ESTUDO QUIMICO E ATIVIDADE BIOLÓGICA DE *Ramalina usnea* (L.) R. Howe

ANTÔNIO SÉRGIO NASCIMENTO MOREIRA

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE
DARCY RIBEIRO
CAMPOS DOS GOYTACAZES
JULHO – 2015

ESTUDO QUIMICO E ATIVIDADE BIOLÓGICA DE *Ramalina usnea* (L.) R. Howe

ANTÔNIO SÉRGIO NASCIMENTO MOREIRA

"Tese apresentada ao Centro de Ciência e Tecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para à obtenção do título de Doutor em Ciências Naturais".

Orientador: Prof. Ivo José Curcino Vieira
Co-Orientador: Prof. Raimundo Braz-Filho

CAMPOS DOS GOYTACAZES - RJ
JULHO – 2015

ESTUDO QUIMICO E ATIVIDADE BIOLÓGICA DE *Ramalina usnea* (L.) R. Howe

ANTÔNIO SÉRGIO NASCIMENTO MOREIRA

"Tese apresentada ao Centro de Ciência e Tecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para à obtenção do título de Doutor em Ciências Naturais".

Aprovado em 02 de julho de 2015

Comissão Examinadora:

Prof. Marcelo Francisco de Araújo (D.Sc., Química Orgânica) - IFF

Prof. Francisco José Alves Lemos (D.Sc., Ciências Biológicas) - UENF

Prof. Vicente Mussi Dias (D.Sc., Produção Vegetal) - ISECENSA

Prof. Raimundo Braz-Filho (D.Sc., Química) – Co-Orientador (No Impedimento)

Prof. Ivo José Curcino Vieira – D.Sc. - Orientador

AGRADECIMENTOS

Ao professor Ivo José Curcino Vieira pela orientação e paciência ao longo da construção desse trabalho.

Ao professor Raimundo Braz-Filho pela orientação e genial colaboração na interpretação dos espectros de RMN e CG/EM.

Aos professores que participaram da banca examinadora, o meu muito obrigado pela colaboração para a melhoria da apresentação desta pesquisa.

Aos técnicos do laboratório de espectroscopia e cromatografia da Universidade Federal Rural do Rio de Janeiro - UFRRJ, pela realização das análises de RMN e CG/EM.

Aos técnicos do laboratório de espectroscopia e cromatografia da UENF, Marcelo e Márcio, pela realização das análises de RMN e CG/EM.

À Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF pela disponibilidade de utilização do espaço acadêmico e laboratórios.

Ao Instituto Federal Fluminense – IFF, pela liberação da jornada de trabalho em tempo parcial e pelo auxílio financeiro para a produção acadêmica.

Aos colegas do laboratório pelo companheirismo.

Aos colaboradores dos laboratórios dos ensaios biológicos das atividades larvicida (LBT-CBB), antimicrobiana (LSA-CCTA) e antineoplásica (LQR-CBB).

Aos órgãos de fomento FAPERJ, CNPQ e CAPES pelas verbas de subvenção concedidas.

Aos meus filhos, netos e esposa pela compreensão nos momentos de ausência.

A Deus, pela luz e força para a realização deste trabalho.

SUMÁRIO

RESUMO vii
ABSTRACT ix
1. INTRODUÇÃO 1
2. REVISÃO DE LITERATURA 5
2.1 O gênero *Ramalina* no Brasil 5
2.1.1 O líquen *Ramalina usnea* 7
3. TRABALHOS 10
3.1 Trabalho 1: Chemistry and Biological Activity of *Ramalina* Lichenized Fungi 9
3.1.1 Introduction 10
3.1.2 Chemical Constituents 11
3.1.2.1 Carbohydrates 11
3.1.2.2 Usnic acid and Derivatives 14
3.1.2.3 Depsides 15
3.1.2.4 Depsidonas 18
3.1.2.5 Fatty Acids 18
3.1.2.6 Other Compounds 20
3.1.3 Biological Activity 27
3.1.3.1 Antimicrobial Activity 28
3.1.3.2 Antioxidant Activity 31
3.1.3.3 Antiviral Activity 35
3.1.3.4 Antitumor and Cytotoxic Activity 36
3.1.3.5 Anti-Inflammatory Activity 42
3.1.3.6 Other Activities 43
3.1.4 Concluding Remarks 45
Acknowledgments 46

