vera*). Table 9 summarizes the volatile compounds from *Pistacia* spp., so that the examination of the data clearly indicates the monoterpenes are the main compounds – especially α- and *β*-pinenes (in *P. lentiscus*), α-pinene, limonene and *β*-ocimene (in *P. terebinthus* and *P. vera*) and myrcene and eudesmol (in *P. khinjuk*).

Table 9. Relative composition (in %) of the most abundant essential oils from *Pistacia* spp

	***P. lentiscus* L.** [12-15]	**Variation (%)**
Monoterpene hydrocarbons [77.0–85.0]	α-pinene	[2.4–70.8]
	β-pinene	[0.3–9.6]
	limonene	[1.0–17.8]
	β-myrcene / myrcene	[2.5–20.1]
	sabinene	[1.0–6.7]
	γ-terpinene	[3.10–6.21]
	α-terpinolene	[2.18–2.20]

	p-cymene	[0.5–7.5]
Oxygenated monoterpenes	terpinen-4-ol	[0.7–21.7]
[6.0–23.1]	α-terpineol	[2.5–4,0]
Sesquiterpene hydrocarbons	*β*-caryophyllene/caryophyllene	[2.6–19,9]
[5.0–28.1]	δ-cadinene	11.7
	α-muurolene	[0.1–6.9]
	P. terebinthus L.[16-17]	
	α-pinene	[12.58–66.29]
	D-limonene // limonene	[13.95–46.29]
	(*E*)-*β*-ocimene // (*Z*)-*β*-ocimene	[40.49–44.85]
Monoterpene hydrocarbons	*β*-pinene	[1.99–20.47]
[63.90–98.94]	sabinene	[5.61–15.43]
	α-phellandrene	2.51
	β-phellandrene	3.21
	β-myrcene	2.79
	τ-terpinene	2.46
	o-cymene	4.72
Oxygenated monoterpenes	terpinen-4-ol	9.65
Sesquiterpene hydrocarbons	*β*-cubebene	2.61
Oxygenated sesquiterpenes	caryophyllene oxide	1.66
	P. khinjuk **Stocks** [18]	
Monoterpene hydrocarbons	myrcene	18.7
Oxygenated sesquiterpenes	α-eudesmol	12.3
[26.5]	*β*-eudesmol	9.3
	δ-eudesmol	4.9
Sesquiterpene hydrocarbons	1,7-di-epi-*β*-cedrene	7.3
[12.9]	bicyclogermacrene	5.6
	P. vera [19]	
	α-pinene	16.07

	β-pinene	2.32
	α-terpinene	[32.44–41.34]
Monoterpene hydrocarbons	limonene	25.10
	α-terpinolene	[1.13–8.02]
	β-myrcene	1.29
	α-phellandrene	3.85
	δ-terpinene	6.99
	α-terpineol	[2.14–4.52]
Oxygenated monoterpenes	terpen-4-ol	1.38
	*iso*bornyl acetate	1.74
	germacrene D	8.4
	β-bourbonene	1.2
	β-elemene	1.3
Sesquiterpene hydrocarbons	*β*-bisabolene	1.6
	α-copaene	1.1
	β-caryophyllene/(Z)-caryophyllene	3.67
	δ-cadinene	1.41
Oxygenated sesquiterpenes	caryophyllene oxide	[1.10–1.51]
	α-cadinol	[1.90–2.12]

Spondias L. is a genus with about ten species, mainly in Asia, three or four species native to the Neotropics, and most of them produce edible fruits. A previous study with *S. pinnata* from east India showed that the major VOCs of whole green fruits were isopropyl myristinate (36.85%), isophorone (6.55%), limonene (4.46%) and linalool (3.57%) (Satpathy, 2011). However, the EOs from fruits of specimens growing in Egypt was composed mainly of long-chain alkanes (51.1%) besides fatty acid esters (25.7%). The relative most predominant component (25.00%) was n-nonacosane (Sameh, 2019). Therefore, these results indicate that the profile of the significant constituents in the green fruits, ripe fruits and fruit peels can change with the plant part

studied, though the extraction methods or geographic locations could also influence such differences, which may partly determine the variation in bioactivity (Ren, 2020).

Variations in the EOs compositions could be related to the investigated species' cultivation, vegetative stage, source or seasonal growing. Furthermore, an increase in the oxygenated monoterpenoid amount as well as a decrease in the sesquiterpenoid hydrocarbons content was observed due to the dehydrating of the leaves, while the contents of some minor metabolites (geraniol, eugenol, borneol, terpinen-4-ol, besides others) were stable in the two oils, although were present in small quantities (< 1.0%) (Oladimeji, 2016). Table 10 summarizes the data and presents additional compounds of some *Spondias* spp.

Table 10. Relative composition (in %) of the most abundant essential oils from *Spondias* spp

	***S. pinnata*[22] (L. Pinn) Kurz (fruit peels)**	**Variation (%)**
	ethyl benzoate	9.05
Aliphatic compounds	methyl salicylate	5.88
[39.42]	(*Z*)-3-hexen-1-ol	4.88
	2-hexenal	4.17
	α-terpineol	13.09
Monoterpene hydrocarbons	γ-terpineol	5.55
[29.62]	terpinen-4-ol	2.66
	limonene	2.04
	*iso*borneol	1.04
Aromatics	furfural	17.14
[22.03]	ethyl cinnamate	3.55

	***S. mombin* Linn[23]**		
		fresh leaves (%)	**dried leaves (%)**
	β-caryophyllene	27.96	30.90

	γ-cadinene	12.30	9.7
	α-humulene	8.1	5.4
Sesquiterpene hydrocarbons	*β*-cadinene	7.8	6.6
[67.40–76.66]	α-gurjunene	6.4	7.4
	α-muurolene	5.9	4.2
	β-elemene	4.2	3.2
	γ-muurolene	4.0	-
Oxygenated monoterpenes	geranial	3.7	3.8
[9.9–13.2]	neral	6.2	9.4
Oxygenated sesquiterpenes	caryophyllene oxide	6.9	6.2
[13.3–15.7]	5-*iso*cedranol	6.4	9.5

Different parts of *Schinus terebinthifolia* Raddi (*sin.: Schinus terebinthifolius* Raddi) and *S. molle* L. are widely studied, probably due to the employment of these species as folk medicines, as well as their fruits are used as spicier (pink pepper). The studies with the composition of *S. terebinthifolia* leaves EOs corroborated with seasonal variation previously observed. The oil obtained from specimens harvested in March showed a high concentration of myrcene (15.4%) and (*E*)-caryophyllene (14.7%); in July, these constituents represented only 0.8% and 2.7% (respectively) of the total oil. Germacrene-D content increased from 8.8% in March to 21.0% in July, whereas *α*-phellandrene, undetectable in oils collected in March, rose to 18.2% in July. The EOs obtained in July contained 15.5% of oxygenated sesquiterpenes, whilst these compounds are present in only 5.8% in the oils obtained from March studies (Barbosa, 2012).

Other *Schinus* species (*S. longifolia, S. fasciculata, S. lentiscifolius* and *S. weinmannifolius*) are frequent sources of essential oils (Murray, 2008; Da Silva, 2019; Hernandes, 2014). Likewise, in the former examples, the differences between the found EOs profiles are related to seasonal factors, extraction methodologies and geographical origin. Table 11 summarizes the last updates in the VOC's content of *Schinus* species

(Lago, 2012; Gazim, 2018; Fernandes, 2012; Sartorelli, 2012; dos Santos, 2009; Pawlowski, 2012; Chaves, 2015; Barbosa, 2012; Simionatto, 2011; Afifi, 2019; Budel, 2019; Rodilla, 2012; Machado, 2018; Descamps, 2011; Murray, 2009).

Table 11. Relative composition (in %) of the most abundant essential oils from different parts of *Schinus* spp

	S. terebinthifolia [24-30, 31a-b]	**Variation (%)**
Monoterpene hydrocarbons [16.44–77.35] (30.9 [31a] ; 46.6 [31b])	α-pinene	[5.7 –44.9] ; [1.2 ± 0.1] [31a] and [4.2 ± 0.1] [31b]
	β-pinene	[1.91 –15.1]
	β-myrcene	[1.56–20.43] ; 15.4 ± 0.9 [31a]
	sylvestrene	3.7
	β-phellandrene	[6.59–7.30]
	limonene	[1.40–20.81] ; [12.0 ± 0.6] [31a] and [16.7 ± 1.1] [31b]
	isosylvestrene	13.87
	α-fenchene	20.75
	p-cymene	[1.45–2.90] ; [3.3 ± 0.2] [31b]
	δ-3-carene	[2.69–12.75]
	sabinene	[2.60–6.20]
	α-phellandrene	[1.35–14.94] ; [18.2 ± 1.2] [31b]
	α-terpinene	[1.17–2.20]
	γ-terpinene	1.81
	trans-ocimene/(*E*)-*β*-ocimene	12.32; [2.3 ± 0.2] [31a] and [2.6 ± 0.1] [31b]
	tricyclene	8.3
	β-longipinene	[4.35–8.1]
	α-humulene	[2.5 ± 0.1] [31a]
	β-camigrene	[7.5 ± 1.0] [31a]
	bicyclogermacrene	[1.01–27.57]

	germacrene D	[2.65–23.8] ; [8.8 ± 0.3] [31a] and [21.0 ± 1.2] [31b]
	δ-cadinene	[1.43–9.21] ; [3.6 ± 0.1] [31a] and [1.6 ± 0.1] [31b]
	aromadendrene	1.1 [24]
Hydrocarbon sesquiterpenes	*β*-elemene	1.4 ; [4.8 ± 0.3] [31a] and [2.1 ± 0.1] [31b]
[3.63–72.1]		
(59.4 [31a] ; 35.4 [31b])	*iso*longifolene	7.11
	(*Z*)-*β*-farnesene	[1.65–6.38]
	α-copaene	[3.4 –7.96] ; [1.7 ± 0.1] [31a]
	δ-elemene	1.68 ; [2.4 ± 0.1] [31a] and 2.0 [31b]
	germacrene A	1.66 ; 2.1 [31a] and 1.3 [31b]
	α-cadinene	1.98
	germacrene B	[1.10–2.01] ; [2.1 ± 0.1] [31a] and 1.6 [31b]
	β-selinene	[4.3 ± 0.1]
	(*E*)-*β*-caryophyllene	[1.03–35.20] ; [14.7 ± 0.8] [31a] and [2.7 ± 0.2] [31b]
	γ-cadinene	1.11 ; [2.3 ± 0.1] [31a]
	α-*trans*-bergamotene	1.80
	α-amorphene	1.41
	α-muurolene	[2.6 ± 0.1] [31a]
	cis-sabinene hydrate	1.03
Oxygenated monoterpenes	terpinen-4-ol	[3.42 –3.63]
[3.10–17.81]	α-terpineol	[2.65–14.39]
	eucalyptol	8.5
	spathulenol	[1.02–1.90] ; 1.1 [31a] and [2.1 ± 0.1] [31b]
	globulol	2.7
	viridiflorol	2.2 ; [2.5 ± 0.2] [31b]

	cedryl acetate	2.6
Oxygenated sesquiterpenes	caryophyllene acetate	2.1
[0.31–27.85]	caryophyllene alcohol	2.41
(5.8 [31a]; 15.5 [31b])	γ-eudesmol	1.49
	α-cadinol	[1.58–11.62]; 1.4 [31a] and [3.1 ± 0.1] [31b]
	τ-cadinol	[1.15–2.67]
	τ-muurolol	5.12
	α-bisabolol	4.37
	elemol	4.07
	cis-cadinen-4-en-7-ol	1.6
	β-caryophyllene oxide	[2.6 ± 0.1] [31b]
	α-muurolol	[1.2 ± 0.1] [31a] and [2.8 ± 0.1] [31b]
	δ-cadinol	[1.3 ± 0.1] [31a] and [2.4 ± 0.1] [31b]

	S. molle [28a-b, 29, 30a-b, 32-36]	**Variation (%)**
	globulol	1.2
	ledol	1.5
	ubenol	27.1
	epi-α-cadinol	[1.7–27.3]
	caryophyllene oxide	[1.02–15.3]
	δ-cadinol	2.11
Oxygenated sesquiterpenes	(+)-spathulenol	[1.97–12.4]
[0.29–63.6]	1,10-di-*epi*-cubenol	[2.5–4.4]
	epi-α-eudesmol	2.3
	guaiol	1.9
	cedrol	1.7
	γ-eudesmol	1.97
	longipinanol	6.08
	α-bisabolol	1.29
	muurolol	11.8

	1,8-cineol	[2.04–7.60]
	terpin-4-ol	6.1
	7-formyloxy-sabinen-2-ol	n.d.a.
	terpin-3-en-1,5-diol	n.d.a.
	terpin-2-en-1,4-diol	n.d.a.
	(-)-*trans*-pinocarveol	[1.9–4.7]
Oxygenated monoterpenes	nopinone	1.7
[0.35–25.6]	*trans*-verbenol	4.2
	pinocarvone	1.1
	myrtenal	5.3
	verbenone	6.2
	trans-linalool oxide	1.3
	myrtenol	4.6
	(-)-*trans*-carveol	1.1
	eugenol	2.96
	piperitone	1.5
	linalool	[1.60–2.98]
	terpinen-4-ol	[1.74–4.93]
	α-selinene	2.7
	alloaromadendrene	1.7
	γ-cadinene	[1.22–9.10]
	δ-cadinene	1.14
	bicyclogermacrene	[1.4–18.12]
Hydrocarbon sesquiterpenes	(*E*)-caryophyllene / *β*-caryophyllene	[1.66–6.08]
[0.10–31.18]	9-*epi*(*E*)-caryophyllene	1.2
	zonarene	1.9
	β-elemene	1.97
	germacrene-B	3.87
	α-bisabolene	2.57

	α-humulene	1.73
Hydrocarbon monoterpenes [4.2–98.5]	α-pinene	[1.21–35.28]
	β-pinene	[1.32–36.3]
	β-phellandrene	[2.90–38.06]
	α-phellandrene	[38.84–55.90]
	myrcene	[1.12–6,43]
	α-terpinene	[1.13–1,46]
	α-thujene	[1.48–5,72]
	terpinolene	[12.23–20.10]
	(L)-limonene	[1.80–32,21]
	sabinene	[2.9–51.74]
	p-cymene	1.46
	γ-terpinene	[1.96–2.39]
	***S. areira* (L.)** [37-38]	**Variation (%)**
Hydrocarbon monoterpenes [61.0–89.8]	α-phellandrene	[16.2–31.8]
	3-carene	[20.8–21.3]
	camphene	[1.8–10.9]
	α-pinene	[3.1–7.1]
	β-myrcene	[3.4–19.7]
	β-pinene	[2.4–5.5]
	o-cymene	[3.9–7.5]
	sabinene	[1.4–4.3]
	α-terpinolene	5.2
	p-cymene	[3.0–5.1]
	β-phellandrene	[17.6–19.9]
Hydrocarbon sesquiterpenes	(*β*)-copaene	[1.5–3.1]
	β-cubebene	[1.6–2.9]
	(*β*)-caryophyllene	[1.9–2.3]
	tricyclene	1.1

[5.0–15.1]	γ-muurolene	5.1
	β-selinene	1.5
	α-muurolene	1.3
	γ-cadinene	1.4
	δ-cadinene	[2.7–4.6]
Oxygenated monoterpenes	terpinen-4-ol	[1.2–2.1]
[2.1–3.9]	bornyl acetate	[1.4–1.9]
	palustrol	1.2
Oxygenated sesquiterpenes	globulol	1.1
[2.9–12.9]	viridiflorol	1.1
	guaiol	6.2
	δ-cadinol	[2.9–3.3]
Esters (2.9)	methyl caprylate	1.6
	ethyl hexanoate	1.3

28: the oils were obtained by two methods: a) using a pilot extractor and b) a Clevenger apparatus.
32: after 6 h extraction by hydrodistillation.
n.d.a. not-determined amount.
31a: *S. terebinthifolia* collected in March (2008); 30b: *S. terebinthifolia* collected in July (2008);
37: all the percentages were reported initially with standard deviation.

Anacardium genus always presents commercially and economically important species, which have justified extensive studies with its main species, including their flavor-related volatile compounds. Studies with Brazilian *A. occidentale* L. oil occurring in different regions indicate differences in the chemical compositions of major compounds, whose differences are probably associated with genetic variability amongst the populations grown at each location. In the leaf species collected in Minas Gerais state (Brazil), (*E*)-caryophyllene (15.4%), germacrene-D (11.5%) and *α*-copaene (10.3%) are the main components. On the other hand, the major compounds from plants cultivated in Pará state (Brazil) were (*E*)-*β*-ocimene (28.8%) and α-copaene (13.6%). Compared with specimens collected in Nigeria, the composition is also different, which were composed mainly of *β*-phellandrene (42.7%) (Barbosa, 2012). Table 12 also includes the composition of the VOCs of other Anacardiaceae species that were

determined for the first time (Cardoso, 2010; Carvalho, 2017; Tintino, 2014; Zoghbi, 2014).

Table 12. Relative composition (in %) of the most abundant essential oils from other Anacardiaceae species

	***Anacardium humile* (St. Hill fruits)[42]**	**Variation (%)**
Hydrocarbon monoterpenes (29.9)	α-pinene	[22.0 ± 0.9]
	β-pinene	[6.6 ± 1.3]
	limonene	[1.3 ± 0.1]
Hydrocarbon sesquiterpenes (60.9)	α-copaene	[2.5 ± 0.3]
	β-selinene	24.0
	(*E*)-*β*-caryophyllene	[31.0 ± 1,8]
	α-humulene	[2.9 ± 0.3]
	germacrene D	[5.9 ± 1.7]
	alloaromadendrene	[1.4 ± 0.2]
	bicyclogermacrene	[7.6 ± 1.2]
	δ-cadinene	[9.3 ± 0.7] ; 5,6
	α-bulnesene	8.0
	γ-cadinene	7.9
	α-neoclovene	7.2
	cyperene	5.3
Oxygenated sesquiterpenes (6.3)	globulol	[1.4 ± 0.4]
	epi-globulol	[1,8 ± 0,2]
	viridiflorol	[1,4 ± 0,3]
	***Myracrodruon urundeuva* (Engl. [43]); (Fr. All.[44]): leaves**	**Variation (%)**
	myrcene / α-myrcene and *β*-myrcene	[4.2 ± 0.3] ; [37.23–42.46]
	δ-3-carene	[78.8 ± 1.7] ; 80.41

Hydrocarbon monoterpenes	*β*-phellandrene	[3.0 ± 0.2]
[80.35–91.00]	α-terpinolene	[4.8 ± 0.6]
	α-pinene	1.90
	α-limonene	1.89
	o-cymene	1.09
	viridiflorene	[3.0 ± 0.2]
Hydrocarbon sesquiterpenes	*β*-selinene	[2.5 ± 0.2]
[7.31–8.10]	(*E*)-caryophyllene	[1.1 ± 0.1] ; 4.28
	α-bergamotene	1.95
Oxygenated sesquiterpenes	caryophyllene oxide	1.81
Carboxylic acids	hexadecanoic acid	3.13
(5.0)	9-hexadecanoic acid	1.34
	***Tapirira guianensis* Aubl. (leaf and branches)** [45]	**Variation (%)**
	(*E*)-caryophyllene	[19.25–66.87]
Hydrocarbon sesquiterpenes	α-selinene	[24.37–31.07]
[58.49-100.00]	*β*-selinene	[42.58–57.56]
	α-zingiberene	[18.61–24.49]
	β-sesquiphellandrene	[17.00–20,00]
	selin-11-en-4α-ol	[1.02–6.55]
	α-cadinol	[3.89–5.91]
	caryophyllene oxide	[1.86–8.29]
Oxygenated sesquiterpenes	spathulenol	[1.53–3.41]
[1.97–28.86]	viridiflorol	[1.44–1.99]
	humulene epoxide II	[1.19–1.59]
	caryophylla-4(12),8(13)-dien-5α-ol	[2.00–2.72]
	epi-α-muurolol	[1.24–2.40]

In conclusion, it have been reported the presence of several essential oils and VOCs in other Anacardiaceae species, such as *Pleyoginium timorense* (Dc.) Leenh

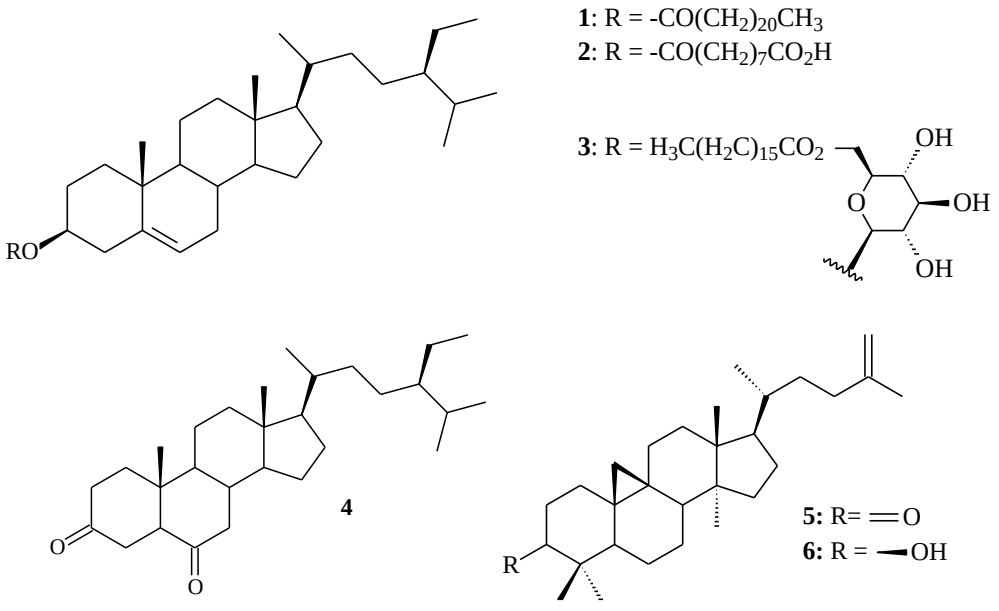
(Said, 2018), *Pseudospondias microcarpa* (A. Rich) Engl. (Babouongolo, 2021) and *Sclerocarya birrea* subsp. *caffra* (Viljoen, 2008; Kpoviéssi, 2011). The most predominant metabolites in *P. timorense* fruits (Said, 2018) were D-limonene (64.51%), γ-terpinene (5.60%), α-copaene and (*E*)-caryophyllene (4.74%). In *P. microcarpa* fruits (Babouongolo, 2021), the main compounds were α-terpinol and borneol (22.9% and 8.2%, for the epicarp), vaccenic acid and ascorbic acid 2,6-dihexadecanoate (20.1% and 29.8%, for the hull), caryophyllene oxide and α-humulene (8.4% and 6.8%, for the seed) and α-humulene and *β*-caryophyllene (9.4% and 6.4%, for the kernel). Lastly, in *Sclerocarya birrea* fruits (Viljoen, 2008), the major compounds of fruit pulp were *β*-caryophyllene and α-humulene (91.3% and 8.3%, respectively). However, in head-space (whole fruit), the most abundant metabolites were heptadecene (16.1%), benzyl 4-methylpentanoate (8.8%), benzyl butyrate (6.7%), (*Z*)-13-octadecenal (6.2%), cyclopentadecane (5.7%) and (*Z*)-3-decen-1-ol (8.4%). Otherwise, it must be highlighted that the EOs compositions of *S. birrea* (A. Rich) Hochst leaves from Benin (Kpoviéssi, 2011) were different according to the season. Thus, in hot period, the major constituents were 7-*epi*-α-selinene (38±0.03%), α-muurolene (25±0.03%), valencene (17±0.06%), *β*-selinene (4.3±0.01%), *β*-caryophyllene (3.2±0.02%), allo-aromadendrene epoxide (1.5±0.03%) and 14-hydroxy-α-humulene (1.5±0.03%), but in cold season the EOs was characterized by 7-*epi*-α-selinene (51.7±0.12%), *β*-selinene (15.1±0.2%), valencene (12.9±0.05%), α-selinene (8.1±0.03%) and *β*-caryophyllene (1.8±0.02%). These results constitute the first report of these components in this species.

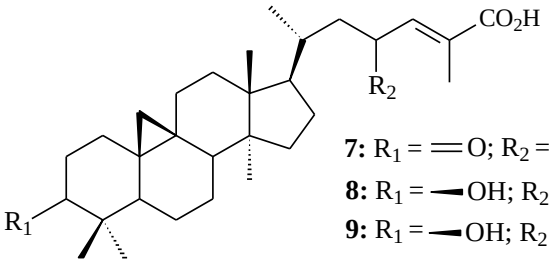
3.2. Terpenoids and steroids

Terpenoids are the most abundant class of natural products found in plants and have particular importance due to their role in plant physiology, biological properties and some industrial uses. They are present in different Anacardiaceae genera, and some isolates from this family are presented in Figure 15. The isolated compounds of these subclasses are well-known in plants in general and in Anacardiaceae *spp*. Compounds **1**-**8** were obtained from *Mangifera mekongensis* (Nguyen, 2016), so that the esters **1** (mekongsterol A), **2** (mekongsterol B) and **3 (**β-sitosteryl-3-*O*-β-D-glucopyranosyl-6-*O*-palmitate) constitute novel derivatives, whereas stigmastane-3,6-dione (**4**), ambonic

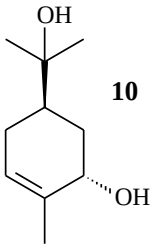
acid (**5**), ambolic acid (**6**), mangiferonic acid (**7**) and mangiferolic acid (**8**) are common in *Mangifera*. Besides, compounds **9**-**10** were obtained from *Mangifera pajang* Kosterm (Sukari, 2015), metabolites **11**-**14** from *Schinus terebinthifolia* (Silva-Júnior, 2015) and daucosterol (**15**) from *Schinopsis brasiliensis* Engl. (David, 2022). It is noteworthy that **14** was named as schinol and possesses a structure different from the previously registered compound named schinol (CAS #6813-07-6). The structure of compound **14** is previously known as the name of 3-epimasticadienolic acid (CAS # 31539-04-5). The substances **11**-**13**, found in the fruit oil of *S. terebenthifolia*, can be associated with the demonstrated antioxidant activity of the species, to the inhibition of NO synthase production and to antimicrobial properties, as well as **14** is related to antifungal activity (Silva-Júnior, 2015) against *Paracoccidioides brasiliensis*. Several sesquiterpenes were obtained from the roots of *Dobinea delavayi* (**16**-**23**) (Cheng, 2012) as well as several ergostane-type compounds (**24**-**27**) from the stem bark of *Antrocaryon klaineanum* Pierre (Fouokeng, 2017). The novel compound antrocarine *E* (**24**) was obtained with the known substances (7α)-7,20-dihydroxyergosta-4,24(28)-dien-3-one (**25**), (6α,7α)-6-methoxyergosta-4,24(28)-dien-7-ol (**26**) and (6α,7α)-ergosta-4,24(28)-diene-6,7-diol (**27**). Lastly, the new steroid-type compound named 3-oxolanosta-1,20(22)-dien-26-oic acid (**28**) was isolated from the galls of *Pistacia integerrima* Stewart (Ahmad, 2010).

