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Avaliação dos Principais Metabólitos Secundários por Espectrometria de Massas e Atividade Hipoglicêmica de *Salacia impressifolia* Miers A. C.Smith

Manuela Merlin Laikowski

Caxias do Sul, Fevereiro de 2015.

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"Dissertação apresentada ao Programa de Pós-Graduação em Biotecnologia da Universidade de Caxias do Sul, visando à obtenção de grau de Mestre em Biotecnologia"

Orientador: Dr. Sidnei Moura e Silva

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MANUELA MERLIN LAIKOWSKI

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Prof. Dr. Ernani Pinto Júnior

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ÍNDICE

RESUMO

A utilização de plantas na cura dos mais diversos males é tradicionalmente conhecida e utilizada há centenas de anos pela humanidade. As células vegetais produzem uma grande variedade de compostos químicos entre o metabolismo primário e secundário. Os produtos do metabolismo secundário são de especial interesse, pois são responsáveis por diversas ações biológicas. Informações etnobotânicas indicam que mais de 800 plantas são usadas como remédios tradicionais para o tratamento da diabetes. Muitas espécies pertencentes ao gênero *Salacia* têm sido descritas com atividade antidiabética, entre elas *Salacia impressifolia.* É uma espécie pertencente à família Celastraceae, conhecida popularmente no Brasil como miraruíra. Este trabalho visou desvendar a composição química desta espécie, utilizando para isso extratos obtidos a partir da casca e do caule. Em seguida, realizou-se análise química dos extratos por espectrometria de massas de alta resolução (EMAR). Nestes foram identificados 32 compostos, os quais haviam sido previamente descritos na literatura para o mesmo gênero. Entre eles destacam-se o kotalanol, salacinol e o mangiferina, com ação hipoglicemiante previamente descrita por outros autores. Para análise *in vivo* do potencial hipoglicemiante, realizou-se ensaio com o extrato hidroalcoólico do caule da planta em ratos com diabetes induzida por estreptozotocina. O extrato apresentou ação semelhante ao fármaco controle glibenclamida. Também foi avaliado o perfil lipídico (triglicerídeos, colesterol total e HDL) e as enzimas hepáticas TGO e TGP, onde pode-se observar aumento de HDL.

ABSTRACT

The use of plants in various ailments healing is traditionally known and used for centuries by mankind. Plant cells produce a large variety of chemical compounds between the primary and secondary metabolism. The secondary metabolism products are of special interest, as they are responsible for several biological activities. Ethnobotanic information indicates that more than 800 plants are used as traditional remedies in the treatment of diabetes. Many species of *Salacia* genus have been described with antidiabetic activity, including *Salacia impressifolia.* This specie belongs to Celastraceae family, popularly known as miraruíra in Brazil. This work aimed to develop the chemical composition of this specie through stem and stem bark extracts. Chemical analysis of the extracts was carried out by high resolution mass spectrometry (HRMS). In these extracts, 32 compounds were identified, previously described for the same genus. Among them, were identified kotalanol, salacinol and mangiferina, with hypoglycemic action previously described by others authors. For in vivo analysis of hypoglycemic activity, it was performed the hydroalcoholic stem extract in streptozotocin induced diabetics rats. The extract presented similar action to control drug glibenclamide. It was also assessed the lipid profile (triglycerides, total cholesterol and HDL) and liver enzymes AST and ALT, and it was observed an increase in HDL level.

1. INTRODUÇÃO

A história da utilização de plantas para a saúde e o bem estar humano é vasta, e tem sido descrita ao longo do tempo. As plantas medicinais vêm sendo utilizadas tradicionalmente para o tratamento de várias enfermidades. Suas aplicações são amplas e abrangem desde o combate ao câncer até microrganismos patogênicos. As plantas, além de seu uso na medicina popular com finalidades terapêuticas, têm contribuído, ao longo dos anos, para a obtenção de vários fármacos até hoje muito utilizados na clínica como a emetina, a vincristina, a colchicina, a rutina, entre outros. A cada momento são relacionadas na literatura novas moléculas, algumas de relevante ação farmacológica proveniente de estudos fitoquímicos.

Até meados do século XX, as plantas medicinais e seus derivados constituíam a base da terapêutica medicamentosa. A síntese química, que teve início no final do século XIX, tinha como objetivo mimetizar moléculas com atividade extraídas de plantas, aumentando vertiginosamente o desenvolvimento de medicamentos e, desta forma, contribuindo para a qualidade de vida da população. Atualmente cerca de 50% dos medicamentos utilizados são de origem sintética e em torno de 25% são de origem vegetal, isolados ou produzidos por semi-síntese. Apesar do grande desenvolvimento da síntese orgânica e dos processos biotecnológicos, cerca de 25 % dos medicamentos prescritos nos países desenvolvidos são originários de plantas, oriundos de nada mais do que 90 espécies (Soejarto, 1996). Durante os últimos 20 anos, os fármacos de origem natural que apareceram no mercado são, quase que na totalidade, oriundos das pesquisas científicas de países como China, Coréia e Japão, sendo que a contribuição dos outros países é bem menor.

Muitas espécies ainda são usadas empiricamente, sem respaldo cientifico quanto à eficácia e segurança. Em todo o mundo, apenas 17% das plantas foram estudadas de alguma maneira quanto ao seu emprego medicinal e, na maioria dos casos, sem grande aprofundamento nos aspectos fitoquímicos e farmacológicos. Esses dados demonstram o grande potencial das plantas para a descoberta de novos fitoterápicos e fitomedicamentos.

Apesar de o Brasil ser o país com a maior diversidade genética vegetal do mundo, com mais de 55.000 espécies catalogadas de um total estimado entre 350.000 e 550.000, apenas 8% das espécies vegetais foram estudadas em busca de compostos bioativos, e apenas 1.100 espécies vegetais foram avaliadas em suas propriedades medicinais (Simões *et al.*, 2003).

Várias empresas nacionais têm empregado matéria-prima vegetal diretamente na elaboração de fitomedicamentos. No Brasil, 20% da população são responsáveis por 63% do consumo dos medicamentos disponíveis, sendo que o restante encontra nos produtos de origem natural, especialmente as plantas medicinais, a única fonte de recursos terapêuticos. Essa alternativa é utilizada tanto dentro de um contexto cultural, na medicina popular, quanto na forma de fitoterápicos.

Atualmente existem várias metodologias para a obtenção de fármacos, dentre elas a abordagem biotecnológica. A pesquisa fitoquímica tem por objetivo conhecer os constituintes químicos de espécies vegetais ou avaliar sua presença. Quando não se dispõe de estudos químicos sobre as espécies de interesse, análise fitoquímica preliminar pode indicar o grupo de metabólitos secundários relevante da mesma. Caso o interesse esteja restrito a uma classe específica de constituintes ou às substâncias responsáveis por certa atividade biológica, a investigação deverá ser direcionada para o isolamento e a elucidação estrutural da mesma.

Desta forma, os objetivos deste estudo foram fornecer dados científicos sobre *Salacia impressifolia* (Miers.) A. C. Smith devido aos escassos estudos fitoquímicos e biológicos desenvolvidos com esta planta, além de verificar o uso a ela atribuído quanto à atividade hipoglicêmica. Para isto, os objetivos específicos tratam da produção de extratos com diferentes solventes (polaridade variada), identificação da composição química através de espectrometria de massas de alta resolução, e do potencial hipoglicemiante através de ensaio *in vivo*. Ainda, uma ampla revisão bibliográfica sobre o gênero *Salacia* foi realizada e será publicada na forma de artigo.

2. REVISÃO BIBLIOGRÁFICA

2.1 Metabólitos secundários das plantas.

Todos os organismos precisam transformar e interconverter um grande número de compostos orgânicos para viver, crescer e se reproduzir. As plantas dependem destas transformações químicas executadas pelo seu metabolismo para garantir sua sobrevivência. O metabolismo nas células vegetais é comumente dividido em primário e secundário (Peres, 2004).

As células vegetais produzem uma grande variedade de compostos que apresentam importância vital às funções celulares, fazendo parte da regulação em atividades essenciais ao vegetal, tais como a fotossíntese, a respiração e o transporte de solutos. Estes são conhecidos como metabólitos primários, possuindo uma distribuição universal nas plantas, como é o caso dos aminoácidos, nucleotídeos, lipídios, carboidratos e clorofila (Briskin, 2000).

Os metabólitos secundários são moléculas cuja função individual não é considerada essencial à sobrevivência das células, mas que contribuem para a adaptação das plantas ao ambiente (Aerts *et al.*, 1991). Em contraste com as vias metabólicas primárias, que sintetizam os compostos comumente encontrados em todos os organismos, o metabolismo secundário diz respeito a compostos que têm uma distribuição mais restrita. Estes compostos são encontrados apenas em organismos específicos, ou grupos de organismos, e são uma expressão da individualidade das espécies (Dewick, 2001).

Já foram desvendadas mais de 200.000 estruturas de metabólitos secundários, e sabe-se que esses compostos têm a função de incrementar o desempenho das plantas frente às condições ambientais a que estão submetidas (Bourgaud *et al.*, 2001; Hartmann, 2007).

De forma simplificada, os metabólitos secundários vegetais podem ser divididos em três grandes grupos: os compostos fenólicos, os terpenóides e os compostos secundários nitrogenados (Figura 1).

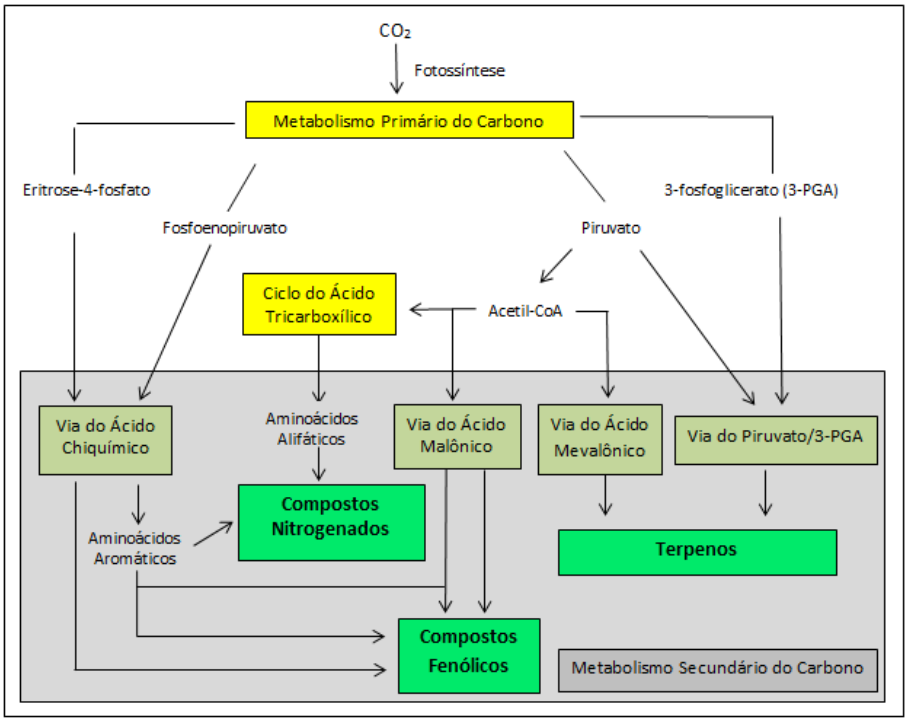


Figura 1. Visão simplificada das principais rotas de biossíntese de metabólitos secundários e suas inter-relações com o metabolismo primário (adaptado de Taiz & Zeiger, 2004).

Dentro dos diferentes grupos de metabólitos secundários em plantas, destacam-se os compostos fenólicos e terpenos, devido à sua ampla distribuição nos vegetais, e por apresentarem atividades importantes em organismos diversos, inclusive em plantas da família Celastraceae. No gênero *Salacia*, geralmente não são encontrados alcalóides de forma significativa. Sendo assim, este tema não será abordado.

2.1.1 Compostos fenólicos

As plantas produzem uma grande variedade de produtos secundários, e entre eles os compostos fenólicos. Estes são formados por um anel aromático com ao menos uma

hidroxila (Taiz & Zeiger, 2004). Eles apresentam uma variedade de funções nos vegetais, e são formados em condições de estresse como infecções, ferimentos, radiação UV, dentre outros. Muitos agem como compostos de defesa contra herbívoros e patógenos, enquanto outros têm função de atrair polinizadores ou dispersores de frutos, e até mesmo reduzir o crescimento de plantas competidoras adjacentes (Taiz & Zeiger, 2004).

Também são encontrados em frutas, verduras, sementes, bem como em chás, derivados de frutas (como o vinho) e outras fontes de alimento, sendo consumidos regularmente como parte da dieta humana (Benavente-García *et al*., 1997). Contribuem no sabor, odor e coloração de diversos vegetais, sendo muito usados como flavorizantes e corantes na indústria alimentícia. As principais classes de compostos fenólicos na dieta são os ácidos fenólicos, flavonoides, lignanas, estilbenos, cumarinas e taninos (Harborne, 1993).

Os compostos fenólicos possuem uma vasta gama de atividades, entre elas a cardioprotetora, antiviral, antitumoral, antibacteriana e antimutagênica (Rice-Evans & Miller, 1996; Benavente-García *et al*., 1997; Sergediene *et al.*, 1999). Além disso, vêm atraindo a atenção dos pesquisadores devido ao seu uso potencial como antioxidantes naturais, representando um papel importante na redução da oxidação lipídica em tecidos (vegetal e animal) (Cuppett, 1998). Quando incorporados à alimentação humana, auxiliam na conservação da qualidade do alimento, e também contribuem reduzindo o risco de desenvolvimento de patologias, como câncer e arteriosclerose (Namiki, 1990; Ramarathnam *et al.*, 1995).

2.1.2 Terpenos

Os terpenos são compostos que ocorrem em todas as plantas e compreendem uma classe de metabólitos secundários com uma grande variedade estrutural (Raven *et al.*, 2001). Os terpenos são formados pela fusão de unidades isoprênicas de cinco carbonos (C_5H_8). Quando submetidos a altas temperaturas, podem se decompor em isoprenos e, desta forma, serem referidos como isoprenóides (Taiz e Zeiger, 2004).

Os terpenos ou terpenóides podem ser classificados de acordo com o número de isoprenos que constituem: hemiterpenóides, monoterpenóides, sesquiterpenóides, diterpenóides, triterpenóides, tetraterpenóides e politerpenóides (Oliveira *et al.*, 2003). Os terpenos são biossintetizados a partir de metabólitos primários por no mínimo duas rotas diferentes, conforme mostra a Figura 01 (Taiz e Zeiger, 2004).

Os triterpenóides (C_{30}) formam os componentes das resinas, látex, ceras e cutícula das plantas. Entre os triterpenos estão uma importante classe de substâncias, os esteróides, os quais são componentes dos lipídios de membrana e precursores de hormônios esteróides em mamíferos, plantas e insetos. Outra classe importante de triterpenos são as saponinas, reconhecidas pela formação de espuma em certos extratos vegetais. Nas plantas, as saponinas desempenham um importante papel na defesa contra insetos e microorganismos (Peres, 2004).

Quase todos os carotenóides são derivados de tetraterpenos (C_{40}) com o esqueleto hidrocarbônico consistindo em oito unidades isoprenóides. De todos os pigmentos naturais, os carotenóides são provavelmente os de maior ocorrência, podendo ser encontrados em animais, plantas e microorganismos. Carotenóides formados somente por átomos de carbono e hidrogênio são chamados de carotenos, enquanto os derivados contendo um heteroátomo como, por exemplo, oxigênio, são chamados de xantofilas (Cardoso, 1997).

Os terpenos apresentam diversas atividades biológicas, como diurética, antibacteriana, colerética, vasodilatadora, analgésica, espasmolítica, anti-inflamatória,

entre outras (Bach, 2010). Triterpenos podem originar heterosídeos cardiotônicos como a digoxina, empregada no tratamento de insuficiência cardíaca congestiva. Em animais, podem originar ácidos biliares e vitamina D (Peres, 2004).

2.2 Metabólitos secundários na Medicina

O homem, ao utilizar-se de plantas para seu sustento, reuniu ao longo da história um conhecimento empírico das ações medicinais e tóxicas das plantas. Desta forma, o conhecimento etnofarmacológico acumulado resultou no desenvolvimento de fármacos importantes na terapêutica atual (Alves, 2001).

O isolamento de compostos ativos a partir de tecidos vegetais surgiu no início do século XIX (Balunas & Kinghorn, 2005). Porém, com a revolução industrial e o desenvolvimento da química orgânica, a indústria de medicamentos passou a priorizar os compostos de origem sintética, por serem de fácil obtenção e purificação. Mesmo assim, atualmente, cerca de um quarto dos medicamentos prescritos mundialmente são de origem vegetal (Rates, 2001; Balunas & Kinghorn, 2005). Além do uso como medicamento, devido às diversas atividades biológicas dos metabólitos secundários, estes também são utilizados em cosméticos, como matéria-prima para a química fina e como nutracêuticos (Fumagali *et al.*, 2008).

Na medicina, produtos secundários de plantas envolvidos na defesa contra patógenos podem ser utilizados como antimicrobianos. Outros, utilizados na defesa contra herbivoria, comumente apresentam atividade sedativa, relaxante muscular ou anestésica e assim por diante. Alguns produtos secundários exercem suas funções pela semelhança com metabólitos endógenos, receptores, hormônios ou neurotransmissores e, por isso, possuem

efeito benéfico nos sistemas fisiológicos humanos como sistema nervoso central e endócrino (Briskin, 2000).

Entre as plantas medicinais utilizadas no tratamento de doenças, pode-se citar o maracujá (*Passiflora edulis* Sims), utilizado como calmante; Malva Santa (*Plectranthus barbatus* Andr.), utilizada para tratamento de acidez estomacal; Guaco (*Mikania glomerata* Spreng), utilizado como expectorante e broncodilatador; Romã (*Punica granatum* L.), usada como antivirótica no tratamento de herpes; Hortelã Rasteira (*Mentha villosa* Huds), usada como amebicida e giardicida, entre outras (Silva, *et al.*,2006).

2.2.1 Plantas medicinais e Diabetes

Os produtos naturais da medicina popular têm sido usados há séculos em todas as culturas por todo o mundo, com o objetivo de tratar doenças e manter a saúde. Há um grande número de plantas medicinais disponíveis na natureza, as quais possuem várias propriedades, dentre elas a hipoglicêmica.

Diabetes mellitus é uma doença metabólica caracterizada pela hiperglicemia crônica com distúrbio do metabolismo de carboidratos, proteínas e gorduras, resultando na secreção insuficiente de insulina, deficiência da ação da insulina ou ambos (OMS, 1999). De acordo com a Organização Mundial da Saúde (2013), 347 milhões de pessoas sofrem com diabetes no mundo, sendo que mais de 80% das mortes por diabetes ocorrem em países de média e baixa renda.

A Organização Mundial da Saúde estima que em 2030, a diabetes será a sétima maior causa de mortes no mundo. Projeta-se que o número de mortes por diabetes vai aumentar 50% nos próximos 10 anos. Estima-se que 90% dos casos de diabetes no mundo

correspondem ao diabetes tipo II, sendo que as doenças cardiovasculares são responsáveis pela morte de 50 a 80% dos pacientes diabéticos. Em países desenvolvidos, a maioria das pessoas que desenvolvem diabetes está em idade de aposentadoria, enquanto nos países em desenvolvimento, os mais afetados estão entre 35 e 64 anos. A diabetes é a principal causa de cegueira, amputação e falência renal (OMS, 2013).

Na medicina moderna ainda não há tratamento efetivamente satisfatório que cure a diabetes. O manejo da doença é feito através da administração de insulina e de agentes hipoglicemiantes. A administração de insulina tem inúmeras desvantagens, como a resistência à insulina, que em tratamentos crônicos pode levar a anorexia nervosa, atrofia cerebral e acúmulo de gordura hepática (Piedrola *et al.*, 2001; Tobias *et al.*, 2001).

Diversos medicamentos hipoglicemiantes orais têm sido utilizados no tratamento da diabetes. Entre eles destacam-se as sulfoniluréias (glibenclamida, glipizide, entre outros) e as glinidas (repaglinida, nateglinida), que estimulam a secreção de insulina pelas células β pancreáticas. As biguanidas como a metformina, por exemplo, agem diminuindo a resistência à insulina. As glitazonas (rosiglitazona e pioglitazona) agem ativando o receptor da insulina no tecido adiposo, muscular e hepático, com aumento da utilização de glicose pelos mesmos. Ainda pode-se contar com a acarbose e o miglitol, fármacos pertencentes à outra classe de hipoglicemiante, e que atuam inibindo as α-glicosidases intestinais, bloqueando assim a entrada de glicose na circulação e controlando a glicemia pós-prandial. Em geral, na prática clínica, associam-se fármacos de diferentes classes e mecanismos de ação no intuito de garantir uma melhor eficácia no controle da glicose sanguínea (Khan *et al.*, 2014).