Author Contributions 46
Conflicts of Interest 46
References 47
Supplementary Material 59
3.2 Trabalho 2. Larvicidal activity of *Ramalina usnea* lichen against Aedes aegypti 90
3.2.1 Introduction 90
3.2.2 Materials and methods 92
3.2.3 Statistical analysis 94
3.2.4 Results 94
3.2.5 Argument 98
3.2.6 Conclusions 99
3.2.7 References 99
Supplementary Material 106
3.3 Trabalho 3. Potencial Antimicrobiano de *Ramalina usnea* 124
3.3.1 Introdução 126
3.3.2 Experimental 128
3.3.2.1 Equipamentos e reagentes 128
3.3.2.2 Coleta e identificação do líquen *Ramalina usnea* 128
3.3.2.3 Extração, obtenção das frações e isolamento dos compostos. 129
3.3.2.4 Avaliação do potencial antimicrobiano 129
3.3.2.4.1 Material biológico 129
3.3.2.4.2 Método de difusão em ágar 130
3.3.2.4.3 Método em meio líquido 131
3.3.3 Resultados e discussão 131
3.3.3.1 Ensaios em meio sólido 131
3.3.3.2 Ensaios em meio líquido 132
3.3.4 Conclusões 136
Referências 137
Material Suplementar 141
4. RESUMO E CONCLUSÕES 151
REFERÊNCIAS BIBLIOGRÁFICAS 153
PESPECTIVAS FUTURAS 156

RESUMO

MOREIRA, Antônio Sérgio Nascimento; D.Sc. Universidade Estadual do Norte Fluminense Darcy Ribeiro. Julho de 2015. Estudo químico e atividade biológica de *Ramalina usnea* (L.) R. Howe. Professor Orientador: Ivo José Curcino Vieira.

Fungos liquenizados são simbiontes entre fungos e algas ou cianobactérias. O fungo liquenizado *Ramalina usnea* pertence ao gênero *Ramalina*, que é composto de cerca de 250 espécies distribuídas em todas as regiões do mundo. Fungos liquenizados também são conhecidos pela diversidade de metabólitos secundários que apresentam e certas classes de compostos são típicos destes organismos, como os meta e para depsídeos e as depsidonas. Atualmente são conhecidos cerca de 1.050 compostos de fungos liquenizados e vários destes metabólitos são biologicamente ativos, possuindo diversas propriedades como agente de proteção contra intensas radiações, como aleloquímicos, ações farmacológicas como, antimicrobiana, antioxidante, antiviral, antitumoral, inseticida, anti-inflamatória entre outras. Desta forma, o objetivo desta pesquisa foi estudar a química e atividade biológica da espécie *Ramalina usnea* coletada na restinga de Iquipari no litoral de São João da Barra estado do Rio de Janeiro. O extrato em metanol do líquen e algumas frações apresentaram atividade larvicida, matando 100% das larvas de 3º instádio do mosquito *Aedes aegypti* após 24 horas de exposição. Dois compostos identificados como 2-hidróxi-4-metóxi-6-propil- benzoato de metila (1) e ácido úsnico (2) apresentaram atividade larvicida, com concentração letal 50% (CL_{50}) de 4,850 e 4,475 µg/mL, respectivamente. O extrato e algumas frações também apresentaram atividade

sobre linhagens de bactérias Gram (+) *Staphylococcus aureus* ATCC e Gram (-) *Burkholderia cepacea*. Ácido úsnico não produziu inibição no crescimento de nenhuma bactéria testada, mas produziu inibição 100 % de crescimento dos fungos *Candida tropicalis* e *Candida kefyr* em baixa concentração. Desta forma, pode-se verificar que esta espécie de líquen apresenta substâncias promissoras como modelos para o desenvolvimento de compostos com diferentes atividades farmacológicas.

Este é o primeiro estudo realizado com o composto 2-hidróxi-4-metóxi-6-propil- benzoato de metila para atividade larvicida com *Aedes aegypti*, o primeiro estudo com o gênero *Ramalina* com os fungos leveduriformes testados nesta pesquisa. Esta é a primeira vez que o composto 2-hidróxi-4-metóxi-6-propil-benzoato de metila é citado no gênero *Ramalina* e na espécie *Ramalina usnea* (L.) R. Howe.

ABSTRACT

MOREIRA, Antônio Sérgio Nascimento; D.Sc. Universidade Estadual do Norte Fluminense Darcy Ribeiro. July 2015. Chemical and biological activity of *Ramalina usnea* (L.) R. Howe. Advisor: Ivo José Curcino Vieira.