Figure 15. Structures of terpenes and terpenoids obtained from plants of different Anacardiaceae species

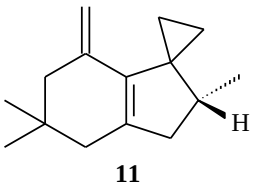




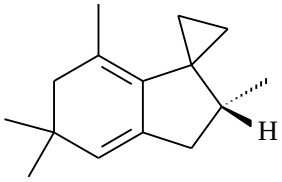
7: R_1 = =O; R_2 = H
8: R_1 = –OH; R_2 = H
9: R_1 = –OH; R_2 = —OH



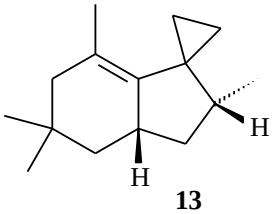
10



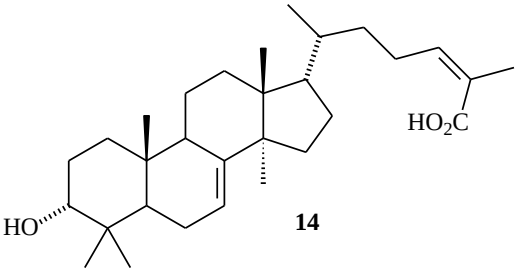
11



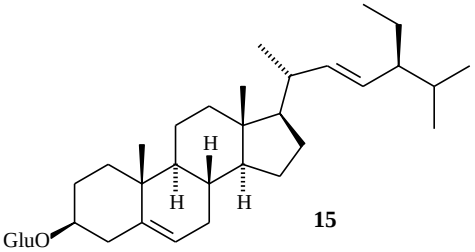
12



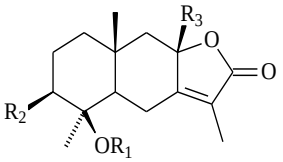
13



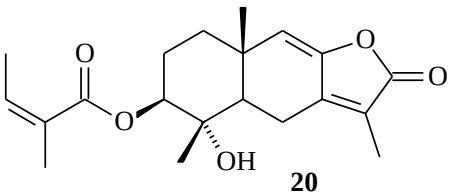
14



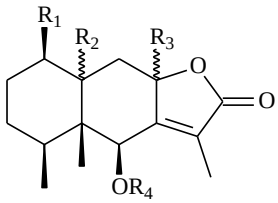
15



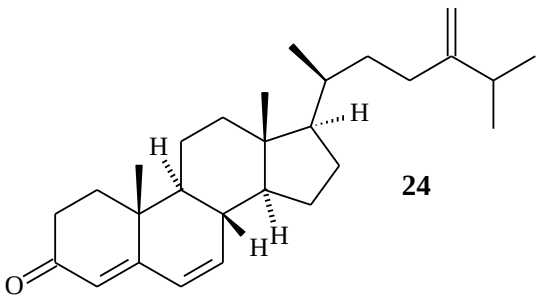
16: R_1 = Ac; R_2 = angeloyl; R_3 = H
17: R_1 = Ac; R_2 = angeloyl; R_3 = -OH
18: R_1 = H; R_2 = angeloyl; R_3 = H
19: R_1 = H; R_2 = angeloyl; R_3 = -OH



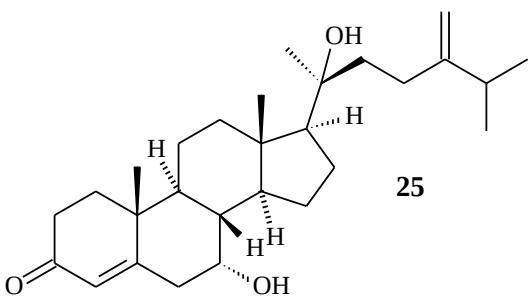
20



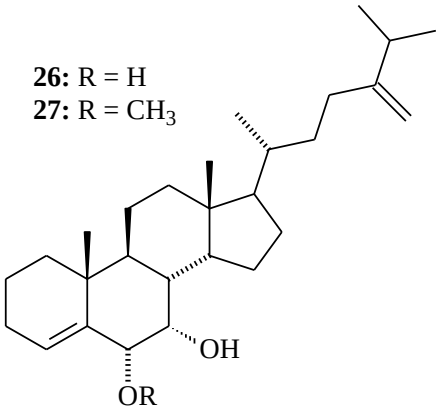
21: R_1 = *O*angeloyl; R_2 = ····OMe; R_3 = ····OH; R_4 = H
22: R_1 = *O*angeloyl; R_2 = –OMe; R_3 = –OH; R_4 = H
23: R_1 = H; R_2 = –OMe; R_3 = –OH; R_4 = Me

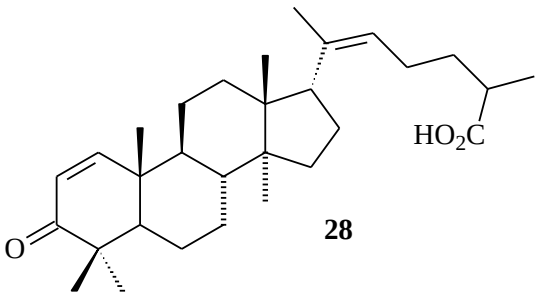


24



25





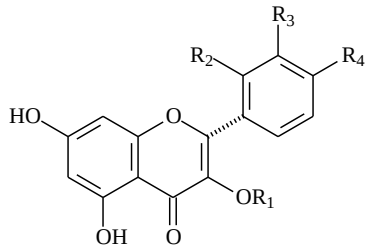
28

3.3. Flavonoids and biflavonoids

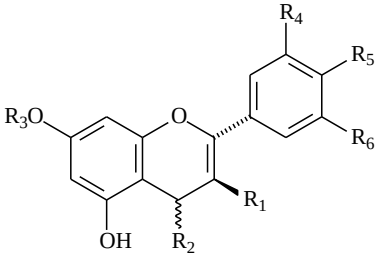
Flavonoids are common in all plant kingdom, but biflavonoids are restricted in some families, including Anacardiaceae (Correia, 2006). Figure 16 presents an update of the presence of this class in species of this family. Isoquercitrin (**29**), morin (**30**), leucocyanidin (**31**), leucodelphinidin (**32**), (2*R*,3*R*)-(+)-4',7-di-*O*-methyldihydroquercetin (**33**), (2*R*,3*R*)-(+)-4',7-di-*O*-methyldihydrokaempferol (**34**), (2*R*,3*R*)-(+)-4'-*O*-methyldihydroquercetin (**35**), 6,7-(2",2’’-dimethyl chromene)-8-g,g-dimethyl allyl flavanone (**36**), 3', 4'dihydroxy-7,8 (2",2’’-dimethyl chromene)-6-g,g dimethyl allyl flavone (**37**), l,7-methyltectorigenin (**38**) and irisolidone (**39**) were isolated from from *Lannea coromandelica* and *L. acida* (Achika, 2018). From *Semecarpus anacardium* Linn. the biflavonoids nallaflavanone (**40**), anacarduflavanone (**41**), jeediflavanone (**42**), galluflavanone (**43**), semecarpuflavanone (**44**) and tetra-hydroamentoflavone (**45**) were obtained (Semalty, 2010). These flavonoid subclass is common in Anacardiaceae *spp*. Robustaflavone (**46**) was obtained for the first time from the leaves of *S. terebinthifolius* (Formagio, 2017) and some chalcone derivatives – schinopsone A and schinopsone B (**47**, **48**), besides two known compounds (David, 2022) (**49**, **50**) – were isolated from the roots of *S. brasiliensis* Engl. Two unknown flavonoids (kaempferol-3-*O*-*β*-(2"-sulphategalactopyranoside) (**51)** and quercetin-3-*O*-*β*-(2"-sulphategalactopyranoside) (**52**) were obtained from aqueous methanol leaf extract of *Harpephyllum caffrum* (Nawwar, 2011). A novel dimer (**53**) C-3/C-3’’ of butin (3',4',7-trihydroxyflavanone) was isolated from *C. coggygria* Scop. wood (Antal, 2010) alongside other known compounds (catechin, fisetin, quercetin, butein, sulfuretin, fustin, dihydroquercetagetin, eriodictyol etc.). From MeOH and EtOH antioxidant

extracts of *Pistacia terebinthus* L. fruits (Topcu, 2007) it was isolated the new flavone 2-(2,4-dihydroxy-5-methoxyphenyl)-5,7,8-trihydroxy-4*H*-1-benzopyran-4-one (**54**) besides other known flavonoids (apigenin, luteolin, quercetin, luteolin-7-*O*-glucoside etc.). On the other hand, the novel hispolone derivative **55** (Me 5-(3,4-dihydroxyphenyl)-3-hydroxypenta-2,4-dienoate) (Yousfi, 2009) was obtained from the mushroom *Inonotus hispidus* growing on *Pistacia atlantica* as well as hispolone, hispidin and other phenolic compounds. The compound **56** (named acuminatanol) (Hu, 2007) was the first 2'2'''-bis-dihydrobiflavonol isolated from the aqueous extract of *Trichoscypha acuminata*, being the first example of a bis-dihydroflavonol linked exclusively via the B-rings at C-2' and C-2''' positions. At last, the phytochemical investigation of the leaves of *Sorindeia juglandifolia* A. Rich. led to the obtention of a new C-glucosylflavone (2",6"-di-*O*-acetyl-7-*O*-methylvitexin) (Ndongo, 2013), alongside other seven known compounds.

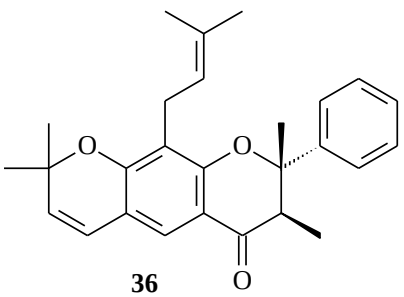
Figure 16. Flavonoids and biflavonoids isolated from plants of different Anacardiaceae species



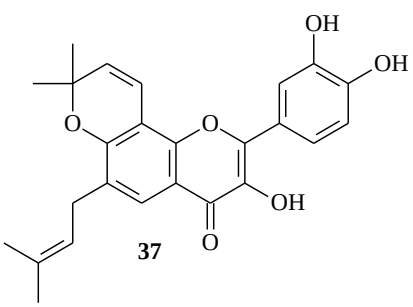
29: R_1 = Glu; R_2 = H; R_3 = R_4 = OH
30: R_1 = H; R_2 = OH; R_3 = H; R_4 = OH



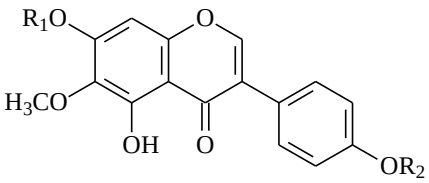
31: R_1 = H; R_2 = OH; R_3 = H; R_4 = R_5 = OH; R_6 = H
32: R_1 = H; R_2 = OH; R_3 = H; R_4 = R_5 = R_6 = OH
33: R_1 = OH; R_2 = =O; R_3 = Me; R_4 = OH; R_5 = OMe; R_6 = H
34: R_1 = OH; R_2 = =O; R_3 = Me; R_4 = H; R_5 = OMe; R_6 = H
35: R_1 = OH; R_2 = =O; R_3 = H; R_4 = OH; R_5 = OMe; R_6 = H



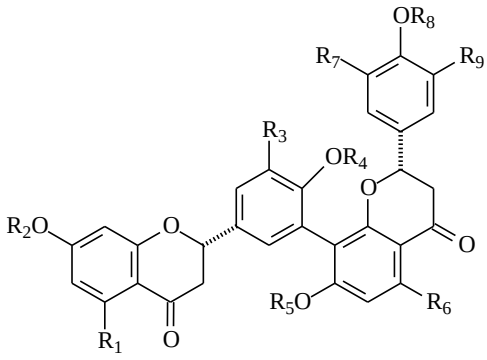
36



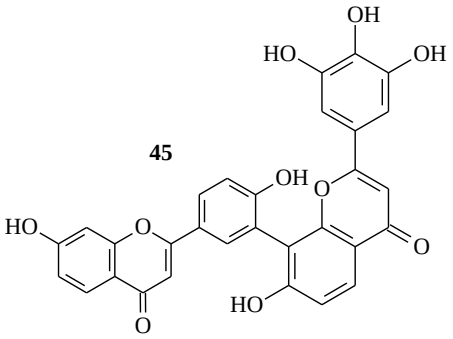
37



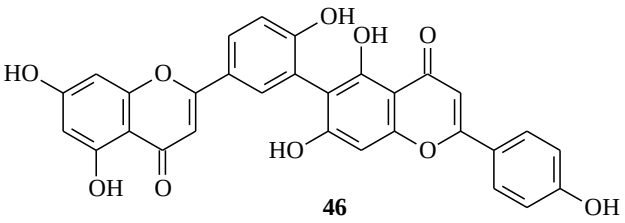
38: R_1 = Me e R_2 = H
39: R_1 = H e R_2 = Me



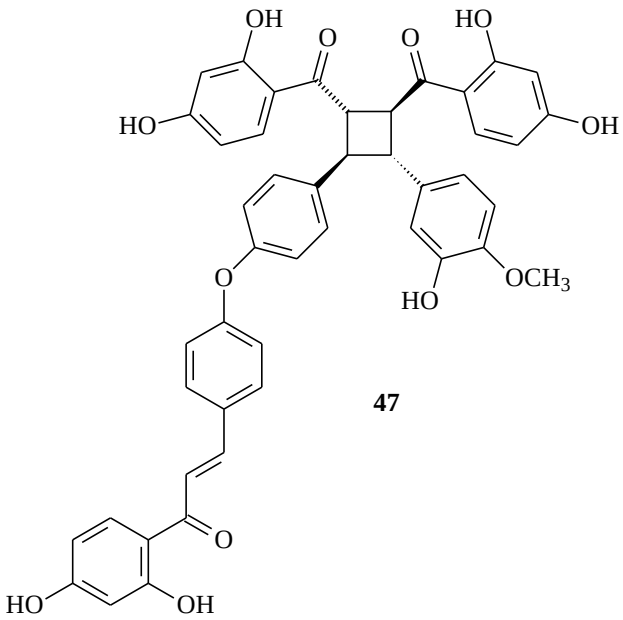
40: R_1=R_6 = OH; R_2=R_4=R_5 = Me; R_3=R_7=R_9 = OMe; R_8 = H
41: R_1=R_6 = OH; R_2=R_4=R_5 = Me; R_3 = OMe; R_7 = H; R_8,R_9 = OCH_2O
42: R_1=R_2=R_3=R_4=R_5 = H; R_6 = OH; R_7=R_8 = H; R_9 = OH
43: R_1=R_2 = H; R_3 = H; R_4=R_5=R_6 = H; R_7=R_9 = OH; R_8 = H
44: R_1 = OH; R_2=R_3=R_4=R_5 = H; R_6 = OH; R_7=R_8=R_9 = H



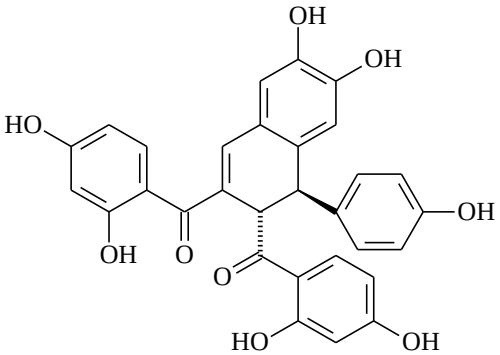
45



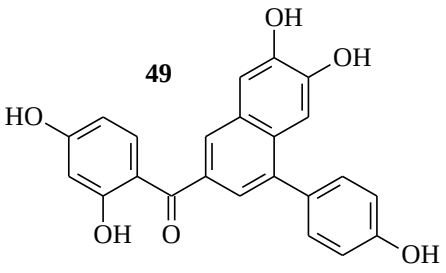
46



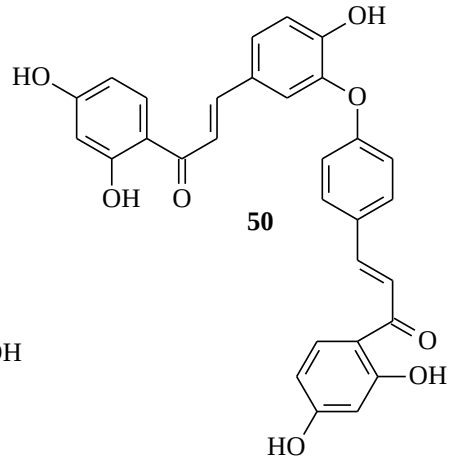
47



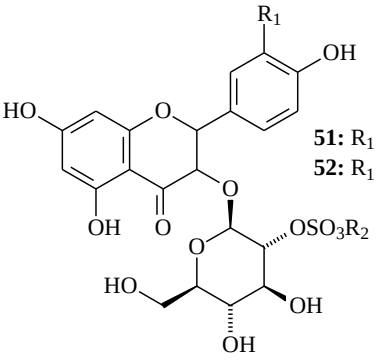
48



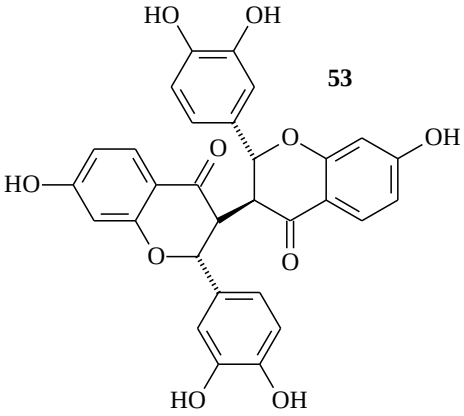
49



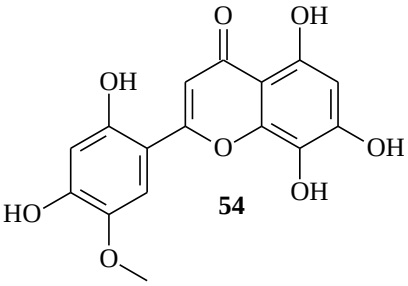
50



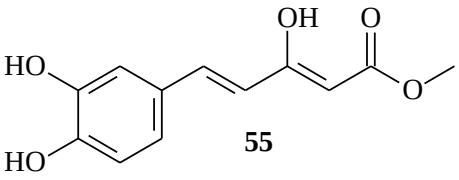
51: R_1 = H; R_2 = H or Na
52: R_1 = OH; R_2 = H or Na



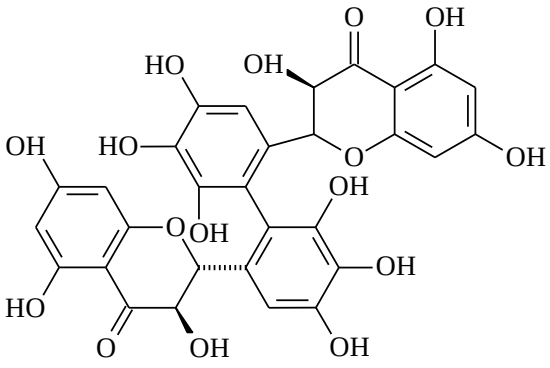
53



54



55

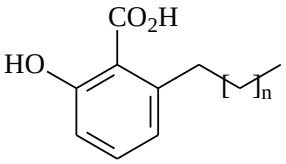


56

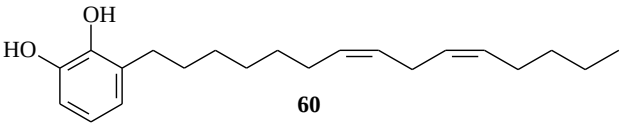
3.4. Alkyl and alkenylphenols

Alkyl and alkenylphenols, also known as phenolic lipids, are chemotaxonomic markers of various species of Anacardiaceae. In general, they present a salicylic acid moiety, but some are decarboxylated structures. Figure 17 represents the structures of several alkyl and alkenylphenols isolated from Anacardiaceae *spp*. Ozorcardic acids A (**57**) and B (**58**), besides anacardic acid (**59**), were obtained for the first time from *Ozoroa pulcherrima* Schweinf. (Christelle, 2011). Moreover, the known compounds 3-((7*Z*-10*Z*)-pentadeca-7,10-dien-1-yl)benzene-1,2-diol (**60**) and 3-((8*Z*)-pentadec-8-en-1-yl)benzene-1,2-diol (**61**) were obtained from *S. anacardium* (Semalty, 2010), and the new alkyl resorcionols **(***Z*,*Z*)-5-(trideca-4,7-dienyl)-benzeno-1,2-diol (**62**), (*Z*)-5-(trideca-4-enyl)-benzeno-1,2-diol (**63**), (*Z*,*Z*)-5-(pentadeca-6,9-dienyl)-benzeno-1,2-diol (**64**), (*Z*,*Z*)-5-(trideca-5,8-dienyl)-benzeno-1,2-diol (**65**) and (*Z*)-5-(heptadec-6-enyl)-benzeno-1,2-diol (**66**) from *Lithraea molleoides* Vell. Eng. (Catalano, 2020). Furthermore, 3-(2-(heptan-2-yl)-3-methylnonyl)phthalic acid (**67**) and 2-hydro-6-[(8'*E*, 11'*E*, 14'*E*)-22'-hydroxydocasa-8',11',14'-trienyl] benzoic acid (**68**) were obtained from sheets (Tokoudagba, 2018) of *S. mombin*. The presence of (*E*)-double bonds and branched alkyl chains in **67** and **68** are unusual, whose detailed analysis of the NMR and MS data published indicates the need of new experiments to corroborate with the published unusual structures for these compounds. Furthermore, three new dihydrobenzofuranoids **[**2-[(10'*Z*)-dodec-10'-enyl]-dihydro-1-benzofuran-5-ol (**69**), 2-[(10'Z)-tridec-10'-enyl]-dihydro-1-benzofuran-5-ol (**70**) and 2-[(10'Z)-pentadec-10'-enyl]-dihydro-1-benzofuran-5-ol (**71**)] were isolated from *T. guianensis* seeds (da Silva, 2020). On the other hand, the new dimeric alkylresorcinol integracin *E* (**72**) was obtained from the stem barks (Dang, 2019) of *Swintonia floribunda*, besides propyl ferulate. At last, the new gentisic acid derivative **73** (mycronic acid) has been isolated from the root of *Micronychia tsiramiramy* (Razakarivony, 2016) with five known compounds (e.g., gallic acid, methyl gallate, moronic acid, masticadienolic acid and masticadienediol) previously isolated.

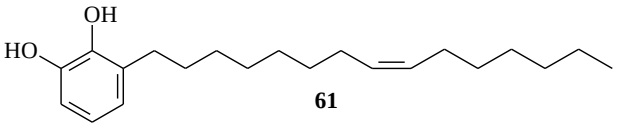
Figure 17. Alkyl and alkenylphenols isolated from several Anacardiaceae species



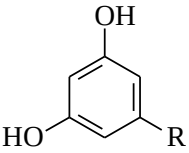
57: n = 21
58: n = 16
59: n = 13

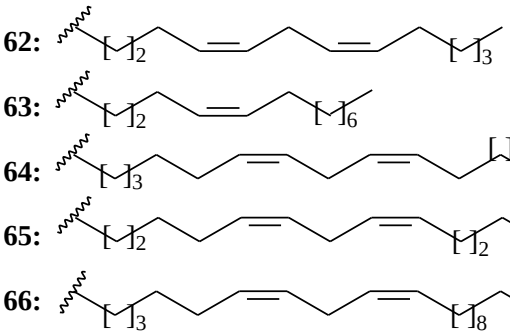


60

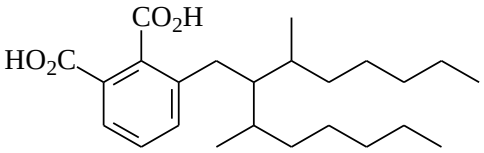


61

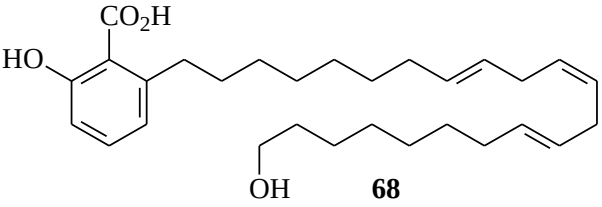




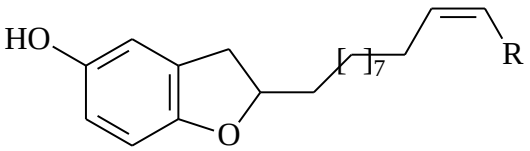
62:
63:
64:
65:
66:



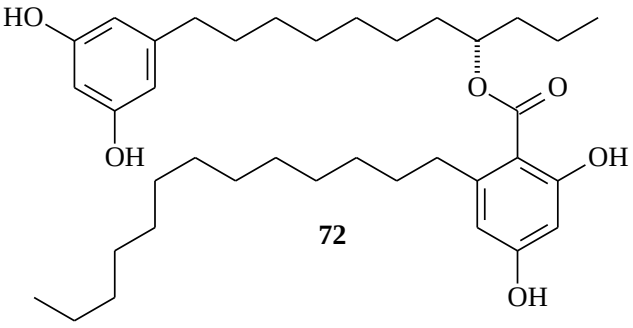
67



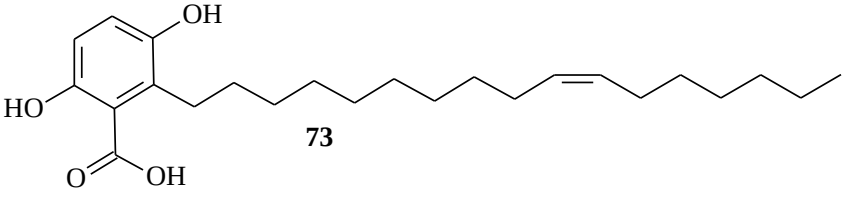
68



69: R = CH_3
70: R = CH_2CH_3
71: R = $CH_2CH_2CH_2CH_3$



72



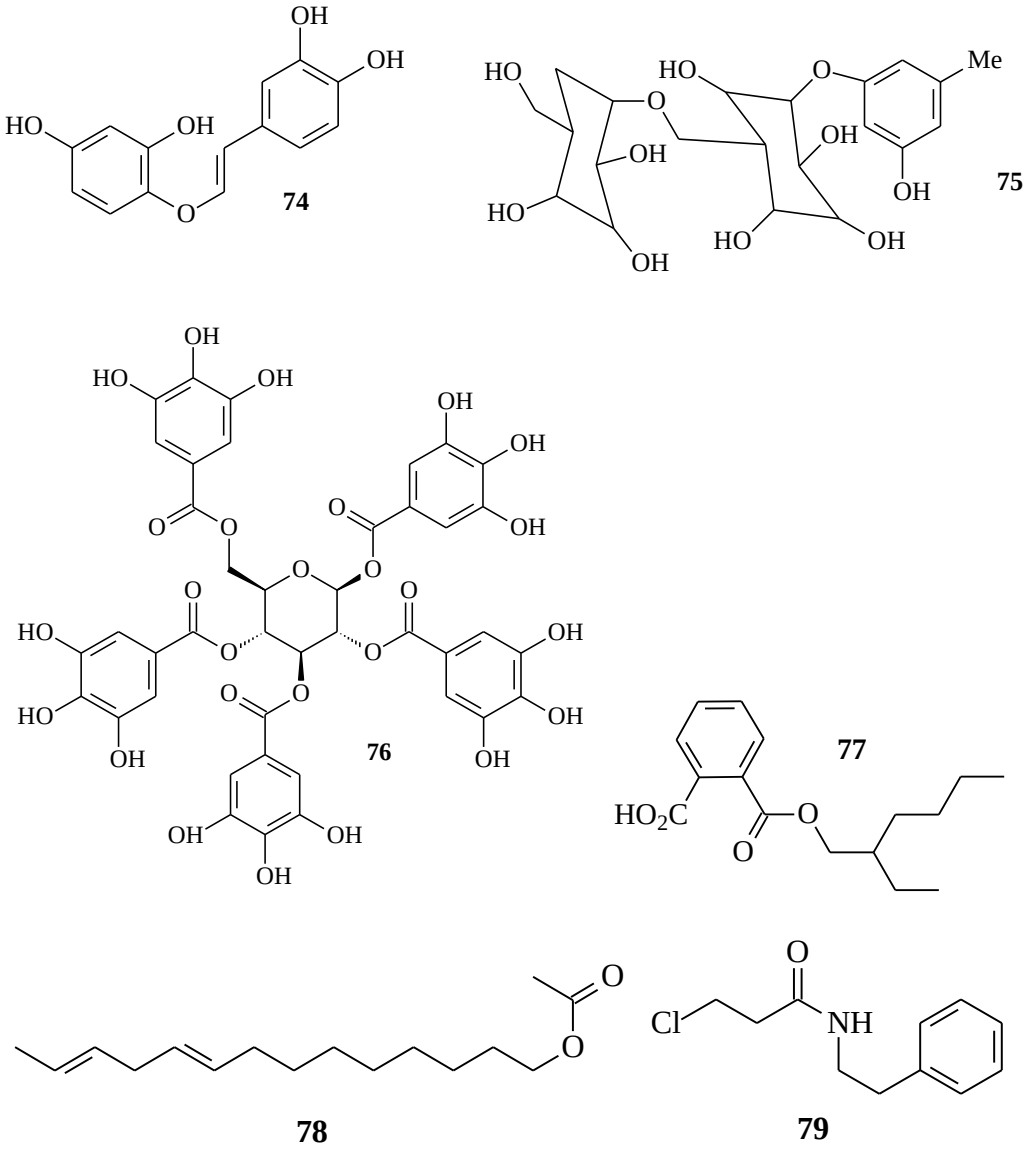
73

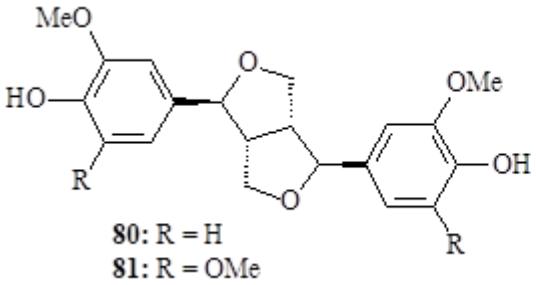
3.5. Miscellaneous compounds isolated from Anacardiaceae

Other types of metabolites that can occur in Anacardiaceae *spp*. are summarized in Figure 18. Butein (**74**) and anacardoside (**75**) from *S. anacardium* Linn (Semalty, 2010) are unusually simple phenolics derivatives. The polyphenol **76** (1,2,3,4,6-penta-*O*-galloyl-glucopyranoside, PGG), was isolated from *S. terebeinthifolius* (Formagio, 2017) and three new metabolites [1,2-benzenedicarboxylic acid-mono(2-ethylhexyl)ester (**77**), (9*E*,12*E*)-tetradeca-9,12-dien-1-yl acetate (**78**) and 3-chloro-*N*-(2-phenylethyl)propanamide (**79**)]**,** both atypical, from *M. indica* (Garg, 2015). Besides, ((+)-pinoresinol (**80**), syringaresinol (**81**) and (+)-*epi*-pinoresinol (**82**) were obtained from the stem barks of *S. floribunda* (Dang, 2019) as well as the antioxidant substances disulfuretin (a novel biaurone, **83**), sulfuretin (**84**) and sulfurein (**85**) were isolated from EtOAc soluble partitions of two separate collections (Kinghorn, 2000) of *C. coggygria* (*R. cotinus*), all of them for the first time in these genera. Moreover, the new lignan **86** ((+)-(8*S*,8'*S*)-5'-methoxy-4,4'-di-*O*-methylsecoisolariciresinol) (Nguyen, 2022) was obtained from the EtOAc-soluble extract of the stems of *Buchanania lucida*. Other several studies reported the isolation of many novel metabolites, as the compounds 2,6,3',4'-tetrahydroxy-4-methoxybenzophenone (**87**), 2,6,4'-trihydroxy-4,3'-dimethoxybenzophenone (**88**) and dobiniside A (**89**) from the roots of *D. delavayi* (Shen, 2021; Cheng, 2013), the substance **90** (3-methoxyellagic acid 4-*O*-galactopyranoside) from the leaves of *H. caffrum* (Nawwar, 2011) and the fatty acid ester **91** from *Cyrtocarpa procera* Kunth. (together other known analogues and some hydrocarbon derivatives) (Rodríguez-Lopez, 2006). Moreover, the new 1,4-benzoquinone derivative **92** was obtained from the root of *M. tsiramiramy* (Razakarivony, 2016) and the novel benzofuran lactone **93** (rhuscholide A) was isolated from the stems of *Rhus chinensis* (Gu, 2007) with other known substances (betulin, betulonic acid, moronic acid etc.). At last, the new bischromanone **94** (e.g., semecarpanone) has been obtained from an $CHCl_3$-soluble fraction of *Semecarpus caudate* stems (Dang, 2018) alongside five known flavonoids (quercetin, naringenin, taxifolin, (+)-eriodictyol and 3,4′,7-trihydroxyflavone) and two novel long-chain alkyl compounds *rel*-(+)-(9*R*,11*R*)-9,11-dihydroxy-7-octadecanone (**95**) and (-)-3-hydroxydecyl eicosanoate (**96**) from the galls of *P. integerrima* Stewart (Ahmad, 2010). The authors signed compound **95** as *rel*-(+)-(9*R*,11*R*) enantiomer, although they did not

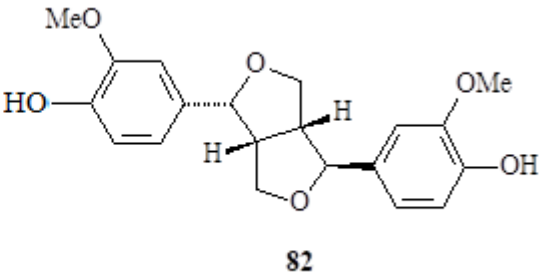
present spectrometric data to support the proposed stereochemistry. Bis(2-ethylhexyl) phthalate is a plasticizer, and compound **77** could not be a natural product, as pointed out by the authors. However, once there is no evidence of optical light deviation of **77**, a partial hydrolysate was synthesized from the commercial phthalate. Finally, regarding the compounds **78** and **92**, there is no spectrometric evidence of the stereochemistry and carbon position of the double bonds of the linear carbon chains.