Informações etnobotânicas indicam que muitas plantas são usadas como remédios tradicionais para o tratamento da diabetes, sendo que a maioria delas não possui estudo científico (Pushparaj *et al.*, 2000). De acordo com Grover e colaboradores (2002), mais de

1123 espécies de plantas têm sido utilizadas para tratar diabetes e mais de 200 compostos puros mostraram atividade hipoglicêmica (Grover *et al.*, 2002). Entre eles, pode-se citar o sesquiterpeno oligoglicosídeo denominado oficinosídeo A2, isolado das flores da *Calendula officinalis* egipcia, o ácido isoferúlico extraído do rizoma da *Cimicifuga dahurica* Maxim, o ácido 4-hidroxibenzóico isolado do extrato aquoso das raízes de *Pandanus odorus* Ridl, o bacuquiol 10 isolado do extrato de *Otholobium pubenscens* L., entre outros (Negri, 2005). A Organização Mundial da Saúde aborda a importância de serem investigados os agentes hipoglicêmicos que se originam nas plantas e que são utilizados pela medicina popular no tratamento da diabettes mellitus (Alarcon-Aguilera *et al.*, 1998).

2.3 Família Celastraceae

A família Celastraceae possui distribuição predominantemente tropical e subtropical, incluindo cerca de 50 gêneros e 1000 espécies (Souza *et al.*, 2008). No Brasil estão distribuídas nas regiões Norte, Nordeste e Sudeste do país (Joly, 1993).

O gênero *Salacia* já foi classificado por alguns autores como pertencente à família Hippocrateaceae que, atualmente, é considerada parafilética e incluída em Celastraceae como duas subfamílias: Hippocrateoideae e Salacioideae (Lombardi, 2010).

A posição taxonômica da família Celastraceae é motivo de debate entre taxonomistas. Há grupos que consideram sua junção com a família Hippocrateaceae, enquanto outros argumentam que deveriam pertencer a uma família distinta (Gunatilaka, 1996). Esse debate é regido pela composição química peculiar dessas famílias. Espécies destas famílias apresentam em comum a ocorrência de sesquiterpenos piridínicos e

triterpenos das classes dos friedelanos e dos quinonametídeos, sendo os últimos de ocorrência restrita às Celastraceae e Hippocrateaceae, e por isso considerados marcadores quimiotaxonômicos dessas famílias. Por outro lado, os diterpenos do tipo 18-(4,3)-abeo-abietanos e sesquiterpenos β-diidroagarofuranos, até o momento só foram isolados de Celastraceae (Gunatilaka, 1996). Na Figura 2, são apresentados alguns representantes dessas classes de substâncias.

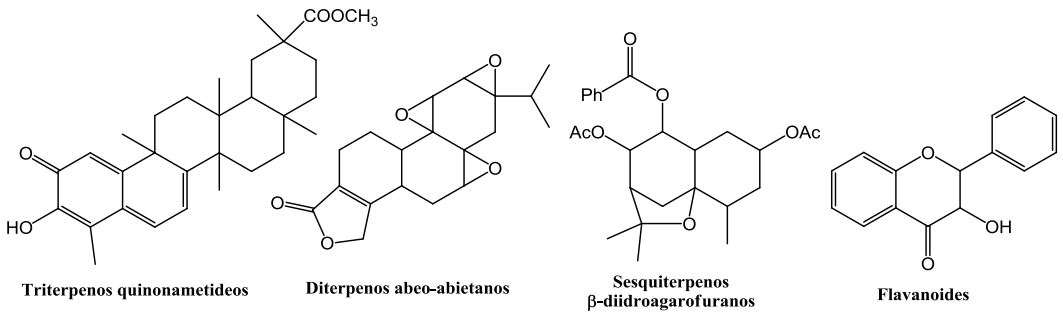
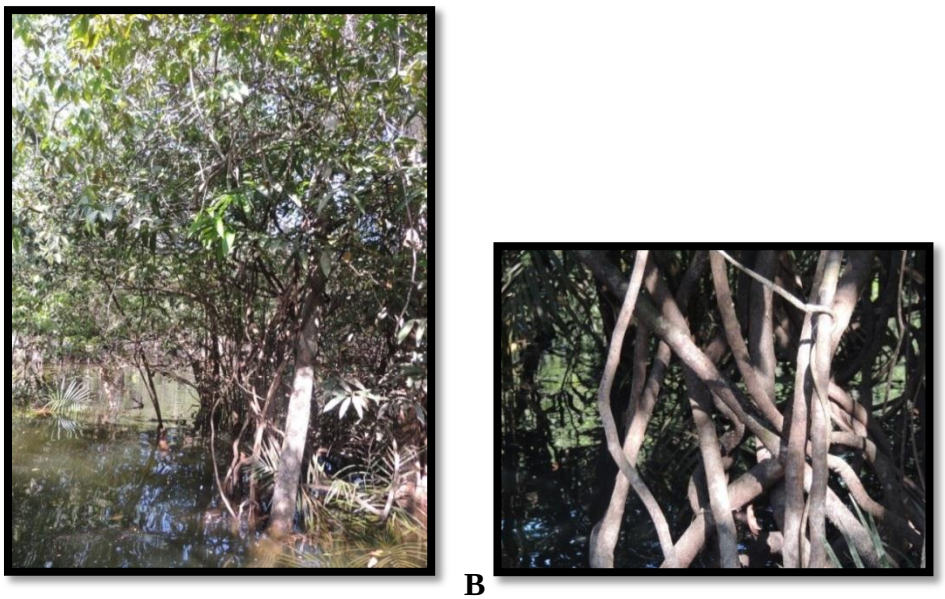


Figura 2. Estruturas químicas representativas das principais classes de metabólitos secundários recorrentes em Celastraceae.

Diante das diversas descrições de atividade biológica das plantas pertencentes à família Celastraceae, observam-se atividades antirreumáticas, antibacterianas, antitumorais, anti-inflamatórias, cicatrizantes, inseticidas, imunossupressoras, antimicrobianas, antimaláricas, e antidiabéticas (Fonseca *et al.*, 2007; Rodrigues *et al.*, 2006; Silva Júnior, 2005). A atividade antidiabética tem sido descrita a muitas espécies pertencentes ao gênero *Salacia*, entre elas *S. chinensis* (Yoshikawa *et al.*, 2003), *S. hainanensis* (Huang *et al.*, 2012), *S. oblonga* (Wolf *et al.*, 2003), *S. prenoides* (Pillai *et al.*, 1979), *S. reticulata* (Karunanayake *et al.*, 1984) e *S. macrosperma* (Venkateswarlu *et al.*, 1993).

2.4 *Salacia impressifolia*

Salacia impressifolia (Miers.) A. C. Smith. é uma espécie do gênero *Salacia* pertencente à família Celastraceae, conhecida popularmente no Brasil como miraruíra, cipó floramira e também muiraruíra. Seu caule é utilizado na medicina popular no combate à diabetes e inflamações (Almeida, 1993). A espécie objeto deste estudo, conhecida popularmente na Bolívia como guaponó, ocorre como uma liana (Figura 3A e B), comum na região Amazônica, podendo ser encontrada em cinco estados: Amazonas, Pará, Rondônia, Roraima e Mato Grosso (Lombardi, 2013). É um cipó escandente, com flores amareladas (Figura 4A), botões esverdeados e fruto comestível rugoso (Figura 4B) (Guia Igapó, USP, 2008).



A **B**

Figura 3: *Salacia impressifolia*: local de coleta (A), detalhe da planta (B).

Salacia impressifolia possui a seguinte classificação taxonômica (Paarakh *et al*., 2008):

Reino: Plantae

Sub-reino: Tracheobionta

Divisão: Magnoliophyta

Classe: Magnoliopsida

Subclasse: Rosidae

Ordem: Celastrales

Familia: Celastraceae

Gênero: *Salacia*

Espécie: *impressifolia*

A  **B** 

Figura 4. *Salacia impressifolia:* Flor **A**; e Fruto **B**. Fonte: Universidade de São Paulo (http://ecologia.ib.usp.br/guiaigapo/familias/hippocrateaceae/salacia_impressifolia/salacia_impressifolia.html).

Até o momento, há poucos estudos referentes à fitoquímica da *Salacia impressifolia*. Porém, já foram reportados o isolamento, a partir do caule, dos esteróis β-sitosterol, estigmasterol e campesterol, dos triterpenos tingenona, tingenina B, friedelan-3-ona, α-amirina, β-amirina, lupeol, (Rocha *et al*., 2000), ácido cinchólico, ácido quinóvico, ácido 3-O-[β-D-quinovopiranosil]-quinóvico (Costa *et al.,* 2007), 3-O-[β-D-fucopiranosil]-quinóvico e ácido 3-oxoquinóvico (Ripardo Filho *et al.,* 2008).

3. RESULTADOS E DISCUSSÃO

Este item será apresentado na forma de um artigo científico experimental denominado capítulo 1. Além deste, encontra-se no Anexo I um artigo de revisão produzido sobre o gênero Salacia.

CAPÍTULO 1

Artigo:

Salacia impressifolia: chemical composition and hipoglycemic activity.

A ser enviado para

Phytomedicine

Autores: Manuela M. Laikowski, Débora M. de Souza, Luciane Minetto, Natália G. Girondi, Camila C. Pires, Gisiele Alano, Leandro Tasso, Sidnei Moura.

Salacia impressifolia: chemical composition and hipoglycemic activity

Laikowski, M.M[1], Souza, D.M,[1], Minetto, L[1]; Girondi, N.G[2], Pires, C.C[2], Alano, G[2], Tasso, L[1,2], Moura, S.[1,*].

[1] *Laboratory of Natural and Synthetics Products, University of Caxias do Sul, Caxias do Sul, Brazil*

[2] *Laboratory of Pharmacology, University of Caxias do Sul, Caxias do Sul, Brazil*

*__Correspondence:__ Prof. Dr. Sidnei Moura, Technology Department, Biotechnology Institute, University of Caxias do Sul, 1130 Francisco Getúlio Vargas st., CEP 95070-560, Caxias do Sul, Brazil.

Phone: + 55 54 3218 2100

E-mail addresses: sidnei.moura@ucs.br; sidmoura@gmail.com

Abstract

Salacia impressifolia (Miers.) A. C. Smith is a liana of the Celastraceae Family, very used to diabetes treatment in folk medicine. In order to evaluate the specie chemical composition, were performed different extracts from bark and steam in ascending order of polarity by ultrasound and under reflux. The extracts were analyzed by High Resolution Mass Spectrometry and 32 compounds were identified, including the important and already reported in the literature as active, Mangiferin, Kotalanol and Salacinol. The hydroalcoolic stem extract (**Ss5**) was administered in STZ-induced diabetes rats to evaluate the potential hypoglycemic activity. Total cholesterol, HDL, triglycerides, ALT and AST were also evaluated. **Ss5** extract had no statistical significance between glibenclamida group (drug standard), exhibiting hypoglycemic effect. The extract was also able to increase de HDL levels. In this way, this work demonstrates the potential use of this specie.

Keywords: *Salacia impressifolia*, diabetes, phytochemical characterization.

INTRODUCTION

Brazil is known for its biodiversity, with 40 to 55 thousand plant species distributed across several biomes. A large number of plants are used by the population in folk medicine and small percentage of these species has been evaluated scientifically for their medicinal and biological potential [1].

There have been many approaches described in order to facilitate the discovery of biological and medicinal agents from natural sources. The approaches to the discovery of bioactive plant secondary metabolites that have been and are still being used include the chemotaxonomical/taxonomical approach. The chemotaxonomical/taxonomical approach to plant collection relies on the premise that related taxa have inherited the genetic ability to produce similar secondary plant metabolites. In an approach to natural product dereplication, a simple method to obtain minimal, significant, mass spectral information has been developed with the advent of the electrospray ionization (ESI) interface [80]. The primary information that can be obtained using an electrospray mass spectrometer is molecular weight, which can be used for comparison with a database of natural products. Using a combined liquid chromatography/mass spectrometry system with an electrospray ionization source (ESI LC/MS), one could therefore crudely separate an extract into its constituents and obtain minimal, structural information (UV and mass) and then use that information to correlate with a database of known natural products [81].

The Celastraceae family is composed by two generous, *Salacia* (about 200 species) and *Hippocrateae* (about 100 species), distributed in tropical and subtropical regions of both hemispheres, and underrepresented in temperate zones. In Brazil, this family is distributed in north, northeast and southeast regions [2].

Studies have reported that various Celastraceae species exhibit important pharmacological constituents, such as: quinone-methide triterpenes, whose occurrence is restricted to the family Celastraceae [3] and have antibiotic, cytotoxic, antitumor, antimalarial, and antioxidant properties [4, 5, 6, 7, 8]; sesquiterpenes, with insecticide activity [9]; flavonoids, with demonstrated antioxidant properties [10]; and alkaloids, which show antitumor and insecticide activities [11, 12].

Salacia impressifolia (Miers.) A. C. Smith popularly known in Brazil as "miraruíra", "cipó miraruíra", and "muiraruíra", is a liana of the Celastraceae Family, common in Amazonic region [2], which is used to diabetes treatment in folk medicine. The same activity was reported to others *Salacia* species such as *S. prenoides* [13], *S. macrosperma* [14], *S. oblonga* [15] and *S. reticulata* [16]. Although these studies involving *Salacia* species, there are few scientific papers focusing *Salacia impressifolia.* In this way, the present work aimed to evaluate the chemical composition of *Salacia impressifolia* stem and bark extracts as well as the hypoglycemic activity of the stem hydroalcoholic extract of this specie.

MATERIAL AND METHODS

Chemical agents

Streptozotocin and glibenclamida was purchased from Sigma-Aldrich (Saint-Louis, Missouri, USA). Accu-check active monitor and test stripes were purchased from Roche Diagnostics (Mannheim, Germany). The solvents P.A. hexane, chloroform, ethyl acetate and ethanol were purchased from Merck. All chemicals were of analytic grade.

Plant material

S. impressifolia Miers A. C. Smith stem, bark and leaf were collected in Manaus (S 02°55'55.031", W 056°49'13.578"), AM, Brazil, under authorization from Ibama number

02001.004236/2013-63. The plant is waiting for identification by the University of Caxias do Sul herbarium.

Extraction

The plant samples were dried in air oven at 45°C. The stem was separated from the bark, and they were powdered individually. Stem and bark were extracted under reflux (10 g of plant material with 200 ml of solvent for 2 h) in ascending polarity order and using ultrasound system (10 g of plant material with 200 ml of solvent for 30 min) (Figure 1). The extracts were nominated according to following condition: S= under reflux extract, U= ultrasound extract, b= bark, s= stem, 1= hexane, 2= chloroform, 3= ethyl acetate, 4= ethanol, 5= ethanol/water (1:1). After the extraction process, the solvent was evaporated under reduced pressure. Each extract resulted in powder, and was stored in the dark.

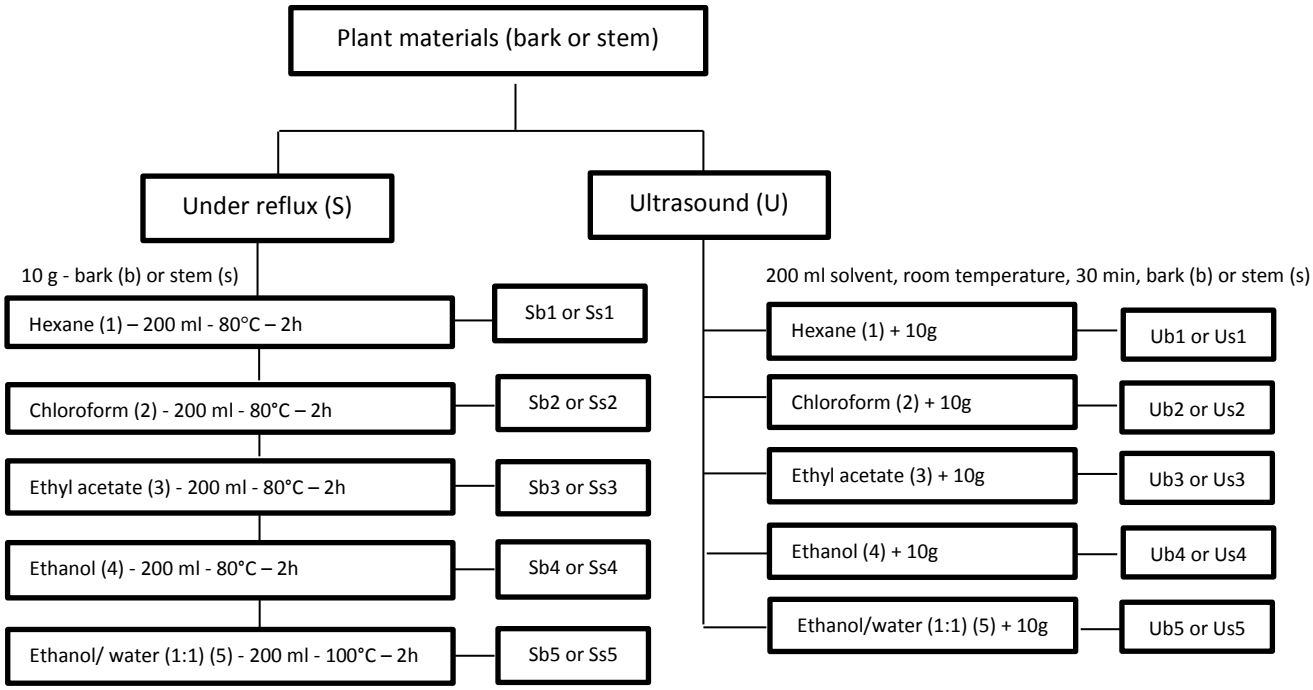


Figure 1. Fluxogram of *S. impressifolia* extracts according to the solvent and extraction condition.

Phytochemical characterization

The powered extracts of *Salacia impressifolia* were dissolved in a solution 50% (v/v) chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA), 50% (v/v) ultrapure water (Milli-Q®) and 0.1% formic acid or 0.1% ammonia hydroxide for ESI(+) or ESI(-) respectively. The samples were separated by liquid chromatography (UFLC system), consisted of a LC-20ADXR pump, a SIL-30AC autosampler (Shimadzu®). Chromatographic separations were performed on a Shim-pack XR-ODS (30 mm × 2.0 mm, 2.2 µm) column. To detection, a hybrid high-resolution and high accuracy microTof (Q-TOF) (Bruker® Scientific) was used, with electrospray ionization (ESI) source (MicrOTOF-QII Bruker® Scientific) in positive and negative mode. The range of mass was 50-1200 *m/z* with two scans per second, providing the resolution of 50,000 (FWHM). Nitrogen was used for drying gas, in a 10 L/min flow. The drying temperature was 200 °C, the ionization energy was 3.0 eV, and the capillary voltage was 4500 eV.

Animals

The study was conducted according to Abeeleh *et al.* (2009) with slight modifications, and it was previously approved by Ethics Committee on Animal Use (CEUA) – University of Caxias do Sul (Project number: 002/2013). Healthy, young male adult Wistar rats, weighing 250 to 350g, purchased from Technology and Science Foundation (Santa Maria, Brazil) were used in the study. The animals were housed under standard conditions and kept on a 12h light: 12h dark cycle. The animals were fed with a commercial rodent diet (Nuvital®) and water *ad libitum.*

***In vivo* study**

For the experiment, male Wistar rats were randomly distributed into 4 groups (n = 5). Diabetes was induced through i.p. administration of 55 mg/kg streptozotocin (STZ). Blood glucose concentration was measured to confirm the development of diabetes mellitus (after 24h). During a 28 days period of diet, normal control rats (G1) were orally administrated 0.6 mL of phosphate buffer (PBS) only. STZ-induced diabetic rats were randomly divided into three groups that orally administrated glibenclamide (G2) 0.7 mg/kg, *S. impressifolia* hydroalcoholic stem extract (**Ss5**) 200 mg/kg (G3) or PBS (G4) using gavage. Basal glycemia was measured in T0 (first measurement), T1 (7 days), T2 (14 days), T3 (21 days) and T4 (28 days). Total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and high density lipoprotein (HDL) were measured before the administration of STZ and at the 28^{th} day. The total cholesterol, triglycerides and HDL content in plasma were estimated by enzymatic colorimetric method [17]. ALT and AST levels were measured by ultraviolet kinetic method [18]. The measurements were processed with Labmax 240 biochemical analyser (Labtest, Japan). The animals were euthanized by decapitation at the end of the experiments.