Lichens are symbionts between fungi and algae or cyanobacteria. The lichen *Ramalina australiensis* (syn. *Ramalina usnea*) belongs to the genus *Ramalina*, which is composed of about 250 species distributed over all regions of the world. Lichens are also known for the diversity of secondary metabolites that (they) have and certain classes of compounds are typical of these organisms, as the meta and para depsides and depsidones. Now days there are about 1050 known lichens compounds and several of these metabolites are biologically active, possessing many properties such as, protective agent against intense radiation, as allelochemicals, pharmacological actions as antimicrobial, antioxidant, antiviral, anti-tumor, insecticide, anti-inflammatory among others. Thus, the objective of this research was to study the chemistry and biological activity of the species *Ramalina australiensis* collected in the restinga (sandbank) of Iquipari in the coastline of São João da Barra, Rio de Janeiro state. The (lichen methanolic extract) and some fractions showed good larvicidal activity, killing 100% of *Aedes aegyti* mosquito in third instar after 24 hour of exposure. Two isolated compounds identified as 2-Hydroxy-4-methoxy-6-propyl-methyl ester benzoic acid (1) and usnic acid (2) showed good larvicidal activity, with lethal concentration 50% (LC_{50}) of 4,850 and 4,475 µg/mL, respectively. The extract and some fractions also showed good activity against (bacteria) lineages of Gram (+) *Staphylococcus aureus* ATCC and Gram (-) *Burkolderia cepacea*. Usnic acid did not produce inhibition on the growth of any bacteria tested, but it produced inhibition of 100% in the growth of fungi lineages *Candida tropicalis* and *Candida kefir* at low concentration. Therefore, it can be concluded that this lichen species present promising substances as models for the development of compounds with different pharmacological activities.

This is the first study carry out with the compound 2-Hydroxy-4-methoxy-6-propyl-methyl ester benzoic acid for larvicidal activity with Aedes aegypti and the first study to Ramalina genre with the yeast-like fungi tested in this research. This is the first time that the compound 2-Hydroxy-4-methoxy-6-propyl-methyl ester benzoic acid is cited in the genus Ramalina and in the specie *Ramalina usnea* (L.) R. Howe.

1. INTRODUÇÃO

Fungos liquenizados constituem uma forma perfeita de mutualismo simbiótico de um fungo (micobionte) e de uma alga ou de uma cianobactéria, portanto um parceiro fotoautotrófico (fotobionte) (Nultsch, 2000; Molnár e Farkas, 2010; Werth, 2012) e constituem uma unidade sistemática independente e estável (Nultsch, 2000; Boustie e Grube, 2005). Extimam-se que existam 13.500 espécies, com mais de 600 gêneros de fungos liquenizados, o que corresponde a 20% dos fungos conhecidos (Honda, Vilegas, 1998). No trabalho publicado por Boustie e Grube (2005) os pesquisadores já mencionam cerca de 18.500 diferentes espécies de liquens por todo o mundo, isso mostra o grande interesse que os estudiosos têm demonstrado pelo estudo destes organismos especiais.

Em geral, um fungo liquenizado é formado por um micobionte e um fotobionte, porém existem também alguns com dois micobiontes e um ou dois fotobiontes ou com um micobionte e até mesmo três fotobiontes (Nultsch, 2000; Honda, Vilegas, 1998). No total são conhecidos aproximadamente 13.500 micobiontes, mas apenas cerca de 30 fotobiontes. Entre os micobiontes, 98% são Ascomicetos e 46% desses são liquenizados, poucos são os Basidiomicetos, como as espécies do gênero *Dictyonema* (Nultsch, 2000; Boustie e Grube, 2005; Honda e Vilegas, 1998).

Os Basidiomicetos formam diversas associações com algas azuis semelhantes a líquens, em regiões tropicais. (Honda e Vilegas, 1998). Entre os fotobiontes, predominam as algas verdes unicelulares (*Chloroccocales*). As algas verde-azuladas (cianofíceas) mais comuns são a *Nostoc* e *Scytonema* (Honda,

Vilegas, 1998). Algumas cianobactérias, como representantes do gênero *Nostoc*, podem fixar nitrogênio elementar (Nultsch, 2000).

Nos fungos liquenizados homômeros, os fotobiontes estão mais ou menos uniformemente distribuídos no talo. Já nos fungos liquenizados heterômeros eles ficam limitados a determinadas camadas. As hifas contribuem para a maior parte da sua massa, envolvendo as algas especialmente nos estágios iniciais da formação do talo (Nultsch, 2000). O talo de um líquen é o produto do desenvolvimento da união da alga e do fungo, e se cultivados em separado não há semelhança com a alga ou com o fungo precursor, perdendo totalmente a característica liquênica. O talo é constituído principalmente pelas hifas do fungo e pelos filamentos da alga, sendo estes em menor proporção. O talo apresenta uma morfologia que varia entre crustosa, fruticosa ou foliosa (Joly, 2002).

O benefício que o fungo tem dessa simbiose consiste, sem dúvida, no fornecimento de produtos de fotossíntese do fotobionte. Medições têm mostrado que até 90% de tais produtos podem chegar aos micobiontes. No caso das algas verdes, esses produtos são, sobretudo, álcoois de açúcares, como ribitol, eritritol ou sorbitol, enquanto das cianobactérias, trata-se da glicose. A vantagem que o fotobionte tem nessa simbiose, por outro lado, ainda não está clara. Entre outras suposições, alguns autores supõem que o micobionte, por possuir pigmentos de líquen, está em condições de proteger o fotobionte da exposição direta à luz solar (Nultsch, 2000).