Figure 18. Structures of miscellaneous compounds obtained from different Anacardiaceae spp

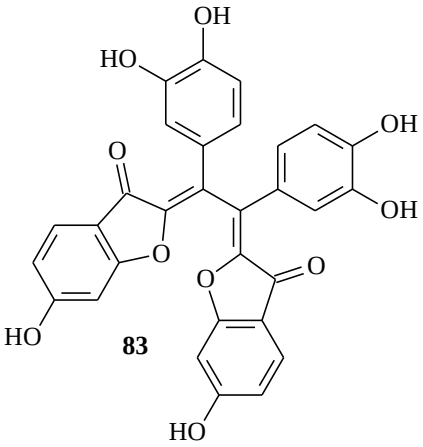




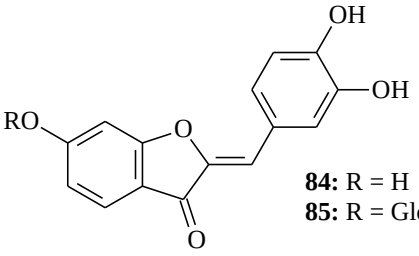
80: R = H
81: R = OMe



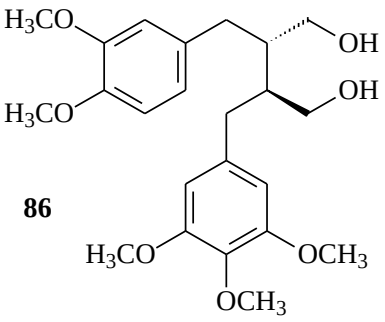
82



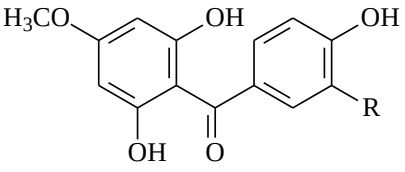
83



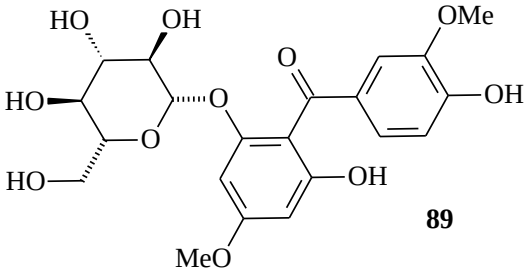
84: R = H
85: R = Glc



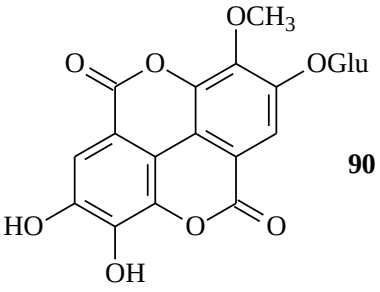
86



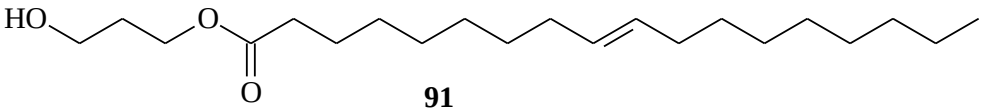
87: R = OH; **88**: R = OCH_3



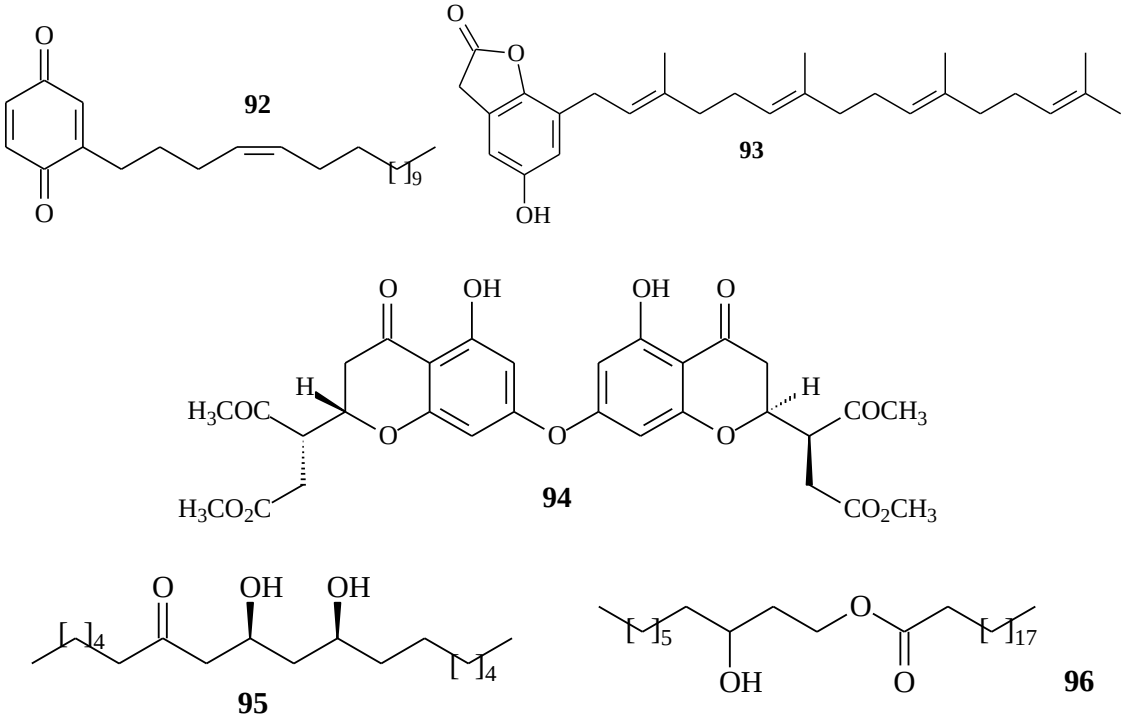
89



90



91



In conclusion, we could highlight the occurrence of *β*-sitosteryl-3*β*-glucopyranoside-6′-*O*-fatty acid esters, *β*-sitosterol, phytol, a mixture of phytyl fatty acid esters and *β*-sitosteryl fatty acid esters, chlorophyll, squalene, the compound **59** and other long-chain constituents in the CH_2CH_2 extract of *Dracontomelon dao* (Merr. & Rolfe) leaves (Ragasa, 2016), as well as the isolation of **15** together with gallic acid and ethyl gallate from the EtOH extract of *Mauria heterophylla* (Mori, 2006).

4. BIOLOGICAL ACTIVITIES

Anacardiaceae family presents several species that produce compounds with different biological properties. Therefore, in the last decades, numerous studies have employed extracts and some isolated metabolites presenting *in vitro* and *in vivo* activities, mainly against microorganisms/strains, cell lines, free radicals, viruses, as well as antinociceptive, anti-inflammatory and other effects.

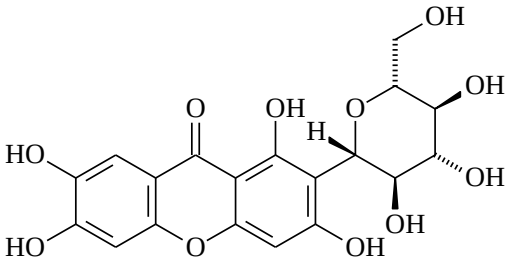
4.1. Extracts of Anacardiaceae spp.

4.1.1. *In vitro* studies

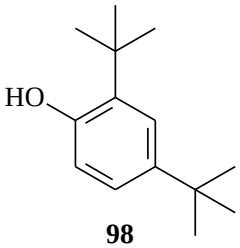
Regarding *Lannea* spp., the aqueous extracts of *L. barteri* Engl. bark (LBE) (Benson, 2018) have presented antibacterial activity against *Pseudomonas aeruginosa* (MIC = 6.25–25.00 mg/mL, LBE 6.25; 12.50; 25.00; 50.00 and 100.00 mg/mL) and *Acinetobacter baumannii* (MIC = 25.00–43.75 mg/mL, LBE 6.25; 12.50; 25.00; 50.00 and 100.00 mg/mL), including MIC/MBC = 1.0 in all cases. These biological properties are probably due to phenolic/polyphenolic compounds in extracts, whose results may justify its traditional use against urinary infections. Moreover, the ethanolic extract of *L. velutina* A. Rich (Pare, 2019) has presented antioxidant (% DPPH inhibition: 52.81±2.16; % Fe^{3+} reducing power/FRAP: 1.74±0.45 mmol EAA/10g extract) and antimicrobial activities (against Gram-positive and Gram-negative bacteria strains, with inhibition diameters greater than 8 mm), which is related to the flavonoid (1.770±0.005 mg eq. quercetin/10g of extract) and polyphenol (969.67±8.23 mg GAE/g extract) contents. Thus, these extracts might be helpful to prevent damage from oxidative stress and several infections.

Concerning the studies with *M. indica*, it is known that compounds from this plant present many biological activities, typically related to mangiferin (**97**) and other polyphenolic compounds, such as flavonoids, benzophenones, carotenoids and tocopherols. The antibacterial activity of (seed) mango kernel extracts (Mirghani, 2011) was attributed to 2,4-*bis*(1,1-dimethylethyl)phenol (**98**) and the inhibitory effect (Pithayanukul, 2009) over PLA_2, hyaluronidase and LAAO is associated with PGG (**77**), which selectively block the PLA_2 and LAAO active sites. Anticancer properties (Kumar, 2021) are possibly due to mangiferin, and other activities (e.g., antidiabetic, antioxidant and antimicrobial) can be associated with different compounds (Kumar, 2021; Jahnavi, 2020), such as aglycones, saponins and terpenes. Figure 19 presents the structures of some bioactive metabolites from *M. indica*.

Figure 19. Some biological active compounds from *Mangifera indica* L



97



98

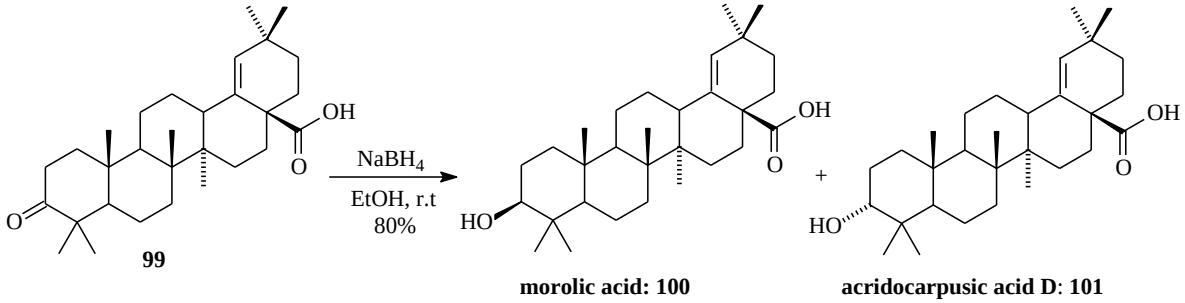
Studies with *Pistacia* spp. reported the crude extract and leaf EtOAc fraction of *P. atlantica* Desf. displayed, simultaneously, a stronger antioxidant activity (Bakka, 2019) (DPPH assay: IC_{50} = 0.0273±0.0001 and 0.0419±0.0010 mg/mL) due to the presence of flavonoids and tannins in comparison to BHA and ascorbic acid (DPPH assay: IC_{50} = 0.080±0.002 and 0.060±0.002 mg/mL). On the other hand, *P. integerrima* stems extracts (Zia, 2012) and fractions exhibited antitumor (with dose-dependent cell viability, low or moderate toxicity and 97.4-100% inhibition of MCF-7 cell lines by EtOAc or $CHCl_3$ fractions at 200 μg/mL) and antifungal activities. However, the crude MeOH extract presented a weak antifungal effect. Moreover, the anti-melanogenic activity of *P. atlantica* subsp. *kurdica* extracts (Tayarani-Najaran, 2021) showed significant inhibition of tyrosinase activity and an ensuing reduction of melanin synthesis, potentially valuable for treatments for skin hyperpigmentation disorders and new advances in the cosmetic industry. In conclusion, EtOH extracts of *in vitro* samples (under NaCl stress) and *in vivo* (grown naturally) of *P. khinjuc* specimens (Tilkat, 2020) were compared regarding their antioxidant and antimicrobial properties and, according to the results, samples from *in vivo* specimens generally presented higher activities than the *in vitro* counterparts.

Aqueous leaf extract of *Rhus parviflora* (Kumar, 2017) was used as a medium (with 0.1M solution of zinc acetate dehydrate) in ZnO nanoparticles synthesis, which exhibited potential antimicrobial activity against *S. aureus*, *P. aeruginosa*, *A. niger* and *C. albicans*. Likewise, the MeOH:CH_2Cl_2 (1:1), MeOH, and aqueous extracts of *R. vulgaris* Meikle stem bark (Mutuku, 2020) were bactericidal/bacteriostatic against different microorganisms, in such a way that MeOH extract showed significant activity toward MRSA (MIC 0.391 mg/mL and MBC 1.563 mg/mL). The authors pointed these results supports traditional use of *R. vulgaris* as a toothbrush. On the other hand, extracts' cytotoxicity and mild skin damage warrant further research, so that *R. vulgaris*

may be recommended to develop effective and safe mouthwashes. Lastly, there are several other *Rhus* spp. that also have shown many biological properties (Rayne, 2007) (antiviral, antimutagenic, antioxidant, hypoglycemic, antitumour, antimalarial etc.), which depend on their constituents, among which phenolic compounds, flavonoids/biflavonoids and glycosides are the primary bioactive metabolites.

Schinus genus is widely present in folk medicine, and some species have been well-studied due to their therapeutic/physiological properties. For instance, in a study with *S. molle* ripe fruits (Al-Naser, 2014), the n-hexane (A) and petroleum ether (B) extracts (oils) showed antifungal activity against *Botrytis cinerea*, and this activity was attributed to a composition of oleic and linoleic acids and monoterpenes. The B extract was weakly active (at 1000 ppm), although there was a higher suppression for the fungi at this concentration (according to the extract). Moreover, even B ripe fruit extract might be recommended as an environment-friendly fungicide by the authors. Likewise, different leaf extracts and fractions of *S. lentiscifolius* (Morel, 2013) were tested for the first time against five Gram-positive, three Gram-negative bacteria and four yeasts, which displayed a broad spectrum of weak antibacterial activity (MIC = 125–250 μg/mL) and a meaningful antifungal activity (e.g., n-hexane extract). The EtOAc fraction was the most antibacterial and various compounds were isolated from it, among which the most active metabolite was the moronic acid (**99**) (MIC = 1.52–3.12 μg/mL). Sequentially, **99** was submitted to 1) a reduction process with $NaBH_4$ (Scheme 10) and 2) a subsequent treatment with diazomethane to evaluate the importance of carbonyl(C-3) and carboxyl(C-28) groups to the activity, whose methyl ester was more active against *Cryptococcus neoformans* (MIC = 50 μg/mL). In summary, *Schinus terebinthifolia* is the species more studied, and the last decade studies have shown its antimycobacterial activity against *Mycobacterium bovis* BCG, alongside a significant inhibitory effect on the nitric oxide production (IC_{50} 19.23 ± 1.64 μg/mL) and mycobacterial growth (IC_{50} 14.53 ± 1.25 μg/mL), what is probably due to the flavonoids present therein (Oliveira, 2014).

Scheme 10. Scheme of reduction of moronic acid (**99**) mediated by $NaBH_4$



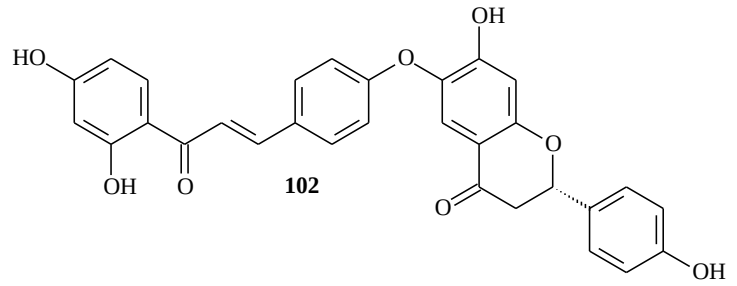
99 **morolic acid: 100** **acridocarpusic acid D**: **101**

Similarly, *Spondias* genus has been reported as a source of medicinal plants. Thus, some species have exhibited many applications and useful therapeutic properties. Stem bark extracts of *S. mombin* (Boni, 2014) (aqueous extract and methanolic extract – AESPM and MESPM, respectively) were evaluated concerning the dose-dependent antioxidant activity, whose results indicated that MESPM presented the highest level of bioactive constituents (total phenolic and flavonoids), being more active than AESPM by DPPH scavenging assay, FRAP and FTC methods. Therefore, it can be suggested that *S. mombin* may act as a chemopreventative agent against free radicals and their related illnesses. Besides, *S. tuberosa* hexane leaf extracts (da Costa Cordeiro, 2018) were studied and presented antioxidant and antifungal activities. Flavonoids (e.g., hyperoside), hydrolysable tannins, saponins and terpenes were identified by TLC and HPLC analysis in the extracts and, likewise, fatty acid methyl esters (saturated and unsaturated) by ^{1}H NMR data as the main components. In this work, the extract showed mild activity in DPPH assay (IC_{50} = 234.00 mg/mL) and moderate by ABTS method (IC_{50} = 123.33 μg/mL) but was reasonably active against *C. albicans* and *glabrata* (MIC_{50} 2.0 and 0.078 mg/mL, respectively), whose results demonstrate its pharmacological potential. Finally, in a study with *S. pinnata* stem bark (Chaudhuri, 2016), several hydromethanolic extracts (70% MeOH) were prepared and an active fraction (water fraction of stem bark raw extract/SPW1) was obtained. In this case, SPW1 exhibited a high antioxidant effect and radical scavenging potential against ROS and RNS, including the reducing power and inhibiting lipid peroxidation (Fe^{2+} *in vitro* chelation and ferritin ion release assays). Therefore, it might be suggested that *S. pinnata* extracts with high phenolic and flavonoid contents can be considered as a possible candidate against iron overload diseases.

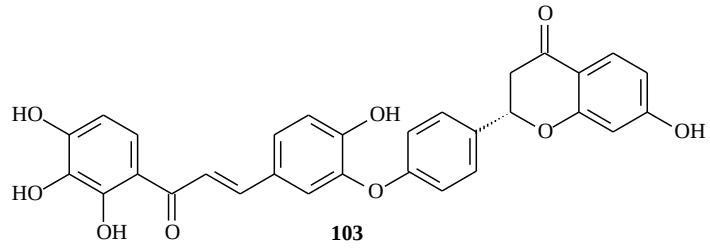
In addition, the study of the phytochemical composition of *Searsia chirindensis* leaf (Madikizela, 2013) crude extract (80% MeOH) and DCM, EtOAc and BuOH respective fractions led to the isolation of bioactive compounds that were tested for their antibacterial activity against Gram-negative (*Campylobacter jejuni*, *E. coli* and *Shigella flexneri*) and Gram-positive (*S. aureus*) bacterial strains. From the EtOAc extract (the most active) were obtained methyl gallate, myricetin-3-*O*-arabinopyranoside, myricetrin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside and quercetin-3-*O*-arabinofuranoside. All compounds showed good antibacterial activity against all bacterial strains tested (MIC = 30–250 μg/mL), whose activity provides credence to the ethnomedicinal use of *S. chirindensis* against diarrhoea. Moreover, it must be highlighted that this is the first report regarding the occurrence of these metabolites in this plant. On the other hand, different phytocompounds from aqueous-MeOH extract (70% methanol) leaf extract of *Searsia lancea* (Vambe, 2021) were separated, whose resultant fractions were evaluated for antibacterial properties (MIC) against four bacterial strains (*Enterococcus faecalis, Klebsiella pneumoniae, Neisseria gonorrhoeae and S. aureus*). In this case, an EtOAc sub-fraction demonstrated potent antibacterial properties (MIC range: 31-61 μg/mL against *E. faecalis* and *S. aureus*) and, based on GC-MS analyses, 81.5% of this sub-fraction consisted of broad-spectrum antibacterial compounds namely tetracosanol (43.98%) and nonadecanol (37.50%). Therefore, these current findings may support the traditional use of *S. lancea* leaves to manage gastro-intestinal disorders as well as gonorrhea.

In conclusion, the study of the total extract (a xanthine oxidase/XO inhibitor *in vitro*) of *Terminthia paniculata* (Sanyeqi) and its active fractions yielded six chalcone-type heterodimers (**102**-**107**, Figure 20), whose structures were elucidated based on extensive spectroscopic analyses involving HRESIMS, 1D and 2D NMR, UV, IR and $[\alpha]_{D.}$ Thus, termipaniculatones A and E showed XO inhibitory activity (IC_{50} = 55.6 and 89.5 μM, respectively), which took effects via a mix-type mode. Regarding to their action mechanisms, a molecular modeling study revealed that termipaniculatone A (**102**) was well located into the active site of XO by interacting with Glu802, Arg880, Thr1010 and Val1011 residues. At last, this is the first time wherein the anti-acute gouty arthritis properties of *T. paniculata* and the characteristic biflavonoids as active constituents were related, what provides valuable information for searching new XO inhibitors from natural sources (Yang, 2019).

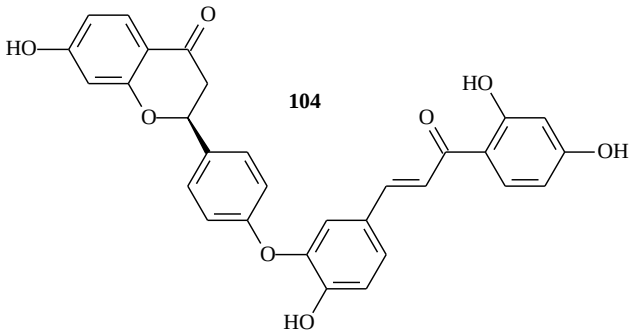
Figure 20. Structures of termipaniculatones isolated from *T. paniculata*



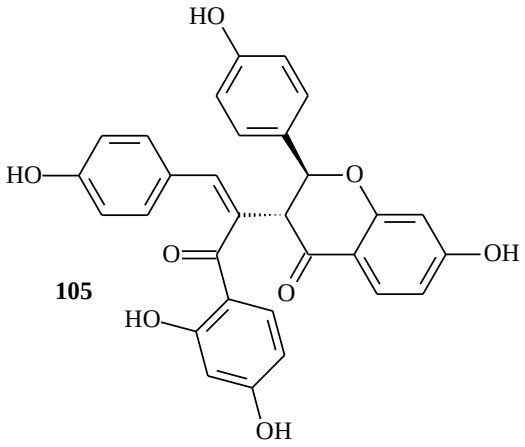
102



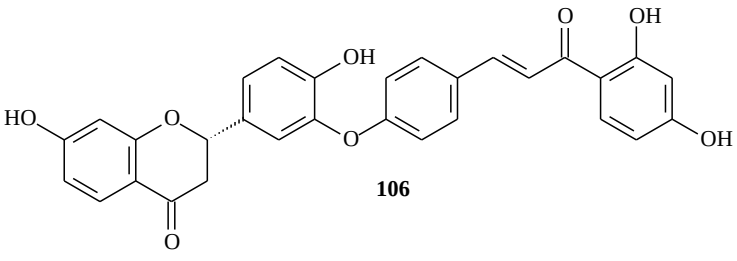
103



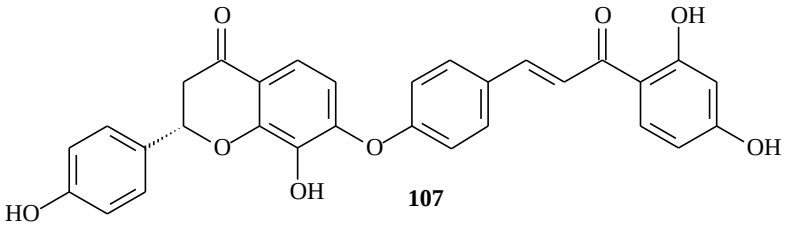
104



105



106



107

In conclusion, other biological properties of different Anacardiaceae species are summarized in Table 13.