Oral Glucose Tolerance Test (GTT)

Glucose tolerance test was performed in all animals before the diabetes induction, in order to evaluate the glucose tolerance. A 25% glucose solution was intraperitoneal administered in a 2 g/kg body weight on the last day of the experiment after an overnight fast. Blood samples were collected from the tail vein at 30, 60, 120, 180 and 240 min after administration.

Statistical Analysis

For *in vivo* experiment, statistical analysis for glucose level was performed through two-way ANOVA followed by Bonferroni test, in order to assess differences between treatment groups and sampling times. For total cholesterol, triglycerides, AST, ALT, and HDL levels statistical analysis was performed employing Student's *t*-test. In all analyses the IBM SPSS 21.0 was used and $p < 0.05$ was considered statistically significant for all tests.

RESULTS AND DISCUSSION

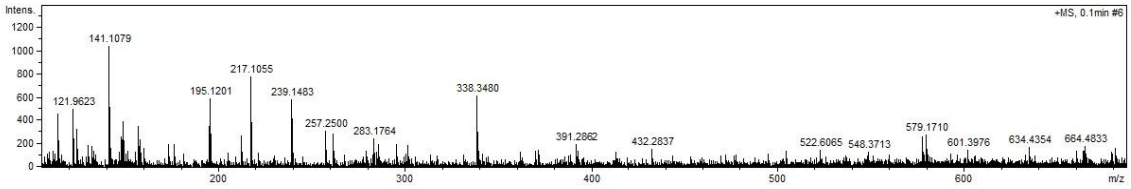
Chemical characterization

With the purpose of the chemical composition overall evaluation of the *S. impressifolia* extracts, different extraction procedures were performed, Figure 1. As can be seen in Figure 1 and Table 1, the phytochemical constituents vary in accordance on the plant part as well as the extraction procedure and solvent.

The chemical composition was performed by High Resolution Mass Spectrometry (HRMS), according to methods described in phytochemical characterization. Based on the complex composition of the plant extracts, the HRMS has been used as a powerful tool for identification of natural metabolites as alkaloids [19] and flavonoids [20] for instance. *Salacia* species are known to elaborate quinonemethides (Celastraceae family chemotaxonomical markers), friedelanes, lupanes and oleananes triterpenes, sequiterpenes and phenolic acids [21]. In agreement with the expected chemical classes, each extract

was analyzed in positive ESI(+) and negative ESI(-) mode. The profile of *S. impressifolia* hydroalcoholic stem extract (**Ss5**) are showed in Figure 2, in ESI(+) (A) and ESI(-) (B).

A)



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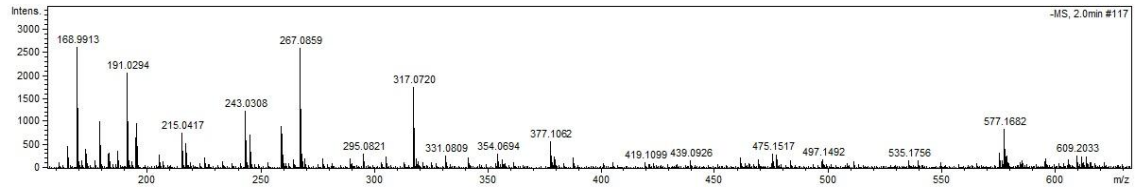

Figure 2. *Salacia impressifolia* hydroalcoholic stem extract (**Ss5**) spectrum by ESI-MS(+) (A) and ESI-MS(-) (B).

Using HRMS, a set of information as exact mass and isotopic ratio can be used in chemical identification. In addition, for unequivocal identification and differentiation of isobaric interferences, the information about fragmentation pathway was determined. However, in this work, for Epicatechin and Catechin (**4**); Tingenine and Maintenine (**12**); Wilforlide and Regelide (**16**) were not possible because the small amount of compound in extract.

By comparison with predetermined for the specie and since the widely accepted accuracy threshold for confirmation of elemental compositions was established as 5 ppm [22], the main chemical compounds are shown in Table 1 and Figure 3.

Table 1. Chemical compounds identified in *Salacia impressifolia* extracts by HRMS in positive and negative mode.

Entry	Precursor ion *m/z*	Extract	Identification	Elem. Comp.	Diff. ppm	Reference
Extracts analysis in positive mode ESI (+): Ultrasound (U); Under reflux (S); stem (s); stem bark (b); hexane (1); chloroform (2); ethyl acetate (3); ethanol (4); and ethanol/water (5).						
1	179.0714	Ss5	Coniferylaldehyde	$C_{10}H_{10}O_3$	2.84	[23]
2	199.0591	Ub3	Lambertic acid	$C_9H_{10}O_5$	4.16	[21]
3	279.1577	Ss5	Dibutyl phtalate	$C_{16}H_{22}O_4$	4.20	[23]
4	291.0877	Ss5	Epicatechin/catechin	$C_{15}H_{14}O_6$	2.63	[24]
5	325.1909 325.1909	Sb4 Us3	Sapatarangi quinine	$C_{20}H_{24}N_2O_2$	2.40 2.40	[25]
6	335.0474 335.0465 335.0468	Ss5 Us5 Sb5	Salacinol	$C_9H_{18}O_9S_2$	0.83 1.87 0.97	[16]
7	343.1238	Us5	Galactinol	$C_{12}H_{22}O_{11}$	0.91	[27]
8	347.1527	Ss5	Ciliarin	$C_{19}H_{22}O_6$	1.84	[28]
9	383.1920 383.1910	Sb5 Ub3	Foliachinenoside I	$C_{16}H_{30}O_{10}$	0.53 2.09	[28]
10	391.2321	Ub3	Foliasalacioside J	$C_{19}H_{34}O_8$	2.99	[29]
11	417.2476	Sb3	Foliachinenoside E, F	$C_{21}H_{36}O_8$	3.17	[28]
12	421.2728	Sb1	Tingenine/Maintenine	$C_{28}H_{36}O_3$	3.68	[30]
13	423.0914 423.0920 423.0926 423.0925	Sb4 Us4 Ub4 Ss5	Mangiferin	$C_{19}H_{18}O_{11}$	3.34 1.92 0.50 0.74	[32]
14	425.0781 425.0779 425.0784 425.0776 425.0796 425.0767 425.0768	Ss5 Sb5 Sb3 Us5 Ss4 Ub4 Ub3	Kotalanol	$C_{12}H_{24}O_{12}S_2$	1.69 2.16 0.99 2.87 1.85 4.99 4.76	[26]
15	441.3026 441.3009	Sb5 Ss4	Regeol A	$C_{28}H_{40}O_4$	4.63 0.77	[23]
16	455.3519	Sb1	Wilforlide/Regelide	$C_{30}H_{46}O_3$	1.53	[23]
17	459.3834	Sb3	Salasone C, D e E	$C_{30}H_{50}O_3$	2.10	[21]
18	463.2851 463.2849 463.2839	Sb3 Ss4 Ub5	Netzahualcoyene	$C_{30}H_{38}O_4$	0.41 0.02 2.18	[28]

19	471.3485	Sb2	Demethyl regelin	$C_{30}H_{46}O_4$	2.10	[34]
20	505.1764	Sb3	Raffinose	$C_{18}H_{32}O_{16}$	1.06	[27]
21	507.2792	Sb3	Foliasalacioside E1	$C_{24}H_{42}O_{11}$	2.79	[28]
22	516.3830	Sb1	Kotalagenin-16-acetate	$C_{32}H_{51}O_5$	2.81	[27] [21]
23	595.2547	Sb3	Salasol B	$C_{33}H_{38}O_{10}$	0.51	[27]

Extracts analysis in negative mode (ESI -): Ultrasound (U); Under reflux (S); stem (s); stem bark (b); hexane (1); chloroform (2); ethyl acetate (3); ethanol (4); and ethanol/water (5);

24	169.0128	Us5	Gallic acid	$C_7H_6O_5$	4.84	[35]
25	181.0706	Ss4	Dulcitol	$C_6H_{14}O_6$	2.96	[23]
26	193.0444	Ss5	Gynuraone	$C_{10}H_{10}O_4$	2.62	[23]
2	197.0450	Ss5	Lambertic acid	$C_9H_{10}O_5$	2.70	[21]
27	255.2320	Ss5	Palmitic Acid	$C_{16}H_{32}O_6$	3.90	[36]
4	289.0725	Ss5	Epicatechin/catechin	$C_{15}H_{14}O_6$	1.0	[24]
28	341.1230	Ss5	Coniferin	$C_{16}H_{22}O_8$	3.60	[28]
29	373.1498	Sb3	Syringin	$C_{17}H_{24}O_9$	0.36	[28]
10	389.2169	Sb4	Foliasalacioside J	$C_{19}H_{34}O_8$	1.46	[29]
12	419.2580 419.2574	Sb4 Ub3	Tingenine/Maintenine	$C_{28}H_{36}O_3$	1.30 2.72	[30]
30	435.2531	Sb4	β-Hidroxytingenone	$C_{28}H_{36}O_4$	0.82	[21]
31	465.1540 465.1553 465.1555	Sb5 Ub5 Ub3	Lehmbachol D	$C_{26}H_{26}O_8$	1.86 0.93 1.36	[34]
20	503.1580	Sb4	Raffinose	$C_{18}H_{32}O_{16}$	1.06	[27]
32	591.1510 591.1493 591.1536 591.1586 591.1495	Sb4 Sb3 Ss5 Ub4 Ub3	Proanthocyanidin	$C_{31}H_{28}O_{12}$	1.39 1.48 0.80 0.88 1.14	[28]

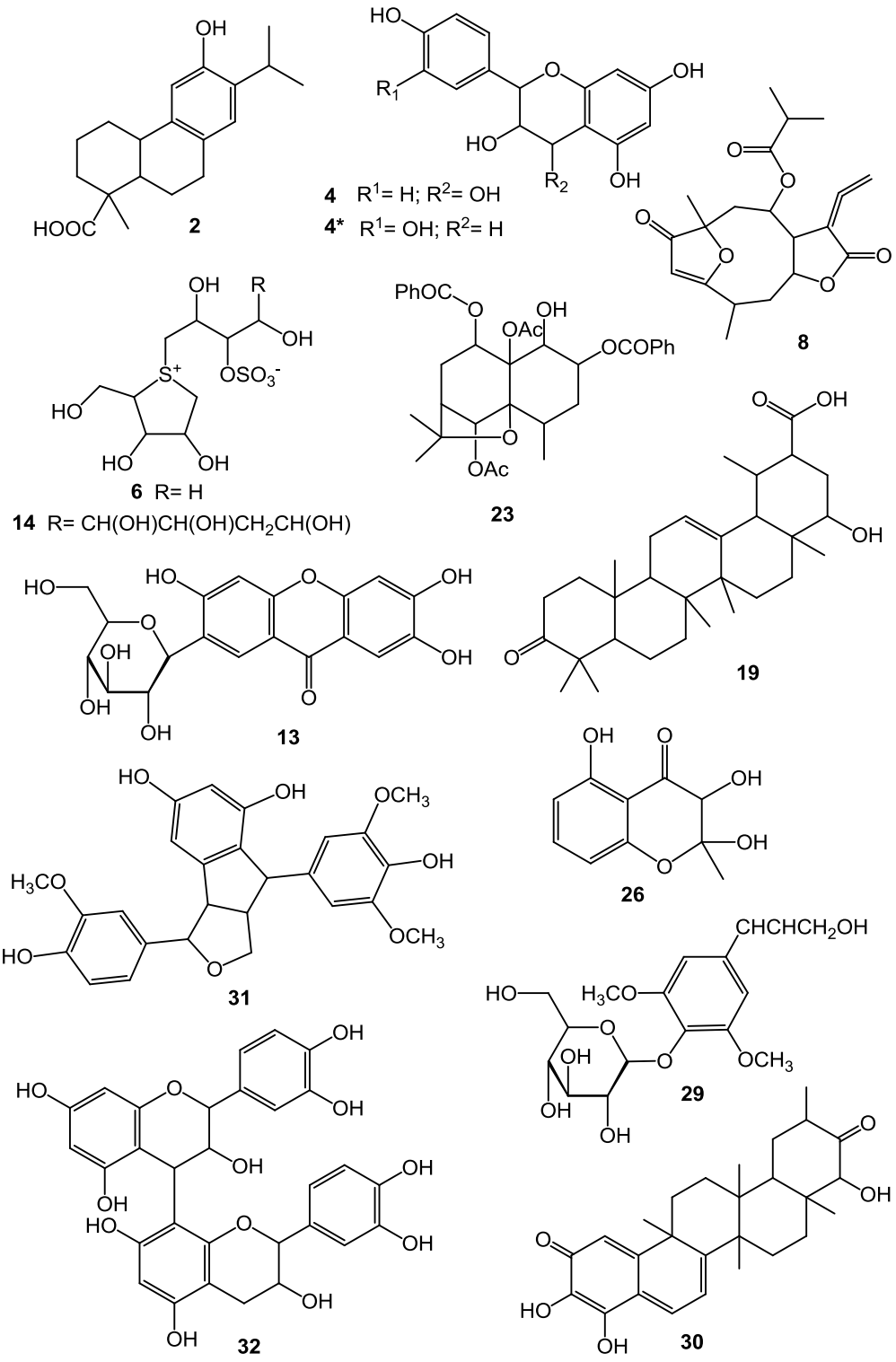

Figure 3. Some chemical structures of the identified compounds from *S. impressifolia* extracts presented in Table 1.

The *S. impressifolia* extracts have shown phenolic compounds and among them, mangiferin (**13**). Studies in rodents have suggested that mangiferin might exhibit antidiabetic properties, by ameliorating postprandial glycemic, mainly due to its ability to inhibit the intestinal *α*-glycosidase activities [37]. According to Girón *et al*. (2009), *S. oblonga* extracts and mangiferin (**13**) showed an increase of glucose uptake and may be considered as a useful therapy to improve the metabolic perturbation in insulin resistance and also under conditions of insulin deficiency [38].

In this way, other two important compounds with hypoglycemic reported activity were identified, kotalanol (**14**) and salacinol (**6**), Figure 2 [26]. They were previously isolated from aqueous methanolic extract of *S. oblonga* roots, and were confirmed as *α*-glucosidase inhibitors [39]. Their activity have been revealed to be as high as those of voglibose and acarbose, which are very used clinically [40]. In order to evaluate the pharmacological properties of this specie, especially the hypoglycemic activity, the **Ss5** extract was tested in an *in vivo* model.

By the way, the comparisons with previous works have demonstrated the presence of the same compounds in different plant parts of Salacia species. The Table 2 summarizes this similarity with *S. impressifolia*.

Table 2. Chemical compounds of *Salacia* species. Comparison between literature and *Salacia impressifolia* extracts.

Salacia species	Isolated Constituents	Parts	*S.impressifolia* extract	References
S. amplifolia	Dibutylphtalate	roots	Ss5	[23], [42]
	Coniferylaldehyde	roots	Ss5	
	Regeol A	roots	Sb5, Ss4	

	Wilforlide A	roots	Sb1	
	Netzahualcoyene	roots	Sb3, Ss4, Ub5	
	Gynuraone	roots	Ss5	
	Dulcitol	roots	Ss4	
S. chinensis	Regeol A	stem	Sb5, Ss4	[28 - 30]
	Kotalagenin 16-acetate	stem	Sb1	[33], [39]
	Salasone	stem	Sb3	[42 - 44]
	Tingenine	stem	Sb1, Sb4, Ub3	
	β-Hidroxytingenone	stem	Sb4	
	Lambertic acid	stem	Ub3, Ss5	
	Salasol B	stem	Sb3	
	Gynuraone	stem	Ss5	
	Foliasalacioside E1	leaves	Sb3	
	Foliasalacioside J	leaves	Sb4, Ub3	
	Leucopelargonidin	stem	Ss5	
	Proanthocyanidin	roots	Sb4, Sb3, Ss5, Ub4, Ub3	
	Foliachinenosides	leaves	Sb5, Sb3, Ub3	
	Dulcitol	stem	Ss4	
	Salacinol	stem	Ss5, Us5, Sb5	
	Maintenin	stem	Sb1,Sb4, Ub3	
	Syringin	leaves	Sb3	
	Coniferin	leaves	Ss5	
	Foliasalaciosides	leaves	Sb3, Sb4, Ub3	
S. campestris	Maintenin	roots	Sb1, Sb4, Ub3	[45]
	Netzahualcoyene	root bark	Sb3, Ss4, Ub5	
S. elliptica	Palmitic acid	leaves, branches	Ss5	[36], [46]
	Maintenin	leaves, branches	Sb1, Sb4, Ub3	
S. hainanensis	Mangiferin	roots	Sb4, Us4, Ub4, Ss5	[3]
S. lehmbachii	Lehmbachol D	bark	Sb5, Ub5, Ub3	[34]
S. madagascariensis	Netzahualcoyene	roots	Sb3, Ss4, Ub5	[47]
S. oblonga	Salacinol	roots	Ss5, Us5, Sb5	[27], [39]
	Kotalanol	roots	Ss5, Sb5, Sb3, Us5, Ss4, Ub4,Ub3	
	Kotalagenin 16-acetate	roots	Sb1	
	Galactinol	roots	Us5	
	Lambertic acid	roots	Ub3, Ss5	
	Raffinose	roots	Sb4, Sb3	
	Dulcitol	roots	Ss4	
	Mangiferin	roots	Sb4,Us4, Ub4, Ss5	
S. prinoides	Salacinol	root bark	Ss5, Us5, Sb5	[35], [49]
	Kotalanol	root bark	Ss5,Sb5, Sb3, Us5,	

			Ss4, Ub4, Ub3	
	Gallic acid	root bark	Us5	
S. reticulata	Netzahualcoyene	root bark	Sb3,Ss4, Ub5	[46],
	Salacinol	roots, stem	Ss5, Us5, Sb5	[50 - 52]
	Kotalanol	roots, stem	Ss5,Sb5, Sb3, Us5, Ss4,Ub4,Ub3	
	Mangiferin	roots	Sb4, Us4,Ub4, Ss5	
	Epichatechin	roots	Ss5	
	Lambertic acid	stem	Ub3, Ss5	
	Kotalagenin 16-acetate	roots	Sb1	

***In vivo* study**

Streptozotocin is used to induce diabetes mellitus by selective cytotoxicity effect on pancreatic β-cells. Thus it affects endogenous insulin release and as a result increases blood glucose level [53]. Due to this, the hyperglycemia induced by STZ in animal is considered an experimental model for the preliminary screening of hypoglycemic agents [54]. The experimental diabetic model used in this study was type 2 since low dose of STZ (55 mg/kg) destroyed some population of pancreatic beta cells [82]. There were residual beta cells which secreted insufficient insulin causing type 2 diabetic model [83].

Diabetes is a chronic carbohydrates, protein and fat metabolism disorder with relative deficiency of insulin secretion and varying degrees of insulin resistance. It causes severe complications including blindness, cardiac and kidney diseases [55]. Diabetes is one of the most important clinical and public health problems in the world today [56]. Insulin deficiency may be due to inadequate secretion or diminished tissue response to insulin in the complex pathway of hormone action, which might lead to disturbance in the metabolism [57, 58].

In this study, 4 groups presented differences in glycemic values obtained through time ($p<0.05$) (Table 3). Statistically significant difference was found in follow up profiles

of glycemic levels between groups ($p<0.05$), with the exception of values between G2 and G3 ($p>0.999$) (Figure 5).

Table 3. Levels of blood glucose in normal and diabetic rats after 1, 7, 14, 21 and 28 days of treatment (Mean ±SD).

	Glucose (mg/dL)				
	1st day	7th day	14th day	21st day	28th day
G1	92.13 ± 6.27	82.00 ± 9.29	92.63 ± 9.91	106.75 ± 16.86	86.63 ± 4.50
G2	330.20 ± 63.36	299.00 ± 210.54	168.60 ± 112.79	227.60 ± 148.67	257.60 ± 158.94
G3	325.33 ± 86.17	221.50 ± 131.04	182.83 ± 123.00	202.33 ± 158.75	218.67 ± 141.14
G4	349.38 ± 33.14	343.88 ± 32.28	414.13 ± 49.57	427.25 ± 52.16	438.25 ± 32.52

n=5 for each group. G1= non diabetic group, G2 = glibenclamide group, G3 = Ss5 extract group and G4 = PBS diabetic group.