Os fungos liquenizados, em geral, multiplicam-se vegetativamente por meio de fragmentos do talo ou por sorédios, que são partes que penetram no tecido vivo do hospedeiro em busca de alimento. Estes órgãos são células de algas envolvidas pelo micélio do fungo, dispersas pelo vento que, sobre um substrato adequado, crescem até se tornarem novamente talo. Via de regra, a forma e a estrutura do talo são decisivamente determinadas pelos micobiontes, embora existam situações em que o fotobionte exerça influência marcante. No entanto, a organização do talo sempre é o resultado da simbiose entre ambos os parceiros (Nultsch, 2000).

Os fungos liquenizados podem ser encontrados em diversos ambientes, sobre troncos de árvores, sobre pedras, nas montanhas e até no gelo. Apesar de serem resistentes às variações de temperatura, esses organismos são muito sensíveis à poluição do ar, funcionando como verdadeiros sinais de alerta

da poluição (Gewandsznajder e Linhares, 2000; Marcelli, 1997). Em ambientes poluídos, a maioria desaparece e os que sobrevivem funcionam como indicadores da poluição atmosférica ao serem analisados. Por não terem raízes e folhas, estruturas que nos vegetais funcionam como filtros contra a poluição, os líquens absorvem os poluentes por toda sua superfície, onde ocorrem as trocas de água, gases e sais minerais (Gewandsznajder e Linhares, 2000).

Os fungos liquenicados produzem um grande número de substâncias naturais, que podem ser classificadas como intra ou extracelulares. Entre os produtos intracelulares encontram-se metabólitos primários como carboidratos, aminoácidos, vitaminas, carotenoides e proteínas, que estão ligados à parede celular ou ao protoplasto, sendo estes produtos normalmente solúveis em água. Além de ocorrerem em fungos e algas de vida livre, muitos desses compostos também ocorrem em plantas superiores, logo não são específicos de liquens. Os produtos extracelulares são resultantes do metabolismo secundário dos liquens, podendo ser corticais ou medulares, sendo a maioria de natureza fenólica, insolúvel em água, e são depositados na superfície das hifas sob a forma de cristais (Honda e Vilegas, 1998).

Fungos liquenizados sintetizam uma grande variedade de metabólitos secundários biologicamente ativos, dos quais certas classes só são produzidas por estes microorganismos, restritos a grupos taxonômicos e/ou áreas geográficas (Marcelli, 1997; Molnár e Farkas, 2010) O desenvolvimento de técnicas analíticas e métodos experimentais resultaram na identificação de cerca de 1.050 substâncias liquênicas, incluindo as produzidas em culturas (Molnár e Farkas, 2010). Dentre esses compostos encontram-se os ácidos alifáticos, meta e para depsídeos, depsidonas, ésteres benzílicos, dibenzofuranos, antraquinonas, xantonas, ácido úsnico, terpenos e derivados do ácido pulvínico (Honda e Vilegas, 1998).

Os compostos secundários apresentam importante papel na sistemática e quimiotaxonomia dos líquens além de possuírem importantes propriedades biológicas, tais como agentes de fotoproteção contra intensas radiações (Urdapilleta e Sampaio, 2006), como aleloquímicos e variadas ações farmacológicas, entre elas antiviral (Fazio et al., 2007; Esimone, 2009), antitumoral (Shibata et al., 1968; Nishikawa et al., 1970; Rankovic et al., 2011; Kosanic et al., 2012) , antimicrobiana (Falcão et al., 2002; Piovano et al., 2002;

Manojlovic et al., 2002; Tay et al., 2004; Cansaran et al., 2006; Mitrovic et al., 2011; Rankovic et al., 2011; Kosanic et al., 2012), anti-Leishmania (Urdapilleta e Sampaio, 2006), anti-inflamatória (Pereira et al., 2010;), antifúngica (Piovano et al., 2002; Manojlovic et al., 2002), antibiótica (Bustinza e López, 1948) e antioxidante (Mitrovic et al., 2011; Rankovic et al., 2011; Kosanic et al., 2012).