Table 13. Several biological activities *in vitro* of different extracts in other Anacardiaceae spp

Species	Obtained extracts or isolated substances	Observed activities
Tapirira guianensis (da Silva, 2020)	EtOAc and BuOH fractions of MeOH flower exts.;	Antioxidant according to DPPH assay;
	CH_2Cl_2 soluble fraction from Hex ext. of seeds;	Moderate cytotoxicity by Brine Shrimp Test (*A. salina* L. lethality);
Anacardium othonianum (Silva, 2016)	EtOH crude ext. of leaves and fractions (n-Hex, EtOAc, n-BuOH, and hydro-MeOH)	Antifungal against *C. albicans* (ATCC 64548) and *Trichophyton rubrum* (Tr1)
Loxostylis alata (Suleiman, 2013)	Leaf acetone ext. (and CCl_4, Hex, $CHCl_3$, aqueous MeOH, BuOH and aq. fractions)	Antimicrobial and antibacterial activities against several strains (mediated by lupeol and β-sitosterol);
		Inhibition of COX-1 (anti-inflammatory activity and antithrombotic effect);

Ozoroa and *Searsia* spp. (Ahmed, 2014)	Crude leaf exts. (0,1% HCl:70% acetone:n-Hex) and fractions (DCM, BuOH, EtOAc, Hex, and resid. aqueous)	Antibacterial and antifungal activities; Antioxidant according to DPPH, ABTS, hydroxyl radical scavenging, and linolenic acid peroxidation assays; Low cytotoxicity against Vero cell lines;
Cyrtocarpa procera (Canales-Martinez, 2015)	MeOH (1 and 2) fruit extracts; Hex fruit extract	Antibacterial against different Gram-positive and Gram-negative strains; High inhibition of DPPH; Cytotoxic against CasKi cell lines (anticancer activity); Reduction of production of pro-inflammatory cytokines (TNF-α and IL-1β) by macrophages;
Schinopsis brasiliensis (Araújo, 2014)	$CHCl_3$ fraction from EtOH:H_2O crude ext. of stem bark	Moderate toxicity against *A. salina*; Larvicidal potential against *A. aegypti* (for dengue); high molluscicidal activity against *Biomphalaria glabrata*;
Bouea macrophylla (Van Giau, 2020)	EtOH leaf crude/raw extract	Antibacterial and antifungal (against *C. albicans*); Antioxidant (DPPH/FRAP assays); Inhibition of proliferation of HeLa and HCT116 cells;
Sorindea warneckei (Adesegun, 2019)	EtOH ext. of leaves and fractions (EtOAc, BuOH, Hex, and aqueous)	Antioxidant according to DPPH and Fe^{3+} / ferricyanide assays; Inhibition of α-amylase;
Anacardium excelsum (Sequeda-Castañeda, 2021)	EtOH ext. and fractions (petroleum ether, CH_2Cl_2, and EtOH/ BuOH)	Antioxidant according to DPPH, ABTS and DMPD assays;

Sclerocarya birrea (Milella, 2018)	MeOH extracts of leaves and bark [112]	Antioxidant according to ABTS, $O_2^{-\bullet}$ and $NO^{\bullet}$ assays; High anticancer activity against HepG2 and normal human dermal fibroblast cell lines (Calcein AM assay); inhibition of lipid peroxidation by β-carotene bleaching assay;
(Njume, 2011)	EtOAc and EtOAc/MeOH/water fractions of stem bark (rich in essential oils: 40.5–86.57%) [113]	Antimicrobial against *H. pylori* (metronidazole- and clarithromycin-resistant strains); MIC_{50} = [310–2500 μg/mL]; terpinen-4-ol and pyrrolidine activities were similar to amoxicillin's ($P > 0.05$);
S. birrea (subsp. *caffra*) (Shai, 2011)	MeOH and acetone stem bark crude extracts; Hex and acetone stem crude bark exts;	Competitive inhibition of α-amylase; Non-competitive inhibition of α-glucosidase;
Holigarna longifolia (Uddin, 2020)	MeOH extract from bark; Aqueous extract from bark;	Antioxidant (DPPH inhibition, against oxidative stress); Moderate clot lysis (compared to streptokinase as standard);
Pleoigynium timoriense (DC.) Leenh. (Al-Sayed, 2010)	EtOH extract of leaves (rich in known phenolic compounds)	Antioxidant (DPPH[a] and super oxide anion[b] radical scavenging assays – IC_{50} = 21.9[a] and 123.5[b] μg/mL); Hypoglycemic and anti-inflammatory effects;

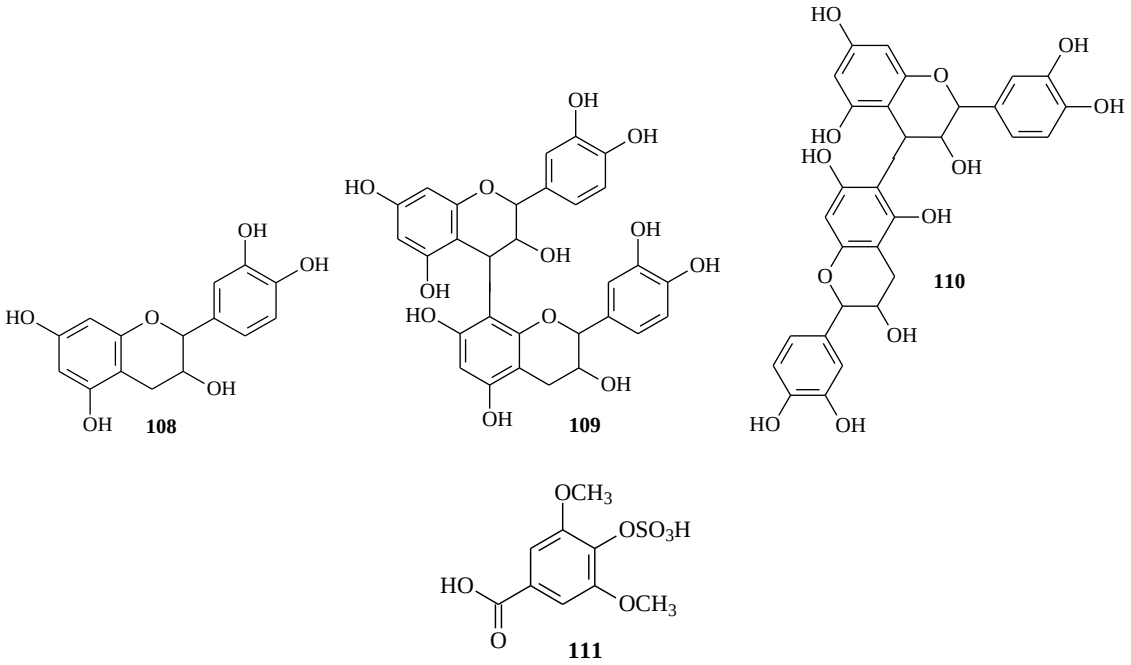
4.1.2. *In vivo* studies

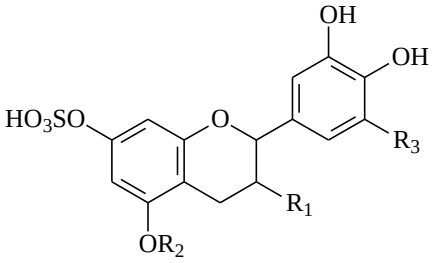
In vivo studies of antioxidant extracts obtained from *Lannea stuhlmannii* and *L. humilis* (Sobeh, 2018) permitted the identification of 22 specialized metabolites (mainly sulphated flavonoids). The antioxidant behaviour of the extracts was observed through the reduction of high levels of AST (serum aspartate aminotransferase) and total bilirubin, wether by attenuation of deleterious histopathologic changes in the liver (induced by D-GalN) or by protection of hepatocytes from apoptosis, including an

increased expression of Bcl-2 (anti-apoptotic protein). Moreover, molecular docking evaluation showed that some identified compounds from both plants (see Figure 21, **108**-**115**) could bind to the Bcl-2:Bim (BH3) interface by hydrophobic interactions (or hydrogen and ionic bonds) with an appreciable binding free energy, whose properties are due to the presence of flavonoids and proanthocyanidins therein. However, the correct stereochemistry of the catechins was not determined.

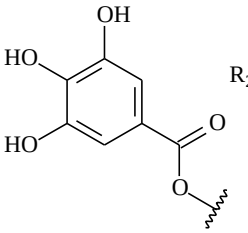
On the other hand, the diuretic and saluretic effects of an aqueous decoction (LMaq) and EtOAc extract of *L. microcarpa* barks (Nitiéma, 2018) compared with amiloride and furosemide were reported, in such a way that their mechanism of action seemed more analogous to the furosemides. In this study, it was verified that the diuretic activity (urinary excretion) of LMaq was dose-dependent and that the administration of extracts provided the selective elimination of Na^+ concerning the stabilizing excretion of K^+, confirming that *L. microcarpa* extracts could be a promising alternative for the therapeutic management of renal and cardiovascular pathologies.

Figure 21. Structures of some detected metabolites from active extracts of *Lannea stuhlmannii* and *L. humilis* docked to the Bcl-2:Bim (BH3) interface: epicatechin (**108**); epicatechin-C-8-epicatechin (**109**); epicatechin-C-6-epicatechin (**110**); syringic acid sulphate (**111**); flavan-3-,4-,5-trihydroxy-5-*O*-methyl-7-*O*-sulphate (**112**); epigallocatechin-5-*O*-methyl-7-*O*-sulphate (**113**); epicatechin-5-*O*-ethyl-7-*O*-sulphate (**114**); epigallocatechin gallate-5-*O*-ethyl-7-*O*-sulphate (**115**)





112: R_1 = H; R_2 = Me; R_3 = OH
113: R_1 = R_3 = OH; R_2 = Me
114: R_1 = OH; R_2 = Et; R_3 = H;

115: R_1 =  R_2 = Me; R_3 = OH

Regarding *in vivo* activities of *M. indica*, the leaf aqueous extract (Shah, 2010) (200 mg/kg body weight, p.o.) significantly decreased the total serum cholesterol, triglycerides (89.75±0.46%), and very low-density lipoprotein (17.95±0.09%) in rats (200 mg/kg body weight, p.o.) but, simultaneously, increased high-density lipoprotein (30.21±2.59%), whose results were almost comparable to those of atorvastatin. Thus, apart from the wide application of mango as an antidiabetic, *M. indica* aqueous extracts can also be used to treat hyperlipidemia. Furthermore, studies of the analgesic properties of CCl_4, EtOH and petroleum ether extracts of *M. indica* dried leaves (Ferdous, 2015) indicated that only EtOH and petroleum ether extracts exhibited a significant antinociceptive activity (oral dose of 200 mg/kg of body weight, with a writhing inhibition of 44.5-51.7% and 41.6-50.0%, respectively), while CCl_4 presented a mild effect (writhing inhibition of 25-30%). Nevertheless, no investigation was performed to lead to the obtention and purification of responsible bioactive compounds.

Concerning *Pistacia* spp., *P. khinjuk* (Shahid, 2019) was studied towards the evaluation of the hypoglycemic effect of its MeOH:H_2O extract in six groups of Swiss albino mice inoculated with alloxan monohydrate (except the normal group). As an outcome, all the mice treated with extract showed hypoglycemic activity and the blood glucose level (daily intake of 250 mg/kg or 500 mg/kg, two weeks) decreased. However, the observed results demand future studies to elucidate this behaviour, for phytochemical isolation and characterization of bioactive metabolites. On the other hand, the aqueous ethanolic extracts of leaves of *S. birrea* (Youl, 2020) were tested on basal plasma glucose (BPG) and oral tolerance glucose in mice, which significantly reduced peak of hyperglycemia at 100mg/kg body ($p<0.001$) but did not have a relevant hypoglycemic effect on BPG. Moreover, this study reported that the co-administration of *S. birrea* aqueous EtOH extracts with analogous extracts of *G. sylvestre*

(Asclepiadaceae) enabled a greater cutback on hyperglycemia (47%) compared to the *S. birrea* extract alone (36%). Therefore, since these species are sources of flavonoids, saponosides, tannins and other bioactive metabolites, the combined use of these plants would be an asset in treating diabetes.

Similarly, aqueous extract (AE) and EtOAc extract/flavonoid fraction (FF) of *Rhus trilobata* (RHTR) were studied as a potential alternative against colorectal adenocarcinoma cells and other types of cancer (Varela-Rodríguez, 2019). The toxicological effect of RHTR was evaluated in female *BALB*/*c* mice after 24 h and 14 days of intraperitoneal administration of 200 mg/kg AE and FF, respectively. Besides, among the most abundant compounds identified in RHTR by UPLC-PDA-MS^E were methyl gallate, epigallocatechin 3-cinnamate, quercetin 3-(2″-alloylglucosyl)-(1→2)-alpha-L-arabinofuranoside, *β*-PGG (**77**, with high relative abundance), 4-*O*-digalloyl-1,2,3,6-tetra-*O*-*β*-D-galloylglucose, myricetin 3-(4″-galloylrhamnoside) and fisetin, which possibly are responsible for the activity. The evaluation of toxicity did not reveal meaningful anatomical changes nor histological damages (which indicates that RHTR might be nontoxic upon acute exposure during i.p. administration in mice), and the high contents of polyphenols and antioxidants in AE may exhibit sound effects as chemoprotective agents against degenerative illnesses associated with oxidative stress, supporting its traditional use.

The total flavonoid content (TFC) of *Rhus cotinus* (e.g. *Cotinus coggygria*) showed a potent antitumor effect *in vivo* (Wang, 2015) in xenograft animal models of ectopic glioblastoma against several lineages of highly malignant cells (IC_{50} = 93.57–128.49 μg/mL). This activity (tumour's volume reduction at 25 and 50 mg/kg TFC) was analogous to that of TMZ (temozolomide, positive control), which could inhibit the growth of tumors in mice according to a day-dependent pattern (7-28 days, $P < 0.05$).

Considering *Spondias* genus, *S. pinnata* stands out regarding *in vivo* bioactivity. The investigation of the antioxidant effect of aqueous bark extract (AE*sp*) (Attanayake, 2015) (through evaluation of the activity of several enzymes in STZ-diabetic rats) showed that AE*sp* decreased 1) the LPO (by 17%) and 2) the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities (by 20, 17 and 36%, respectively). On the other hand, 1) the liver reduced glutathione (GSH) content and 2) the activities of glutathione reductase, glutathione peroxidase and

glutathione S-transferase were increased by 43, 44, 69 and 52%, respectively ($P < 0.05$). Thus, treatment with *S. pinnata* aqueous extract (at a dose of 1.00 g/kg) suggests an amelioration of oxidative stress (due to the increasing of GSH concentration) as well as an acceleration of the regeneration of hepatocytes (by decreasing leakage of ALT, AST, and ALP), confirming its significant *in vivo* antioxidant properties in diabetic rats. Furthermore, the EtOAc extract of *S. pinnata*'s stem heartwood (E*shw*) exhibited a hepatoprotective effect (Rao, 2009) in rats under CCl_4-injury induction. The results showed that E*shw* brought back the altered serum levels of some biochemical markers (SGPT/serum glutamyl pyruvate transaminase, SGOT/serum glutamyl oxalacetic acid transaminase, ALKP/alkaline phosphatase and bilirubin) to near normal range according to a dose-dependent mechanism. At last, the antipyretic potential (Panda, 2014) of the acetone and EtOH extracts of *S. pinnata* stem bark was evaluated. The ethanol extract (at 200-400 mg/kg p.o) presented a substantial reduction in yeast-induced elevated temperature (along 1 h up to 5 h) in a dose-dependent manner (by Brewer's yeast method applied in albino rats), whose outcomes were compared to the standard drug paracetamol. Thus, many plant extracts' antipyretic behavior has been attributed to their triterpenes, saponins, flavonoids, alkaloids and steroids, so that the possible mechanisms of action of *S. pinnata* stem bark extracts (and isolation of the active compounds) need to be further elucidated.

Moreover, two works (with *Buchnania lanzan* and *Sclerocarya birrea*) should be stood out, in which the MeOH leaf extract of *B. lanzan* exhibited a meaningful neuroprotective activity (against $AlCl_3$ induced Alzheimer's in Albino Wistar rats) in two different doses (200 and 400mg/kg/day, orally for four weeks). Since the phytochemical investigation of *B. lanzan* (Chaturvedi, 2021) showed the presence of phenolic compounds, a suggestion that the extract has a high antioxidant activity is reliable, which ratifies that the extract could prevent or slow down aging/age-associated oxidative process stress-related neurological illnesses. Thus, the evaluation of learning and memory outcomes indicated that the leaf part of *B. lanzan* was more active in attenuating memory deficits than other parts (as well as its mechanism of memory retention seems to be similar as compared to standard drugs), which might allow its use to treat various cognitive dysfunctions connected with neurodegenerative disorders.

Finally, the crude MeOH extract of *Holigarna longifolia* Roxb. bark (Uddin, 2020) and the respective bioactive fractions were investigated, whose analyses

demonstrate a meaningful neuroprotective activity (according to the increasing phenobarbitone-induced sleeping time of mice), as well as a substantial inflammation inhibitory efficacy compared to standard diclofenac sodium. In addition, only MeOH extract provoked a significant antinociceptive activity by inhibiting abdominal writhes produced by AcOH compared to standard analgesic diclofenac sodium, what indicates that *H. longifolia* might be a promising neuroprotective plant.

4.2. Biological activities of isolated compounds from Anacardiaceae spp.

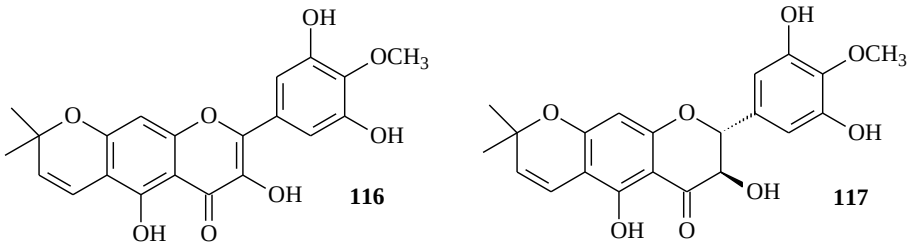
4.2.1. *In vitro* studies

Firstly, it was reported that the compound catechin-3-*O*-rhamnoside, isolated from *Lannea kerstingii* (EtOAc stem bark extract) for the first time, has exhibited antimicrobial (diffusion and broth dilution methods) and antioxidant (by DPPH scavenging assay) activities. The flavonoid presented a selective activity against several bacteria and fungi (*Candida* spp.) with MIC ranging from 6.25 μg/mL (for *S. aureus* and *MRSA*, *B. subtilis*, *E. coli*, *K. pneumonia* and *S. dysentariae*) to 12.5 μg/mL (for *S. typhi*, *C. albicans* and *C. tropicalis*), while the MBC/MFC (minimum bactericidal/fungicidal concentrations) ranged from 12.5 to 50.0 μg/mL. Moreover, these activities are higher than chloramphenicol and positive nystatin controls, probably due to the flavonoid skill to complex with bacterial cell walls and extracellular soluble proteins (Stanislaus, 2021).

In addition, two new prenylated flavonoids (**116** and **117**, Figure 22) from *Lannea alata* Engl. roots – alongside four known compounds (myricitrin, betmidin, lupeol and sitosterol) – might be associated to a good antibacterial and dose-dependent DPPH scavenging activity. Both glycosides presented better antioxidant activity than **116** and **117** and betmidin showed the best antimicrobial activity among all metabolites. The presence of 3-*O*-arabinose glucoside might be linked to the high activity of betmidin against Gram-positive bacteria. Similarly, the arabinofuranoside's antioxidant effect (followed by the rhamnopyranoside), which was compared to ascorbic acid in high concentrations, corroborates with the ethnomedicinal uses of *L. alata* in the

management of Gram-positive bacteria sicknesses. Structural features of **116** and **117** are narrowly related to their properties, such that the lower antioxidant (compared to the glycosides) behavior may be due to the presence of cyclized prenyl moieties in their structures. Nevertheless, flavonol **116** is more active than **117** against Gram-negative strains (*Pseudomonas* spp.), what can be related with its planar C2-C3 double bond and suitability to this activity (Okoth, 2013).

Figure 22. Structures of lanneaflavonol (**116**) and dihydrolanneaflavonol (**117**) isolated from *L. alata* roots



Lupeol and a mix of phenolic lipids (mainly urushiols, with minor amounts of an alkenylphenol) from *Schinopsis* spp. showed antifungal (against *Fusarium graminearum* and *F. verticillioides*) and antimicotoxigenic effects. In this study, the phenolic lipids were more active than lupeol against *Fusarium* spp. with MIC_{50} 31-28 μg/g on *F. graminearum* and 165-150 μg/g on *F. verticillioides* and, besides, the antimicotoxigenic activity was higher than that of ferulic acid, since the fumosinin and deoxynivalenol production was thoroughly inhibited by all bioactive metabolites, even at lower concentrations. Thus, both lupeol and phenolic lipids were inhibitors of fungal growth and mycotoxin production of *Fusarium* spp., which is relevant to controlling these toxigenic fungi, owing to the stimulation of mycotoxin biosynthesis by several commercial antifungals. These activities suggest the use of lupeol as a food preservative and of phenolic lipids as fungal growth inhibitors without an increase in mycotoxin accumulation. In conclusion, further research is needed to elucidate the effect of these substances (from *Schinopsis* spp.) under different environmental conditions and characterize the substrates related to the control of *Fusarium* species (Ficoseco, 2014).

The *in vitro* activities of several EOs of branches, fruits and leaves of *Rhus typhina* L. wood (from Northeast Italy) exhibited high antimicrobial activity *in vitro*

against *C. albicans* (inhibition zone 22.60-35.00 mm, MIC 0.02 mg/mL), although only the EO from leaves and fruits were active against *E. coli* ATCC (inhibition zone 17.6-22.5 mm, MIC 0.064 mg/mL). Moreover, the antioxidant effect (DPPH assay) of leaf and fruit EOs was superior to that of branches' EOs, as it is indicated by their respective IC_{50} values (2.29 ± 0.10 μg/mL, leaves; 2.54 ± 0.10 μg/mL, fruits; 5.80 ± 0.18 μg/mL, branches). Lastly, given the chemical profiles of major essential oils in each plant part (cyclic sesquiterpenes in branches; aldehydes and other oxygenated derivatives in leaves; monoterpenes in fruits), it might be suggested that their specific bioactivities are probably due to these different groups of compounds (Beretta, 2021).

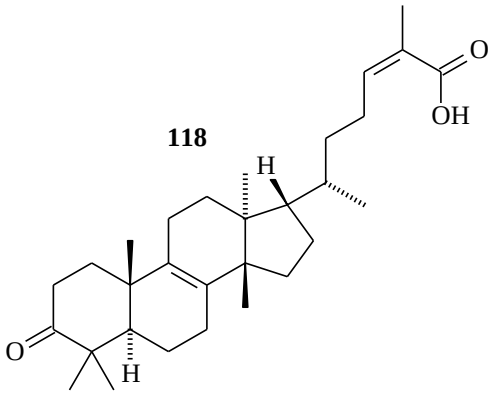
The biological studies with *M. indica* have shown a wide range of applications of active extracts and isolated substances. Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-*β*-D-glucoside, **97**) is a pharmacologically active phytochemical present in high yields in *M. indica* (bark, roots, fruits and leaves), which has many biological properties, among which are the antibacterial and cytotoxic/anticancer activities. A study reports that the solution of **97** was found to exert promising activity against both Gram-positive and Gram-negative bacteria (Maji, 2015), which is particularly relevant because drug resistance to human pathogens has increased worldwide due to the indiscriminate use of antibiotics. The cytoprotective potential of **97** for hematopoietic cells from leukemogenesis was also verified based on the decreased olive tail moment (OTM) and micronucleus (MN) frequency, so that **97** probably reduces DNA damage in the etoposide-treated mononuclear cells (Zhang, 2015).

Moreover, a study for the characterization of epicuticular leaf extracts (dichloromethane extract) and several derivatives of *Lithrea caustica* (Molina) Hook and Arn. showed that litreol (and some derivatives) behave as inhibitors of 15 soybean and 5 human lipoxygenases (15-sLOX and 5-hLOX). The highest activities were exhibited by litreol (IC_{50} = 54.77 μM against s-LOX; 2.09 μM against h-LOX) and 3-pentadecylcathecol (IC_{50} = 55.28 μM against s-LOX; 2.74 μM against h-LOX), in such a way that the respective kinetic studies indicated a mixed and selective inhibition mechanism to 5-hLOX (Muñoz-Ramírez, 2020).

Besides, the pistagremic acid (PA, **118**, Figure 23), isolated from the dried galls of *P. integerrima* Stewart (Uddin, 2012), exhibited an inhibitory effect against α-glucosidase *in vitro* in yeast (IC_{50} = 89.12 ± 0.12 μM), confirming former molecular docking simulations. Thus, a molecular binding mode was explored and the results

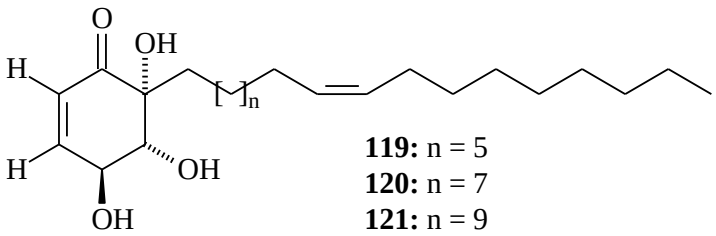
indicated hydrogen bonding interactions between PA and significant amino acid residues – Asp60, Arg69 and Asp 70 (3.11 Å) – surrounding the catalytic site of the α-glucosidase, which could be mainly responsible for their role in potent inhibitory activity of PA. Therefore, **118** showed a promising potential to be further investigated as a new lead compound for better management of diabetes.

Figure 23. Structure of pistagremic acid (isomasticadienonic acid)



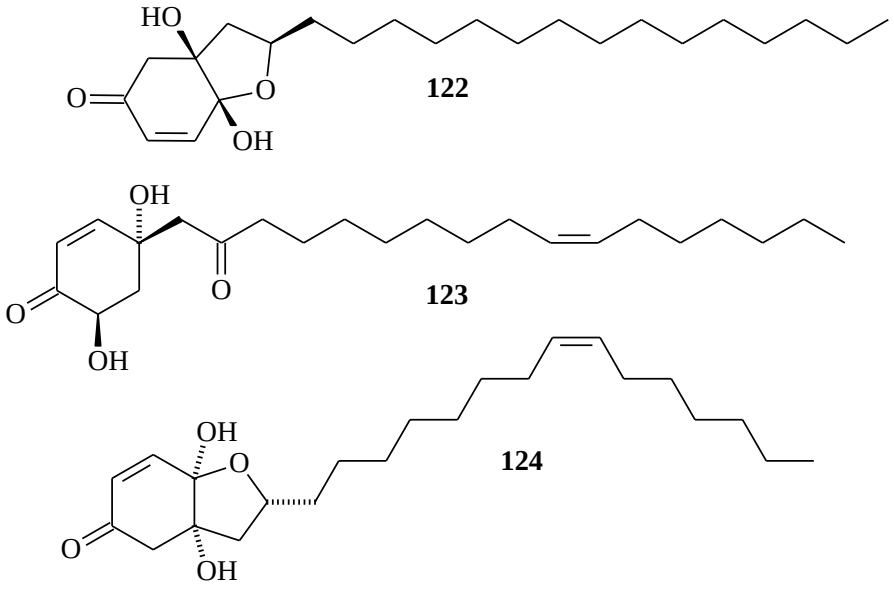
The investigation of a DCM extract of the bark of *Pleiogynium timoriense* (Eaton, 2015) indicated that it was active with an IC_{50} value of 1.3 μg/mL against the A2780 ovarian cancer cell line (A2780 OCCL). Bioassay-directed fractionation of this extract yielded the three new bioactive trihydroxyalkenylcyclohexenones **119-121** (named pleiogenones A, B and C, respectively), which showed a higher submicromolar antiproliferative activity against the A2780 human OCCL than the raw DCM extract (IC_{50} **119-121**: 0.8, 0.7 and 0.8 μg/mL), whose structures are presented in Figure 24:

Figure 24. Structures of pleiogenones A-C (**119-121**) obtained from *P. timoriense*



Likewise, the bioactivity-guided fractionation of EtOAc leaf extract of *Poupartia borbonica* (Ledoux, 2017) allowed the obtention of three novel alkyl cyclohexenone derivatives (**122-124**), whose structural elucidation was performed by 1D and 2D NMR (as well as MS) to determine their absolute configurations. These compounds were active against 3D7 and W2 *Plasmodium falciparum* strains (IC_{50} = 0.55–1.81μM) and exhibited *in vitro* cytotoxicity against WI38 human fibroblasts and the human cervical cancer (HeLa) cell line (WST-1 assay), but no hemolytic activity was observed for the extract and pure metabolites. Besides, the methanol extract was also evaluated and it displayed moderate antiplasmodial properties *in vitro*, which might be attributed to its flavonoid content (including the unknown compound 3'-*O*-hydroxysulfonylquercetin). The structures of **122-124** are showed in Figure 25:

Figure 25. Structures of new alkyl cyclohexenone derivatives from *P. borbonica*

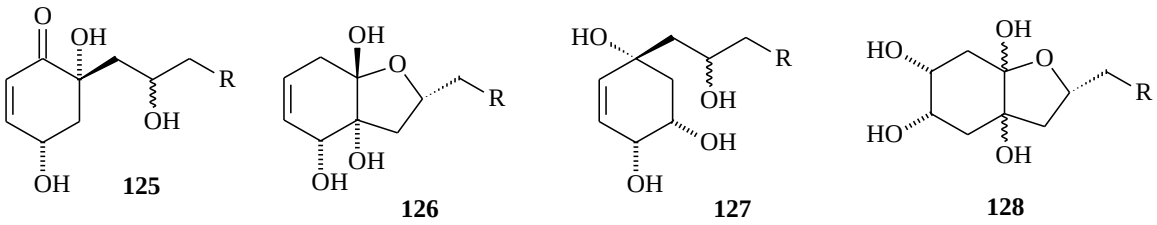


A further study with *P. borbonica* evaluated the cytotoxicity and pharmacological interest of poupartone B (**122**). For that purpose, a real-time live-cell imaging of different human cancer cell lines and normal fibroblasts, treated or not treated with **122,** was performed. Thus, a potent inhibition of cell proliferation associated with the induction of cell death was verified, in such a way that **122** (at 1-2 μg/mL) induced a rapid retraction of cellular protrusions associated with cell rounding, massive cytoplasmic vacuolization, loss of plasma membrane integrity and plasma

membrane bubbling, ultimately leading to paraptosis-like cell death. These results highlight the cytotoxicity of **122** against several *in vitro* cancer cell lineages (Ledoux, 2021). At last, it should be highlighted that compound **72** (integracin *E*), obtained from the stem barks of *Swintonia floribunda*, has presented a potent tyrosinase inhibitory activity with an IC_{50} value of 48.2 μM (Dang, 2019).