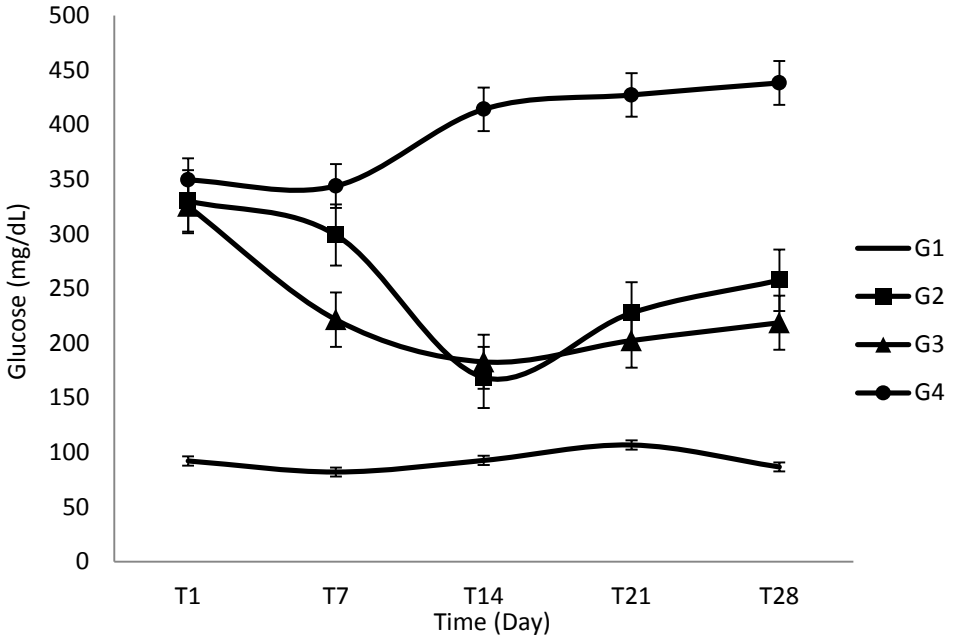

Figure 5. Effect of hidroalcoholic stem extract (Ss5) of *Salacia impressifolia* on fasting blood glucose level of type 2 diabetic rats (Mean ±SD). G1= non diabetic group, G2 = glibenclamide group, G3 = Ss5 extract group and G4 = PBS diabetic group.

The continuous administration of hidroalcoholic extract of *S. impressifolia* (**Ss5**) at 200 mg/kg or glibenclamide for 28 days significantly reduced the blood glucose concentration in STZ induced diabetic rats. The plant extract showed a comparable activity

with the glibenclamide treated group. This is a standard antidiabetic drug that stimulates insulin secretion from β cells of islets of Langerhans [59]. According to Figure 5, there was no statistically significant difference of glycemic levels between G2 and G3 ($p>0.999$).

The hypoglycaemic effect of plant extracts is generally dependent upon the degree of β-cell destruction. Treatment of moderate STZ-diabetic rats with medicinal plant extract can result in the activation of β-cells, showing an insulinogenic effect [60].

Many authors have described antidiabetic activity for others species of Salacia genus, such as *S. chinensis* [33], *S. hainanensis* [61], *S. oblonga* [62], *S. prinoides* [13], *S. reticulata* [31] and *S. macrosperma* [14]. The hypoglycemic effect of **Ss5** can be due to the presence of salacinol (**6**), kotalanol (**14**), and mangiferin (**13**). Salacinol (**6**) and kotalanol (**13**), which occur naturally in *Salacia spp*., such as *S. reticulata, S. oblonga*, and *S. chinensis*, were identified as potent inhibitors of α-glucosidase, showing better activity than that of acarbose (a clinically used α-glucosidase inhibitor) [31, 21]. The antihyperglycemic potential of mangiferin (**14**) from methanolic root extract of *Salacia chinensis* was previously described by Sellamuthu and coworkers (2009) [63]. The same author (2013) described that mangiferin has protective effects against pancreatic β-cell damage and on the antioxidant defense systems in streptozotocin (STZ)-induced diabetic rats. The oral administration of mangiferin and glibenclamide to diabetic rats significantly decreased the level of blood glucose and increased levels of insulin. Additionally, mangiferin treatment significantly modulated the pancreatic nonenzymatic antioxidants status and other oxidative stress biomarkers. The histoarchitecture of diabetic rats showed degenerated pancreas with lower β-cell counts, but mangiferin treatment effectively regenerated insulin secreting islet cells. In accordance with that, mangiferin is probably one of the compounds responsible for the hypoglycemic activity of **Ss5** extract [64].

Furthermore, the presence of epicatechin (**4**) may contribute to the hypoglycemic effect. As a phenolic constituent, it is a moderate *α*-glucosidase inhibitor [52]. This compound is also presented in **Ss5**, and can corroborate for the hypoglycemic effect.

It was also described that *S. oblonga* extract induces a dose-dependent reduction of insulin concentration in fasting states, indicating an improvement in peripheral insulin action, and the extract significantly improves glucose tolerance in a model of insulin resistance [65]. This anti-hyperglycemic action is exerted not only at the luminal level by inhibiting *α*-glucosidase activities but also at the systemic level by increasing tissue insulin sensitivity and stimulating *β*-cells response to glucose. Due to the similarity of extract chemical composition in both species (*S. impressifolia* and *S. oblonga*) action mechanism can have resembling. However, future studies are necessary.

The assessment of biochemical parameters can be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, hematology, normal functioning and histomorphology of the organs [66]. In order to evaluate if **Ss5** changes total cholesterol, triglycerides, ALT, AST, and HDL levels, the experiment appraised the individual values before the administration of STZ and at the end of the experiment. The results are expressed in Table 4. The body weight at the beginning and at the end of the experiment, and the food intake were also evaluated, and can be seen on Table 5.

Table 4. Serum levels of cholesterol, triglycerides and other related parameters (Mean ± SD).

		Triglycerides mg/dL	HDL mg/dL	Total Chol. mg/dL	AST U/L	ALT U/L
G1	Initial	64 ± 20.82	29.4 ± 6.58	94.6 ± 10.97	162.0 ± 82.91	26.0 ± 8.46
	Final	135.40 ± 22.60	33.0 ± 8.72	129.80 ± 23.97	93.0 ± 48.42	28.0 ± 10.42
	Sig.	p<0.05	NS	NS	NS	NS
G2	Initial	88.4 ± 56.21	33.4 ± 6.43	123.0 ± 69.09	333.2 ± 43.30	25.8 ± 17.67
	Final	122.20 ± 107.84	45.50 ± 9.10	168.0 ± 111.51	161.80 ± 111.51	113.00 ± 8.22
	Sig.	NS	p<0.05	NS	NS	p<0.05
G3	Initial	76.8 ± 24.28	31.4 ± 7.96	103.4 ± 22.94	253.0 ± 156.31	55.6 ± 33.62
	Final	147.60 ± 47.84	40.40 ± 7.64	105.0 ± 22.67	49.00 ± 26.36	82.00 ± 34.89
	Sig.	p<0.05	p<0.05	NS	NS	NS
G4	Initial	65.8 ± 13.35	26.0 ± 6.48	107.4 ± 19.75	194.6 ± 27.94	21.6 ± 14.33
	Final	213.60 ± 13.43	38.60 ± 1.95	128.0 ± 37.97	222.0 ± 82.42	78.60 ± 17.74
	Sig.	p<0.05	NS	NS	NS	p<0.05

Level of significance between groups is determined from initial and final values and is considered significant for $p < 0.05$. NS= not significant ($p > 0.05$). *n*=5 for each group.

Table 5. Body weight and food consume (Mean ± SD). Level of significance between groups is determined from initial and final values and is considered significant for $p < 0.05$. NS= not significant ($p > 0.05$).

		G1	G2	G3	G4
Initial body weight (g)		342. 87 ± 32.23	200.80 ± 17.75	215.0 ± 15.46	317.63 ± 27.92
Final body weight (g)		374.37 ± 30.54	242.60 ± 32.59	238.5 ± 24.24	231.87 ± 32.91
	Sig.	p<0.05	NS	NS	p<0.05
Dietary intake (g/day)		30.98± 12.68	33.62± 13.28	31.52± 8.65	36.60 ± 9.66

Diabetes is associated with hyperlipidemia, and it is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. It is also well established that dyslipidemia plays an important role in the development of diabetic complication [67]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [68, 69]. Hypertriglyceridemia is also associated with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin

resistance and glucose intolerance [70]. In addition, STZ rats show an important lipolytic activity, due to the insulinopenic state which contributes to maintain the abnormally elevated plasma triglycerides and cholesterol levels [71]. In STZ-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol and triglycerides, and decreases in HDL [69]. Increased levels of triglycerides and cholesterol during diabetes lead to cardiovascular risk. In this way, the effects on diabetic complication were assessed by measuring the atherogenic lipids (total cholesterol and triglycerides) after chronic feeding of **Ss5** to diabetic rats.

The results demonstrated that total cholesterol level did not change by *S. impressifolia* treatment. In this study, all groups exhibited significantly elevated triglyceride levels at the end of the experiment, except group 2, treated with glibenclamide. The **Ss5** extract seems not be able to control the triglycerides levels, unlike the glibenclamide group. The elevated triglyceride level in non diabetic group (group 1) was an unspected result, and it can be assigned to biological variability.

As follows, repeated extract administration for 28 days, significantly ($p<0.05$) increased HDL levels, Table 4. The same result was observed in glibenclamide group. HDL is inversely associated with coronary heart disease and its elevation is considered as an anti-atherosclerotic factor [72].

Serum enzymes including AST and ALT are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels [73, 74]. According to Zafar and coworkers, STZ in rats can produce alterations in the hepatic functions as well as structure of hepatocytes [75]. The effect of STZ on the levels of diagnostic enzymes in the liver has remained unraveled. While some authors reported

increased activities of AST and ALT [76, 77] in the liver of STZ diabetic rat models, another group reported no alteration in the levels of these enzymes in the liver of diabetic rats ([78]. In our study, ALT was significantly higher (G4 and G2). On the other hand, treatment of the diabetic rats with the **Ss5** extract had no significance changes of the ALT enzyme activity in plasma compared to the beginning of the experiment (T0). More studies are necessary in order to evaluate if **Ss5** extract can have a hepatoprotector activity against liver damage in STZ diabetic rats.

The decrease in body weight of STZ diabetic rats (Table 5), as seen in the present study in G4, is owing to gluconeogenesis which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins [79]. STZ diabetic rats treated with **Ss5** had no significant difference in body weight at the end of the experiment compared to the initial time (G3) as well as the glibenclamide treated group (G2). This can be related to a protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis. To investigate if **Ss5** extract has the ability of regulating gluconeogenesis, more studies are necessary.

In conclusion, the chemical composition of *S. impressifolia* was similar to others *Salacia* species, with 32 compounds identified herein by HRMS in different extracts, which are representatives of the sesquiterpenes, flavonoids, quinone-methide triterpenes and alkaloids class as expected. About the activity, the **Ss5** extract presented increased of HDL levels and hypoglycemic effect.

Further studies designed to isolate, characterize, and test the compounds of *S. impressifolia* should provide a better understanding of the action mechanisms observed in the present study.

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4. DISCUSSÃO GERAL

As plantas têm sido utilizadas pelo homem como medicamentos desde os primórdios da humanidade. Triagens etnofarmacológicas têm direcionado pesquisas que relacionam o potencial das espécies vegetais com o tratamento de determinadas patologias. Entre as doenças popularmente tratadas com plantas medicinais destaca-se a diabetes, que atinge grande parte da população mundial. Por ser uma doença crônica e exigir tratamento contínuo, é interessante que se busquem novos métodos de tratamento, oferecendo maior eficácia e segurança a estes pacientes, bem como um melhor controle da doença.

A *Salacia impressifolia*, popularmente conhecida como miraruíra, é um cipó comum da região amazônica, e vem sendo utilizada pela população brasileira na forma de chá para o tratamento da diabetes. Pela ausência de dados científicos sobre esta espécie, o objetivo deste trabalho foi avaliar a composição química desta planta e confirmar o potencial efeito antiglicêmico da mesma.

Para este trabalho, foi realizada extensa pesquisa de revisão bibliográfica a respeito das demais espécies de *Salacia* com o intuito de apurar dados científicos a cerca dos compostos produzidos pelo metabolismo secundário deste gênero e do uso destas espécies no tratamento da diabetes. Os dados coletados constituíram a base da busca de compostos presentes nos extratos a serem analisados e serão publicados na forma de um artigo de revisão.

Sob licença do Conselho de Gestão do Patrimônio Genético (CGEN, n° 02001.004236/2013-63), foi coletado material vegetal na cidade de Parintins-AM (S02°55'55.031", W056°49'13.578"), e encaminhado ao laboratório. A exsicata foi encaminhada no herbário desta universidade para confirmação da espécie. Procedeu-se a secagem do material vegetal em estufa com circulação de ar, e posteriormente, foi separada a casca do caule. Ambos foram moídos separadamente em moinho de faca. Após a obtenção de um pó fino, foram realizados extrações em ultrassom e sob refluxo de ambos os materiais separadamente, em ordem crescente de polaridade de solventes. Desta forma, obtiveram-se 20 extratos, sendo dez da casca e dez do caule (Figura 5).

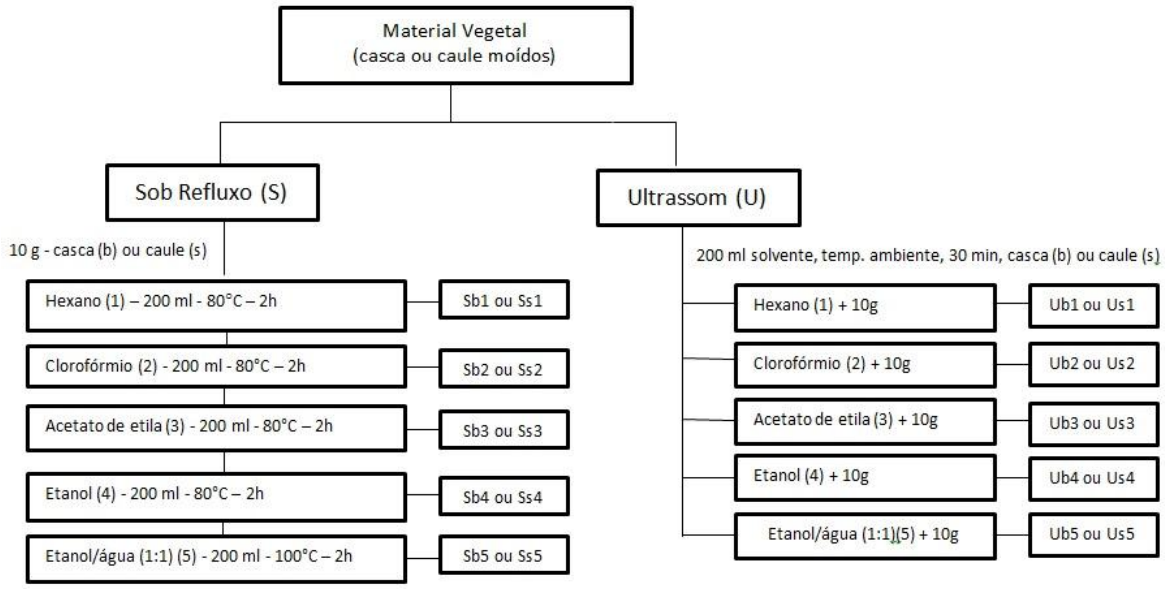

Figura 5. Fluxograma de extração de *S. impressifolia* de acordo com o material vegetal, método e solvente utilizado.

Os extratos foram avaliados por Cromatografia Líquida de Alta Eficiência (CLAE) acoplada a Espectrômetro de Massas de Alta Resolução (EMAR) nos modos positivo e negativo. Para isso, foram utilizados solventes com características ácidas (0,1% de ácido

fórmico em acetonitrila/água (1:1)) e básicas (0,1% formiato de amônia em acetonitrila/água (1:1)) respectivamente. Desta forma, foram identificados 32 compostos previamente descritos em outras espécies de *Salacia* (Tabela 1, Figura 6).

Tabela 1. Composição química de *Salacia impressifolia* por CLAE-EMRS nos modos ESI-MS(+) e ESI-MS(-).

Cód.	Íon precursor m/z (%)	Extrato (%)	Identificação	Fórmula molecular	Diff. ppm	Referência
Extratos analisados no modo positivo ESI (+): Ultrassom (U); Sob refluxo (S); caule (s); casca do caule (b); hexano (1); clorofórmio (2); acetato de etila (3); etanol (4); e etanol/água (5);						
1	179.0714	Ss5	Coniferilaldeído	$C_{10}H_{10}O_3$	2.84	Wang *et al.*, 2011;
2	199.0591	Ub3	Ácido lambértico	$C_9H_{10}O_5$	4.16	Matsuda *et al.*, 2005;
3	279.1577	Ss5	Dibutilftalato	$C_{16}H_{22}O_4$	4.20	Wang *et al.*, 2011;
4	291.0877	Ss5	Epicatequina ou Catequina*	$C_{15}H_{14}O_6$	2.63	Koga *et al.*, 2013;
5	325.1909 325.1909	Sb4 Us3	Sapatarangi quinina	$C_{20}H_{24}N_2O_2$	2.40 2.40	Paarakh *et al.*, 2008;
6	335.0474 335.0465 335.0468	Ss5 Us5 Sb5	Salacinol	$C_9H_{18}O_9S_2$	0.83 1.87 0.97	Yoshikawa *et al.*, 1997;
7	343.1238	Us5	Galactinol	$C_{12}H_{22}O_{11}$	0.91	Chawla *et al*., 2013;
8	347.1527	Ss5	Ciliarina	$C_{19}H_{22}O_6$	1.84	Nakamura *et al.*, 2011;
9	383.1920 383.1910	Sb5 Ub3	Foliachinenoside I	$C_{16}H_{30}O_{10}$	0.53 2.09	Nakamura *et al.*, 2011;
10	391.2321	Ub3	Foliasalacioside J	$C_{19}H_{34}O_8$	2.99	Zhang *et al.*, 2008;
11	417.2476	Sb3	Foliachinenoside E, F	$C_{21}H_{36}O_8$	3.17	Nakamura *et al.*, 2011;
12	421.2728	Sb1	Tingenina/Maintenina	$C_{28}H_{36}O_3$	3.68	Morikawa *et al.*, 2003;
13	423.0914 423.0920 423.0926 423.0925	Sb4 Us4 Ub4 Ss5	Mangiferina	$C_{19}H_{18}O_{11}$	3.34 1.92 0.50 0.74	Karunanayake, 1984;
14	425.0781 425.0779 425.0784 425.0776	Ss5 Sb5 Sb3 Us5	Kotalanol	$C_{12}H_{24}O_{12}S_2$	1.69 2.16 0.99 2.87	Yoshikawa *et al.*, 1997;

	425.0796 425.0767 425.0768	Ss4 Ub4 Ub3			1.85 4.99 4.76	
15	441.3026 441.3009	Sb5 Ss4	Regeol A	$C_{28}H_{40}O_4$	4.63 0.77	Wang *et al.*, 2011;
16	455.3519	Sb1	Wilforlida/Regelida	$C_{30}H_{46}O_3$	1.53	Wang *et al.*, 2011;
17	459.3834	Sb3	Salasone C, D e E	$C_{30}H_{50}O_3$	2.10	Matsuda *et al.*, 2005;
18	463.2851 463.2849 463.2839	Sb3 Ss4 Ub5	Netzaualcoiene	$C_{30}H_{38}O_4$	0.41 0.02 2.18	Nakamura *et al.*, 2011;
19	471.3485	Sb2	Demetilregelina	$C_{30}H_{46}O_4$	2.10	Kawazoe *et al.*, 1997;
20	505.1764	Sb3	Rafinose	$C_{18}H_{32}O_{16}$	1.06	Chawla *et al.*, 2013;
21	507.2792	Sb3	Foliasalacioside E1	$C_{24}H_{42}O_{11}$	2.79	Nakamura *et al.*, 2011;
22	516.3830	Sb1	Kotalagenina-16-acetato	$C_{32}H_{51}O_5$	2.81	Chawla *et al*., 2013; Matsuda *et al.*, 2005;
23	595.2547	Sb3	Salasol B	$C_{33}H_{38}O_{10}$	0.51	Chawla *et al.*, 2013;

Extratos analisados no modo negativo ESI (-): Ultrassom (U); Sob refluxo (S); caule (s); casca do caule (b); hexano (1); clorofórmio (2); acetato de etila (3); etanol (4); e etanol/água (5);