Estudos sobre a química, estrutura e atividade biológica sobre as comunidades liquênicas brasileiras são escassos, além de terem sido realizados a quase vinte anos, para o caso de *Ramalina usnea* (Kashiwadani e Kalb, 1993), quando as técnicas de isolamento e identificação ainda não eram tão evoluídas como atualmente. Desta forma, o presente trabalho teve como objetivos: 1. Realizar o estudo químico do fungo liquenizado *Ramalina usnea*; 2. Avaliar a atividade antimicrobiana do extrato bruto, de frações e dos compostos identificados contra três linhagens ATCC de *Staphylococcus aureus* e três linhagens do fungo *Candida*; 3. Avaliar a atividade do extrato, frações e substâncias identificadas contra larvas de 3º estádio do mosquito *Aedes aegypti.*

A tese será apresentada na forma de artigos para publicação. O primeiro trabalho apresentado corresponde ao artigo de revisão cujo título é: “Chemistry and Biological Activity of *Ramalina* Lichenized Fungi”, que foi o objeto do estudo defendido como qualificação, em que foram apresentados estudos sobre a química e as atividades biológicas desenvolvidas com o gênero *Ramalina* nos últimos cem anos. Este artigo foi publicado no periódico Suiço *Molecules* produzido pela Molecular Diversity Preservation International (MDPI) enviado em 10 de março de 2015 e aceito em 04 de maio de 2015.

O segundo trabalho corresponde ao artigo científico intitulado “Larvicidal activity of *Ramalina usnea* lichen against *Aedes aegypti*” e foi escrito de acordo com a instruções para elaboração de manuscrito do periódico *Parasitology Research* produzido pela organização mundialmente reconhecida Springer SBM, com sede em Berlim.

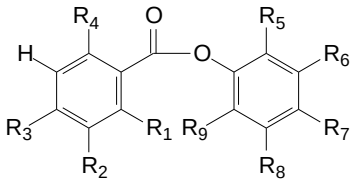
O terceiro trabalho intitulado “Potencial Antimicrobiano de *Ramalina usnea”* foi escrito segundo as instruções para autores da Revista Virtual de Química, uma publicação eletrônica (ISSN 1984-6835) bimestral, sem fins lucrativos, com difusão gratuita na Internet.

2. REVISÃO DA LITERATURA

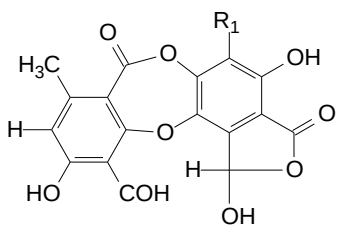
2.1 O gênero *Ramalina* no Brasil

O primeiro relato de espécies brasileiras de *Ramalina* foi feito por Eschweiler, em Martius 1833, que descreveu *Parmelia denticulata* Eschw. [= *Ramalina denticulata* (Eschw.) Nyl.]. Meyen e Flot. (1843) descreveram *R. costata* Meyen & Flot. e *R. costata* var. compressa Meyen & Flotow, com base em espécimes coletadas no Rio de Janeiro. Nylander (1870) relatou 11 espécies do Brasil: *R. complanata* (Sw.) Ach., *R. dasypoga* Tuck., *R. denticulata* (Eschw.) Nyl., *R. gracilis* (Pers.) Nyl., *R. hypodectodes* Nyl., *R. lanceolata* Nyl., *R. pumila* Mont., *R. rectangularis* Nyl., *R. subpollinaria* Nyl., *R. usneoides* (Ach.) Mont., e *R. yemensis* (Ach.) Nyl. Vainio (1890) descreveu *R. complanata f. reagens* Vain., *R. denticulata var. subolivacea* Vain., *R. flagellifera* Vain. e acrescentou *R. anceps* Nyl., *R. denticulata var. canalicularis* Nyl., *R. geniculata* (Hook. f. & Taylor), *R. inflata* (Hook. f. & Taylor), *R. peranceps* Nyl., e *R. rigida* Ach. à flora de líquens do Brasil. Vainio (1891-1892) também distribuiu 16 táxons de *Ramalina* brasileira em sua obra *Lichenes Brasilienses Exsiccati*. Zahlbruckner (1902) descreveu três táxons, *R. denticulata var. stephanophora* (Zahlbr. Kashiw. & Kalb), *R. cochlearis* Zahlbr. e *R. yemensis var. minima* Zahlbr. e, adicionou, *R. farinacea* (L.) Ach., *R. farinacea var. multifida* Ach., e *R. yemensis var. ecklonii* (Spreng.) Vain. para a flora do Brasil. Malme (1934) descreveu *R. ecklonii f. lobuligera* Malme e acrescentou *R. bicolor* Müll. Arg., *R. dendriscoides* Nyl., *R. ecklonii var. sublinearis* (Nyl.) Malme, *R. laevigata* Fr., *R. linearis* (Sw.) Ach., *R. peruviana* Ach., *R. prolifera* Taylor e *R. usnea* (L.) R. Howe (Kashiwadani e Kalb, 1993).