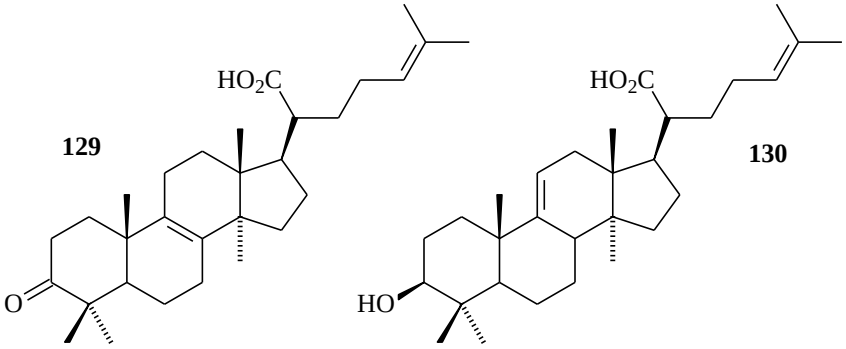
On the other hand, studies with *Tapirira guianensis* (Roumy, 2009) leaves also led to the obtention several compounds that seems to be precursors of alkyl and alkenyl phenols (**125-128**, Figure 26). The cyclohexene derivatives **125** and **126** were in mixture and showed against *P. falciparum* strains (IC_{50} = 4.7±0.3 and 5.4±1.7μM) against F32 and FcB1 strains. This mixture was also active against *Leshimania amazonensis* (IC_{50} = 1.0±0.1μM), *S. aureus* (IC_{50}: 75.4 μM) and *S. epidermidis* (IC_{50}= 17.6 μM).

Figure 26. Some antiprotozoal compounds from *Tapirira guianensis*



Moreover, two bioactive triterpenes – 3-oxo-5α-lanosta-8,24-dien-21-oic acid (**129**) and 3*β*-hydroxylanosta-9,24-dien-24-oic acid (**130**) – were isolated from the stem bark $CHCl_3$ extract of *Protorhus longifolia* (Figure 27), which were screened for several activities (antioxidant, cytotoxic and anti-platelet aggregation) *in vitro*. Although both metabolites exhibited low antioxidant and cytotoxic activities, they showed satisfactory concentration dependent anti-platelet aggregation activity, so that **129** showed the highest activity (IC_{50} = 0.99 mg/mL) on the thrombin-induced platelet aggregation (Mosa, 2011).

Figure 27. Structures of 3-oxo-5α-lanosta-8,24-dien-21-oic (**129**) and 3*β*-hydroxylanosta-9,24-dien-24-oic (**130**) obtained from *P. longifolia*



Two studies using *Semecarpus anacardium* afforded the obtention and the characterization of two bioactive metabolites – e.g., 3-(8'(*Z*),11'(*Z*)-pentadecadienyl)-catechol (Nair, 2009) (SA-3C, isolated from the plant kernel) and tetrahydroamentoflavone (THA, yielded from the seeds) (Arimboor, 2011). Concerning to SA-3C, the results indicated 1) a cytotoxic activity against tumor cell lines with lower IC_{50} values than doxorubicin as well as that 2) multidrug resistant tumor cell lines were equally sensitive to SA-3C. Besides, SA-3C induced apoptosis in human leukemia cell lines in a dose-dependent manner, showed synergistic cytotoxicity with doxorubicin and induced the cell cycle arrest at S- and G_2/M-phases, what was correlated with inhibition of checkpoint kinases. Thus, SA-3C can be developed as an important anticancer agent for single and/or multiagent cancer therapy (Nair, 2009). Similarly, THA exhibited a strong inhibitory effect against xanthine oxidase (XO), what was investigated through a Lineweaver-Burk (LB) plot for the XO inhibition of THA and allopurinol constructed from the kinetic data. In this case, IC_{50} values of THA and allopurinol for XO inhibition were 92 and 100 nM and their corresponding values for K_i were 0.982 and 0.612 μM. These results indicated that THA could be considered a promising drug candidate or chemopreventive agent, what can also ratify the claim of the traditional medicine with respect to the efficacy of *S. anacardium* seed against inflammation and gout (Arimboor, 2011).

Besides, an anti-malarial activity-driven investigation of the fruits of *Sorindeia juglandifolia* (Kamkumo, 2012) allowed the obtention of different fractions and two bioactive compounds (2,3,6-trihydroxybenzoic acid (**A**) and methyl 2,3,6-trihydroxybenzoate (**B**)). After several analyses *in vitro*, nine fractions tested against *P. falciparum* W2 and falcipain-2 were active (IC_{50} = 2.3-11.6 μg/ml for W2 and 1.1-21.9 μg/ml for falcipain-2). Purified compounds **A** and **B** also showed inhibitory effects

against *P. falciparum* W2 (IC_{50} 16.5 μM and 13.0 μM) and falcipain-2 (IC_{50} 35.4 and 6.1 μM). In studies of *P. falciparum* isolates from Cameroon, the plant fractions exhibited IC_{50} values of 0.14-19.4 μg/ml and the compounds (**A**) and (**B**) showed IC_{50} values of 6.3 and 36.1 μM. Thereafter, it is suggested that further investigation of the anti-malarial activities of natural products from *S. juglandifolia* would be appropriate.

4.2.2. *In vivo* studies with pure compounds

Although there are few examples of *in vivo* biological properties of common metabolites of Anacardiaceae spp., we can highlight the study with the leaves of *Schinus polygamous* C. (El Sayed, 2016). In this work, eight substances were obtained from EtOH extracts and identified as 3-*O*-acetyllupeol, *β*-sitosterol and lupeol, besides the gallic acid (GAc), methyl gallate, quercetin-3-α-*O*-rhamnoside, kaempferol and quercetin. Afterward, these compounds were submitted to hepatoprotective, antioxidant and curative activities. Lupeol and GAc were evaluated by oral administration in adult male albino rats of 50-100 mg/kg body weight of compounds, whose results showed a) significant protection against CCl_4-induced liver damage (indicated by an increase in AST, ALT and ALP enzymes in adult male albino mice) and b) a remarkable antioxidant effect (>90% for both compounds, measured by the activity of enzyme reduced glutathione).

In addition, we may stand out the study of *in vivo* antihyperglycemic activity of methyl-3*β*-hydroxylanosta-9,24-dien-21-oate (RA-3), a lanosteryl triterpene isolated from *P. longifolia* stem bark (Mosa, 2015). RA-3 antihyperglycemic behaviour was evaluated in an STZ-induced diabetes rat model, wherein the animals were orally administered with RA-3 (100 mg/kg body weight) daily for 14 days. RA-3 showed hypoglycemic effect by reducing blood glucose levels by 37% and improved glucose tolerance in diabetic rats. Furthermore, a relatively higher hepatic glycogen content, alongside the hexokinase and glucokinase activities (with a decrease in glucose-6-phosphatase activity), were also observed in the triterpene-treated diabetic group when compared with the diabetic control group. The treatment increased antioxidant status of the diabetic animals, as well as the activity of superoxide dismutase and catalase along

with a decrease in malondialdehyde content. Thus, RA-3 presents potential pharmaceutical effects in the management of diabetes mellitus.

At last, different compounds aforementioned have also exhibited *in vivo* bioactivities, as termipaniculatone A (Yang, 2019) (anti-hyperuricemic and anti-inflammatory activities in mice), 2,3,6-trihydroxy benzoic acid (Kamkumo, 2012) (activity against *P*. *berghei* strain B, with mean parasitaemia suppressive dose and curative dose of 44.9 mg/kg and 42.2 mg/kg), 3*β*-hydroxylanosta-9,24-dien-24-oic acid (Mosa, 2011) (strong inhibition of the acute inflammation of rat paw) and pourpatone B (Ledoux, 2017) (antimalarial/*P*. *berghei* growth inhibition at a dose 15 mg/kg/day i.p).

5. PROCESSES AND PRODUCT PATENTS BASED ON ANACARDIACEAE spp.

The latest advances in research in bioactive molecules have been characterized by their versatility in their deployments and applications. Thus, a significant part of the most recent studies in the Chemistry of Natural Products has enabled the discovery of alternative uses of extracts, isolated compounds and formulations published in patents, highlighting its relevance for the industry, medicine and biotechnology. This way, some examples of patents resulting from studies with Anacardiaceae *spp*. are presented herein, highlighting their impact on cosmetic and pharmaceutical industries, health sciences and other therapeutic or technological applications.

5.1. Employment in cosmetics

Different cosmetic compositions were developed based on compounds isolated from (or present in) Anacardiaceae *spp*., such as ellagic acid or its derivatives, essential oils or foaming agents. Thus, although such cosmetic formulations have different proportional amounts of bioactive substances, surfactants (anionic, non-anionic, zwitterions or amphoteric), thickening agents and other constituents, all evaluate compositions showed a good anti-dandruff effect. Therefore, this invention was related to a cosmetic treatment process using these formulations as well as to washing and/or

treatment of keratin fibers (e.g., of the scalp, whether followed by rinsing with water) to eliminate or reduce dandruff – mainly caused by Malassezia (*Pityrosporum* spp. yeasts) –, which may be helpful for hair and dermatological health (Poletti, 2010).

Likewise, a hair styling composition (in the form of foam) relating to a process for shaping keratin fibers (Dubief, 2010) was also developed, comprising the application of at least one "mousse" composition, including one or several fresh fruit juices, some Anacardiaceae species and/or surfactants with sugar moieties. This type of cosmetic formulation was mainly made in an aqueous medium or organic hydrophilic solvent (linear or ramified alcohols), in such a way as to allow to take advantage of some active constituents that are naturally present in fresh fruits (vitamins, α- and *β*-hydroxyacids, antioxidants, anti-inflammatories etc.), which have beneficial properties for the hair and scalp. In this product, various fruits such as mango, apple, lemon and others were employed to prepare the juices, whose constituents were in different proportions. However, similarly to the study as mentioned earlier, several styling mousses were obtained, which allowed the hair to be fixed and a satisfactory styling.

On the other hand, it was elaborated a topical cosmetic formulation (Ennamany, 2016) in which the dedifferentiated plant cells are elicited *in vitro* following a cycle of successive darkness and lighting periods under a CO_2 atmosphere (1-10%). The compositions with Anacardiaceae *spp*. and other plants permitted to observe an anti-aging effect, a protective effect for the skin, an antioxidant effect, as well as antifungal and antiradical properties.

5.2. Patents of medical and other biological uses

Several formulations comprising a hydroxylated fatty acid (such as ricinoleic acid) or a triglyceride containing hydroxylated fatty acid (e.g., castor oil) were combined with the liquid from the cashew nut peel (*A. occidentale* or Anacardiaceae *spp*.) and/or its agents or organic acids (cardol, cardanol, anacardic acid and derivatives), which have presented broad antimicrobial against Gram-positive and Gram-negative bacteria, fungi and protozoans. In this case, for the first time was presented that ricinoleic acid might behave as an oral antibiotic and an antiprotozoal agent, which was followed by extremely low toxicity in comparison with other antibiotics. Therefore, these formulations could be applied to the prevention and

treatment of pathogenic processes in people and animals (by oral, topical or parenteral administration), as well as to control fermentation (made with yeast – *S. cerevisiae*) and as an antifungal for food and seeds (Campmany, 2013).

Moreover, an antiviral composition with antiretroviral properties for treating HIV patients, which was made with acetic acid and coconut extracts, a solution of mineral salts (e.g., seawater) and other plant extracts from Anacardiaceae (*S. mombin* bark), Liliaceae (*Smilax medica* roots) and Euphorbiaceae (roasted castor beans, *Ricinus* spp.), was developed (Commin, 2011). The results of these formulations indicated an inhibition of the HIV-1 reverse transcriptase activity (which may exceed 90% compared to the activity measured in the controls) and a decrease in the cytopathogenic effect of HIV-1 in infected cells after the treatment with the antiviral compositions. Besides, the absence of toxicity was observed in mice essays. The amount of active ingredient varied according to the patient's condition, whose dosage in humans is most often from 1–500 mg/day of the active ingredient, which suggests the efficacy *in vitro* and *in vivo* of these compositions against HIV-1 virus.

Notwithstanding it, an invention based on a *M. indica* preparation as sirtuin 1 (SIRT1) activating agent was developed for *in vivo* and *in vitro* applications (Buter, 2020), which may be used to reduce the risk of obesity, type-II diabetes, high blood lipid levels, arteriosclerosis and heart illnesses, as well as a cellular and DNA protector. In this work, the powder of fruits of *M. indica* is preferably the fruit aqueous extract, being comprised in a food product, a dietary supplement or a medicament, wherein the active ingredient's concentration varied according to three different ranges in each case. Furthermore, this preparation may be administered twice a day (topically or preferably orally), so that the SIRT-1 activation was not dose-dependent but was similar to resveratrol’s at the highest tested concentration. Thus, these formulations are opening a wide application in therapeuticacl field by the improvement the general condition of individuals – including healthy body composition, glucose and lipid metabolism, energy homeostasis, physical strength, muscle mass, cell protection, and, thereby, slowing down the aging process or preventing age-related chronic diseases.

In addition, an aqueous ethanolic dilution of juices or extracts derived from some plants (Pushpangadan, 2006) were transformed into a paste (or a jelly, a jam, a cake, a cream puff or a chocolate) to be used as functional foods and had anti-stress (induced by gastric ulcers) and adaptogenic activities. The extracts of *M. indica* (65.0-

75.0 wt. %), *Withania somnifera* (3.5–5.0 wt. %, Solanaceae), *Aspargus recemosus* (3.5–5.0 wt. %, Asparagaceae), *Amaranthus hypochondriacus* (10.0–20.0 wt. %, Amaranthaceae) and *Evolvulus alsinoides* (0.2–0.6 wt. %, Convolvulaceae) did not provoke mortality in any of the rats' treated groups, as well as behaviour's abnormalities in the animals exposed with herbal preparations. The results of these formulations showed antiulcer properties (since they reduce the ulcer index and decrease its severity) and exhibited an antioxidant effect by the decrement of lipid peroxidation, the rising of catalase levels and the enhancement of superoxide dismutase activity.

A novel formulation related to an herbal formulation prepared with several plants (including *M. indica*) developed for the diabetes prevention and treatment, as well as associated damages, was proposed in the period (Krishnan, 2009). The invention developed therein might not only control Type 2 diabetes but also offer reversal possibilities in prediabetes and, thereby, a possible prevention for diabetes mellitus and its complications. The plant extract composition provides good glycemic management and reduces the glycosylation of hemoglobin, controls the total cholesterol levels, improves cardiovascular health by decreasing hypertension and enhances wound healing of diabetic ulcers. Besides, another minor effects are the reduction of systolic and diastolic blood pressures, prevention of oxidative stress and minimization of hypertensive drug dependency.

Extracts and compositions of *Schinus* species (in particular, *S. terebinthifolia* Raddi) were investigated to prevent bacterial infections, acne and other corresponding applications (Quave, 2021). Their main compounds (flavonoids, triterpenoids and steroidal sapogenins) were identified by LC-FTMS, which have many known biological properties (anti-inflammatory, antidiabetic, antimicrobial etc.). In this case, compositions based on the oleanolic acid C-3 ketone (or a salt thereof) in the form of a lotion, liquid or gel were developed, so that the treatment/prevention of a bacterial disease (e.g., caused by *Staphylococci* spp.) includes administering an adequate amount of the formulation against other skin damages, venous and diabetic ulcers, meningitis and pneumonia. The organic extractions of these plants yielded an active butanolic fraction (named 430D-F5), which quenched quorum sensing and toxin production, affected biofilm formation and abated dermonecrosis in mice.

Similarly, an effective formulation for treating anemia and low blood pressure comprising type-anacardic acids (**59** and the $\Delta^{8,9}$ alkene derivative) isolated from

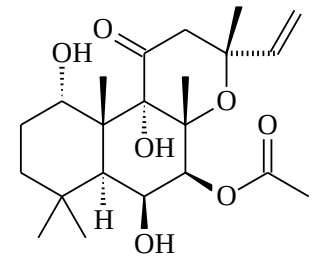
powdered roots/barks aqueous extract of *Ozoroa paniculosa* was developed. The composition was active in oral, rectal, nasal, vaginal or parenteral (subcutaneous, intramuscular, intravenous or intradermal) administration, both for humans and animals (Bogoshi, 2014).

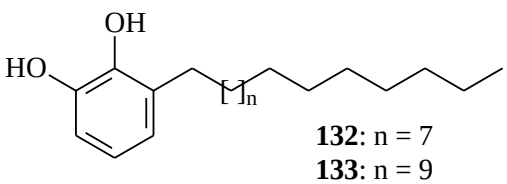
Besides, six active fractions were employed to elaborate herbal compositions using plants of Anacardiaceae and Asteraceae *spp*. (Nandeshwar, 2017) to treat infertility in men. Thus, these simple, cost-effective and no-side effects formulations can be related to a restored vigor and an increased sexual libido, exhibiting a healing effect on X and Y chromosome-related diseases and granting other corresponding benefits in synergistic plant compositions.

Lastly, some like-urushiol derivatives – 3-n-pentadecyl catechol (poison ivy saturated congener) and/or 3-n-heptadecyl catechol (poison oak saturated congener) – were synthesized (Elsohly, 2019) for the prevention and/or prophylactic treatment of contact dermatitis induced by poison ivy and poison oak. The compounds were effective for tolerizing and desensitizing a subject against allergens contained in Anacardiaceae/Gingkoaceae *spp*. comprising urushiol esters, as it is shown in Figure 28 (compounds **131**-**149**):

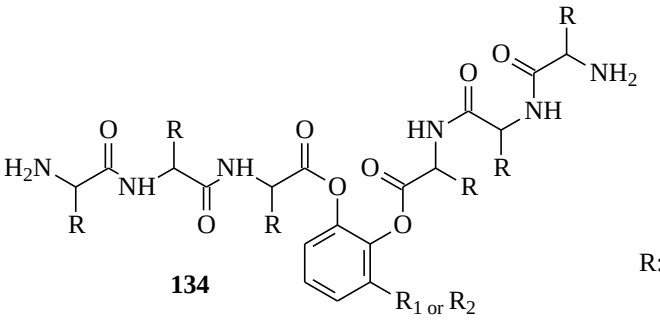
Figure 28. Molecular structures of forskolin (**131**), pentadecyl catechol (PDC, **132**), heptadecyl catechol (HDC, **133**), an urushiol derivative (tri-amino acid, **134**), urushiol-allyl carbamates (**135**), sulfuric acid mono-(3-heptadecyl-2-sulfooxy-phenyl) ester (**136**), HDC amino acid derivatives (**137-141**), PDC amino acid derivatives (**142-146**) and PDC allyl derivatives (**147-149**)

131



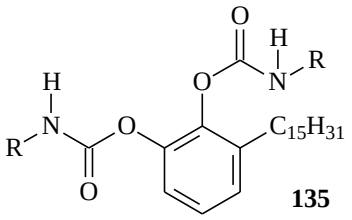


132: n = 7
133: n = 9



134

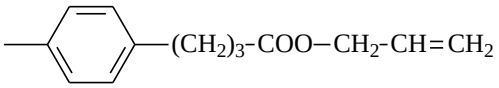
R: amino acid residue
R_1: $C_{15}H_{31}$; R_2: $C_{17}H_{35}$



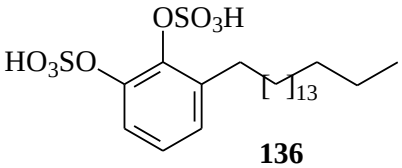
135

R: —$(CH_2)_3$-COO—CH_2-CH=CH_2

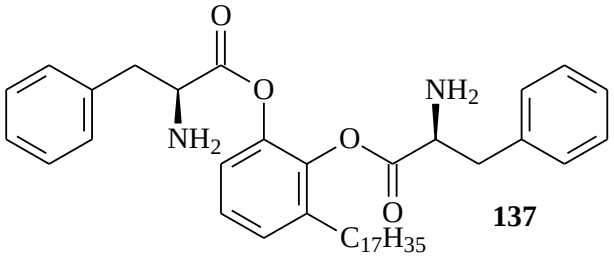
—$(CH_2)_5$-COO—CH_2-CH=CH_2



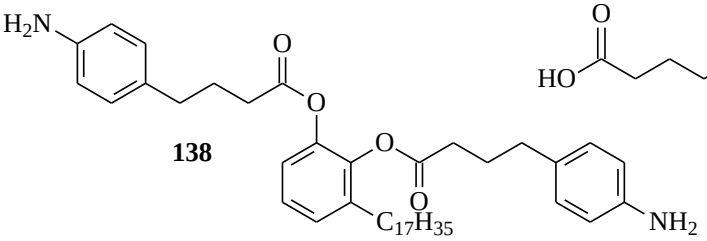
(allyl protected carbamates)



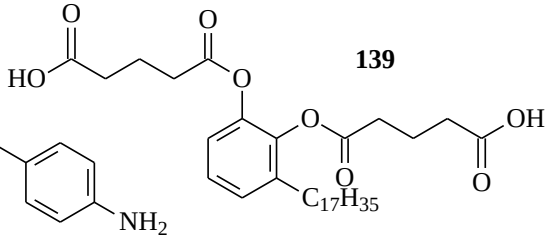
136



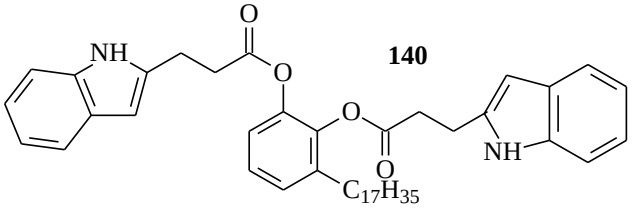
137



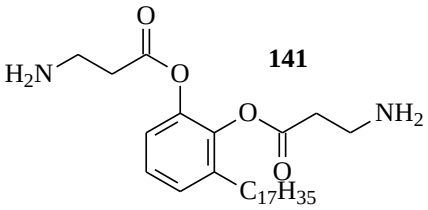
138



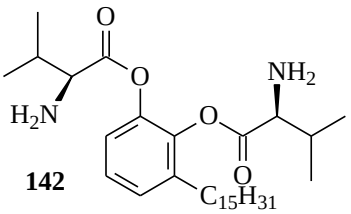
139



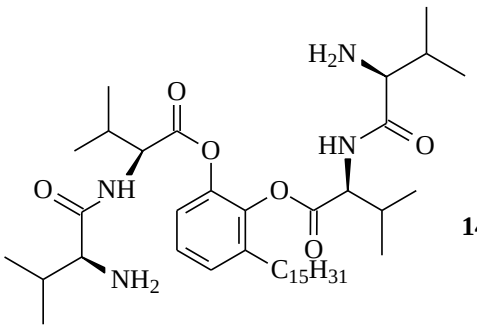
140



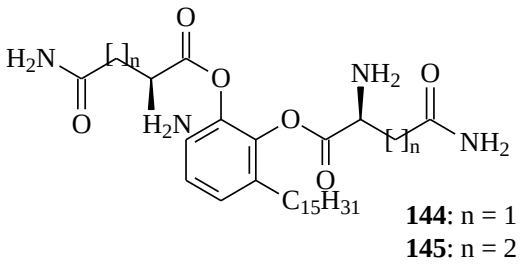
141



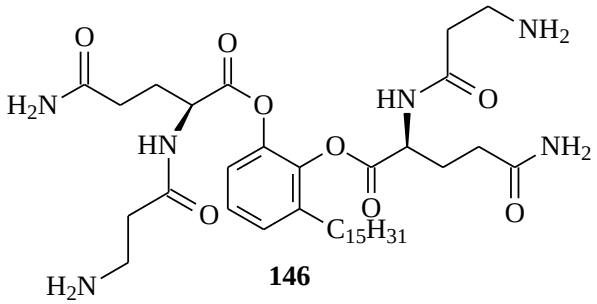
142



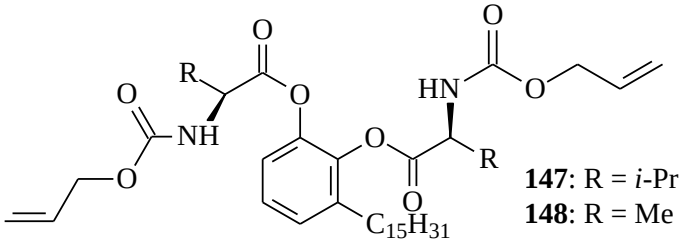
143



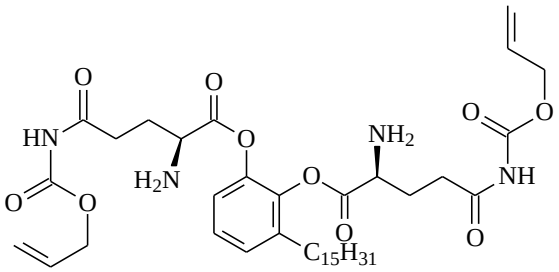
144: n = 1
145: n = 2



146



147: R = *i*-Pr
148: R = Me



149

5.3. Technological uses

Different insecticidal compositions were prepared in liquid, dehydrated and lyophilized forms, wherein several plants of various families (e.g., *Pistacia vera*, Anacardiaceae) were included, whose constituents (polypeptides alone vs. binary systems) that were expressed as polypeptides/polynucleotides showed pesticidal properties. These combinations were usually more active than the components individually, whose results are promising for agriculture, ecology, biotechnology and other scientific applications (Rotem, 2021).

The efficacy of the gum of *Odina wodier* Roxb. (i.e., *Rhus odina*) (Mukherjee, 2003), an Asian plant that presents many applications in folk medicine, was evaluated for the first time as a tablet binder. Hitherto, the potential binding capability and an emulsifier have already been studied to stabilize emulsions. Chemical analyses appointed to the presence of carbohydrates alongside the absence of tannins and

peroxidase enzyme in the "gum odina" compositions, what removed the possibility of oxidative degradation of gum as excipient. The gum was stable in liquid conditions and no toxicity was observed. These results showed that "gum odina" could be used as pharmaceutical excipients (e.g., tablet binder or emulsifier), being effective in minimal amounts compared to the standard tablet binders.

Furthermore, other studies with Anacardiaceae *spp*. were developed with new compositions or products that might be applied as (**i**) antimicrobial coating films for filters and air conditioning equipment (e.g., branch/leaf/rhizome/bark aqueous extracts of different plants, including *Pistacia* spp.) (Tomioka, 2006), (**ii**) in procedures for a concentration of xanthones at high pressure on a semi-industrial scale (Fernández-Ponce, 2017) using extracts of several plants (e.g., *M. indica*) or (**iii**) to increase the content of desired ingredients in crops (such as fruits and vegetables) (Rieck, 2011) by applying succinate dehydrogenase inhibitors (SDIs). Thus, the embodiments of invention (**i**) were intensely active against harmful microorganisms in the living environment, promoting its cleanliness and preventing microorganisms' degradation. On the other hand, the use of a hydrophobic stationary phase mixed to a supercritical eluent (pure CO_2 vs. a mix of CO_2 with a polar cosolvent in isocratic or gradient mode) in (**ii**) allowed the obtention of phenolic acids, benzophenones, flavonoids and xanthones in high amounts (mainly **97**, 5x higher than in original leaf extract), whose process was efficient and avoided losses in the bioactivity of the fractionated substances. At last, the study (**iii**) showed the behavior of several SDIs against various plant species (including mango, sumac and pistachio), whose results indicated that different types of natural metabolites might have their contents increased thereon, since that the SDI is applied to the crop prior to the harvest and at a rate ranging from 1 to 250 g/ha.