24	169.0128	Us5	Ácido gálico	$C_7H_6O_5$	4.84	Gao *et al.*, 2008;
25	181.0706	Ss4	Dulcitol	$C_6H_{14}O_6$	2.96	Wang *et al.*, 2011;
26	193.0444	Ss5	Ginuraona	$C_{10}H_{10}O_4$	2.62	Wang *et al.*, 2011;
2	197.0450	Ss5	Ácido lambértico	$C_9H_{10}O_5$	2.70	Matsuda *et al.*, 2005;
27	255.2320	Ss5	Ácido palmítico	$C_{16}H_{32}O_6$	3.90	Duarte *et al.*, 2010;
4	289.0725	Ss5	Epicatequina Ou Catequina*	$C_{15}H_{14}O_6$	1.0	Koga *et al.*, 2013;
28	341.1230	Ss5	Coniferin	$C_{16}H_{22}O_8$	3.60	Nakamura *et al.*, 2011;
29	373.1498	Sb3	Siringin	$C_{17}H_{24}O_9$	0.36	Nakamura *et al.*, 2011;
10	389.2169	Sb4	Foliasalacioside J	$C_{19}H_{34}O_8$	1.46	Zhang *et al.*, 2008;
12	419.2580 419.2574	Sb4 Ub3	Tingenina/Maintenina	$C_{28}H_{36}O_3$	1.30 2.72	Morikawa *et al.*, 2003;
30	435.2531	Sb4	*β*-Hidroxitingenona	$C_{28}H_{36}O_4$	0.82	Matsuda *et al.*, 2005;
31	465.1540 465.1553 465.1555	Sb5 Ub5 Ub3	Lehmbachol D	$C_{26}H_{26}O_8$	1.86 0.93 1.36	Kawazoe *et al.*, 1997;
20	503.1580	Sb4	Rafinose	$C_{18}H_{32}O_{16}$	1.06	Chawla *et al.*, 2013;
32	591.1510 591.1493 591.1536 591.1586 591.1495	Sb4 Sb3 Ss5 Ub4 Ub3	Proantocianidina	$C_{31}H_{28}O_{12}$	1.39 1.48 0.80 0.88 1.14	Nakamura *et al.*, 2011;

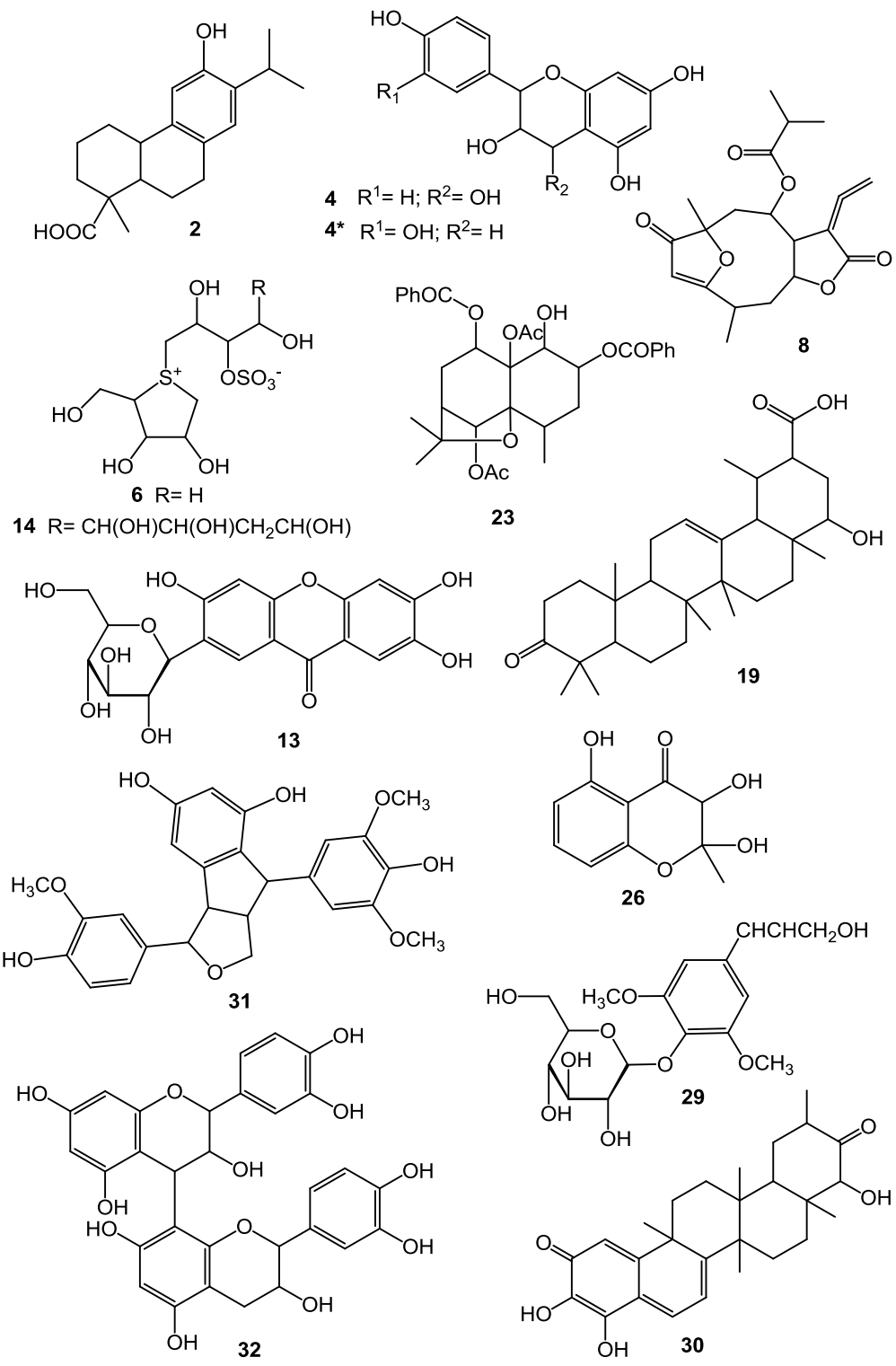

Figura 6. Principais compostos químicos identificados nos extratos de *Salacia impressifolia* apresentados na Tabela 1.

Destes compostos, destacam-se Salacinol (**6**), Kotalanol (**14**) e Mangiferina (**13**), os quais têm sido descritos na literatura como sendo agentes hipoglicemiantes (Matsuda *et al*, 2005). Para confirmar o potencial uso no tratamento da diabetes, o extrato hidroalcoólico de *S. impressifolia* foi escolhido para o teste *in vivo* devido à presença destes três compostos neste extrato (Tabela 1).

O ensaio *in vivo* foi realizado de acordo com o modelo experimental de indução à diabetes por administração de estreptozotocina em ratos. Para estimativa da dose a ser administrada, foi realizado teste piloto com as doses de 100, 200 e 400 mg/kg, sendo que a dose de 200 mg/kg foi a mais efetiva. Na etapa seguinte, o experimento foi realizado com 4 grupos de 5 ratos cada. Antes do experimento, todos os ratos passaram pelo teste de tolerância à glicose para avaliação de possível resistência à insulina. Após, foi coletado sangue por punção caudal de cada animal antes da indução a diabetes para avaliação inicial do perfil lipídico (triglicerídeos, HDL, colesterol total) e perfil hepático (TGO e TGP). Os grupos foram constituídos da seguinte forma: G1 = grupo não diabético, G2 = diabético tratado com glibenclamida (0,7 mg/kg), G3 = grupo diabético tratado com extrato (200 mg/kg) e G4 = grupo diabético não tratado. Com duração de 28 dias, a glicemia foi medida em jejum semanalmente e, ao final do período, foi coletado novamente amostra de sangue da cauda para avaliação dos parâmetros bioquímicos. Ao final do experimento, os ratos foram eutanasiados por decapitação.

Após tratamento estatístico dos resultados, observamos que o extrato hidroalcoólico de *S. impressifolia* teve um efeito similar ao fármaco de referência glibenclamida (Figura 6). Não houve diferença estatística significativa ($p>0.999$) entre os grupos 2 e 3.

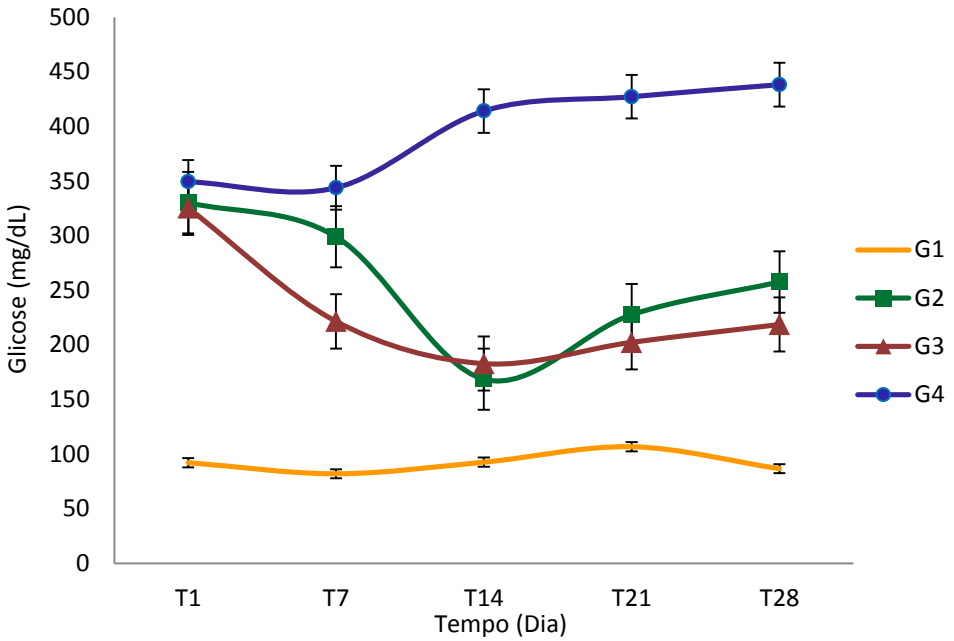

Figura 6. Efeito do extrato hidroalcoólico do caule de *S. impressifolia* (Ss5) em jejum sob o nível de glicose no sangue de ratos diabéticos tipo 2 (Média ± DP).

O efeito hipoglicemiante do extrato Ss5 pode ser atribuído principalmente à presença de salacinol, kotalanol, e mangiferina. Salacinol e kotalanol, que ocorrem naturalmente em *Salacia spp.*, tais como *S. reticulata, S. oblonga,* e *S. chinensis*, foram identificados como inibidores potentes da α-glicosidase, mostrando melhor atividade do que a acarbose (fármaco utilizado clinicamente como inibidor desta enzima) (Karunanayake *et al.*, 1984; Matsuda *et al.*, 2005).

O efeito antiglicêmico da mangiferin foi descrito por Sellamuthu e colaboradores (2009). Previamente isolada do extrato metanólico da raiz de *Salacia chinensis*, a mangiferina apresentou efeitos protetores contra danos de células β-pancreáticas e sobre os sistemas de defesa antioxidante em ratos diabéticos induzidos por estreptozotocina (STZ). Ainda de acordo com o mesmo autor, a administração oral de mangiferina em ratos diabéticos diminuiu significativamente o nível de glicose no sangue e aumentou os níveis

de insulina (Sellamuthu *et al.*, 2009). Desta forma, o efeito hipoglicêmico do extrato deve-se ao sinergismo dos compostos apresentados.

5. CONCLUSÕES

- A extração em ordem decrescente de polaridade foi eficiente na separação dos diferentes compostos da planta, tendo em vista que cada extrato apresentou um perfil diferente, o que foi confirmado através da análise de espectrometria de massas de alta resolução (EMRS);
- A EMRS mostrou-se uma ferramenta eficiente para a identificação de compostos químicos em extratos de *S. impressifolia*. A partir dos resultados foram identificados 32 compostos previamente descritos para outras espécies de *Salacia*, porém nunca antes descritos para *Salacia impressifolia*. Entre os compostos identificados, destacam-se a mangiferina, o salacinol e o kotalanol;
- No ensaio *in vivo* observou-se que não houve diferença significativa ($p>0.999$) entre o grupo que recebeu o extrato (G3) e o grupo que recebeu o fármaco de referência glibenclamida (G2), evidenciando o efeito antiglicêmico do extrato hidroalcoólico na dose de 200mg/kg. Este pode estar relacionado com um sinergismo dos compostos químicos presentes;
- Quanto aos parâmetros bioquímicos, o extrato não alterou os níveis de colesterol total do grupo tratado, também não sendo capaz de controlar os níveis de triglicerídeos neste grupo (G3), diferente do fármaco controle (G2) onde se observou a diminuição dos níveis do mesmo. O extrato, assim como a glibenclamida, possibilitou o aumento dos níveis de HDL ao longo dos 28 dias de tratamento. O extrato não causou aumento da enzima TGP no grupo tratado (G3), evidenciando uma possível diminuição no dano hepático causado. Ainda, o extrato, assim como a glibenclamida, foi capaz de inibir a perda de peso nos ratos tratados.

- Dessa forma, com esse estudo concluímos que *S. impressifolia* possui compostos químicos similares a outras espécies do mesmo gênero. Ainda, que o extrato hidroalcoólico possui atividade hipoglicêmica e uma possível atividade anti-aterosclerótica. Também há um possível efeito hepatoprotetor e reversor de perda muscular em pacientes diabéticos, entretanto, estes devem ser investigados e confirmados com novos estudos.

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ANEXO 1

Artigo de revisão:

Genus *Salacia*: chemical composition, antidiabetic effect and other bioactivities.

Artigo enviado para o

Current Medicinal Chemistry

Autores: Manuela M. Laikowski, Débora M. de Souza, Sidnei Moura

Genus *Salacia*: chemical composition, antidiabetic effect and other bioactivities

Manuela M. Laikowski, Débora M. de Souza, Sidnei Moura*

Laboratory of Natural and Synthetics Products, University of Caxias do Sul, Caxias do Sul, Brazil

*__Corrrespondence:__ Prof. Dr. Sidnei Moura, Technology Department, Biotechnology Institute, University of Caxias do Sul, 1130 Francisco Getúlio Vargas st., CEP 95070-560, Caxias do Sul, Brazil.

Phone: + 55 54 3218 2100

E-mail addresses: sidnei.moura@ucs.br

Abstract

The plants of *Salacia* genus (Celastraceae) have distribution in tropical areas, especially in regions of Asia as in India, Sri Lanka, Thailand and Indonesia, and in South America, as in Brazil. Around the world, about 120 species are known. Salacia`s species are known to elaborate secondary metabolites like anthocyanidines, catechins, phenolic acids, quinones, friedo-oleanones, quinonemethide and related triterpenoids (celastroloids). Biological activities have been reported to crude extracts, fractions and isolated compounds, which are the main antidiabetic, antioxidant, anti-inflammatory. This review aims to identify key species, their chemical components, and the reported bioactivity as a guide to future research about this genus.

Keywords: Salacia genus, diabetes, phytochemistry, biological actions.

Introduction

The use of plants as medicament to diseases treatment and cure is the oldest form of medicinal practice [1]. For centuries, many medicinal plants have been used all over the world, sometimes through unknown mechanisms. The number of herbal used in medicine has increased every year, and the phytotherapeutic market trades billions of dollars annually [2]. Moreover, most compounds present in plants may cause serious side effects. Therefore, the correct separation and identification of compounds is crucial, making the use of active medicinal plants safe.

The *Salacia* genus plants (Celastraceae) are climbing shrubs, distributed in tropical regions in countries like Thailand, Indonesia, India, Sri Lanka in Asia and Brazil in South America. About 120 species are known [3]. The macroscopical characteristics of the genus are scandent or sarmentosa shrub or small tree [4-6].

Approximately 120 species are known, and more than 200 different chemical compounds were identified as secondary metabolites. The main classes are the terpenes and derivatives, and phenolic compounds, which are responsible for the plant bioactivity.

This review aims to identify key species, their chemical components and the main herbal medicine action reported as a guide to future research on the Salacia genus.

Chemical composition

All organisms need to interconvert a large number of organic compounds to live, grow and reproduce. Despite the extremely varied characteristics of plants, the way of general modification and synthesis of carbohydrates, proteins, fats and

nucleic acids is almost the same. These processes demonstrate the fundamental unity, and are collectively described as primary metabolism [7]. In contrast, there is also an area of metabolism that refers to compounds which has a limited distribution in nature. The molecules, called secondary metabolites, are found only in specific organisms or groups, being essential to plants survival due its innumerable functions as defense or attract agents, for example [7-9].

Salacia species are known to elaborate secondary metabolites like anthocyanidines, catechins, phenolic acids, quinones, friedo-oleanones, quinonemethide and related triterpenoids (celastroloids), as summarized in Table 1.

Table 1: Some compounds already isolated from Salacia genus.

Species	Isolated Substance	Reference
S. amplifolia	Pristimerin; Dibutyl phthalate; Wilforlide A; Wilforlide B; *β*-amyrin; Regeol A; Netzahualcoyene;	[10-14].
S. campestris	Maytenin; Pristimerin; 3-friedelanol; Friedelin; Celastrol; Netzahualcoyene; Salacin; Wilforine;	[10], [15-16].
S. chinensis	Salacinol; Leucoplargonidin; Salasone A, B; Salasone C, D, E; Salasol A; Salasol B; Salaquinone A; Salaquinone B; Proanthocyanidin; Mangiferin; 3-friedelanol; Betulinic acid; Coniferin; Syringin; Foliachinenoside E, F; Foliachinenoside G; Foliachinenoside H; Foliachinenoside I; Foliasalacioside J; Tingenin B; Salaspermic acid;	[17-20].
S. oblonga	Salacinol; Kotanolol; Kotalagenin 16 acetate; Mangiferin; Lambertic acid; Mangiferin; Kotanolol;	[3], [21].
S. reticulata	Epicatechin; Salacinol; Sitosterol; Pristimerin; Maytenfolic acid; Iguesterin; Isoigesterol; Epigalocatechina; Epicatechina/ Catechina; Netzahualcoyene; Kotalagenin16 acetate; Neokotalanol; Ponkoranol; Salacianone; Salacianol; Kokoonol;	[22-30].
S. elliptica	Dulcitol; Palmitic acid; Mangiferin; Sitosterol	[31]
S. hainanensis	Friedelin; β- sitosterol; Ursolic acid; Mangiferin;	[32]
S. Krausii	Celastrol; Isoigesterol; Pristimerin;	[33]
S. macrosperma	Tingenone; Pristimerin; Salaspermic acid;	[34]

S. petenensis	Tingenone; Netzahualcoyonol; Friedelanol;	[35]
S. verrucosa	Kokoonol; Friedelanol; 21*α*-hydroxyfriedelane-1,3-dione;	[36]
S. prinoides	Friedelin; Friedelane-1,3-dione	[37]

The common biomarkers are the mangiferin and the thiosugars, salacinol and kotalanol [38]. Also, some sesquiterpene, pyridines, alkaloids, friedelans and quinones, are restricted to this family [39]. The mains classes of compounds present in Salacia genus are illustrated as follows.

Sesquiterpenes

A large number of highly oxygenated sesquiterpenes have been isolated from Celastraceae family [40]. Some of these have shown bioactivity as, for instance, antitumor [41] and immunosuppressive [42], including the celahin C (1), salasol B (2) and salasol A (3), Figure 1, which were carried-out from *S. chinensis* by Yoshikawa, 2003 [18].

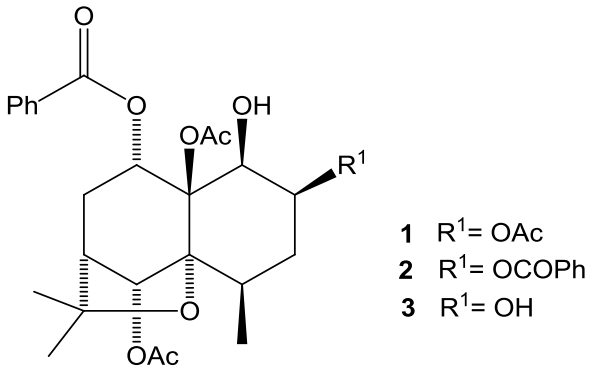


Figure 1. Sesquiterpenes isolated from Salacia genus: celahin C (1), salasol B (2), salasol A (3).

Triterpenes

Meanwhile, there are also triterpenes related in the genus [43]. Because its biological activity related, some of triterpenes has been considered as candidates or prototypes of new drugs [7]. The Figure 2 shows some triterpenes isolated from Salacia genus, as the Kokoonol (**4**), carried-out from *S. oblonga* by Matsuda et coworkers (1999) [21], Figure 2. As well as, the Tingenone (**5**), a triterpene isolated from *S. macrosperma* roots [34], has showed activity against *Trypanosoma cruzi* [44]; *Giardia intestinalis* [45]; and tubulin protein inhibition, which can justify cytotoxic and antitumor activity of the compound [46].