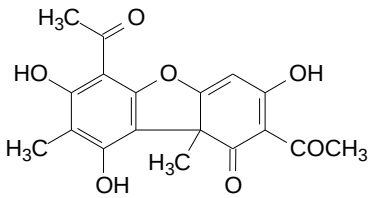
Kashiwadani em 1987 descreveu *Ramalina grumosa* Kashiw. do Brasil. Portanto, 30 espécies, 8 variedades, e 2 formas de *Ramalina* (s. str.) são conhecidas do Brasil. No entanto, a maioria dos táxons mencionados do Brasil têm sido pouco revistos por princípios taxonômicos modernos. No estudo, realizado por Kashiwadani e Kalb (1993), atenção especial foi dada à morfologia interna do talo e apotécio e também à composição química. Esta pesquisa também apresentou a composição química das espécies do gênero *Ramalina* identificadas no Brasil. Entre os compostos, encontram-se depsídeos derivados do orcinol (ácido orsenílico), depsidonas derivadas do β-orcinol (ácido β-metil-orsenílico) como os ácidos sequicáico (1), divaricático (2), homossequicáico (3), criptoclorofeico (4), bonínico (5) e atranorina (6) (depsídeos), ácidos salazínico (7) e norstítico (8), (depsidonas) e ácido úsnico (9) (Figura 1).



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
1	OH	H	OCH_3	*n*-C_3H_7	OH	COOH	*n*-C_3H_7	H	OCH_3
2	OH	H	OCH_3	*n*-C_3H_7	H	OH	COOH	*n*-C_3H_7	H
3	OH	H	OCH_3	*n*-C_3H_7	OH	COOH	*n*-C_5H_{11}	H	OCH_3
4	OCH_3	H	OH	*n*-C_5H_{11}	OH	COOH	*n*-C_5H_{11}	H	OH
5	*n*-C_3H_7	H	OCH_3	OCH_3	OH	COOH	*n*-C_5H_{11}	H	OCH_3
6	OH	CHO	OH	CH_3	CH_3	OH	$COOCH_3$	CH_3	H



	R_1
7	CH_2OH
8	CH_3



9

Figura 1: Estruturas químicas dos compostos encontrados no gênero *Ramalina*

2.1.1 O fungo liquenizado *Ramalina usnea*

O líquen *Ramalina usnea* foi descrito pela primeira vez, como Lichen usnea L., por Linnaeus (1767) e está amplamente distribuído nas partes mais quentes do mundo. Nas Índias Ocidentais, este tipo de líquen tem sido usado como material de cama de aves e do homem (Imshaug, 1972).

R. usnea é uma espécie de fungo liquenizado amplamente distribuída, podendo ser encontrada em todo Hemisfério Ocidental - Índia, Coréia na América do Sul - Argentina, Brasil, Paraguai e Uruguai, Ilhas Galápagos, América do Norte - Texas e Flórida (Rundel, 1978). Possui hábito fruticoso e é caracterizada por possuir talos compostos por ramos que crescem preso por um ou poucos pontos. Os ramos (Figura 2) são pendulosos, com aproximadamente 30 cm de comprimento, possuem ramificações sólidas irregulares, achatadas e cilíndricas (Rundel, 1978; Kashiwadani e Kalb, 1993).

Figura 2: Ramos pendulosos do fungo liquenizado *Ramalina usnea* sobre vegetação de restinga em Iquiparí, município de São João da Barra, RJ (Foto: Moreira, A. S. N.; 2012)

Os constituintes químicos pertencentes às classes de compostos depsideos e depsidonas desempenham um papel importante na taxonomia de líquen e Asahina (1938) relata a presença dos ácidos ramalinólico e sequicáico em *Ramalina usnea*; estas também são as únicas substâncias relatadas por Follmann e Huneck (1969) e Culberson (1969,1970) nesta espécie (apud Imshaug, 1972).

O estudo de Imshaug (1972), “Tipificação de *Ramalina usnea* (L.) R. Howe” mostra que o herbário “Linnean” contém três espécimes de *Ramalina usnea* catalogadas em 1945. Linnean herb. 1273-278 - Da Martinica onde cresceu em árvores. Componentes: ácido úsnico e ácido divaricatico (verificado por cristais em GAW).

Linnean herb. 1273-279 - Localidade não mencionada, aparentemente determinada por Dickson. Constituintes: ácidos úsnico e salazínico.

Linnean herb. 1273-280 - Do Brasil. Componentes: ácido divaricático (verificado por cristais em GAW).

Os componentes foram determinados por cromatografia em camada fina completada por testes de microcristal. Os cromatogramas foram executados em Folhas Eastman Chromagram (n. 6060) em um sistema de solvente contendo benzeno (90): dioxano (25): ácido acético glacial (4).

Nos estudos de Kashiwadani e Kalb (1993), “O Gênero Ramalina no Brasil”, os autores mencionam que a espécie *Ramalina usnea* apresenta duas raças, raça 1 e raça 2 que são constituídas pelos depsideos: ácidos divaricático, homossequicáico e sequicáico e pelo ácido úsnico. A diferença na composição química das duas raças é que a raça 2 possui o ácido divaricático enquanto a raça 1 não o possui.