5.4. Other uses

Different formulations, including at least one plant of Asteraceae, Lamiaceae, Anacardiaceae (*P. lentiscus*) and Cistaceae families in treating varroatosis (by *Varroa destructor*, varroa mite) in bees (Bellei, 2021) were elaborated. This invention achieves a composition that is harmless for bees and humans and effective in a short time on mites in both phoretic and reproductive phases in opened/operculated cells. Moreover, an antioxidant formulation from Anacardiaceae *spp*. (in particular, *Sclerocarya birrea*)

(Cyril, 2006) were obtained by maceration/extraction of roots, bark, leaves, fruits or its parts (endocarp, mesocarp, epicarp) and using different solvents, whose results suggested that the prepared extracts had outstanding oxidative stability and showed a good antiradical behavior.

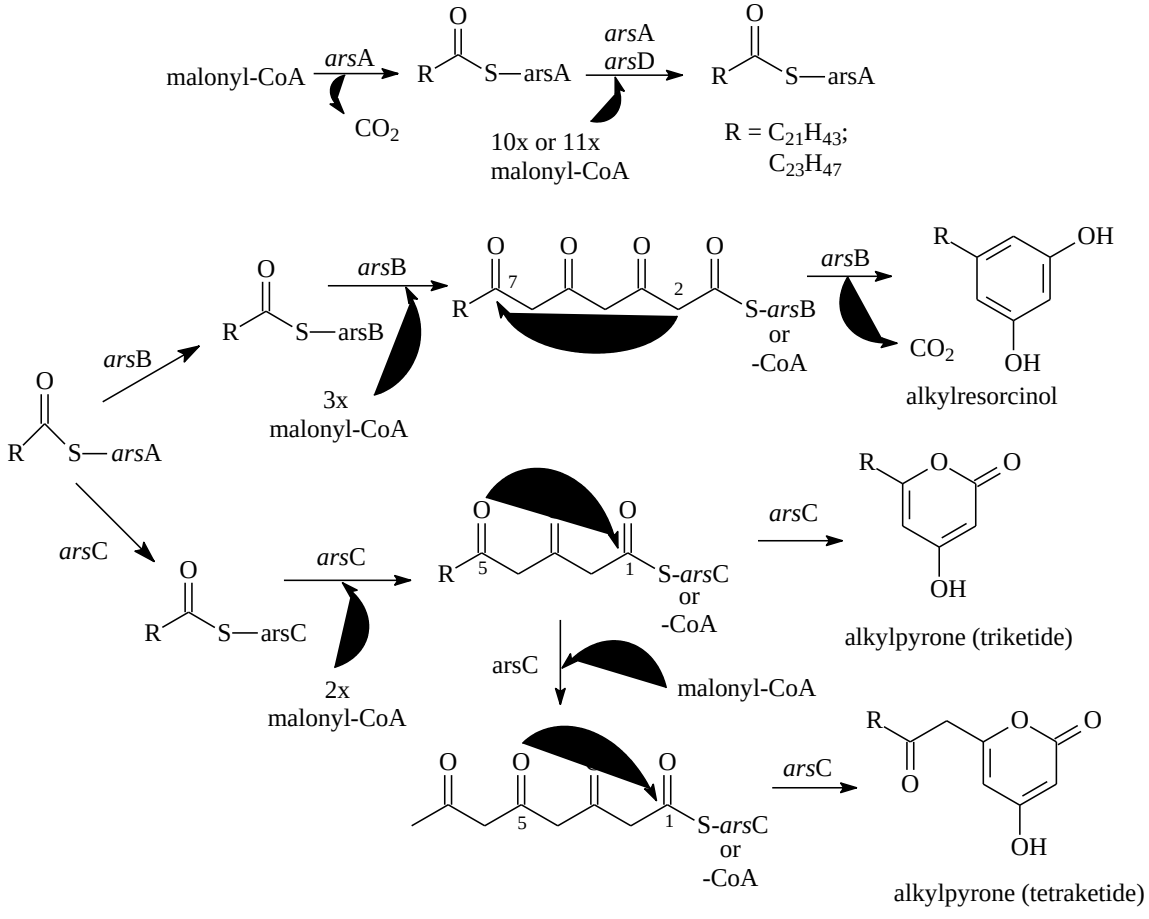
Other plant compositions, including Anacardiaceae species and using unrefined oils (from natural seedlings) free of phorbolic esters and *trans* fatty acids, were developed for cosmetic, dermatologic, dietetic, insecticide, pharmaceutical, veterinary and eating uses (Boucher, 2006). These compositions exhibited satisfactory outcomes as an antimicrobial/antifungal, germ inhibitor and for the management of cellular functions, including the potential to be employed in external and internal medications. In conclusion, formulations comprising sumac (*Rhus* spp.) and oregano can be highlighted (Falco, 2021), once they can be used as preservative agents to prevent or slow down the deterioration of food products (e.g., for wet and dry baked products). These formulations allowed the storage time of the baked products to be significantly extended, substantially delaying both the appearance of mold (mainly on wet baked goods) and the rancidity, whose properties can be associated with the presence of high polyphenol quantities.

6. PROGRESS IN BIOSYNTHESIS OF PHENOLIC LIPIDS

More recent publications have given new contributions to elucidate phenolic lipids biosynthesis possible mechanisms. For instance, the structure and function of polyketide biosynthetic enzymes (PKSs) and the strategies for producing several polyketides were investigated (Myianaga, 2017), whose results indicated that the type III PKSs have involved in the processes of polyphenols and phenolic lipids biosynthesis in plants, bacteria and fungi. Hence, type III PKSs synthesize a broad group of metabolites, since they differ in their preference of starter and extender substrates, the number of condensation steps and the mechanism of intramolecular cyclization of poly-*β*-keto intermediates.

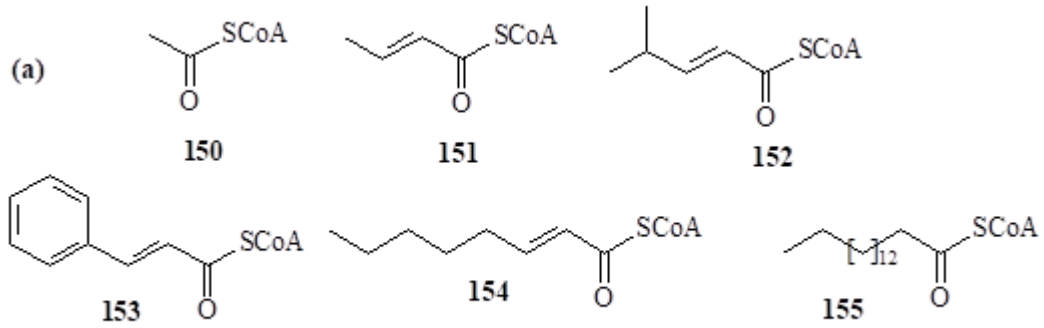
The elucidation of the biosynthesis of phenolic lipids and biochemical analysis *in vitro* using *Azotobacter vinelandii* bacterium strains was formerly conducted, once alkylresorcinols and alkylpyrones are the major lipids of *A. vinelandii* cyst membranes. Gene disruption analyses showed that the *ars* gene cluster is essential for biosynthesis, which consists of two types I fatty acid synthase (FAS) genes (*arsA*, *arsD*) and two types III PKSs (*arsB*, *arsC*). Thus, it was observed that the reactions of *arsA*, *arsB*, and *arsD* gave alkylresorcinols. In contrast, the reactions of *arsA*, *arsC*, and *arsD* gave alkylpyrones, once *arsB* catalyzes the decarboxylative C2-C7 aldol condensation to produce alkylresorcinols and *arsC* catalyzes the C5 oxygen-C1 lactonization to synthesize alkylpyrones. These features are due to the specific amino acid residues at the type III PKSs active site cavity (Trp281 to alkylresorcinols and Gly284 to alkylpyrones), as it is presented in Scheme 11 (Myianaga, 2017).

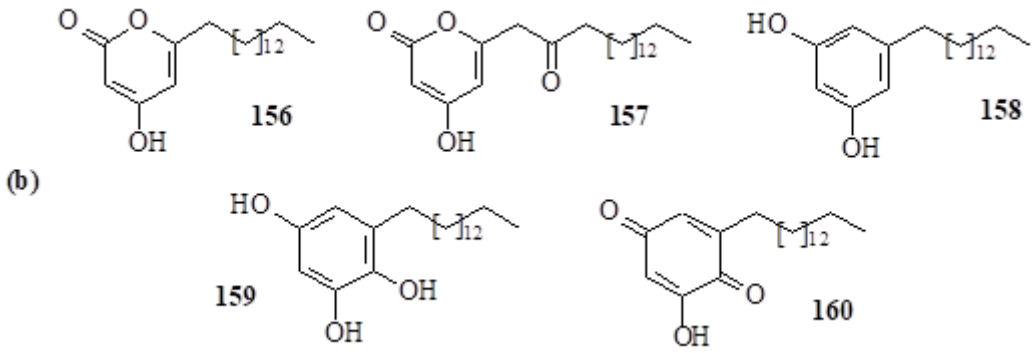
Scheme 11. The biosynthetic pathway of phenolic lipids according to the experiments with *Azotobacter vinelandii* bacterium



Likewise, the characterization of an orphan Type III polyketide synthase (PKS/CepA) in uncultivated Entotheonella (*Theonella* spp.) sponge symbionts provided new information regarding the enzymatic activity in phenolic lipids' biosynthesis, as well as the metagenomic features related. Thus, the three PKS18 residues (Thr144, Cys205 and Ala209) were crucially involved in its substrate preference (i.e., alkylresorcinols vs. alkylpyrones, according to the long-chain alkyl units binding). However, for the enzyme BpsA the PKS-like substrate-binding tunnel is composed of Thr, Cys and Phe residues at the corresponding positions. Based on their bioinformatic analyses, they suggested that CepA was most likely a resorcinol synthase that accepted long-chain fatty acid starters (Figure 29a, **150**-**155**) directly from several coenzyme-A precursors. In contrast, the presence of alkylpyrones might indicate an apparent incoherence with the bioinformatic prediction. Therefore, according to optimized enzymatic assays, two alkylresorcinols and three alkylpyrones were obtained (Figure 29b, **156**-**160**), whose structures were determined by NMR (^{1}H, ^{13}C, COSY, HMBC and HSQC) and HPLC-ESI-MS/MS, including isotope-labeling analyses. At last, the *in vitro* experiments demonstrate that CepA factor acts as a phenolic lipid synthase, processing long-chain fatty acid acyl-CoA and malonyl-CoA thioesters, wherein the product range includes tetraketide resorcinols as well as tri- and tetraketide α-pyrones, which were detected for the first time in theonellid sponges of *Entotheonella* species (Piel, 2020).

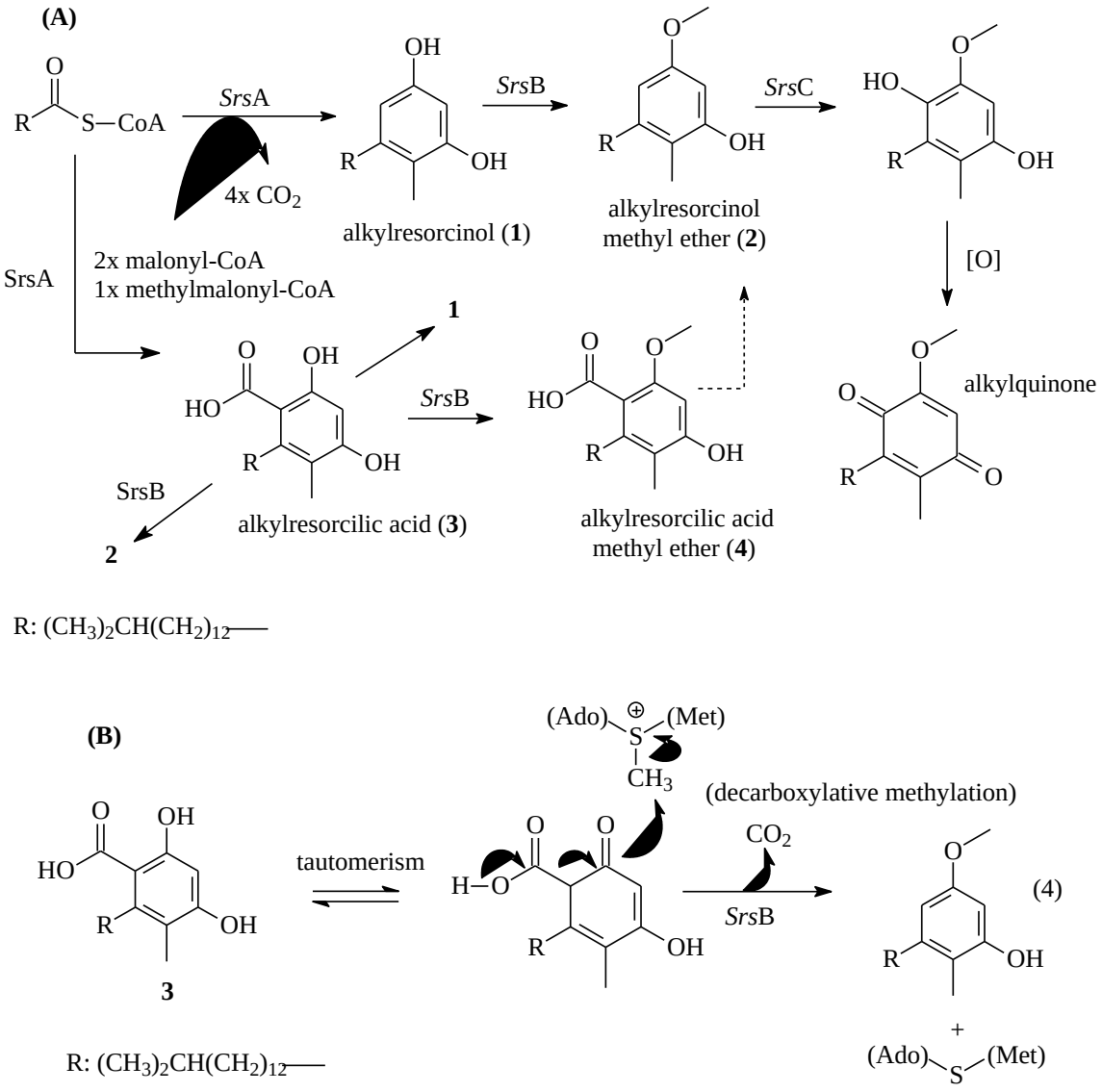
Figure 29. (a): Structures of potential PKS starters used as test substrates. (b): Proposed structures to the tri- or tetraketides obtained by optimized enzymatic assays using theonellid sponges (CepA pathway).





In conclusion, the catalytic activity of *O*-methyltransferase *Srs*B in the decarboxylative methylation of alkylresorcylic acid (ARA) along phenolic lipid biosynthesis by *Streptomyces griseus* (or *S. lividans*) was investigated, whose operon (*srs*) encodes a type III PKS and a flavoprotein hydroxylase (Nakano, 2012). Former studies have reported that *Srs*A enabled the production of an ARA as a direct product rather than a corresponding alkylresorcinol (ARC), while *Srs*B produced alkylresorcinol methyl ether (ARME) in the presence of *S*-adenosyl-l-methionine (SAM). However, *Srs*B has been shown incapable of catalyzing the *O*-methylation of ARC, suggesting that ARA was the substrate of *Srs*B, whose conversion to ARME might take place by (**i**) the *O*-methylation of the OH-group (C-6) or (**ii**) the decarboxylation of the neighboring carboxyl group (C-1). These studies proposed that *O*-methylation was coupled with decarboxylation, so that *Srs*B catalyzed the feasible SAM-dependent decarboxylative methylation of ARA, which is the first report of a methyltransferase with this catalytic behavior in an *in vitro* assay. After that, the *Srs* biosynthetic pathway (A) and the mechanism of *Srs*B reactions (B) are represented in Scheme 12.

Scheme 12. Scheme of the Srs biosynthetic pathway (A) and proposed mechanism of biosynthesis of alkylresorcinol methyl ether by SrsA and SrsB (B) with the mediation of S-adenosyl-L-methionine (SAM).



7. CONCLUSION AND FURTHER PERSPECTIVES

This review presents a detailed report regarding the Anacardiaceae family. The chemical composition, the wide range of their biological properties *in vitro* and *in vivo*, the applicability of Anacardiaceae *spp*. extracts or isolated metabolites in promising products through patents and new significant information about phenolic lipids biosynthesis were pointed out. This botanical family is significant to pharmacology, the chemistry of natural products and to corresponding scientific fields. The relevance of

this study must be highlighted, since it is the first work wherein such an investigation concerning Anacardiaceae *spp*. was undertaken. Therefore, based on the data and the new findings shown hither, further research with Anacardiaceae *spp.* is needed, both to isolate new bioactive compounds and elucidate the compounds responsible for the biological activities, as well as towards alternative contributions to biosynthetic studies of chemotypes/chemotaxonomic markers in this family.

REFERENCES

[1] Correia, S. de J.; David, J. P.; David, J. M., 2006. **Metabólitos secundários de espécies de Anacardiaceae**. *Quim. Nova*, *29*, p. 1287. DOI: https://doi.org/10.1590/S0100-40422006000600026

[2] Baer, H., 1979. **The poisonous Anacardiaceae**. Edited by Kinghorn, A. Douglas. *Toxic Plants* [Symp.], p. *161-170*.

[3] Schulze-Kaysers, N.; Feuereisen, M. M.; Schieber, A., 2015. **Phenolic compounds in edible species of the Anacardiaceae family – a review**. *RSC Adv.*, 5(*89*), p. *73301*. DOI: http://dx.doi.org/10.1039/C5RA11746A

[4] Singh, V. K.; Sharma, S.; Dhyani, S. K.; Kumar, S. S.; Sinha, R. K., 2009. **Phytochemical and Pharmacological investigation on Mangiferin**. *Herba Polonica*, 55(*1*), p. *126*.

[5] El-Nashar, H. A. S.; Mostafa, N. M.; Abd El-Ghffar, E. A.; Eldahshan, O. A.; Singab, Abdel Nasser B., 2022. **The genus Schinus (Anacardiaceae): a review on phytochemicals and biological aspects**. *Nat. Prod. Res.*, 36(*18*), p. *4839*. DOI: https://doi.org/10.1080/14786419.2021.2012772

[6] https://www.mobot.org/mobot/research/apweb/genera/anacardiaceaegen.html, accessed on November 2022.

[7] Simionatto, E.; Peres, M. T. L. P.; Hess, S. C.; da Silva, C. B.; Chagas, M. O.; Poppi, N. R.; Prates, C. B.; Matos, M. de F. C.; Santos, E. C. S.; de Carvalho, J. E., 2010. **Chemical Composition and Cytotoxic Activity of Leaves Essential Oil from *Mangifera indica* var. *coquinho* (Anacardiaceae).** *J. Essent. Oil Res.*, *22*, p. *596*. DOI: http://dx.doi.org/10.1080/10412905.2010.9700408

[8] Nyegue, M. A; Kemegne, G. A; Kamdem, S. L. S.; Etoa, François-Xavier; Menut, C., 2018. ***Mangifera indica* Bark Essential Oil: Chemical Composition and Biological Activities in Comparison with Aqueous and Ethanol Extracts.** *Nat. Prod. Commun.*, 13(*7*), p. *903*.

[9] Joshi, S. C.; Mathela, C. S., 2014. **Essential oil composition of *Rhus cotinus* and its antioxidant activity.** *J. Nat. Prod. Plant Resour*., 4(*3*), p. *39*.

[10] Ulukanli, Z.; Karabörklü, S.; Bozok, F.; Çenet, M.; Öztürk, B.; Balcilar, M., 2014. **Antimicrobial, insecticidal and phytotoxic activities of *Cotinus coggyria* Scop. essential oil (Anacardiaceae).** *Nat. Prod. Res.*, 28(*23*), p. *2150*. DOI: https://doi.org/10.1080/14786419.2014.928879

[11] Fraternale, D.; Ricci, D., 2018. **Chemical Composition and Antifungal Activity of the Essential Oil of *Cotinus coggygria* from Marche Region (Italy).** *Nat. Prod. Commun.*, 13(*9*), p. *1175*. DOI: http://dx.doi.org/10.1177/1934578X1801300919

[12] Negro, C.; De Bellis, L.; Miceli, A, 2015. **Chemical composition and antioxidant activity of *Pistacia lentiscus* essential oil from Southern Italy (Apulia).** *J. Essent. Oil Res.*, 27(*1*), p. *23*. DOI: http://dx.doi.org/10.1080/10412905.2014.973614

[13] Tabanca, N.; Nalbantsoy, A.; Kendra, P. E; Demirci, F.; Demirci, B., 2020. **Chemical Characterization and Biological Activity of the Mastic Gum Essential Oils of *Pistacia lentiscus* var. *chia* from Turkey.** *Molecules, 25*, p. *2136*. DOI: https://doi.org/10.3390/molecules26247504

[14] Abed, A.; Rachid, D.; Catherine, M; Ismahene, S.; Aicha, L.; Nadia, B.; Hichem, D.; Charef, R.; Mehdi, E.; Farida, T.; Mounira, O. K.; Lahouari, D., 2017. **Chemical composition and antibacterial activity of essential oil from leaves and twigs of *Pistacia lentiscus* growing in Mostaganem Province (Algeria).** *Int. J. of Biosci.*, *10*(*5*), p. *146*. DOI: http://dx.doi.org/10.12692/ijb/10.5.146-158

[15] Komaitis, M.; Gardeli, C.; Papageorgiou, V.; Mallouchos, A.; Theodosis, K., 2008. **Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts.** *Food Chem.*, 107, p. *1120*. DOI: http://dx.doi.org/10.1016/j.foodchem.2007.09.036

[16] Hadjari, A.; Pulaj, B.; Mustafa, B.; Nelson, K.; Quave, C. L., 2016. **Chemical composition and *in vitro* antibacterial activity of *Pistacia terebinthus* essential oils derived from wild populations in Kosovo.** BMC *Complementary Altern. Med.*, 16:*147*, p. *1*. DOI: https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-016-1135-8

[17] Ulukanli, Z.; Karabörklü, S.; Öztürk, B.; Çenet, M.; Balcilar, M., 2014**. Chemical Composition, Antibacterial and Insecticidal activities of the essential oil from the *Pistacia terebinthus* L. spp. *palaestina* (Boiss.) (Anacardiaceae).** *J. Food Process. Preserv.*, 38, p. *815*. DOI: http://dx.doi.org/10.1111/jfpp.12035

[18] Abolghasemi, A.; Shojaaddini, M.; Tajabadipour, A.; Sefidkon, F., 2018. **Composition of *Pistacia khinjuk* (Anacardiaceae) Leaf Essential Oil and its Insecticidal Activity on Common Pistachio Psyllid, *Agonoscena pistaciae* (Hem., Psylloidea).** *Journal of Essential Oil Bearing Plants*, 21(*3*), p. *796*. DOI: http://dx.doi.org/10.1080/0972060X.2018.1498397

[19] Ismail, A.; Lamia, H.; Mohsen, H.; Samia, G.; Bassem, J., 2013. **Chemical composition and antifungal activity of three Anacardiaceae species grown in Tunisia.** *Sci. Int.*, 1(*5*), p. *148*. DOI: http://dx.doi.org/10.5567/sciintl.2013.148.154

[20] Satpathy, G.; Tyagi, Y. K.; Gupta, R. K., 2011. **Preliminary evaluation of nutraceutical and therapeutic potential of raw *Spondias pinnata* K., an exotic fruit of India.** *Food Res. Int.*, 44, p. *2076*. DOI: https://doi.org/10.1016/j.foodres.2011.01.040

[21] Sameh, S.; Al-Sayed, E.; Labib, R. M.; Singab, A. N. B., 2019. **Comprative metabolic profiling of essential oils from *Spondias pinnata* (Linn. F.) Kurz and**

characterization of their antibacterial activities. *Ind. Crop. Prod*., 137, p. *468*. DOI: http://dx.doi.org/10.1016/j.indcrop.2019.05.060

[22] Ren, L.; Jing-Jing, Y.; Xing-Zhen, S.; Yuan-Fei, W.; Corlett, R. T.; You-Kai, X.; Hua-Bin, H., 2020. **Chemical Composition and the Cytotoxic, Antimicrobial, and Anti-Inflammatory Activities of the Fruit Peel Essential Oil from *Spondias pinnata* (Anacardiaceae) in Xishuangbanna, Southwest China.** *Molecules*, 25, p. *343*. DOI: https://doi.org/10.3390/molecules25020343

[23] Oladimeji, A. O.; Aliyu, M. B.; Ogundajo, A. L.; Babatunde, O.; Adeniran, O. I.; Balogun, O. S., 2016. **Identification and comparison of the volatile constituents of fresh and dried leaves of *Spondias mombin* found in North-central Nigeria: *in vitro* evaluation of their cytotoxic and antioxidant activities.** *Pharm. Biol.*, (54):*11*, p. *2674*. DOI: https://doi.org/10.1080/13880209.2016.1178308

[24] Lago, J. H. G.; Santana, J. S.; Sartorelli, P.; Guadagnin, R. C.; Matsuo, A. L.; Figueiredo, C. R.; Soares, M. G.; da Silva, A. M., 2012. **Essential oils from *Schinus terebinthifolius* leaves: chemical composition and *in vitro* cytotoxicity evaluation.** *Pharm. Biol.*, 50(*10*), p. *1248*. DOI: https://doi.org/10.3109/13880209.2012.666880

[25] Gazim, Z. C.; Bortolucci, W. de C.; de Oliveira, H. L. M.; Silva, E. S.; Vilas Boas, M. R.; de Carvalho, T. M.; Campo, C. F. de A. A.; Gonçalves, J. E.; Júnior, R. P., 2018. **Acaricidal and larvicidal activity of leaves and fractions of rose pepper (*Schinus terebinthifolius* Raddi. (Anacardiaceae) essential oil against *Rhipicephalus* (*Boophilus*) *microplus*.** *Aust. J. Crop Sci.*, (12):*10*, p. *1645*. DOI: http://dx.doi.org/10.21475/ajcs.18.12.10.pne1346

[26] Fernandes, M. Z. L. C. M.; Zanini, S. F.; Affonso, C. R. G.; Fernandes, R. M.; de Oliveira, J. M. G.; de Carvalho e Martins, M. C.; de Lima, S. G.; Júnior, G. R. de S., 2012. **Effects of the Essential Oil from Fruits of *Schinus terebinthifolius* Raddi (Anacardiaceae) on Reproductive Functions in Male Rats.** *J. Braz. Chem. Soc.*, (23):*1*, p. *180*. DOI: http://dx.doi.org/10.1590/50103-50532012000100025

[27] Sartorelli, P.; Lago, J. H. G.; Guadagnin, R. C.; Santana, J. S.; Pinto, E. G.; Tempone, A. G.; Stefani, H. A.; Soares, M. G.; da Silva, A. M., 2012. ***In Vitro* Trypanocidal Evaluation of Pinane Derivatives from Essential Oils of Ripe Fruits from *Schinus terebinthifolius* Raddi (Anacardiaceae).** *Quim. Nova*, 35(*4*), p. *743*.

[28] Atti dos Santos, A. C.; Rossato, M.; Agostini, F.; Serafini, L. A.; dos Santos, P. L.; Molon, R.; Dellacassa, E.; Moyna, P., 2009. **Chemical Composition of the Essential Oils from Leaves and Fruits of *Schinus molle* L. and *Schinus terebinthifolius* Raddi from Southern Brazil.** *Journal of Essential Oil Bearing Plants*, (12):*1*, p. *16*.