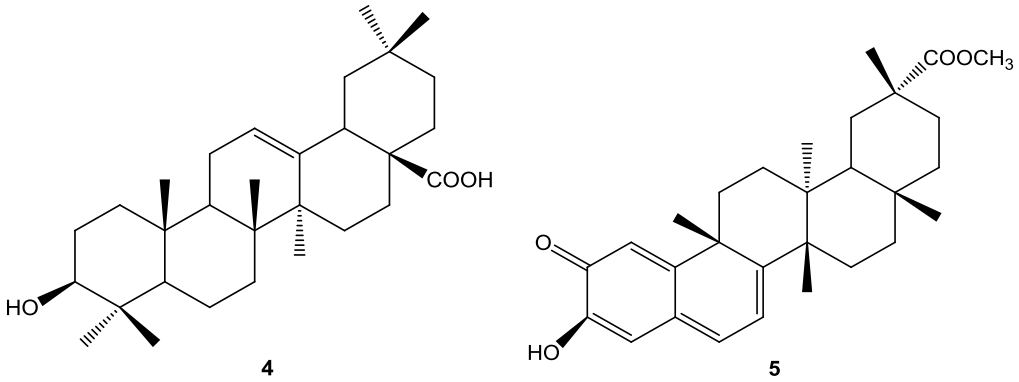


Figure 2. Triterpenes isolated from Salacia genus: Kokoonol (4) and Tingenone (5).

Phenolic compounds

The phenolic are defined as substances which have aromatic ring with one or more hydroxyl substituents, including its functional groups [47]. They are responsible for the color, astringency, aroma and oxidative stability, [48]. Naturally occurring conjugated as mono-and polysaccharides, and may also occur as functional derivatives such as esters and methyl esters. There are about 8.000 different phenolic compounds which, according to their chemical structure, are divided into classes: phenolic acids, flavonoids, stilbenes and tannins [49].

In Salacia genus, some flavonoids are founded as catechin (**6**) isolated by Yoshikawa and coworkers (2001) [26] from *S. reticulata* and Mangiferin (**7**) carried-out by Sellamuthu (2013) [50] from *S. chinensis*, Figure 3. These molecules are knows as antioxidant action demonstrating high inhibition of cell proliferation [51], [52].

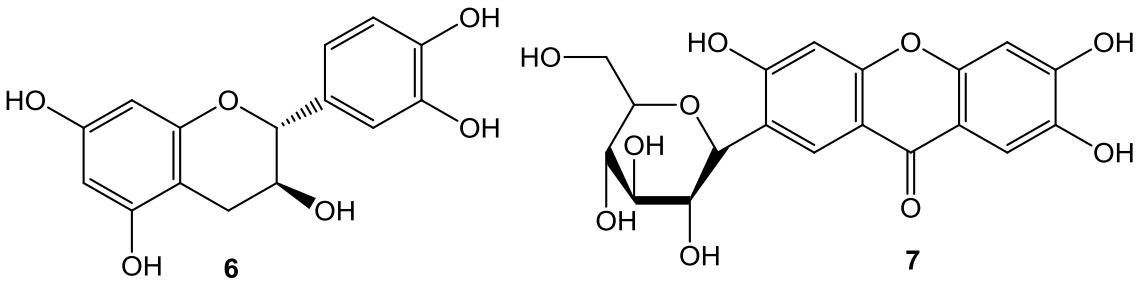


Figure 3. Flavanoids compounds isolated from Salacia genus: Catechin (6), and Mangiferin (7).

Bioactivities

Medicinal plants play an important role in maintaining public health, mainly due to their low cost and availability. Many Salacia's species have been used in folk and also in Ayurvedic medicine, against various metabolic diseases, such as diabetes, obesity, anti-inflamatory, depurative, liver tonic, thermogenic, etc [54]. A resume of the pharmacological activities discovered so far are described in Table 2.

Table 2. Resume of species and pharmacological potential uses.

Species	Potential use	Reference
S. prenoides	Antidiabetic	[55]
S. reticulata	Antidiabetic; Inhibitory effects against sucrose (Kotalanol);	[3], [22-23],

	Inhibitory effects egainst α-glucosidase; Decrease serum triglycerides; Inhibitory effect against α-amilase; Inhibitory effect against sucrose and maltose; Anti-Inflammatory; Antimicrobial; Immunostimulator; Antiobesity	[26], [56-59], [60-69].
S. macrosperma	Antidiabetic, Antimicrobial	[70]
S. oblonga	Antidiabetic ; Inhibitory effects against α -glucosidase and aldose-reductase, Reduced plasma glucose and serum insulin, Inhibition of excess cardiac lipid accumulation in diabetes and obesity; Decrease plasma triglyceride and total cholesterol, Anti-Inflammatory; Antioxidant and Nephroprotective; Anti-peroxidative; Antitumor	[21], [71-74], [75-83].
S. chinensis	Antidiabetic, Inhibitory effects against α-glucosidase and aldose-reductase; Antimutagenic; Nephroprotective	[18], [14], [84-85].
S. hainanensis	Antidiabetic, Inhibitory effects against α-glucosidase	[86-87].
S. kraussii	Antimalarial	[33].
S. beddomei	Antibacterial; Insecticidal and Antifeedant	[88-89].
S. microsperma	Antimicrobial	[91].
S. longipes	Antiplasmodial	[92].
S. leptoclada	Antiplasmodial	[93].
S. madagascariensis	Antitumor	[94].

Antidiabetic activity

Between the proved activities for Salacia genus, the antidiabetic is the main. Several authors have been conducted experiments with this plant species in order to evaluate this feature [54]. In the first reported, Pillai and coworkers in 1979, have demonstrated the hypoglycemic activity of root bark of *Salacia prenoides* against alloxan induced diabetes in rats proving its potential as antidiabetic plant [55].

After, an experiment with the aqueous decoction of 40 plants was conducted by Karunanayake and coworkers (1984). In order to investigate the

hypoglycemic activity were used Sprague-Dawley rats. Maximum reduction in blood glucose level (30%) was observed after 3 hours of *Salacia reticulata* administration, which persisted up to 5 hours suggesting its hypoglycemic potential [23].

In sequence, Serasinghe and coworkers (1990) have demonstrated the activity in streptozotocin induced diabetic rats. Reduction in plasma glucose levels were observed with 0.5, 1.0 and 5.0 g/kg doses of *Salacia reticulata* by 42.8, 45.4 and 87.5% respectively [56].

In 1993, Venkateswarlu and coworkers investigated the antidiabetic activity of *Salacia macrosperma* roots alcoholic extracts in alloxan-diabetic rats. A significant activity was showed by the methanolic and one of ethanolic fractions. The authors have associated the result to extract insulin-like properties [70].

From the petroleum ether extract of the *Salacia oblonga* root bark were carried-out two active fractions by column and thin layer chromatography [71]. A methanol eluted fraction showed 100% of cytotoxicity on Ehrlich ascites tumour cells. The chloroform eluted fraction had demonstrated about 60% and 76% hypoglycemic potency of an equal dose of tolbutamide (250 mg/kg) in albino rats.

The most potent natural *α*-glucosidase inhibitor named salacinol had been isolated from *S. reticulata* through bioassay-guided separation, by Yoshikawa and coworkers, in 1997 [96]. The chemical structure of salacinol (**8**), Figure 4, was determined by NMR and X-ray crystallographic analysis, and the molecular conformation showed the unique spirt-like configuration of the inner salt comprised of 1-deoxy-4- thioarabinofuranosyl cation and l'-deoxyerythrosyl-3'-sulfate anion.

After, those authors in 1998, isolated also the kotanalol (**9**) by fractionation which showed potent inhibitory activity against enzimes, Figure 4. The IC_{50} values were 2.8 µg/ml, 0.58 µg/ml and 1.9 µg/ml for maltase, sucrase and isomaltase respectively [25], [3].

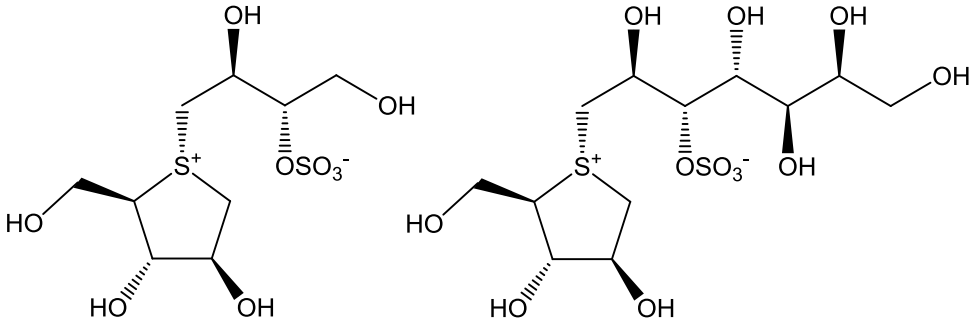
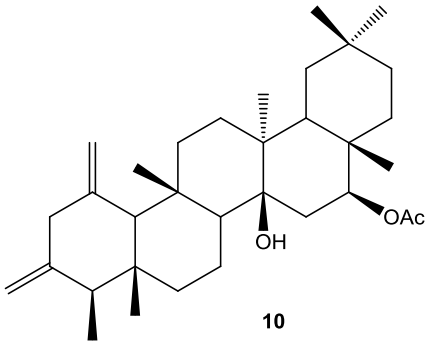


Figure 4. Thiosugar sulfonium sulfate structure.

In the same line, was investigated the effect of the aqueous extract of *S. reticulata* stems in rats and humans post prandial hyperglycemia. The authors reported a dose-dependent suppressed in serum glucose levels. In addition, the same extract strongly inhibited the activities of *α*-glucosidase prepared from the yeast and rat jejunum with IC_{50} of 5 and 8 µg/ml respectively. In the sucrose tolerance test, the aqueous extract (200 mg), given five minutes before sucrose loading (50 mg), significantly suppressed post prandial hyperglycemia in healthy human volunteers [57].

In an animal model (KK-Ay mice) of type 2 diabetes, mangiferin (**7**) Figure 3, lowered blood glucose level at a dose of 30 mg/kg p.o. for two weeks and significantly improved the insulin level, which concluded that mangiferin probably decrease blood sugar level through reducing insulin resistance [97].

Matsuda and coworkers, 1999, have evaluated the inhibitory activity of aqueous-methanolic extract of *Salacia oblonga* roots which increased serum glucose level in sucrose- and maltose-loaded rats. The water and ethyl acetate soluble portions from the extract showed inhibitory activities on *α*-glucosidase and aldose reductase respectively. From the water-soluble portion, potent *α*-glucosidase inhibitors, salacinol (**8**) and kotalanol (**9**), Figure 4 were isolated together with nine sugar related components. A new friedelane-type triterpene, kotalagenin 16-acetate (**10**), Figure 5, was isolated from the ethyl acetate-soluble portion. This compound had been examined as inhibitor of aldose reductase, and it was responsible for the inhibitory activity [21].



10

Figure 5. Kotalagenin 16-acetate (10) from *Salacia oblonga.*

Krishnakumar and coworkers, also in 1999, have studied the effect of petroleum ether extract of *S. oblonga* root bark (SOB) in streptozotocin (STZ) induced diabetic rats. The SOB prevented significantly the streptozotocin-induced hyperglycemia and hypoinsulinemia suggesting that this plant extract possesses anti-diabetic activity [73].

Kajimoto and coworkers (2000) have investigated the effect of the *S. reticulata* stem aqueous extract for the prevention of type 2 diabetes in a placebo

controlled cross-over trial. There was significant reduction in fasting plasma glucose level, glycosylated haemoglobin A ($HbA1_C$) and body mass index [59].

In the same year, Shimoda and coworkers (2000) have showed that an aqueous extract from *S. reticulata* when administered in diet (0.05 or 0.10%) for 3 weeks, lowered the serum triglycerides level which is attributed to decrease in sugar absorption, the main source of triglycerides *in vivo* [58].

Yoshikawa and coworkers, in 2001, have also studied the inhibitory effect of mangiferin against carbohydrate metabolizing enzymes, sucrase, maltase, isomaltase, *α*-amylase and aldose reductase and compared it with salacinol (**8**) and kotanolol (**9**). Mangiferin (**7**) inhibited *α*- glucosidase, sucrase, isomaltase and also aldose reductase activities which were not seen with kotanolol and salacinol. *S. reticulata* extract effectively inhibited α-amylase activity (derived from procaine pancreas) in a dose dependent with 68% inhibition at a concentration of 35 µg/ml [26].

Williams and coworkers, also in this year, have studied the *S. oblonga* extract (240 and 480 mg) effects on postprandial glycemia and insulinemia in patients with type 2 diabetes after ingestion of a high carbohydrate meal. Both doses of the extract significantly lowered the postprandial positive area under the glucose curve (14% and 22% for the 240 and 480 mg respectively) and the adjusted peak glucose response (19% for the lower dose and 27% for the higher dose) compared to the control meal. The results suggested that *S. oblonga* may be beneficial for postprandial glucose control [79].

Yoshikawa and coworkers, in 2002 discovered that a water soluble fraction (25-100 mg/kg p.o.) prepared from the roots and stems of *S. reticulata* inhibited the elevation of serum glucose level after the administration of sucrose or maltose. In addition, the fraction inhibited rat intestinal maltose and sucrose with IC_{50} value of 35 µg/ml and 26 µg/ml respectively. To confirm, was also performed a bioassay guided separation to isolate salacinol (**8**) which showed competitive inhibition of intestinal *α*-glucosidase *in vitro*. The IC_{50} were 3.20, 0.84, and 0.59 µg/ml for maltase, sucrase and isomaltase respectively. In comparison, the inhibitory action against maltase and sucrose was almost equal to that of acarbose (clinically used α-glucosidase inhibitor), however stronger than acarbose against isomaltase. In addition, the salacinol inhibitory effect on serum glucose levels in maltose and sucrose loaded rats were also more efficient that acarbose [22].

The syntheses of two selenium analogues of salacinol (**8**), are described in 2002 by Johnston and coworkers, Figure 6. With the new compounds, the authors tested glucoamylase, barley-*α*-amylase and porcine pancreatic-*α*-amylase inhibition. Was reported that the selenium analogue A (**12**) had a better performance of glucoamylase inhibition than salacinol (Ki 1.7 mM). In contrast, selenium analogue B (**13**) showed no significant inhibition of glucoamylase. Both showed no significant inhibition of barley-R-amylase and porcine pancreatic-R-amylase [98].

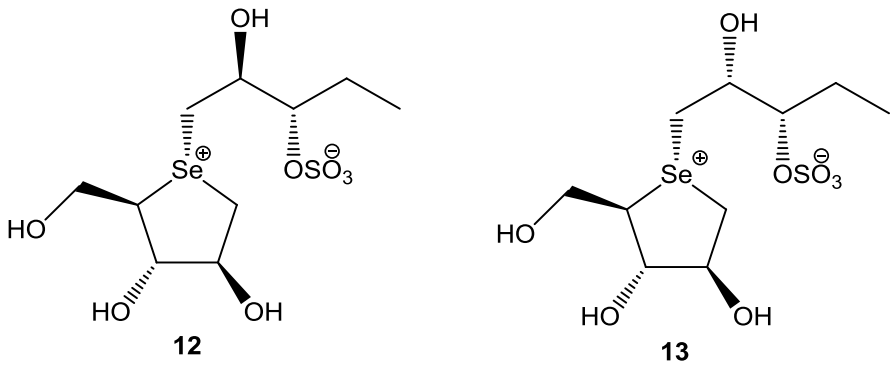
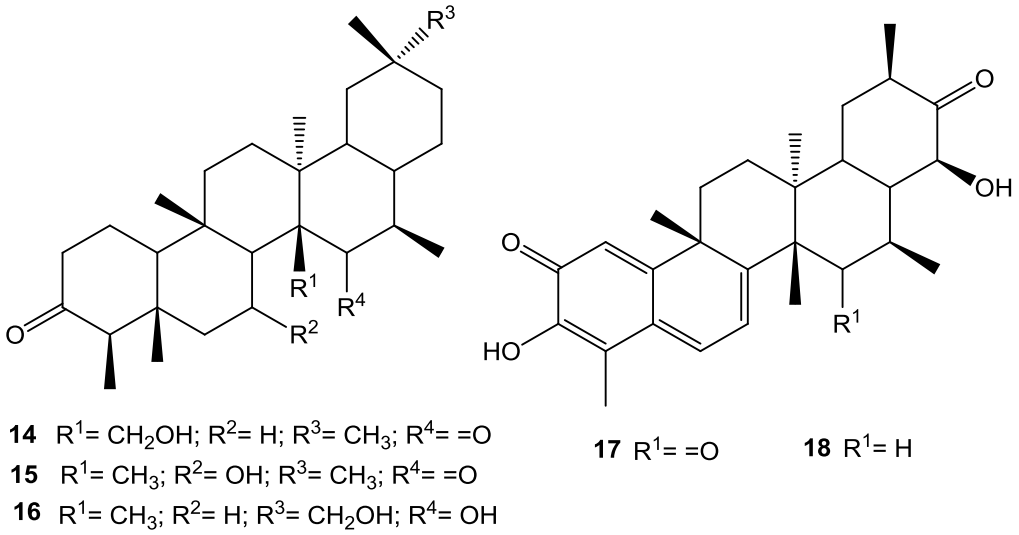


Figure 6. Salacinol selenium analogues.

Yoshikawa and coworkers (2003) have studied the antidiabetogenic activity of *S. chinensis* stems methanolic extract, which showed potent effects in oral sucrose or maltose-loaded rats, inhibitory effects on intestinal α-glucosidase and rat aldose reductase [18]. Aldose reductase (AR), a cytosolic, monomeric oxidoreductase, is a key enzyme in the polyol pathway which controls the conversion of glucose to sorbitol [99].

Morikawa and coworkers (2003) have showed that three new friedelane-type triterpenes named salasones A (**14**), B (**15**), and C (**16**), a new norfriedelane-type triterpene, salaquinone A (**17**), Figure 7, and a new acylated eudesmane-type sesquiterpene, salasol A (**3**), Figure 1, were isolated from the 80% aqueous methanolic extract of *Salacia chinensis* stems. In addition, six constituents, 3*β*,22*β*-dihydroxyolean-12-en-29-oic acid, tingenone, tingenine B (**18**), regeol A, triptocalline A, and mangiferin (**7**), were found to show an inhibitory effect on rat lens aldose reductase, suggesting its antidiabetic potential [14].



14 R^1= CH_2OH; R^2= H; R^3= CH_3; R^4= =O

15 R^1= CH_3; R^2= OH; R^3= CH_3; R^4= =O

16 R^1= CH_3; R^2= H; R^3= CH_2OH; R^4= OH

17 R^1= =O **18** R^1= H

Figure 7. New friedelane-type triterpenes isolated from *S. chinensis*

Matsuura and coworkers (2004) have studied the inhibitory effects of sugar intestinal digestion and absorption from health teas which are used for diabetes controlling. 110 min was the duration of the inhibitory effect on the sucrose load of *S. oblonga* tea [75].

Also in 2004, Li and coworkers have studied the effect of *S. oblonga* water extract on cardiac fibrosis and hyperglycemia in a genetic model of type 2 diabetes, the obese Zucker rat (OZR). The extract chronic administration, markedly improved interstitial and perivascular fibrosis in the hearts of the OZR. It also reduced plasma glucose levels in non-fasted OZR, whereas it had little effect in the fasted animals, suggesting inhibition of postprandial hyperglycemia in type 2 diabetic animals, which might play a role in improvement of the cardiac complications of OZR. Extract dose-dependently inhibited the increase of plasma glucose in sucrose, but not in glucose-loaded mice. The extract demonstrated a strong inhibition of α-glucosidase activity *in vitro*, which is suggested to contribute to the improvement of postprandial hyperglycemia [100].

After, the same authors have evaluated antidiabetic and antiobesity activity on chronic oral administration of the water extract of *S. oblonga* root in zucker diabetic fatty (ZDF) rat. The extract lowered plasma triglyceride and total cholesterol levels, increased plasma high-density lipoprotein levels and reduced the liver contents of triglyceride, non-esterified fatty acids and the ratio of fatty droplets to total tissue. These findings suggest that *S. oblonga* extract functions as a PPAR-alpha activator, providing a potential mechanism for improvement of postprandial hyperlipidemia and hepatic steatosis in diabetes and obesity [78].

Jayawardena and coworkers (2005) conducted a randomized double blind clinical trial to investigate the effect of a tea containing *S. reticulata* in patients with type II Diabetes mellitus as assessed by glycosylated haemoglobin A ($HbA1_C$). The $HbA1_C$ at the end of drug treatment was significantly lower than after treatment with placebo (6.29 ± S.D. 1.02 versus 6.65 ± S.D. 1.04; P = 0.008). A statistically significant fall in HBA1c was seen with the active drug compared to a rise in $HbA1_C$ with the placebo group (0.54 ± S.D. 0.93) versus − 0.3 ± S.D. 1.05; P < 0.001. The daily mean dose of Glibenclamide fell by 1.89 (S.D. 6.2) mg in the drug treated group but increased 2.25 mg in the placebo treated group (P = 0.07). The differences in the metformin dose were not significant in the two groups. The tea was effective treatment for type II Diabetes [60].