3. TRABALHOS

Molecules **2015**, *20*, 8952-8987; doi:10.3390/molecules20058952

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Review

Chemistry and Biological Activity of *Ramalina* Lichenized Fungi

Antônio Sérgio Nascimento Moreira [1,3,*], RaimundoBraz-Filho [1,†], Vicente Mussi-Dias [2,†] and Ivo José Curcino Vieira [1]

[1] Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro, UENF, Avenida Alberto Lamego 2000, Campos dos Goytacazes, 28013-602 Rio de Janeiro, Brazil; E-Mails; braz@uenf.br (R.B.-F.); curcino@uenf.br (I.J.C.V.)

[2] Laboratório de Entomologia e Fitopatologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, UENF, Avenida Alberto Lamego 2000, Campos dos Goytacazes, 28013-602 Rio de Janeiro, Brazil; E-Mail: vicmussi@uenf.br

[3] Instituto Federal Fluminense, IFF, Avenida Souza Mota 350, Parque Fundão, Campos dos Goytacazes, 28060-010 Rio de Janeiro, Brazil

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: ansernam@iff.edu.br;
Tel.: +55-22-2738-6504; Fax; +55-22-2739-7248.

Academic Editor: Derek J. McPhee

Received: 10 March 2015 / Accepted: 4 May 2015 / Published:

Abstract: Lichens are a form of symbiont between a fungus and an alga or cyanobacterium, which contains a wide variety of organic compounds with certain secondary metabolite classes typical of these organisms. The *Ramalina* genus has approximately 246 species distributed around the World, of which in this review approximately 118 species with published chemical or biological activity studies of extracts or isolated compounds were cited. From the 153 mentioned compounds, only 27 passed were tested for biological activity, being usnic acid the most studied compound and the one showing the best results in almost all *in vitro* tests performed, although other compounds also presented excellent results as antimicrobial, antitumor and anti-inflammatory agents, among others. Extracts of several species also presented significant results in performed biological tests, demonstrating the potential that these organisms have, in particular, the gender *Ramalina*, to produce bioactive molecules that can be used as a model for the production of pharmaceuticals.

Keywords: lichen; *Ramalina*; biological activitiy; usnic acid; antitumoral

1. Introduction

Lichenized fungi (syn. Lichens) constitute a form of symbiotic mutualism between a fungus (micobiont) and an alga or cyanobacteria (photobiont) that contains a large number of organic compounds, some of them specific to these organisms [1], and constitute a stable and independent systematic unit belonging to the Kingdom Fungi (phylum Ascomycota-Basidiomycota) [2]. Until 1998, it was estimated that there was 13,500 species (about 600 genera), which corresponds to 20% of fungi known [3]. In 2005 this estimate rose to 18,500 different species around the World. This demonstrates, in addition to the diversity, the great interest that researchers have been increasing in the study of these special organisms [2].

Lichenized fungi are well known for the diversity of secondary metabolites that they produce [1,4]. This diversity of compounds has made the study of their chemistry attractive since the beginning of organic chemistry, from 1830 to the present day [1]. Many of the metabolites are typical of this class of organisms [1,5,6]. More than 800 compounds have been reported, and among the different classes of compounds are ones containing nitrogen, phosphorus and sulfur, polyols, carbohydrates, aliphatic and cycloaliphatic compounds, aromatic compounds, meta- and para-depsides, depsidones, dibenzofurans, diphenylethers, naphtopyrans, biphenyls, diphenylmethanes, nostoclides, xanthones, quinones, naphthoquinones and usnic acid. Esters, terpenes, steroids, terphenylquinones and derivatives of pulvinic acid also occur [1,6]. Currently 246 species at the genus *Ramalina* are described, which are widely distributed worldwide [5,7]. In this review paper about 110 species were cited, for which the chemistry or biological activity of its crude extracts or any of its isolated compounds was studied. The goal of this review paper is to verify which are the main biological activities and which metabolites have been isolated from lichens of the genus *Ramalina*.

2. Chemical Constituents

In the researched species of the genus *Ramalina*, a diversity of chemical compounds was found, including both primary and secondary metabolites. Among primary metabolites, carbohydrates were the most abundant ones; amino acids, glycolipids, glycosphingolipids and polyols were also detected. Among secondary metabolites, usnic acid deserves attention because of its frequent mention. Derivatives of this acid (usimines) [8–11], (+)-*iso*-usnic acid [12] and usninic acid [13] were also found. Depsides, depsidones, fatty acids, sterols and monocyclic aromatic compounds were found among the most frequent ones, besides other classes of compounds at very low frequency. Each compound and its respective origin (lichenized species) are listed in Tables S1–S10 (Supplementary Material), and their structures are represented in Figures 1–10.