[29] Pawlowski, Â.; Kaltchuk-Santos, E.; Zini, C. A.; Caramão, E. B.; Soares, G. L. G., 2012. **Essential oils of Schinus terebinthifolius and S. molle (Anacardiaceae): Mitodepressive and aneugenic inducers in onion and lettuce root meristems.** *S. Afr. J. Bot.*, 80, p. *96*. DOI: http://dx.doi.org/10.1016/j.sajb.2012.03.003

[30] Chaves, D. S. de A.; Cavalcanti, A. dos S.; Alves, M. de S.; da Silva, L. C. P.; Patrocínio, D. dos S.; Sanches, M. N.; de Souza, M. A. A., 2015. **Volatiles composition**

and extraction kinetics from *Schinus terebinthifolius* and *Schinus molle* leaves and fruit. *Rev. Bras. Farmacogn.*, 25:(*4*), p. *356*. DOI: http://dx.doi.org/10.1016/j.bjp.2015.07.003

[31] Barbosa, L. C. A.; Montanari, R. M.; Demuner, A. J.; Silva, C. J.; Andrade, N. J.; Ismail, F. M. D.; Barbosa, M. C. A., 2012. **Exposure to Anacardiaceae volatile oils and their constituents induces lipid peroxidation within food-borne bacteria cells.** *Molecules*, 17, p. *9728*. DOI: https://doi.org/10.3390/molecules17089728

[32] Simionatto, E.; Chagas, M. O.; Peres, M. T. L. P.; Hess. S. C.; da Silva, C. B.; Ré-Poppi, N.; Gebara, S. S.; Corsino, J.; Morel. A. F.; Stuker, C. Z.; Matos, M. de F. C.; de Carvalho, J. E., 2011. **Chemical Composition and Biological Activities of Leaves Essential Oil from *Schinus molle* (Anacardiaceae).** *Journal of Essential Oil Bearing Plants*, (14):*5*, p. *590*. DOI: https://doi.org/10.1080/0972060X.2011.10643976

[33] Afifi, F. U.; Aboalhaija, N. H.; Awwad, O.; Khalil, E; Abbassi, R.; Abaza, I. F., 2019. **Chemodiversity and Antiproliferative Activity of the Essential Oil of *Schinus molle* Growing in Jordan.** *Chem. Biodiversity*, 16:(*11*), e1900388. DOI: http://dx.doi.org/10.1002/cbdv.201900388

[34] Budel, J. M.; Machado, C. D.; Raman, V.; Rehman, J. U.; Maia, B. H. L. N. S.; Meneghetti, E. K.; Almeida, V. P.; Silva, R. Z.; Farago, P. V.; Khan, I. A., 2019. ***Schinus molle*: anatomy of leaves and stems, chemical composition and insecticidal activities of volatile oil against bed bug (*Cimex lectularius*).** *Rev. Bras. Farmacogn.*, 29:(*1*); p. *1*. DOI: http://dx.doi.org/0.1016/j.bjp.2018.10.005

[35] Rodilla, J. M.; Rocha, P. M. de M.; Diéz, D.; Elder, H.; Guala, M. S.; Silva. L. A.; Pombo, E. B., 2012. **Synergistic Antibacterial Activity of the Essential Oil of Aguaribay (*Schinus molle* L.).** *Molecules*, 17, p. *12023*. DOI: https://doi.org/10.3390/molecules171012023

[36] Machado, M. M.; de Campos, M. M. A.; Duarte, J. A.; Zambrano, L. A. de B.; Quintana, L. D.; Rocha, M. B.; Schmitt, E. G.; Boligon, A. A.; de Oliveira, L. F. S., 2018. **Immunotoxicological Evaluation of *Schinus molle* L. (Anacardiaceae) Essential Oil in Lymphocytes and Macrophages.** *J. Evidence-Based Complementary Altern. Med.*, 1, p. *1*. DOI: https://dx.doi.10.1155/2018/6541583

[37] Descamps, L. R.; Chopa, C. S.; Ferrero, A. A., 2011. **Activity of *Schinus areira* (Anacardiaceae) Essential Oils against the Grain Storage Pest *Tribolium castaneum*.** *Nat. Prod. Commun.*, 6(*1*), p. *887*. DOI: http://dx.doi.org/10.1177/1934578X1100600632

[38] Murray, A. P.; Gurovic, M. S. V.; Rodríguez, S. A.; Murray, M. G.; Ferrero, A. A., 2009. **Acetylcholinesterase Inhibition and Antioxidant Activity of Essential Oils from *Schinus areira* L. and *Schinus longifolia* (Lindl.) Speg.** *Nat. Prod. Commun.*, (4):*6*, p. *873*. DOI: http://dx.doi.org/10.1177/1934578X0900400628

[39] Murray, A. P.; Rodríguez, S. A.; Murray, M. G., 2008. **Antioxidant Activity and Chemical Composition of Essential Oils from *Schinus fasciculata* (Griseb.) I.M.**

Johnst and *S. praecox* (Griseb.) Speg. *Nat. Prod. Commun.*, (3):*9*, p. *1551*. DOI: http://dx.doi.org/10.1177/1934578X0800300931

[40] da Silva. E. R.; Lazarotto, D. C.; Pawlowski, Â.; Soares, G. L., 2019. **Antioxidant activity of essential oils from leaves of *Schinus lentiscifolius* Marchand.** G. *Rev. Cubana Plant. Med.*, 24:(*2*), p. *783*.

[41] Hernandes, C.; Taleb-Contini, S. H.; Bartolomeu, A. C. D.; Bertoni, B. W.; França, S. C.; Pereira, A. M. S., 2014. **Chemical Composition and Antifungal Activity of the Essential Oils of *Schinus weinmannifolius* Collected in the Spring and Winter.** *Nat. Prod. Commun.*, (9):*9*, p. *1383*. DOI: http://dx.doi.org/10.1177/1934578X1400900940

[42] Cardoso, C. A. L; Jeller, A. H.; Ré-Poppi, N.; Coelho, R. M.; Yasunaka, D. S.; Schleder, El. J. D., 2010. **Identification of the Volatile Compounds of Fruit Oil of *Anacardium humile* (Anacardiaceae).** *J. Essent. Oil Res.*, 22, p. *469*. DOI: https://doi.org/10.1080/10412905.2010.9700374

[43] Carvalho, C. E. S.; Sobrinho-Júnior, E. P. C.; Brito, L. M.; Nicolau, L. A. D.; Carvalho, T. P.; Moura, A. K. S.; Rodrigues, K. A. F.; Carneiro, S. M. P.; Arcanjo, D. D. R.; Citó, A. M. G. L.; Carvalho, F. A. A., 2017. **Anti-Leishmania activity of essential oil of *Myracrodruon urundeuva* (Engl.) Fr. All.: Composition, cytotoxity and possible mechanisms of action.** *Exp. Parasitol.*, 175, p. *59*. DOI: https://doi.org/10.1016/j.exppara.2017.02.012

[44] Tintino, S. R.; Figueredo, F. G.; Lucena, B. F. F.; Matias, E. F. F.; Leite, N. F.; Andrade, J. C.; Nogueira, L. F. B.; Morais, E. C.; Costa, J. G. M.; Coutinho, H. D. M.; Rodrigues, F. F. G., 2014. **Chemical composition and evaluation of modulatory of the antibiotic activity from extract and essential oil of *Myracrodruon urundeuva*.** *Pharm. Biol.*, 52(*5*), p. *560*. DOI: https://doi.org/10.3109/13880209.2013.853810

[45] Zoghbi, M. G. B.; Pereira, R. A.; de Lima, G. S. L.; Bastos, M. de N. do C., 2014. **Variation of essential oil composition of *Tapirira guianensis* Aubl. (Anacardiaceae) from two sandbank forests, North of Brazil.** *Quim. Nova*, (37):*7*, p. *1188*. DOI: http://dx.doi.org/10.5935/0100-4042.20140197

[46] Said, A.; Omer, E. A.; El Gendy, M. A. M.; Fawzy, G.; Abd El-Kader, A. E.; Fouad, R., 2018. **Volatile constituents and cytotoxic activity of the fruits of *Pleyoginium timorense* (Dc.) Leenh.** *J. Mater. Environ. Sci.*, 9(*8*), p. *2274*.

[47] Babouongolo, S. G.; Nkounkou Loumpangou, C.; Dao, E.; Simon, V.; Elouma Ndinga, A. M.; Ouamba, J. M., 2021. **Variability in Aromatic Composition of Different Fruit Parts of *Pseudospondias microcarpa* (A. Rich.) Engl. from Congo.** *J. Essent. Oil-Bear. Plants*, 24(*3*), p. *421*. DOI: https://doi.org/10.1080/0972060X.2021.1936203

[48] Viljoen, A. M.; Kamatou, G. P. P.; Baser, K. H. C., 2008. **Head-space volatiles of marula (*Sclerocarya birrea* subsp. *caffra*).** *S. Afr. J. Bot.*, 74(*2*), p. *325*. DOI: http://dx.doi.org/10.1016/j.sajb.2007.10.005

[49] Kpoviéssi, D. S. S.; Gbaguidi, F. A.; Kossouoh, C.; Agbani, P.; Yayi-Ladekan, E.; Sinsin, B.; Moudachirou, M.; Accrombessi, G. C.; Quetin-Leclercq, J., 2011. **Chemical composition and seasonal variation of essential oil of *Sclerocarya birrea* (A. Rich.) Hochst subsp. birrea leaves from Benin.** *J. Med. Plants Res*., 5(*18*), p. *4640*.

[50] Nguyen, H. X.; Le, T. C.; Van Do, T, N.; Le, T. H.; Nguyen, N. T.; Nguyen, M. T. T., 2016. **α-Glucosidase inhibitors from the bark of *Mangifera mekongensis*.** *Chem. Cent. J*., (10):*45*, p. *1*. DOI: https://doi.org/10.1186/s13065-016-0193-9

[51] Sukari, M. A.; Ahmad, S.; Ismail, N.; Ismail, I. S.; Abdul, A. B.; Abu Bakar, M. F.; Kifli, N.; Ee, G. C. L., 2015. **Phytochemicals from *Mangifera pajang* Kosterm and their biological activities.** BMC *Complementary and Alternative Medicine*, 21(*1*), p. *105*. DOI: http://dx.doi.org/10.1186/s12906-015-0594-7

[52] Silva-Júnior, E. F.; Aquino, P. G. V.; Santos-Júnior, P. F. S.; Nascimento, I. J. S.; Gomes, E. A.; Silva, A. L. L.; Verissimo, R. C. S. S.; Aquino, T. M.; Araújo-Júnior, J., 2015. X. **Phytochemical compounds and pharmacological properties from *Schinus molle* Linnaeus and *Schinus terebinthifolius* Raddi (Anacardiaceae).** *J. Chem. Pharm. Res.*, 7(*12*), p. *389*.

[53] David, J. M.; Moreira, B. O.; Vilar, V. L. S.; de Almeida, R. N. S.; Morbeck, L. L. B.; Andrade, B. S.; Barros, R. G. M.; Neves, B. M.; de Carvalho, A. L.; Cruz, M. P.; Yatsuda, R., 2022. **New dimer and trimer of chalcone derivatives from anti-inflammatory and antinociceptive extracts of *Schinopsis brasiliensis* roots.** *J. Ethnopharmacol.*, 289, p. *1-12*. DOI: https://doi.org/10.1016/j.jep.2022.115089

[54] Cheng, Zhong-Quan; Yang, Dan; Ma, Qing-Yun; Dai, Hao-Fu; Huang, Sheng-Zhuo; Yi, Xiang-Hui; Zhou, Jun; Zhao, You-Xing., 2012. **Three new sesquiterpenes with cytotoxic activity from *Dobinea delavayi***. *Planta Med.*, (78)*17*, p. *1878*. DOI: http://dx.doi.org/10.1055/s-0032-1315369

[55] Fouokeng, Y.; Akak, C. M.; Tala, M. F.; Azebaze, A. G. B.; Dittrich, B.; Vardamides, J. C.; Laatsch, H., 2017. **The structure of antrocarine E, an ergostane isolated from *Antrocaryon klaineanum* Pierre (Anacardiaceae).** *Fitoterapia*, 117, p. *61*. DOI: https://doi.org/10.1016/j.fitote.2017.01.002

[56] Ahmad, S.; Ali, M.; Ansari, S. H.; Ahmed, F., 2010. **Phytoconstituents from the galls of *Pistacia integerrima* Stewart**. *J. Saudi Chem. Soc.*, 14(*4*), p. *409*. DOI: http://dx.doi.org/10.1016/j.jscs.2010.05.003

[57] Achika, J. I., 2018. **A Review of the Phytochemistry and Pharmacology of *Lannea* species.** *Trop. J. Nat. Prod. Res.*, 2(*10*), p. *442*. DOI: http://dx.doi.org/10.26538/tjnpr/v2i10.1

[58] Semalty, M.; Semalty, A.; Badola, A.; Joshi, G. P.; Rawat, M. S. M., 2010. ***Semecarpus anacardium* Linn.: A review.** *Pharmacogn. Rev.*, 4(*7*), p. *88*. DOI: https://doi.org/10.4103/0973-7847.65328

[59] Formagio, A. S. N.; da Silva, M. M.; Iriguchi, E. K. K.; Kassuya, C. A. L.; Vieira, M. do C.; Foglio, M. A.; de Carvalho, J. E.; Ruiz, A. L. T. G.; Souza, K. de P., 2017. ***Schinus terebinthifolius*: phenolic constituents and in vitro antioxidant, antiproliferative and in vivo anti-inflammatory activities.** *Rev. Bras. Farmacogn.*, 27, p. *445*. DOI: https://doi.org/10.1016/j.bjp.2016.12.007

[60] Nawwar, M.; Hussein, S.; Ayoub, N.; Hashim, A.; El-Sharawy, R.; Lindequist, U.; Harms, M.; Wende, K., 2011. **Constitutive phenolics of *Harpephyllum caffrum* (Anacardiaceae) and their biological effects on human keratinocytes**. *Fitoterapia*, 82(*8*), p. *1265*. DOI: https://doi.org/10.1016/j.fitote.2011.08.014

[61] Antal, D. S.; Schwaiger, S.; Ellmerer-Mueller, E. P.; Stuppner, H., 2010. ***Cotinus coggygria* wood: novel flavanone dimer and development of an HPLC/UV/MS method for the simultaneous determination of fourteen phenolic constituents.** *Planta Med.*, 76(*15*), p. *1765*. DOI: http://dx.doi.org/10.1055/s-0030-1249878

[62] Topcu, G.; Ay, M.; Bilici, A.; Sarikuerkcue, C.; Oezturk, M.; Ulubelen, A., 2007. **A new flavone from antioxidant extracts of *Pistacia terebinthus***. *Food Chem.*, 103(*3*), p. *816*. DOI: https://doi.org/10.1016/j.foodchem.2006.09.028

[63] Yousfi, M.; Djeridane, A.; Bombarda, I.; Chahrazed-Hamia; Duhem, B.; Gaydou, E. M., 2009. **Isolation and characterization of a new hispolone derivative from antioxidant extracts of *Pistacia atlantica***. *Phytother. Res.*, 23(*9*), p. *1237*. DOI: https://doi.org/10.1002/ptr.2543

[64] Hu, Jin-Feng; Garo, E.; Hough, G. W.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R., 2007. **Acuminatanol, the first 2',2''-bis-dihydrobiflavonol from the aqueous extract of *Trichoscypha acuminata***. *Tetrahedron Lett.*, 48(*33*), p. *5747*. DOI: https://doi.org/10.1016/j.tetlet.2007.06.094

[65] Ndongo, J. T.; Mbing, J. N.; Bikobo, D. N.; Atchade, A. de T.; Shaaban, M.; Pegnyemb, D. E.; Laatsch, H., 2013. **A New *C*-Glucosylflavone from *Sorindea juglandifolia*.** *Z. Naturforsch., C: J. Biosci.*, 68(*5/6*), p. *169*.

[66] Christelle, T. D.; Hussain, H.; Dongo, E.; Hermine, J. M. B.; Ahmed, I.; Krohn, K., 2011. **Two New Alkylanacardic Acids, Ozorcardic A and B, from *Ozoroa pulcherrima*.** *Nat. Prod. Commun.*, (6):*8*, p. *1133*.

[67] Catalano, A.; Lantaño, B.; Fabián, L.; López, P., 2020. **New 5-Alkenyl Resorcinols from *Lithraea molleoides* (Vell). Eng.** *Am. J. Plant Sci.*, 11, p. *861*. DOI: http://dx.doi.org/10.4236/ajps.2020.116062

[68] Tokoudagba, J. M.; Gandonou, C. D.; Hougbeme, A.; Gom, S. N.; Auger, C.; Schini-Kerth, V.; Lobstein, A., 2018. **Phytochemistry and activity vasodilator of sheets of *Spondias mombin* L. (Anacardiaceae).** *World J. Pharm. Pharm. Sci.*, 7(*10*), p. *309*.

[69] da Silva, E. P.; David, J. M.; David, J. P.; Garcia, G. H. T.; Silva, M. T., 2020. **Chemical composition of biological active extracts of *Tapirira guianensis***

(Anacardiaceae). *Quim. Nova*, (43)*9*, p. *1216*. DOI: http://dx.doi.org/10.21577/0100-4042.20170605

[70] Dang, P. H.; Nguyen, L. T. T.; Nguyen, H. T. T.; Le, T. H.; Do, T. N. V.; Nguyen, H. X.; Le, N. D.; Nguyen, M. T. T.; Nguyen, N. T., **2019**. **A new dimeric alkylresorcinol from the stem barks of *Swintonia floribunda* (Anacardiaceae).** *Nat. Prod. Res.*, 33, p. *2883*. DOI: https://doi.org/10.1080/14786419.2018.1509329

[71] Razakarivony, A. A.; Lenta, B. N.; Andriamihaja, B.; Michalek, C.; Razanamahefa, B.; Razafimahefa, D. R.; Rakotondramanga, M. F.; Randrianasolo, R.; Lannang, A. M.; Randriamiaramisaina, R.; Boyom, F. F.; Rosenthal, P. J.; Sewald, N., 2016. **Long-chain alkyl-substituted gentisic acid and benzoquinone derivatives from the root of *Micronychia tisramiramy* (Anacardiaceae).** *J. Chem. Sci.*, 71(*4*), p. *297*. DOI: http://dx.doi.org/10.1515/znb-2015-0188

[72] Garg, A. N.; Singh, R.; Singh, S. K.; Maharia, R. S., 2015. **Identification of new phytoconstituents and antimicrobial activity in stem bark of *Mangifera indica* (L.).** *J. Pharm. Biomed. Anal.*, 105, p. *150*. DOI: https://doi.org/10.1016/j.jpba.2014.12.010

[73] Kinghorn, A. K.; Westenburg, H. E.; Lee, K. J.; Lee, S. K.; Fong, H. H. S.; van Breemen, R. B.; Pezzuto, J. M., 2000. **Activity-guided isolation of antioxidative constituents of *Cotinus coggygria***. *J. Nat. Prod.*, 63, p. *1696*. DOI: https://doi.org/10.1021/np000292h

[74] Nguyen, M. T. T.; Nguyen, N. T.; Dang, P. H.; Nguyen, H. X.; Le, T. H.; Van Do, T. N., 2022. **A new lignan from the stems of *Buchanania lucida* Blume (Anacardiaceae)**. *Nat. Prod. Res.*, 36(*14*), p. *3737*. DOI: https://doi.org/10.1080/14786419.2020.1871341

[75] Shen, Y.; Chen, H.; Lang, Li-Juan; Dong, X.; Xiao, Chao-Jiang; Jiang, B., 2021. **Two new benzophenones from *Dobinea delavayi***. *Phytochem. Lett.*, 46, p. *172*. DOI: https://doi.org/10.1016/j.phytol.2021.10.017

[76] Cheng, Zhong-Quan; Yang, D.; Ma, Qing-Yun; Yi, Xin-Hui; Zhou, J.; Zhao, You-Xing., 2013. **A new benzophenone from *Dobinea delavayi***. *Chem. Nat. Compd.*, 49, p. *46*. DOI: http://dx.doi.org/10.1007/s10600-013-0502-7

[77] Rodriguez-Lopez, V.; Aguirre-Crespo, F.; Salazar, L.; Estrada-Soto, S., 2006. **Identification of fatty acid esters and hydrocarbon derivatives from *Cyrtocarpa procera* Kunth by GC–MS.** *Nat. Prod. Res.*, 20(*1*), p. *1*. DOI: https://doi.org/10.1080/14786410500045358

[78] Gu, Q.; Wang, R-R.; Zhang, X-M.; Wang, Y.-H.; Zheng, Y-T.; Zhou, J.; Chen, J-J., 2007. **A New Benzofuranone and Anti-HIV Constituents from the Stems of *Rhus chinensis.*** *Planta Med.*, 73(*3*), p. *279*. DOI: https://doi.org/10.1055/s-2007-967113

[79] Dang, P. H.; Nguyen, T. T.; Le, T. H.; Nguyen, H. X.; Nguyen, M. T. T.; Nguyen, N. T., 2018. **A new bischromanone from the stems of *Semecarpus caudata.*** *Nat. Prod. Res.*, 32(*15*), p. *1745*. DOI: https://doi.org/10.1080/14786419.2017.1399391

[80] Ragasa, C. Y.; Vivar, J. L. A.; De Los Reyes, M. M.; van Altena, I. A., 2016. **Secondary Metabolites from Dracontomelon dao (Merr. & Rolfe).** *Pharma Chem.*, 8(*19*), p. *257*.

[81] Mori, T.; Chang, C.; Maurtua, D.; Hammond, G. B., 2006. **Isolation of the active compound in *Mauria heterophylla*, a peruvian plant with antibacterial activity.** *Phytother. Res.*, 20(*2*), p. *160*. DOI: https://doi.org/10.1002/ptr.1825

[82] Benson, B. B.; Brice, B. J.; Fernique, K. K.; Mida, K. G. R.; Christian, K. K.; Nathalie, G. K; Akhanovna, M. B. J.; Alain, B. Y., 2018. ***Lannea barteri* Engl. (Anacardiaceae) plant used in the treatment of urinary tract infections in Ivory Coast: biological and chemical studies of the aqueous extract.** *Int. J. Pharm. Pharm. Sci.*, 10:*12*, p. *64*.

[83] Pare, D.; Guenne, S.; Nikiema, M.; Hilou, A.; N'do, J. Y. P., 2019. **Phytochemical Study and Biological Activities of Two Medicinal Plants used in Burkina Faso: Lannea velutina A. Rich (Anacardiaceae) and Ximenia americana L. (Olacaceae).** *Asian J. Chem. Sci.*, 6(*3*), p. *1*. DOI: http://dx.doi.org/10.9734/ajocs/2019/v6i318997

[84] Mirghani, M. E. S.; Al-Shwyeh, H. A.; Jamal, P., 2011. **Antibacterial activity of Malaysian mango kernel.** *Afr. J. Biotechnol.*, 10(*81*), p. *18739*. DOI: https://doi.org/10.5897/AJB11.2746

[85] Pithayanukul, P.; Bavovada, R.; Saparpakorn, P.; Leanpolchareanchai, J., 2009. **Molecular Docking Studies and Anti-enzymatic Activities of Thai Mango Seed Kernel Extract Against Snake Venoms.** *Molecules*, 14, p. *1404*. DOI: https://doi.org/10.3390/molecules14093198

[86] Kumar, M.; Saurabh, V.; Tomar, M.; Hasan, M.; Changan, S.; Sasi, M.; Maheshwari, C.; Prajapati, U.; Singh, S.; Prajapat, R. K.; Dhumal, S.; Punia, S.; Amarowicz, R.; Mekhemar, M., 2021. **Mango (Mangifera indica L.) Leaves: Nutritional Composition, Phytochemical Profile and Health-Promoting Bioactivities.** *Antioxidants*, 10(*2*), p. *299*. DOI: https://doi.org/10.3390/antiox10020299

[87] Jahnavi, C. H.; Jyothsna, K.; Geetika, D. L.; Keerthi, G. S.; Santhosha, D.; Ramesh, A., 2020. **Review on pharmacological activities of *Mangifera indica* and *Zingiber officinale***. *J. Pharmacogn. Phytochem.*, 9(*3*), p. *1166*.

[88] Bakka, C.; Hadjadj, M.; Smara, O.; Dendougui, H.; Mahdjar, S., 2019. ***In vitro* antioxidant activities and total phenolic content of extracts from *Pistacia atlantica* desf.** *J. Pharm. Sci. & Res.*, 11(*11*), p. *3634*.

[89] Zia, M.; Bibi, Y.; Nisa, S.; Waheed, A.; Ahmed, S.; Chaudhary, M. F., 2012. **The Study of Anticancer and Antifungal Activities of *Pistacia integerrima* Extract *in vitro*.** *Indian J. Pharm. Sci.*, 74, p. *375*. DOI: https://doi.org/10.4103/0250-474x.107085

[90] Tayarani-Najaran, Z.; Taleghani, A.; Eghbali-Feriz, S.; Shokouhnam, P.; Emami, S. A.; Farhadi, F.; Asili, J.; Hasanzadeh, S., 2021. **Anti-Melanogenic Activities of Different Extracts from *Pistacia atlantica* subsp. *kurdica*.** *Jundishapur Jour. Nat. Pharm. Prod.*, 16(*2*), p. *1*. DOI: http://dx.doi.org/10.5812/jjnpp.69844

[91] Tilkat, E. H.; Kuru, I. S.; Süzerer, V.; Haşimi, N., 2020. **Antioxidant and antimicrobial responses associated with *in vitro* salt stress of *in vitro* and *in vivo* grown *Pistacia khinjuk* stocks.** *Not. Bot. Horti Agrobot. Cluj-Napoca*, 48(*4*), p. *1885*. DOI: http://dx.doi.org/10.15835/48412016

[92] Kumar, G.; Badoni, P. P., 2017. **Antimicrobial Activity of Rhus parviflora Roxb.: Leaves Extract Mediated Synthesized ZnO Nanoparticles.** *Int. J. ChemTech Res.*, 10(*7*), p. *377*.

[93] Mutuku, A.; Mwamburi, L.; Keter, L.; Ondicho, J.; Korir, R.; Kuria, J.; Chemweno, T.; Mwitari, P., 2020. **Evaluation of the antimicrobial activity and safety of *Rhus vulgaris* (Anacardiaceae) extracts.** *BMC Complementary Med. Ther.*, 20:(*272*), p. *1*. DOI: https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-020-03063-7

[94] Rayne, S.; Mazza, G., 2007. **Biological activities of extracts from Sumac (*Rhus* spp.): a review.** *Plant Foods Hum. Nutr.* (*Nature Precedings*), 62(*4*), p. *165*. DOI: https://doi.org/10.1007/s11130-007-0058-4

[95] Al-Naser, Z.; Ibrahim, B., 2014. **Analysis of fruits Schinus molle extractions and the efficacy in inhibition of growth the fungi in laboratory.** *Int. J. ChemTech Res.*, 6(*5*), p. *2799*.

[96] Morel, A. F.; Gehrke, I. T. S.; Neto, A. T.; Pedroso, M.; Mostardeiro, C. P.; da Cruz, I. B. M.; Silva, U. F.; Ilha, V.; Dalcol, I. I., 2013. **Antimicrobial activity of *Schinus lentiscifolius* (Anacardiaceae).** *J. Ethnopharmacol.*, 148, p. *486*. DOI: https://doi.org/10.1016/j.jep.2013.04.043

[97] Oliveira, D. B.; Bernardes, N. R.; Heggdorne-Araújo, M.; Borges, I. F. J. C.; Almeida, F. M.; Amaral, E. P.; Lasunskaia, E. B.; Muzitano, M. F., 2014. **Nitric oxide production, inhibitory, antioxidant and antimycobacterial activities of the fruits extract and flavonoid content of *Schinus terebinthifolius.*** *Rev. Bras. Farmacogn.*, 24, p. *644*. DOI: http://dx.doi.org/10.1016/j.bjp.2014.10.012

[98] Boni, A. N. R.; Ahua, K. M.; Kouassi, K.; Djaman, A. J.; Nguessan, J. D., 2014. **Comparison of *In-Vitro* Antioxidant Activities and Total Phenolic Contents in Water and Methanol Extracts of Stems Bark of *Spondias mombin*.** *Res. J. Pharm., Biol. Chem. Sci.*, 5(*3*), p. *1457*.