The effect of different doses of *S. oblonga* extract (0, 500, 700, or 1000 mg) on postprandial glycemic, insulinemic and breath hydrogen responses in healthy adults were studied by Heacock and coworkers. When compared with the control, the plant extract at 1000 mg reduced the plasma glucose and serum insulin (0 to

120 minutes postprandial) by 23% and 29% respectively. However, others doses did not have significant result [76].

In a similar study, Collene and coworkers (2005) have evaluated the postprandial glycemic, insulinemic and breath hydrogen responses to a liquid nutritional product containing *S. oblonga* extract (100 mg; SOE) and two insulinogenic amino acids, phenylalanine and leucine. The extract was a promising nutraceutical in glycemia control (decreasing in plasma glucose and insulin level) and breath hydrogen excretion was 60% greater in the SOE-containing meals. Supplementation with amino acids had no significant effect on glycemia [77].

Rabbani and coworkers (2006) showed that hydroalcoholic extract of *S. reticulata* at the dose of 500 mg/kg reduced significantly the serum glucose level when compared to the control group in hydrocortisone induced hypoglycemia model [61].

In 2008, Huang and coworkers have investigated the effect of the *Salacia oblonga* (SOE) water extract on obesity and diabetes-associated cardiac hypertrophy and discussed the modulation role on cardiac angiotensin II type 1 receptor (AT(1)) expression. The SOE treatment suppressed cardiac over expression of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), AT (1) mRNAs and protein in ZDF rats. SOE (50-100 µg/ml) and mangiferin (25 µmol) suppressed angiotensin II-induced ANP mRNA over expression and protein synthesis in H9c2 cells. They also inhibited angiotensin II-stimulated [3H] thymidine incorporation by cardiac fibroblasts which demonstrated that SOE decreases cardiac hypertrophy in ZDF rats at least in part by inhibiting cardiac AT(1) over expression [83].

Minami and coworkers (2008) demonstrated that desulfonated derivative of salacinol carried-out from the *S. oblonga* roots is a potent inhibitor of isomaltase with IC_{50} value of 0.64 mM [101].

A polyhydroxylated cyclic 13-membered sulfoxide (**19**) (Figure 8) was isolated from the *S reticulata* aqueous extract by Ozaki, Oe and Kitamura (2008) [63]. The compound *α*-glucosidase inhibitory activity (IC_{50}: maltase, 0.227 μM; sucrase, 0.186 μM; isomaltase, 0.099 μM) was significantly higher than the inhibitory activity of salacinol (**8**) and kotalanol (**9**), which were previously isolated from the same species. Also, the inhibitory activity was investigated by maltose and sucrose loading on Wistar rats. The study found significant lowering of postprandial glucose level, and the potency of the molecule was confirmed *in vivo* [102].

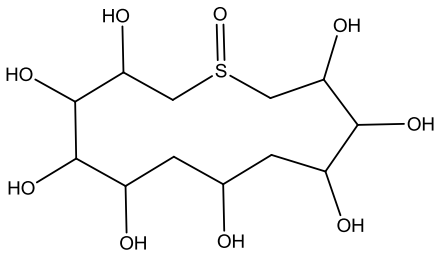


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Figure 8. Polyhydroxylated cyclic 13-membered sulfoxide from *S. reticulata.*

The effects of water extract of *S. reticulata* leaves on the absorption of sugars in normal and type 1 diabetic mice were investigated by Yoshino and coworkers in 2009. The simultaneous oral administration of the extract at a dose of 1.0 mg/mouse with maltose or sucrose inhibited the postprandial elevation of the

plasma glucose and insulin levels and intestinal *α*-glucosidase activities in mice. In addition, the supply of 0.01% solution of the extract in drinking water, prevented the plasma glucose level elevation and intestinal *α*-glucosidase activities in type 1 diabetic mice. This treatment also prevented the elevation of the plasma, pancreatic, and kidney lipid peroxide levels, lowering of the plasma insulin level, and elevation of the kidney aldose reductase activities in diabetic mice. According to the authors, these results suggested that *S. reticulata* extract could be a beneficial food material for the prevention of diabetes and obesity because of its multiple effects [64].

Also in 2009, Im and coworkers studied the mechanisms of blood glucose lowering effect of aqueous extract from *S. reticulata* stems (KTE) in mouse. DNA microarray and RT-PCR analyses (real time polymerase chain reaction) revealed that gluconeogenic fructose-1,6-bisphosphatase (FBP) was decreased compared with the control in KTE-treated KK-Ay mice. RT-PCR analysis using cultured liver cells treated with KTED (freeze-dried aqueous extract) and/or actinomycin D or cycloheximide, revealed that KTED directly decreased fructose-1,6-bisphosphatase (FBP) mRNA levels via destabilization of the mRNA. The authors have demonstrated the dose-dependently down-regulate FBP mRNA of mangiferin, carried-out from KTE. These findings suggested that the mangiferin in KTE acts directly on liver cells and down-regulates the gluconeogenic pathway through regulation of FBP expression, thereby decreasing fasting blood glucose levels in mice. Their results demonstrated that gluconeogenic gene regulation was one possible mechanism by which *Salacia reticulata* exerts its effects in traditional diabetic medicine [62].

In the same year, Sellamuthu and coworkers evaluated the antihyperglycemic potential of mangiferin purified from methanolic root extract of *S. chinensis* in streptozotocin (STZ)-induced diabetic rats. The treated animals had significantly decreased the glucose level, glycosylated hemoglobin as well as increased insulin and hemoglobin level. The activities of hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, glycogen synthase, and glycogen content level were increased to almost normal. The activities of lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-diphosphatase and glycogen phosphorylase were significantly decreased in liver tissue also. These results demonstrated that the compound has antidiabetic activity against STZ-induced rats. The mangiferin antidiabetic effect was compared with standard drug glibenclamide [103].

Radha and Amrithaveni (2009) have conducted an experiment with 60 type 2 diabetics patients, consisting of experimental group (30) and control group (30). Experimental group received 2 grams of *S. reticulata* powder daily for a period of 90 days and control group did not receive any supplements. Blood glucose levels before and after medications were estimated in both groups at baseline and at 90 days. However, there was no significant reduction in fasting blood glucose, $HbA1_c$ and lipid levels at the end of 90 days in the supplemented group [104].

An approach to controlling blood glucose levels in individuals with type 2 diabetes is to target R-amylases and intestinal glucosidases using R-glucosidase inhibitors acarbose and miglitol. An intestinal glucosidase targeted is the *N*-terminal catalytic domain of maltase-glucoamylase (ntMGAM), which is part of the set intestinal glycoside hydrolase. Sim and coworkers in 2010, presented the X-ray

crystallographic studies of ntMGAMin (N-terminal catalytic domain of maltase-glucoamylase (ntMGAM) complex) with a new class of *α*-glucosidase inhibitors derived from *S. reticulata* extracts. About the evaluated compounds, salacinol, kotalanol, and de-O-sulfonated kotalanol showed activity. The study revealed that de-O-sulfonated kotalanol is the most potent ntMGAM inhibitor reported (Ki= 0.03 μM) which is about 2000-fold better than the compounds currently used in the clinic, and highlights the potential of the salacinol class of inhibitors as future drug candidates [105].

Recently the hypoglycemic activity of *S. hainanensis* was carried out with roots extract by Huang and coworkers in 2012. By means of a bioassay-guided method, three new triterpenoids (2b,3b-dihydroxylup-20(29)-ene (**20**), 30-hydroxy-D:A-friedo-olean-1-en-3-one, and 24,25,26-trihydroxytirucall-7-en-3-one (**21**) along with three known compounds (olibanumol J (**22**), 21a-hydroxy-D:A-friedo-olean-3-one (**23**), and 29-hydroxy-D:A-friedo-olean-3-one (**24**), Figure were isolated from the EtOAc part and were shown to have effective *α*-glucosidase inhibitory activity. All molecules exhibited similar activity against *α*-glucosidase than did the positive control (acarbose, IC50= 1.02 mM) [86].

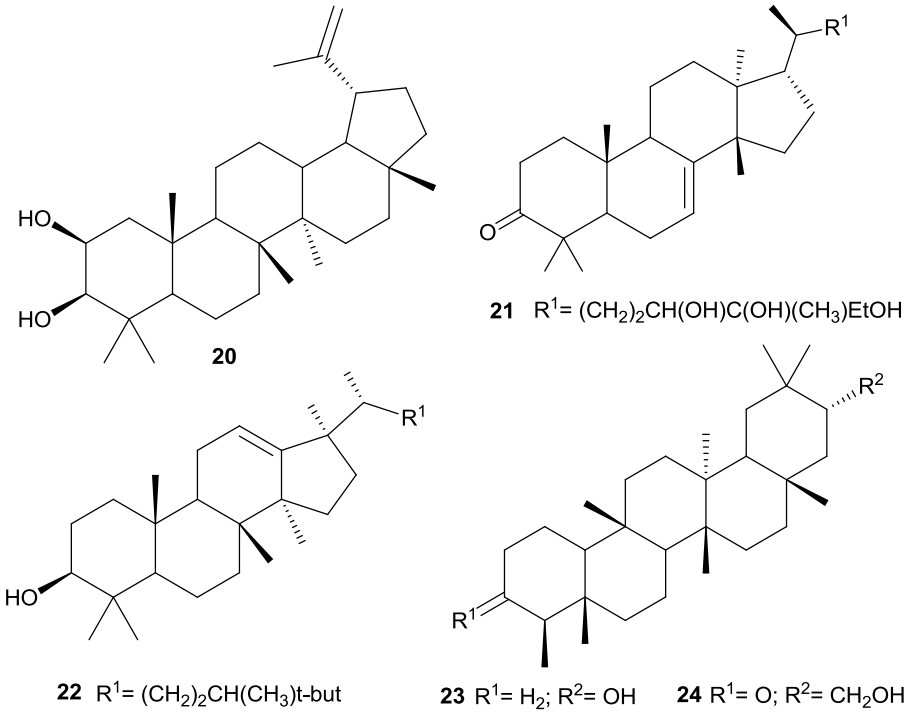

Figure 9. Compounds carried-out from *S. hainanensis*.

The three new compounds, 24,26-dihydroxy-25-methoxy-tirucall-9-en-3-one (**25**), 3b-hydroxy-2-carbonyl-lupan-29-oic acid (**26**) and 2b,3b,22a-trihydroxy-lup-20(29)-ene (**27**) (Figure 10) were carried out from *S. hainanensis* roots with others six known triterpenoids. Some of them resulted in higher inhibitory activity in control comparison (acarbose, IC_{50} 10.2 lM) towards α-glucosidase [87].

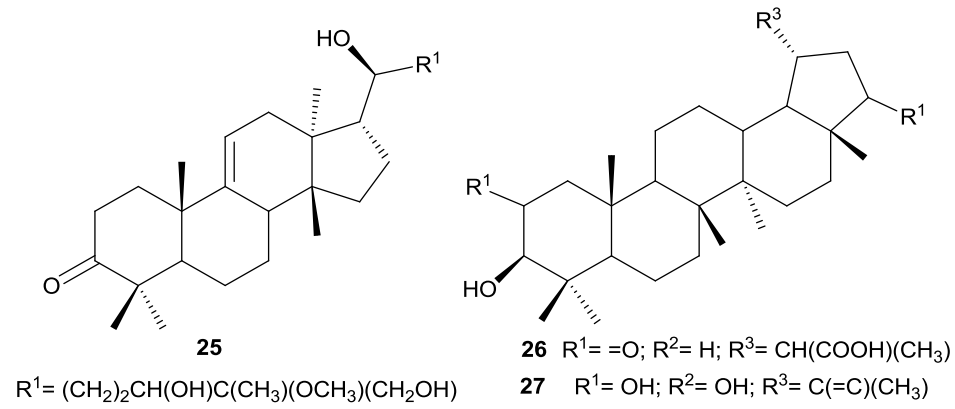

Figure 10. New *S. hainanensis* compounds.

Hypolipidemic and hypotriglyceridemic activity

A study was undertaken for evaluation of the hypoglycemic and hypolipidemic effects of an ayurvedic medicine “Rajanyamalakadi” containing *Curcuma longa*, *Emblica officinalis* and *Salacia oblonga* in type 2 diabetic patients over 3 months. The ongoing antidiabetic medications were stopped under medical supervision and the patients were provided with “Rajanyamalakadi” tablets (500 mg, 1-2 tablets). The ayurvedic medicine has showed significant antidiabetic, hypolipidemic and antioxidant effects [106].

The aqueous extract of *S. reticulata* and cyclodextrin (SRCD) mixture effects on several metabolic parameters and cecal fermentation in obese male Wistar rats, a model of type II diabetes mellitus, was examined [107]. The animals were fed 0% (control group) or 0.2% SRCD-supplemented diets and weighed weekly. The plasma glucose, triacylglycerol, total cholesterol, insulin, and adiponectin were measured at weeks 0, 2, 4, and 5. As a result, SRCD supplementation suppressed the time-dependent increase in the plasma total cholesterol and insulin concentrations. After 6 weeks of a 0.2% SRCD-supplemented diet, the body weight gain, food intake, visceral fat mass, liver mass, and liver triacylglycerol were significantly lower, whereas the plasma adiponectin concentrations were considerably higher than those of the control group. The SRCD had no significant effect on plasma glucose and triacylglycerol concentrations. However, there was a significantly increased cecum mass, whereas it significantly decreased the cecal butyrate and short-chain fatty acid (sum of the acetate, butyrate, and propionate) concentrations.

According to Nakata and coworkers (2011), lifestyle-related diseases are some of critical health issues worldwide. It was reported that lipopolysaccharide derived from a Gram-negative bacteria (IP-PA1) symbiotic with wheat exhibited several advantageous effects, such as the reduction of plasma glucose levels in non-obese diabetic (NOD) mice and low-density lipoprotein (LDL) levels in Watanabe heritable hyperlipidemic (WHHL) rabbits. The authors reported the beneficial effects on plasma glucose and lipids of a tea (SI tea, a combination of *Salacia oblonga*, fermented flour and vitamins) were investigated in the KK-Ay/TaJcl type 2 diabetic model mice and in human subjects with premetabolic syndrome in a randomized double-blind study. SI tea decreased plasma glucose levels in KK-Ay/TaJcl mice. A clinical trial of SI tea was performed with 41 subjects aged 40 and 69, who belonged either to a high plasma glucose group (HG: FPG 100-125 mg/dl) or to a hyperlipidemia group (HL: TG ≥ 150 mg/dl, or LDL ≥ 120 mg/dl, or HDL < 40 mg/dl). The patients ingested either *S. oblonga* without IP-PA1 (the control) or SI tea. Blood samples were collected at 0, 30, and 60 days after initiating SI tea treatment, and were measured for FPG, HbA1c, TG, LDL, and HDL. The results showed that SI tea reduced FPG and HbA1c more rapidly than the control in the HL group, and also significantly improved LDL and HDL levels in the HG group. Thus, SI tea may be helpful in preventing lifestyle-related diseases [108].

The effects of *S. oblonga* root (SOR) extract on lipid metabolism using laying hen, a unique animal model with a very high rate of triglyceride synthesis in the liver, were investigated [80]. The animals were treated with the layer ration containing 0%, 0.5%, or 1% SOR water extract for 4 weeks. Laying hens showed

higher fasted triglyceride concentrations (increased by 5-13 folds) in plasma, liver, skeletal muscle and heart than pullets. 1% SOR extract treatment inhibited body weight increase without affecting food intake. The treatment substantially attenuated hypertriglyceridemia and inhibited increases in triglyceride contents in the non-adipose tissues. However, SOR did not induce change in plasma glucose concentration. Moreover, did not alter all variables in pullets. The results demonstrated that SOR ameliorated a hypertriglyceridemia and excessive ectopic fat accumulation in laying hens and suggested that the triglyceride-lowering property is one of the primary effects, possibly via hepatic mechanisms [80].

To investigate the molecular mechanisms of *S. oblonga* root extract in the treatment of dietary-induced fatty liver, an experiment was conduct by Liu and coworkers (2013). Male rats were co-administered with fructose in drinking water and vehicle or the aqueous ethanolic extract (SOR) (by gavage, once daily) for 10 weeks. Biochemical variables were determined enzymatically or by ELISA. Gene expression was analyzed by Real-Time PCR and/or Western blot. SOR treatment (20mg/kg) diminished fructose-induced fatty liver indicated by decreases in excess triglyceride accumulation and the increased vacuolization and Oil Red O staining area in the livers of rats. Importantly, Hepatic gene expression profile revealed that SOR suppressed fructose-stimulated over expression of sterol regulatory element-binding protein (SREBP)-1/1cmRNA and nuclear protein. In accord, over expression of SREBP-1c-responsive genes, such as fatty acid synthase, acetyl-CoA carboxylase-1 and stearoyl-CoA desaturase-1, was also down regulated. In contrast, over expressed nuclear protein of carbohydrate response element binding protein and mRNA of its target gene liver pyruvate kinase were not altered.

Additionally, SOR also did not affect expression of peroxisome proliferator-activated receptor-gamma-and-alpha, as well as their target genes, such as carnitine palmitoyltransferase-1a, acyl-CoA oxidase and CD36. According to the authors, these results suggested that modulation of hepatic sterol regulatory element-binding protein-1c-mediated gene expression contributes to SOR-elicited improvement of fructose-induced fatty liver in rats [109].

Anti-peroxidative activity

Hypoglycaemic activity of the root bark of *S. oblonga* (SOB) petroleum ether extract was studied in streptozotocin hyperglycaemic rats. In addition, the anti-lipid peroxidative activity of the same extract was also studied. The extract showed significant hypoglycemia ($p<0.001$), which was supported by an insulin assay. A detailed biochemical study (thiobarbituric acid reactive substances, hydroperoxides, conjugated dienes, glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) in the renal tissue of diabetic animals treated with SOB demonstrated promising anti-lipid peroxidative activity. These results suggested that *S. oblonga* root bark possesses anti-diabetic and anti-peroxidative principles, and may be used in the diabetes treatment associated with renal complications [74].

Antiobesity activity

Researchers attempted to clarify the antiobesity mechanism and the safety of *S. reticulata in vivo* and *in vitro*. They gave ordinary feed, alone or mixed with *S. reticulata* (0.3 or 1.0%), to Tsumura Suzuki Obesity Diabetes (TSOD) mice

(spontaneous obese type II diabetes model mice) and Tsumura Suzuki non-obese (TSNO) mice (the corresponding reference animals), ad- libitum for 2 months. As compared with the TSNO control mice, the TSOD control mice became obese due to fat accumulation and developed various signs of metabolic diseases. The TSOD mouse group receiving the plant showed suppression of body weight increase and fat accumulation, alleviation of abnormal lipid metabolism and abnormal glucose tolerance, and suppression of intrahepatic fat accumulation, and also prevented the mesenteric adipocyte hypertrophy recognized in TSOD mice. In the TSNO controls, the feed containing 1.0% *S. reticulata* exerted a suppressing effect on body weight increase and fat accumulation, but the feed containing 0.3% did not show any effect at all. In an *in vitro* experiment using mouse-derived adipocyte precursor 3T3-L1 cells, *S. reticulata* significantly suppressed fat accumulation in the differentiation induction phase and maturation phase. This suggested that the metabolic disease-preventing effects, including the antiobesity, may involve suppression of differentiation and accumulation in the adipocytes [68].

A study was performed to elucidate the mechanism of action of *S. reticulata* with special attention to the adipocytes as the tissue primarily involved in the pathology of metabolic diseases. Mouse-derived adipocyte precursor 3T3-L1 cells were treated with differentiation inducers in the presence or absence of the extract (SRCD). The researchers determined triacylglycerol accumulations, differentiation makers, released glycerol and adiponectin. Mangiferin, the primary component of SRCD, was also used to treat 3T3-L1 cells. Concurrent administration of SRCD extract and differentiation inducers resulted in a significant inhibition of differentiation into mature adipocytes. SRCD also exhibited significant inhibitory

action on the expression of genes and proteins of peroxisome proliferator-activated receptor (PPAR)γ and CCAAT-enhancer binding protein (C/EBP)α, as well as on the activity of glycerol-3-phosphate dehydrogenase (GPDH), a differentiation marker, and caused a reduction in the concentration of released adiponectin. However, SRCD had no influence on lipolysis as indicated by the release of glycerol into the culture medium. Mangiferin (**7**) was investigated for its effect on adipocytes; the compound caused no suppression of fat accumulation, suggesting that a component of SRCD, other than mangiferin, may be involved in the inhibition of adipocyte differentiation. The results suggest that the inhibitory action of SRCD on adipocyte differentiation, and not the promotion of lipolysis, is involved in the suppression of fat accumulation [69].

Anti-inflammatory activity

The anti-inflammatory activity of *S. oblonga* root bark powder and *Azima tetracantha* leaf powder were assayed in male albino rats using carrageenan-induced rat paw edema (acute inflammation) and cotton pellet granuloma (chronic inflammation) methods. Both the crude drugs were maximally active at a dose of 1000 mg/kg. In the cotton pellet granuloma assay, these drugs were able to suppress the transudative, exudative and proliferative components of chronic inflammation. Furthermore, were able to lower the lipid peroxide content of exudate and liver, γ-glutamyl transpeptidase activity in the exudate of cotton pellet granuloma. The increased acid and alkaline phosphatase activity and decreased serum albumin in cotton pellet granulomatous rats were normalized after treatment with these drugs. It is likely that these drugs may exert their activity by antiproliferative, antioxidative and lysosomal membrane stabilization [72].

Sekiguchi and coworkers (2010) have investigated both *in vivo* and *in vitro*, whether the leaf of *S. reticulata* (SRL) could ameliorate collagen antibody-induced arthritis (CAIA) in mice as the rheumatoid arthritis (RA) model. The mice were fed lard containing chow diet (AIN-93G) or the same diet containing 1% (w/w) SRL powder. All mice were bred for 23 days. On day 7 or 14 after lipopolysaccharide injection, mice were sacrificed, and tissue and blood samples were collected. Histological analysis was performed, and serum levels of inflammatory mediators and the mRNA levels of inflammation- related genes and osteoclast-related genes were measured. SRL treatment ameliorated the rapid initial paw swelling, inflammatory cells infiltration, skeletal tissues damage, osteoclast activation and the mRNA levels for osteoclast-related genes compared with the CAIA mice. However, the serum and mRNA levels of inflammatory mediators did not differ between the CAIA mice and the SRL-treated mice. SRL might reduce the inflammatory cells induction and skeletal tissue degradation by CAIA by the regulating osteoclastogenesis [65].

Antioxidant and nephroprotective activity

The effect of *S. chinensis* on stabilization of renal functions, and markers of endothelial dysfunction in diabetic chronic kidney disease patients were tested [85]. Thirty stable diabetic with chronic kidney disease patients were randomized into 2 groups; group A and B of 15 patients each. Group A was given trial drug *S. chinensis* 1000 mg twice-daily while group B received placebo. Rating of renal function was done, like serum creatinine and creatinine clearance, as well as markers of endothelial dysfunction as Interleukin-6 and serum Homocysteine. The lipid profile was evaluated at baseline and during follow-up period of 6 months.

There was stabilization of renal function as measured by serum creatinine and creatinine clearance in patients who received *S. chinensis* compared to placebo (p value < 0.05), suggesting that the plant may retard the progression of chronic kidney disease. Similarly, there was significant decline in both serum homocysteine and IL-6 levels (p value < 0.05 for both).

The investigation of the nephroprotective and antioxidant activity of the *S. oblonga* ethanolic extract was studied with two dose levels (250 and 500 mg/kg rats) on acetaminophen induced toxicity. The results showed that acetaminophen significantly increases the levels of serum urea, creatinine and decrease levels of uric acid. The reduction was by increasing of anti-oxidative responses as assessed by biochemical and histopathological parameters. The results also suggested a nephroprotective and antioxidant activity of the extract against acetaminophen induced toxicity in rats [81].

The *in vitro* antioxidant activity of the *S. oblonga* methanolic-aqueous extract (SO) was analyzed through Reducing Power assay, H_2O_2 Scavenging activity, Superoxide Radical Scavenging activity, and Nitric Oxide Radical Scavenging activity assays. The Phenolic, Flavonoid, Flavonol and Tannin content of SO were 65.80, 8.89, 5.78 and 83.60 mg Gallic acid equivalent (GAE)/g of dry weight respectively. Statistical analysis revealed that, the total Phenolic and Tannin content of SO significantly enhanced its Reducing Power, while the total Flavonoid content contributed significantly to the H_2O_2 and O_2^- Scavenging activity. The Phenolic compounds of SO brought about significant scavenging of NO^- radical. In this way, the author proved that SO extract behaves like a good radical

scavenger which might be considered as a natural source of antioxidants for medicinal and commercial uses [82].

Antitumor activity

An investigation of *S. madagascariensis* extract, after an extensive fractionation procedure, yielded a new pentacyclic bisnortriterpene isoiquesterin (**28**), Figure 11, with activity against the P388 lymphocite-leukemia *in vivo* [94].

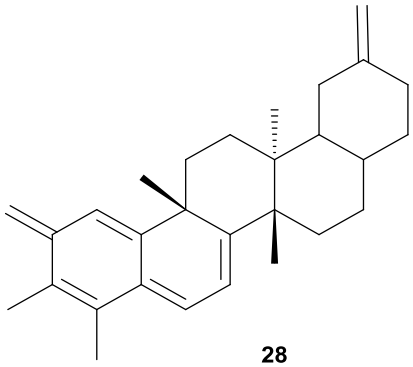

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Figure 11. Chemical structure of Isoiguesterin from *S madagascariensis.*

From the petroleum ether extract of *S. oblonga* wall root bark, after a bioguide fractionation a methanol, showed 100% cytotoxicity on Ehrlich ascites tumor cells at 50µg/ml [71].

Immunostimulatory activity

Oda and coworkers (2010) evaluated the gene expression profiles in the small intestinal epithelium of rats witch were administered a *S. reticulata* water extract. In detail, DNA microarray analysis was performed to evaluate the gene expression profiles in the rat ileal epithelium. The intestinal bacterial flora was also

studied using T-RFLP (Terminal Restriction Fragment Length Polymorphism, Nagashima method). Expressions of immune-related genes, especially Th1-related genes, associated with cell-mediated immunity, increased in the small intestinal epithelium. Therefore, the plant extract exerts bioregulatory functions by boosting intestinal immunity [67].

Safety Evaluation, Antimutagenic, Genotoxic, cytotoxic and protective effects

The safety of hot water *S. oblonga* extract supplemented to or processed into a medical food was evaluated. In this way, thirty male Sprague–Dawley rats were assigned among one of three treatments: (A) EN-0178 (control, liquid diet), (B) EN-0178+salacinol (as 1 plus 500 mg of salacinol extract per 253 g diet, which was added to product immediately prior to feeding), (C) EN-0195 (as 1 plus 500 mg of salacinol extract per 253 g diet, which was added during product manufacture). After 14 days of free access to dietary treatments, the rats were sacrificed, blood collected and organs weighed. The treated animals had reduced ($P<0.05$) weight gain and feed intake. The relative (% of body weight) testicular weight was higher ($P<0.05$), whereas, the relative liver and spleen weight was lower ($P<0.05$) for rats consuming salacinol extract. Also the serum chemistries analyzed, blood urea nitrogen and alkaline phosphatase was lower ($P<0.05$) for those treated. No differences in blood hematology were found. The authors concluded that salacinol extract, in a medical food consumed for 2 weeks in amounts estimated at ten-fold greater than proposed for human intake, did not result in clinical chemistry or histopathologic indications of toxic effects in male Sprague–Dawley rats [110].

As part of a safety evaluation of novel ingredients for use in blood glucose control, the potential genotoxicity of a *S. oblonga* root extract (SOE) was evaluated using the standard battery of tests (reverse mutation; chromosomal aberrations; mouse micronucleus assays). SOE was determined not to be genotoxic under the conditions of the reverse mutation and mouse micronucleus assay, and weakly positive for the chromosomal aberrations test. A weak but reproducible positive chromosomal aberrations response in human lymphocytes concerns, and further toxicity research was recommended by the author. Use of SOE is presently expected to be safe, as anticipated intake is small compared to the doses administered in the genotoxicity assays and may, after further toxicity research, prove to be a useful ingredient in foodstuffs [111].

Also, the toxicity of a *S. oblonga* root extract (SOE) was evaluated in a subchronic 90-day feeding study in rats. An *in vivo* – *in vitro* rat peripheral blood lymphocyte chromosomal aberrations assay was added after the subchronic study. The outcomes indicated that SOE was negative for the induction of chromosomal aberrations in cultured rat peripheral blood lymphocytes. There was not observable adverse effect level with 2.500 mg/kg/day following daily subchronic oral gavage administrations to rats [112].

The mangiferin (**7**), was purified from methanolic root extract of *S. chinensis*. The compound was evaluated for antimutagenicity studies in order to confirm the safety of its usage. It showed no mutagenicity up to 5 mg/plate when tested with *Salmonella typhimurium* TA97a, TA98,TA100, TA102 and TA1535 strains with or without metabolic activation. On the other hand, the mangiferin showed a significant protective effect against mutagenicity induced by mutagen in

S. typhimurium TA98 and TA100 strain with or without metabolic activation. The results of these studies indicated that the compound is non-mutagenic in Ames test, and exhibited protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain [84].

The genotoxic, cytotoxic, antigenotoxic, and anticytotoxic effects of *S. crassifolia* stem bark fractions (hexane, ethyl acetate, and hydroalcoholic) were evaluated using the mouse bone marrow micronucleus test [113]. The results showed that none of the fractions led to a significant increase in the frequency of micronucleated polychromatic erythrocytes (MNPCE) ($P > 0.05$), suggesting the absence of genotoxicity. In the antigenotoxicity testing, a significant decrease in the MNPCE frequency was observed in all fractions ($P < 0.05$). Thus was demonstrated its protective action against genotoxicity induced by mitomycin C (MMC) (positive control). Only the hexane fraction showed significantly decreased the poly- and normochromatic erythrocyte ratio (PCE/NCE) in all doses tested ($P < 0.05$), demonstrating its cytotoxic activity. In association with MMC, both ethyl acetate and hydroalcoholic fractions significantly increased the PCE/NCE ratio in almost all doses tested ($P < 0.05$), demonstrating the protective action of against the cytotoxic effect of the positive control (MMC). In contrast, the hexane fraction presented a significant decrease in the PCE/NCE ratio in all treatments ($P < 0.05$), demonstrating an increase in this plant's cytotoxicity in mouse bone marrow cells.

The protective effects of mangiferin, isolated from *S. chinensis* against pancreatic *β*-cell damage and on the antioxidant defense systems in streptozotocin (STZ)-induced diabetic rats was investigated. Oxidative stress

biomarkers such as tissue malondialdehyde, hydroperoxides, reduced glutathione (GSH), and nonenzymatic antioxidants were evaluated. Biochemical observations were further substantiated with histological examination and structural studies in the pancreas of diabetic, glibenclamide and mangiferin-treated diabetic rats (dosage of 40 mg/kg body weight daily for 30 days). Oral administration of mangiferin and glibenclamide to diabetic rats significantly decreased the level of blood glucose and increased levels of insulin. Additionally, mangiferin treatment significantly modulated the pancreatic nonenzymatic antioxidants status (vitamin C, vitamin E, ceruloplasmin, and reduced GSH content) and other oxidative stress biomarkers. The histoarchitecture of diabetic rats showed degenerated pancreas with lower *β*-cell counts, but mangiferin treatment effectively regenerated insulin secreting islet cells. The electron microscopic study revealed damaged nuclear envelope and mitochondria and fewer secretory granules in pancreas of diabetic rats; however, mangiferin treatment nearly normalized pancreatic architecture. Mangiferin decreased oxidative stress due to its antioxidative properties, what leads to protection pancreatic *β*-cell against damage [50].

Reproductive outcome

The root extract of *S. reticulata* had been used in Sri Lanka as an herbal therapy for glycemic control even during pregnancy. Ratnasooriya and coworkers (2003) determined the effects of the *S. reticulata* root extract on the reproductive outcome of Wistar rats when administered orally (10 g/kg) during early (days 1-7) and mid- (days 7-14) pregnancy. The root extract significantly enhanced post-implantation losses. Gestational length was unaltered but the pups had a low birth

weight. However, the root extract was non-teratogenic. They concluded that this plant extract can be hazardous to successful pregnancy in women and should not be used in pregnancy complicated by diabetes [114].

The effects of *S. chinensis* extract on reproductive functions and the effects on survival and growth were examined using Sprague-Dawley rats. The extract was administered at dose levels of 0, 500 1000 and 2000 mg/Kg/day orally to groups consisting of 25 males and 25 females. In all treatment groups, no toxic signs were noted on reproductive outcome as estrous cycle of females or any parameters for reproductive function or survival, growth, sensory reflex or function development of pups [53].

Antimalarial activity

Three novel quinone methides, 28-nor-isoiguesterin-17-carbaldehyde (**29**), 17-(methoxycarbonyl)-28-nor-isoiguesterin (**30**), and 28-hydroxyisoiguesterin (**31**), together with the known celastrol (**32**), pristimerin, and isoiguesterol, were isolated from the roots of *S. kraussii* by bioassay-guided fractionation, Figure 12. These molecules showed antimalarial activity 30-50-fold greater than their cytotoxicity (in HT-29 cells) *in vitro,* and positive synergic effect when combined. In vivo, the compound **30** was inactive against blood stages of *Plasmodium berghei* in mice after oral and parenteral administration, and was toxic with increasing concentrations [33].

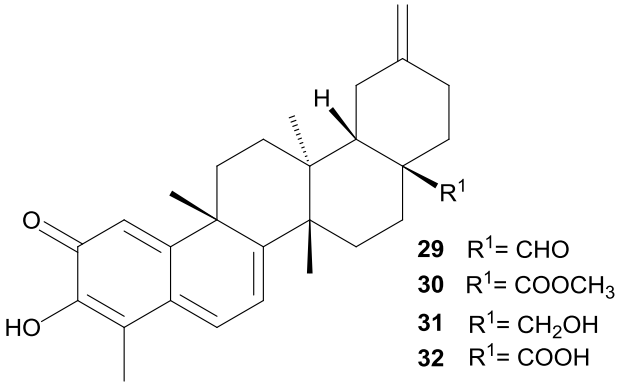


Figure 12. Quinone methides from *Salacia kraussi.*

Antiplasmodial activity

Phytochemical investigation of *S. longipes* seeds resulted in four sesquiterpenoid derivatives, salaterpene A (**33**), salaterpene B (**34**), salaterpene C (**35**) and salaterpene D (**36**), Figure 13, together with two known compounds, 1α,6β-diacetoxy-8β,9β-dibenzoyloxy-4β-hydroxy-2-oxo-dihydro-β-agarofuran e 2β-acetoxy-1α,6β,9β-tribenzoyloxy-4β-hydroxy-dihydro-β-agarofuran. The *in vitro* antiplasmodial activity against *Plasmodium falciparum* chloroquine-resistant strain W2 of the salaterpenes A, B, C, D and 2β-acetoxy-1α,6β,9β-tribenzoyloxy-4β-hydroxy-dihydro-β-agarofuran were tested. All the tested compounds exhibited a moderate potency with IC_{50} below 2.7 µM [92].

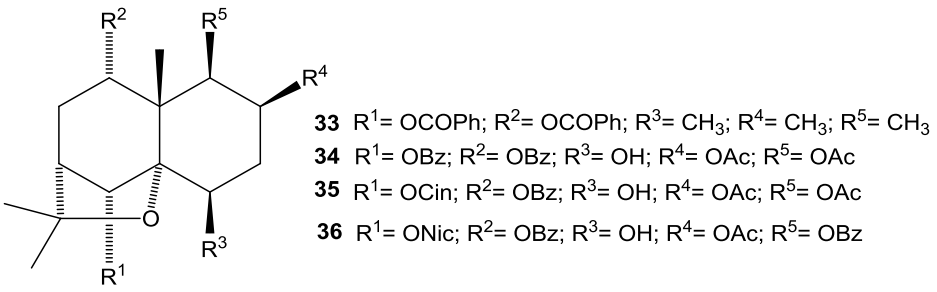
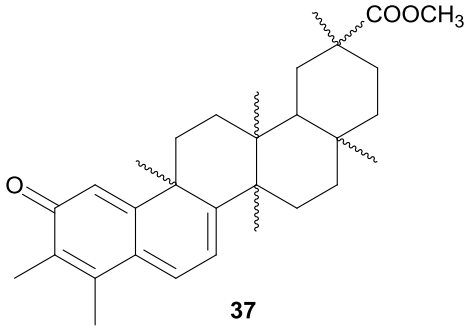


Figure 14. Salaterpenes A, B, C and D from *S. longipes.*

To validate the traditional use of *S. leptoclada*, a bioassay-guided fractionation of the stem barks acetone extract was carried [93]. Were evaluated the activity against *Plasmodium falciparum* and in P388 leukemia cell lines. The biological screening resulted in the isolation of a not yet named pentacyclic triterpenic quinone methide (**37**), Figure 15. The pure compound exhibited both in vitro a cytotoxic effect on murine P388 leukemia cells with IC_{50} value of (0.04±0.020) µg/mL and an antiplasmodial activity against the chloroquine-resistant strain FC29 of *Plasmodium falciparum* (IC_{50} 0.052±0.030 µg/mL).



37

Figure 15. Pentacyclic triterpenic quinone methide from *Salacia leptoclada.*

Antimicrobial activity

The ethanolic extract of *S. macrosperma* roots, exhibited antimicrobial activity *in vitro*. The fractions, with different solvents, were screened for their antimicrobial spectrum against eight gram-positive, five gram-negative and ten fungal strains. The gram positive bacteria were *Staphylococcus aureus, Staphylococcus albus, Bacillus cereus, Bacillus subtilis, Sarcine lute, Lactobacillus casei, Streptococcus fecalis* and *Bacillus pumulis.* The gram negative bacteria tested were *Fusarium devorans, Escherichia coli, Klebsiella aerogens, Proteus*

vulgaris and *Salmonella typhi.* The fungal strains were: *Aspergillus niger, Aspergillus flavus, Acremonium terricola, Culvularia lunata, Drechslera specifier, Fusarium solani, Fusarium oxysporum, Lasiodipoldia theobroma, Trichoderma viride* and *Pencillium viridicatum.* Chloroform and benzene fractions in addition of ethanolic crude extract showed significant antimicrobial effect against all the microorganisms tested. The dose-dependent activity of fractions were observed, and compared with appropriate standards [95].

Leaves and stems of *S. beddomei* were extracted successively with petroleum ether, ethyl acetate and chloroform and antibacterial activity was tested. Ethyl acetate extracts was the most effective against the tested organisms, which were: *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas phosphorescence* and *Aeromonas hydrophylla* [90].

Antimicrobial activity of chloroform and methanolic extracts of *S. reticulata* were tested against gram positive, gram negative and fungus strains using zone of inhibition and minimum inhibitory concentrations (MIC). It was observed that both extracts had inhibitory effect towards all microorganisms used in the test (*Staphylococcus aureus, Bacilus subtilis, Pseudomonas aeroginosa, Escherichia coli, Cryptococcus neoformans, Candida tropicalis, Candida albicans* and *Epidermophyton floccosum*). Chloroform extract was more effective than methanolic extract [66].

Samy (2005) reported that the methanolic extract of *S. microsperma* showed promising results against *Burkholderia pseudomallei*, and also exhibited maximum inhibition zone against *Proteus vulgaris* compared to others plants [91].

Insecticidal and Antifeedant properties

From the leaves of *S. beddomei,* Deepa and coworkers (2003) reported a new compound having a benzoic skeleton. The antifeedandt activity of the compound was tested against the cotton leaf worm *Spodoptera litura.* The compound at 0.5% and 1% concentration showed maximum antifeedant activity against the IV instar larvae of *S. litura* and 70% mortality was recorded with 1% extracts [88]. The same researchers, in 2004, reported an oxaza cyclohexane derivative from the nutrient stress stem callus of *S. beddomei*, which exhibited a significant antifeedant and insecticidal activity with 100% mortality on *Spodoptera litura* [89].

Summary and future perspectives

Out of 120 Salacia species identified from Celastraceae family, many of them provide over 80 different chemical compounds, among terpenes and derivatives, some alkaloids and phenolic compounds. From its extensive use as herbal medicine, it was identified several of these compounds as being of highly pharmacological interest due to its actions. Besides the activities already identified, other actions were not tested yet. Once new studies are carried-out, it may indicate other interesting features of the molecules already extracted from this genus. In the future, widespread interest in this species seems certain to ensure continued research with this family. Moreover, interdisciplinary research and the

development of modern combinatorial techniques make possible the discovery of novel agents from these species.

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