[99] da Costa Cordeiro, B. M. P.; Paiva, P. M. G.; Santos, N. D. de L.; Ferreira, M. R. A.; de Araújo, L. C. C.; Júnior, A. R. C.; Santos, A. D. da C.; de Oliveira, A. P.; da Silva, A. G.; Falcão, E. P. da S.; Correia, M. T. dos S.; Almeida, J. R. G. da S.; da Silva, L. C. N.; Soares, L. A. L.; Napoleão, T. H.; da Silva, M. V., 2018. **Hexane extract from *Spondias tuberosa* (Anacardiaceae) leaves has antioxidante activity and is an anti-*Candida* agent by causing mitochondrial and lysosomal damages.** *BMC Complementary Altern. Med.*, 18:*284*, p. *1*. DOI: https://doi.org/10.1186/s12906-018-2350-2

[100] Chaudhuri, D.; Mandal, N.; Ghate, N. B.; Panja, S.; Basu, T.; Shendge, A. K., 2016. **Glycoside rich fraction from *Spondias pinnata* bark ameliorate iron overload induced oxidative stress and hepatic damage in Swiss albino mice.** *BMC*

Complementary Altern. Med., 16:*262*, p. *1*. DOI: https://doi.org/10.1186/s12906-016-1244-4

[101] Madikizela, B.; Aderogba, M. A.; Van Staden, J., 2013. **Isolation and characterization of antimicrobial constituents of *Searsia chirindensis* L. (Anacardiaceae) leaf extracts.** *J. Ethnopharmacol.*, 150(*2*), p. *609*. DOI: https://doi.org/10.1016/j.jep.2013.09.016

[102] Vambe, M.; Naidoo, D.; Aremu, A. O.; Finnie, J. F.; Van Staden, J., 2021. **Bioassay-guided purification, GC-MS characterization and quantification of phyto-components in an antibacterial extract of *Searsia lancea* leaves.** *Nat. Prod. Res.*, 35(*22*), p. *4658*. DOI: http://dx.doi.org/10.1080/14786419.2019.1700251

[103] Yang, T. H.; Yan, De-Xiu; Huang, X. Y.; Hou, B.; Ma, Yun-Bao; Peng, H.; Zhang, X. M.; Chen, Ji-Jun; Geng, C. A., 2019. **Termipaniculatones A-F, chalcone-flavonone heterodimers from *Terminthia paniculata*, and their protective effects on hyperuricemia and acute gouty arthritis.** *Phytochemistry* (Elsevier), 164, p. *228*. DOI: https://doi.org/10.1016/j.phytochem.2019.05.019

[104] Silva, F. G.; Curado, F. M. L. M. J.; Gazolla, A. P.; Pedroso, R. C. N.; Pimenta, L. P.; de Oliveira, P. F.; Tavares, D. C.; Andrade e Silva, M. L.; Cunha, W. R.; Pietro, R. C. L. R.; Januário, A. H.; Pauletti, P. M.; Sales, J. de F., 2016. **Antifungal and cytotoxicity activities of Anacardium othonianum extract.** *J. Med. Plants Res.*, 10(*29*), p. *450*. DOI: http://dx.doi.org/10.5897/JMPR2016.6115

[105] Suleiman, M. M.; Elgorashi, E. E.; Samuel, B. B.; Naidoo, V.; Eloff, J. N., 2013. **Biological Activities of Compounds Isolated from Loxostylis alata (Anacardiaceae) Leaf Extract.** *Biochemistry & Pharmacology*, (2):*4*, p. *1*. DOI: http://dx.doi.org/10.4172/2167-0501.1000123

[106] Ahmed, A. S.; McGaw, L. J.; Moodley, N.; Naidoo, V.; Eloff, J. N., 2014. **Cytotoxic, antimicrobial, antioxidant, antilipoxygenase activities and phenolic composition of *Ozoroa* and *Searsia* species (Anacardiaceae) used in South African traditional medicine for treating diarrhea.** *S. Afr. J. Bot.*, 95, p. *9*. DOI: http://dx.doi.org/10.1016/j.sajb.2014.07.013

[107] Canales-Martinez, M.; Martinez-Elizalde, K. S.; Jimenez-Estrada, M.; Flores, C. M.; Hernández, L. B.; Rosas-López, R.; Duran-Diaz, Á.; Nieto-Yañez, O. J.; Barbosa, E.; Rodríguez-Monroy, M. A., 2015. **Evaluation of the medicinal properties of *Cyrtocarpa procera* Kunth fruit extracts.** *BMC Complementary Altern. Med.*, 15:*74*, p. *1*. DOI: http://dx.doi.org/10.1186/s12906-015-0602-y

[108] Araújo, B. S.; Santos, C. C. S.; Araújo, S. S.; Santos, A. L. L. M.; Almeida, E. C. V.; Dias, A. S.; Damascena, N. P.; Santos, D. M.; Santos, M. I. S.; Júnior, K. A. L. R.; Pereira, C. K. B.; Lima, A. C. B.; Shan, A. Y. K. V.; Sant'ana, A. E. G.; Estevam, C. S., 2014. **Evaluation of the toxicity and molluscicidal and larvicidal activities of *Schinopsis brasiliensis* stem bark extract and its fractions.** *Rev. Bras. Farmacogn.*, 24, p. *298*. DOI: http://dx.doi.org/10.1016/j.bjp.2014.07.006

[109] Van Giau, V.; Nguyen, N. H.; Thuc-Huy, D.; Nguyen, T. T.; Ma, P. C.; Ta, Q. T. H., 2020. **Potential Antimicrobial and Anticancer Activities of an Ethanol Extract**

from *Bouea macrophylla*. *Molecules*, 25(*8*), p. *1996*. DOI: https://doi.org/10.3390/molecules25081996

[110] Adesegun, S. A.; Badejo, M. V.; Odebumni, S. O.; Ojobo, P. D.; Coker, H. B., 2019. **Alpha Amylase Inhibitory and Antioxidant Activities of Leaf Extract and Fractions of *Sorindea warneckei* Engl. (Anacardiaceae).** *Trop. J. Nat. Prod. Res.*, 3(*2*), p. *58*. DOI: http://dx.doi.org/10.26538/tjnpr/v3i2.7

[111] Sequeda-Castañeda, L. G.; Celis-Zambrano, C. A.; Torrenegra-Guerrero, R. D., 2021. **Bioassay-guided fractionation in *Anacardium excelsum* (Bert. & Balb. Ex Kunth) Skeels (Anacardiaceae).** *Pharmacology Online*, 1, p. *426*.

[112] Milella, L.; Russo, D.; Miglionico, R.; Carmosino, M.; Bisaccia, F.; Andrade, P. B.; Valentão, P.; Armentano, M. F., 2018. **A Comparative Study on Phytochemical Profiles and Biological Activities of *Sclerocarya birrea* (A. Rich.) Hochst Leaf and Bark Extracts.** *Int. J. Mol. Sci*., 19(*1*), p. *186*. DOI: https://doi.org/10.3390/ijms19010186

[113] Njume, C.; Afolayan, A. J.; Green, E. Ndip, R. N., 2011. **Volatile compounds in the stem bark of *Sclerocarya birrea* (Anacardiaceae) possess antimicrobial activity against drug-resistant strains of *Helicobacter pylori*.** *Int. J. Antimicrob. Agents*, 38(*4*), p. *319*. DOI: http://dx.doi.org/10.1016/j.ijantimicag.2011.05.002

[114] Shai, L. J.; Mogale, M. A.; Lebelo, S. L.; Thovhogi, N., de Freitas, A. N., 2011. **α-Amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* [(A. Rich.) Hochst.] subspecies *caffra* (Sond) Kokwaro (Anacardiaceae) stem-bark extracts.** *Afr. J. Biotechnol.*, 10(*66*), p. *15033*. DOI: http://dx.doi.org/10.5897/AJB11.1408

[115] Uddin, Z. M.; Rana, S. M.; Hossain, S.; Ferdous, S.; Dutta, E.; Dutta, M.; Bin-Emran, T., 2020. ***In vivo* neuroprotective, antinociceptive, anti-inflammatory potential in Swiss albino mice and *in vitro* antioxidant and clot lysis activities of fractionated *Holigarna longifolia* Roxb. bark extract.** *J. Complementary Integr. Med.*, 17(*1*), p. *1-11* (20190102). DOI: https://doi.org/10.1515/jcim-2019-0102

[116] Al Sayed, E.; Martiskainen, O.; Sinkkonen, J.; Pihlaja, K.; Ayoub, N.; Singab, Abd-El Naser; El-Azizi, M., 2010. **Chemical Composition and Bioactivity of *Pleiogynium timorense* (Anacardiaceae).** *Nat. Prod. Commun.*, 5(*4*), p. *545*. DOI: http://dx.doi.org/10.1177/1934578X1000500410

[117] Sobeh, M.; Mahmoud, M. F.; Hasan, R. A.; Abdelfattah, M. A. O.; Sabry, O. M.; Ghareeb, M. A.; El-Shazly, A. M.; Wink, M., 2018. **Tannin-rich extracts from *Lannea stuhlmannii* and *Lannea humilis* (Anacardiaceae) exhibit hepatoprotective activities *in vivo via* enhancement of the anti-apoptotic protein Bcl-2.** *Sci. Rep.* (*Nature*), 8, p. *9343*. DOI: https://www.nature.com/articles/s41598-018-27452-8

[118] Nitiéma, M.; Belemnaba, L.; Ouédraogo, S.; Ouédraogo, N.; Ouédraogo, S.; Gissou, I. P., 2018. **Diuretic activity of aqueous decoction extract and ethyl acetate fraction of *Lannea microcarpa* Engl. And K. Krause (Anacardiaceae) trunk barks in Wistar rats.** *World J. Pharm. Res.*, 7(*19*), p. *39*. DOI: http://dx.doi.org/10.20959/wjpr201819-13750

[119] Shah, K. A.; Patel, M. B.; Chauhan, K. N.; Shah, S. S.; Parmar, P. K.; Patel, N. M., 2010. **Antihyperlipidemic activity of *Mangifera indica* l. leaf extract on rats fed with high cholesterol diet.** *Der Pharmacia Sinica*, 1(*2*), p. *156*. (ISSN: 0976-8688).

[120] Ferdous, R. U.; Jami, S. I.; Begum, M.; Begum, T.; Rahman, M. A.; Haque, T.; Khan, A.; Hossain, B.; Tarek, H.; Alam, N. A., 2015. **Potential In-Vivo Evaluation of Analgesic Investigation of *Mangifera indica* and Antimicrobial Activity of *Areca catechu*.** *American Journal of BioScience* (*The American Journal of Police Science*), 3(*2-1*), p. *19*. DOI: http://dx.doi.org/10.11648/j.ajbio.s.2015030201.14

[121] Shahid, S.; Taj, S., 2019. **Antidiabetic activity of extracts of *Pistachia khinjuk* on alloxan monohydrate induced diabetic mice.** *MATEC Web of Conferences* 272:01006, Lahore, Pakistan.

[122] Youl, E. N. H.; Nassouri, S.; Ilboudo, S.; Ouédraogo, M.; Sombié, C. B.; Ilboudo, S.; Dakuyo, Z. P.; Gissou, I. P., 2020. **Hypoglycemic and antihyperglycemic activities of the aqueous ethanolic extracts of *Gymnema sylvestre* (RETZ) R. Br. Ex SCHULT and *Sclerocarya birrea* (A RICH) HOCHST.** *Afr. J. Pharm. Pharmacol.*, 14(*9*), p. *339*. DOI: https://doi.org/10.5897/AJPP2020.5187

[123] Varela-Rodríguez, L.; Sánchez-Ramírez, B.; Rodríguez-Reyna, I. S.; Ordáz-Ortiz, J. J.; Chávez-Flores, D.; Salas-Muñoz, E.; Osorio-Trujillo, J. C.; Ramos-Martínez, E.; Talamás-Rohana, P., 2019. **Biological and toxicological evaluation of *Rhus trilobata* Nutt. (Anacardiaceae) used traditionally in Mexico against cancer.** *BMC Complementary Altern. Med.*, 19(*1*), p. *153*. DOI: https://doi.org/10.1186/s12906-019-2566-9

[124] Wang, G.; Li, F.; Du, L.; Wang, J. J., 2015. **Effect and mechanism of total flavonoids extracted from *Cotinus coggygria* against glioblastoma cancer *in vitro* and *in vivo*.** *BioMed Res. Int.*, 1, p. *1-9*. DOI: http://dx.doi.org/10.1155/2015/856349

[125] Attanayake, A. P.; Jayatilaka, K. A. P. W.; Pathirana, C.; Mudduwa, L. K. B., 2015. **Potential antioxidant activities of *Spondias pinnata* (family Anacardiaceae) bark extracts in rats with streptozotocin induced diabetes mellitus.** *Int. J. Pharmacogn.*, 2(*4*), p. *166*. (E-ISSN: 2348-3962; P-ISSN: 2394-5583).

[126] Rao, B. G.; Raju, N. J., 2009. **Hepatoprotective Activity of Stem Heart Wood of *Spondias pinnata*.** *Asian J. Chem.*, 21:*9*, p. *7416*.

[127] Panda, B. K.; Patro, V. J.; Mishra, U. S.; Kar, S., 2014. **Comparative Study of Anti-Pyretic Activity between Acetone and Ethanol Stem Bark Extracts of *Spondias pinnata* (Linn.F) Kurz.** *J. Pharm., Chem. Biol. Sci.*, 1(*1*), p. *26*.

[128] Chaturvedi, B.; Chaturvedi, D. S., 2021. **Antialzheimer activity of methanolic extract of *Buchnania lanzan* leaves by using behaviour models on albino Wistar rat.** *Int. J. Pharm. Sci. Res.*, 12(*7*), p. *3744*. DOI: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3744-49

[129] Stanislaus, N. N.; Ibrahim, S. M.; Usman, P. U; Sa'adyia, H. H.; Garba, M. M.; Toyin, A. S.; Moji, B. O. T.; Ndifor, A. R.; Osas, E. G.; Oyetunji, S. A.; Salawu, S. M., 2021. **Antimicrobial and antioxidant activity of catechin-3-o-rhamnoside isolated from the stem bark of *Lannea kerstingii* Engl. and K. Krause (Anacardiaceae).** *Pak. J. Pharm. Sci.*, 34:*2*, p. *629*.

[130] Okoth, D. A; Koorbanally, N. A.; Chenia, H. Y., 2013. **Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae).** *Phytochem. Lett.*, 6, p. *476*. DOI: https://doi.org/10.1016/j.phytol.2013.06.003

[131] Ficoseco, M. E. A.; Sampietro, D. A; Vattuone, M. A.; Audenaert, K.; Catalán, C. A. N., 2014. **Antifungal and antimycotoxigenic metabolites in Anacardiaceae species from northwest Argentina: isolation, identification and potential for control of *Fusarium* species.** *J. Appl. Microbiol.*, 116:(*5*), p. *1262*. DOI: https://doi.org/10.1111/jam.12436

[132] Beretta, G.; Arlandini, E.; Gelmini, F.; Testa, C.; Angioletti, S., 2021. **GC-MS analysis and biological activity of hydroalcholic extracts and essential oils of *Rhus typhina* L. wood (Anacardiaceae) in comparison with leaves and fruits.** *Nat. Prod. Res.*, 35:(*22*), p. *4764*. DOI: https://doi.org/10.1080/14786419.2020.1721486

[133] Maji, H. S.; Biswas, T.; Sen, A.; Roy, R.; Maji, S., 2015. **Isolation of Mangiferin from Flowering Buds of *Mangifera indica* L . and its Evaluation of *in vitro* Antibacterial Activity.** *J. Pharm. Anal.*, 4(*3*), p. *49*.

[134] Zhang, B.; Fang, J.; Chen, Y.; Zhao. J. Li, S.; Zeng, L., 2015. **Mangiferin activates the Nrf2-ARE pathway and reduces etoposide-induced DNA damage in human umbilical cord mononuclear blood cells.** *Pharm. Biol.*, 53(*4*), p. *503*. DOI: https://10.3109/13880209.2014.927890

[135] Muñoz-Ramírez, A.; Torrent-Farías, C.; Mascanayo-Collado, C.; Urzúa-Moll, A., 2020. **Study of two isoforms of lipoxygenase by kinetic assays, docking and molecular dynamics of a specialised metabolite isolated from the aerial portion of *Lithrea caustica* (Anacardiaceae) and its synthetic analogs.** *Phytochemistry*, 174, p. *112359*. DOI: https://doi.org/10.1016/j.phytochem.2020.112359

[136] Uddin, G.; Rauf, A.; Al-Othman, A. M.; Collina, S.; Arfan, M.; Ali, G.; Khan, I., 2012. **Pistagremic acid, a glucosidase inhibitor from *Pistacia integerrima*.** *Fitoterapia*, 83(*8*), p. *1648*. DOI: https://doi.org/10.1016/j.fitote.2012.09.017

[137] Eaton, A. L.; Rakotondraibe, L. Harinantenaina; B., Peggy J.; Goetz, M.; Kingston, D. G. I., 2015. **New antiproliferative trihydroxyalkylcyclohexenones from *Pleiogynium timoriense***. *Planta Med.*, 78(*7*), p. *1752*. DOI: http://dx.doi.org/10.1055/s-0032-1320970

[138] Ledoux, A.; St-Gelais, A.; Cieckiewicz, E.; Jansen, O.; Bordignon, A.; Illien, B.; Di Giovanni, N.; Marvilliers, A.; Hoareau, F.; Pendeville, H.; Quetin-Leclercq, J. Frederich, M., 2017. **Antimalarial Activities of Alkyl Cyclohexenone Derivatives**

Isolated from the Leaves of *Poupartia borbonica*. *J. Nat. Prod.*, 80(*6*), p. *1750*. DOI: http://dx.doi.org/10.1021/acs.jnatprod.6b01019

[139] Ledoux, A.; Beriot, D.; Mamede, L.; Desdemoustier, P.; Detroz, F.; Jansen, O.; Frederich, M.; Maquoi, E., 2021. **Cytotoxicity of Poupartone B, an Alkyl Cyclohexenone Derivative from Poupartia borbonica, against Human Cancer Cell Lines.** *Planta Med.*, 87(*12*/*13*), p. *1008*. DOI: https://doi.org/10.1055/a-1532-2384

[140] Roumy, V.; Fabre, N.; Portet, B.; Bourdy, G.; Acebey, L.; Vigor, C.; Valentim, A.; Moulis, C., 2009. **Four anti-protozoal and anti-bacterial compounds from *Tapirira guianensis*.** *Phytochemistry*, 70, p. *305*. http://dx.doi.org/10.1016/j.phytochem.2008.10.003

[141] Mosa, R. A.; Oyedeji, A. O.; Shode, Fr. O.; Singh, M.; Opoku, A. R., 2011. **Triterpenes from the stem bark of Protorhus longifolia exhibit anti-platelet aggregation activity.** *Afr. J. Pharm. Pharmacol.*, 5(*24*), p. *2698*. DOI: https://doi.org/10.5897/AJPP11.534

[142] Nair, P. K. R.; Melnick, S. J.; Wnuk, S. F.; Rapp, M.; Escalon, E.; Ramachandran, C., 2009. **Isolation and characterization of an anticancer catechol compound from *Semecarpus anacardium***. *J. Ethnopharmacol.*, 122(*3*), p. *450*. DOI: https://doi.org/10.1016/j.jep.2009.02.001

[143] Arimboor, R.; Rangan, M.; Aravind, S. G.; Arumughan, C., 2011. **Tetrahydroamentoflavone (THA) from *Semecarpus anacardium* as a potent inhibitor of xanthine oxidase**. *J. Ethnopharmacol.*, 133(*3*), p. *1117*. DOI: https://doi.org/10.1016/j.jep.2010.10.027

[144] Kamkumo, R. G; Ngoutane, A. M; Tchokouaha, L. R. Y; Fokou, P. V. T.; Madiesse, E. A. K; Legac, J.; Kezetas, J. J. B.; Lenta, B. N; Boyom, F. F.; Dimo, T. *et al*., 2012. **Compounds from *Sorindeia juglandifolia* (Anacardiaceae) exhibit potent antiplasmodial activities *in vitro* and *in vivo*.** *Malar. J.*, 11, p. *382*. DOI: http://dx.doi.org/10.1186/1475-2875-11-382

[145] El Sayed, A. M., 2016. **Leaves of *Schinus polygamous* (Cav.) Cabrera (Anacardiaceae) are a potential source of hepatoprotective and antioxidant phytochemicals.** *J. Med. Plants Res.*, 10(*17*), p. *223*. DOI: http://dx.doi.org/10.5897/JMPR2016.6061

[146] Mosa, R. A.; Cele, N. D.; Mabhida, S. E.; Shabalala, S. C.; Penduka, D.; Opoku, A. R., 2015. ***In vivo* Antihyperglycemic Activity of a Lanosteryl Triterpene from *Protorhus longifolia***. *Molecules*, 20(*7*), p. *13374*. DOI: https://doi.org/10.3390/molecules200713374

[147] Poletti, M.; Marion, C., 2010. **Composition cosmétique moussante à base d'acide éllagique ou d'un de ses dérivés et d'huile essentielle.** *L'Oréal Societé Anonyme, Institut National de la Proprieté Industrielle, FR pat. 2,959,666* A1.

[148] Dubief, C.; Nadia, K.; Boche, B., 2010. **Composition coiffante sous forme de mousse.** *L'Oréal Societé Anonyme, Institut National de la Proprieté Industrielle, FR pat. 2,961,693* A1.

[149] Ennamany, R., 2016. **Procédé de préparation d'une composition topique à base de cellules végétales dédifférentiées et élicitées en culture *in vitro*.** *Office Européen des Brevets, EP 3,250,295* B1.

[150] Campmany, J. T., 2013. **Antimicrobial composition and use thereof.** *Oligo Basics Indústria e Comércio de Ração* LTDA/Paraná (BR), *US pat*. 8,337,485 B2.

[151] Commin, A. R., 2011. **Composition Antivirale et Retrovirale Notamment pour le Traitment du SIDA.** *Organisation Mondiale de la Proprieté Intellectuelle, WO pat.2011/01000* A1.

[152] Buter, K. B.; Buchwald-Werner, S., 2020. ***Mangifera indica* as a sirtuin-1 activating agent.** *Vital Solutions Swiss* AG/Basel (CH), *US pat*. *10,596,212* B2.

[153] Pushpangadan, P.; Rawat, A. K. S.; Rao, C. V.; Srivastava, S. K.; Govindarajan, R., 2006. **Novel health promoting functional foods fortified with herbs.** *Council of Scientific and Industrial Reserach, WO pat*. 2006/*061849* A1.

[154] Krishnan, G., 2009. **An herbal formulation for prevention and treatment of diabetes and associated complications.** *Innoveda Biological Solutions (P) Ltd.*, New Delhi-110048 (IN), *EP pat*. *2,326,338* B1.

[155] Quave, C. L.; Lyles, J.; Tang, H.; Porras-Brenes, G., 2021. **Botanical extracts and compounds from *Schinus* plants and methods of use.** *Emery University*, Atlanta (GA), *US pat*. *2021/0315906* A1.

[156] Bogoshi, M. N., 2014. **Composition for use in the treatment of anaemia and low blood pressure.** *Indigenous Therapies Holdings* (PTY) LTD, *PAT*035/*TDP 2014/03582*.

[157] Nandeshwar, W. M., 2017. **Herbal compositions and process to treat infertility in men.** *IN pat*. 21,007,662, A23K1/18.

[158] Elsohly, M.; Gul, W.; Ashfaq, M. K., 2019. **Compositions for prevention /prophylactic treatment of poison ivy dermatitis.** *The University of Mississipi*, *US pat*. 10,322,103 B2.

[159] Rotem, M. O.; Elbaz, M.; Etkes, A. S.; Meihls, L. N.; Ben Naim, N.; Ghosh, D., 2021. **Insecticidal polypeptides and use thereof.** *Evogene LTD.*, Rehovot (IL), *US pat*. *2021/0171976* A1.

[160] Mukherjee, B.; Samanta, A., 2003. **Gum Odina as new pharmaceutical excipients.** *The Gazette of India*/*The Patent Rules*, *61*/KOL/*2006*.

[161] Tomioka, T., 2006. **Coating-type antimicrobial composition, antimicrobial composition, antimicrobial coating film, filter and electric air-quality conditioning equipment.** *Matsushita Electric Industrial* CO., LTD, *US pat*. *2006/0008539* A1.

[162] Fernández Ponce, M. T.; Fernández, E. J. M. de la O.; Cardoso, L. C.; Gay, D. A., 2017. **Procedimiento de concentración de xantonas a alta presión a escala semi-industrial.** *Oficina Española de Patentes y Marcas, ES pat. 2,567,530* B1.

[163] Rieck, H.; Lachaise, H.; Steiger, D.; Labourdette, G., 2011. **Use of succinate dehydrogenase inhibitors for increasing the content of desired ingredients in crops.** *Bayer CropScience* AG/Monheim (DE), *US pat. 2011/0065580* A1.

[164] Bellei, S.; Melis, L.; Peloso, A.; Betti, N., 2021. **Composition useful in the treatment of varroatosis.** *Nurares S.R.L.* [Pula, IT/IT], *WO pat. 2021/009379* Al.

[165] Cyril, N. L.; Raynard, M.; de Chily, P. C., 2006. **Nouveaux antioxydants à base d'espèces d'anacardiacées, plus particulièrement de *Sclerocarya birrea*, leurs procédés d'obtention et leurs applications.** *ALDIVIA Societé Anonyme, FR pat. 2,883,003* A1.

[166] Boucher, C.; Ghislain, H. M., 2006. **Composition cosmétique diététique, pharmaceutique obtenue à partir d'huile non rafinée exempte d'acides gras trans, d'esters phorboliques, extraite sans solvant.** *Institut National de la Proprieté Industrilelle, FR pat.* 2,886,547 A1.

[167] Falco, G., 2021. *Redess S.R.L.* **Preservative composition comprising sumac.** [Termini Imerese, IT/IT], *WO pat. 2021/124167* Al.

[168] Miyanaga, A., 2017. **Structure and function of polyketide biosynthetic enzymes: various strategies for production of structurally diverse polyketides.** *Biosci., Biotechnol., Biochem.*, 81:*12*, p. *2227*. DOI: https://doi.org/10.1080/09168451.2017.1391687

[169] Piel. J.; Reiter, S.; Cahn, J. K. B.; Wiebach, V.; Ueoka, R., 2020. **Characterization of an Orphan Type III Polyketide Synthase Conserved in Uncultivated "Entotheonella" Sponge Symbionts.** *ChemBioChem Full Papers*, 21, p. *564*. DOI: http://dx.doi.org/10.1002/cbic.201900352

[170] Nakano, C.; Ohnishi, Y.; Funa, N.; Horinouchi, S., 2012. **The *O*-Methyltransferase SrsB Catalyzes the Decarboxylative Methylation of Alkylresorcylic Acid during Phenolic Lipid Biosynthesis by *Streptomyces griseus*.** *J. Bacteriol.*, 194(*6*), p. *1544*. DOI: https://doi.org/10.1128/JB.06406-11

CONSIDERAÇÕES FINAIS

O presente trabalho contém contribuições relativas a diferentes áreas da Química (especialmente a Química de Produtos Naturais e a Síntese Orgânica), além da farmacologia, da quimiotaxonomia de plantas e outros campos da ciência e tecnologia. Nesse sentido, destacam-se os resultados concernentes 1) à obtenção do ácido betulínico (AB) em quantidades consideráveis a partir de plantas de famílias distintas (Dilleniaceae e Lamiaceae) juntamente com a validação do método cromatográfico aplicado na separação e determinação simultâneas do AB e seus análogos (ácido oleanólico/AO e ácido ursólico/AU), 2) à versatilidade associada à síntese de diferentes derivados bioativos do AB através de estratégias eficientes e simplificadas (tendo em vista o uso da betulina ou do lupeol como principais precursores, o que pode evitar a necessidade de metodologias de síntese mais complexas e dispendiosas quanto à estereoquímica) e 3) ao estudo minucioso e pioneiro dos aspectos químicos, farmacológicos e dos variados usos de plantas da família Anacardiaceae, cujos desdobramentos possuem significativa relevância para um conhecimento mais preciso desta família, de modo que poderão impulsionar novas pesquisas que contribuam para a descoberta de novos gêneros botânicos, de novas substâncias bioativas e de outros usos comerciais e tecnológicos.

Finalmente, vale salientar que um dos capítulos que constituem este trabalho já foi publicado em revista/periódico internacional (com fator de impacto 4.927) bem como os demais capítulos foram igualmente submetidos e estão em processo de publicação, conforme listado a seguir:

1. “Chemical Strategies towards the Synthesis of Betulinic Acid and Its More Potent Antiprotozoal Analogues” (*Molecules* **2021**, *26*, 1081) – publicado e com 7 citações;
2. “Development of methods employing MAE for extraction and quantification of triterpenic acids from *Davilla rugosa* and *Eriope blanchetii*” (*Phytomedicine* PLUS, **2023**) – submetido e aguardando publicação;

3. “Chemical composition, biological activities and uses of Anacardiaceae species: an updated review” (*Química Nova*, **2023**) – submetido e aguardando publicação;

Desse modo, deve-se ressaltar a importância prática deste trabalho, uma vez que o seu conteúdo estará disponível de modo praticamente integral em inglês e, portanto, suas subsequentes contribuições poderão exercer um impacto mais abrangente na comunidade científica que atua nas áreas a ele relacionadas.