2.1. Carbohydrates

The main polysaccharides of *Ramalina* are linear glucans and heteropolysaccharides. The first group consists of compounds with α and β

configurations that possess in their structures (1→3)- and (1→4)- (**1**) bonds in different proportions. The α-configuration corresponds to the the isolichenan class and β to the lichenan one. The second group is heteropolysaccharidic branched-chain-containing galactose and mannose (galactomannan), where the most abundant feature bonds (1→6)- containing α-D-Man*p* (**2**) as the main chain and α- or β-D-Gal*p*, β-D-Gal*f* and α-D-Man*p* as side chains [14–21].

Takahashi *et al.* (1979) [22] extracted water-soluble and insoluble homoglucans from *R. crassa* Delise ex Nyl. (currently *R. siliquosa* (Huds.) A.L. Sm.). Separately, from the mycobiont, the hydrolysis of homoglucan produced glucose (**3**) similar to the symbiotic association, although galactose (**4**) was produced from the alga (phycobiont) itself by hydrolysis of its galactomanan [22]. Polysaccharides of *R. sinensis* were hydrolyzed with sulfuric acid and monosaccharides were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) producing the monosaccharides glucose, mannose (**5**), rhamnose (**6**) and galactose in a molar ratio of 5.05:3.89:0.14:0.09 [23].

Kosugi *et al.* [24] isolated D-arabitol (**7**) from the green alga *Trebouxia* sp., photobiont from *R. yasudae* lichen. The aqueous extract of the lichen stem produced several sugars, among them D-arabitol, which was identified by nuclear magnetic resonance (NMR), mass spectrometry (MS) and gas chromatography (GC) [24]. From the extract of the *R. fraxinea* stem in acetone, D-arabitol and mannitol (**8**) were isolated, and from the aqueous extract glucose, galactose, glucosamine (**9**), arabinose (**10**), xylose (**11**), rhamnose and glucuronic acid (**12**) were obtained [25]. D-Arabitol was also isolated from the species *R. reticulata* [26], *R. calicaris* and *R. sinensis* [27], *R. siliquosa* [28], *R. tayloriana* [29], *R. geniculata* and *R. scopulorum* [30].

Six species of *Ramalina,* and separately, phycobionts and mycobionts of some of them, were studied [14,16–21,31,32], where it was concluded that all the studied species presented components structurally similar to the ones from the isolichenan, laminaran, nigeran and galactomannan classes [18]. These include the species, *R. usnea* (L.) R. Howe (currently, *R. australiensis* Nyl.) [21,32,33], *R. ecklonii* (Spreng.) Meyen & Flot. (currently *R. celastri*) [14,16,17,33,34], *R. dendriscoides* Nyl. [18], *R. fraxinea* (L.) Ach. [18], *R. gracilis* (Pers.) Quél. [18,20,31] and *R. peruviana* Ach. [18,19]. From the *R. complanata* mycobiont were extracted the mentioned polysaccharides, in addition to a type of β-glucan, a

lentinan (**13**) and a heteropolysaccharide whose composition is Man:Gal:Glc in the ratio of 21:28:51 [35].

From the alga *Trebouxia puymaly*, a photobiont of *R. gracilis,* a polysaccharide containing bonds (1→5)-β-galactofuranosyl as the main chain was extracted, with replacement at *O*-6 by β-gal*f* units. Amylose (**14**) has also been found in small quantities in this photobiont and also in the symbiont of *R. celastri* (Spreng.) Krog & Swinscow, probably originating from its photobiont [16,17,20]. These polysaccharides were not found in symbiotic stems of the species *R. gracilis* [20]. However, several complex side chain structures, composed primarily of D-Man*p* units were found with replacements at the positions *O*-4, *O*-2,4, *O*-2,3 and *O*-3,6 [20,31]. In the *Trebouxia* phycobiont stems of the species *R. maciformis* starch was found distributed in the chromatophores [36].

The lichen species of Antarctic *R. terebrata*, whose photobiont is a *Trebouxia* species, produced hemicelluloses and cellulose/lignin. Neutral and acidic monosaccharides components were derivatized with tetramethylsilane (TMS), producing glucose as most abundant neutral monosaccharide, but also the derivatives revealed the presence of galacturonic acid (**15**) as the most abundant among fatty acids [37].

Komiya and Shibata (1971) [38] studied the metabolism of polyols of *R. crassa* and *R. subbreviuscula* from phyco and mycobionts grown, and observed that ribitol (**16**) was produced by phycobiont and was converted to arabitol and mannitol in the mycobiont [38]. The principal *Ramalina* polysaccharides are compiled in Figure 1 and Table S1 (supplementary material).

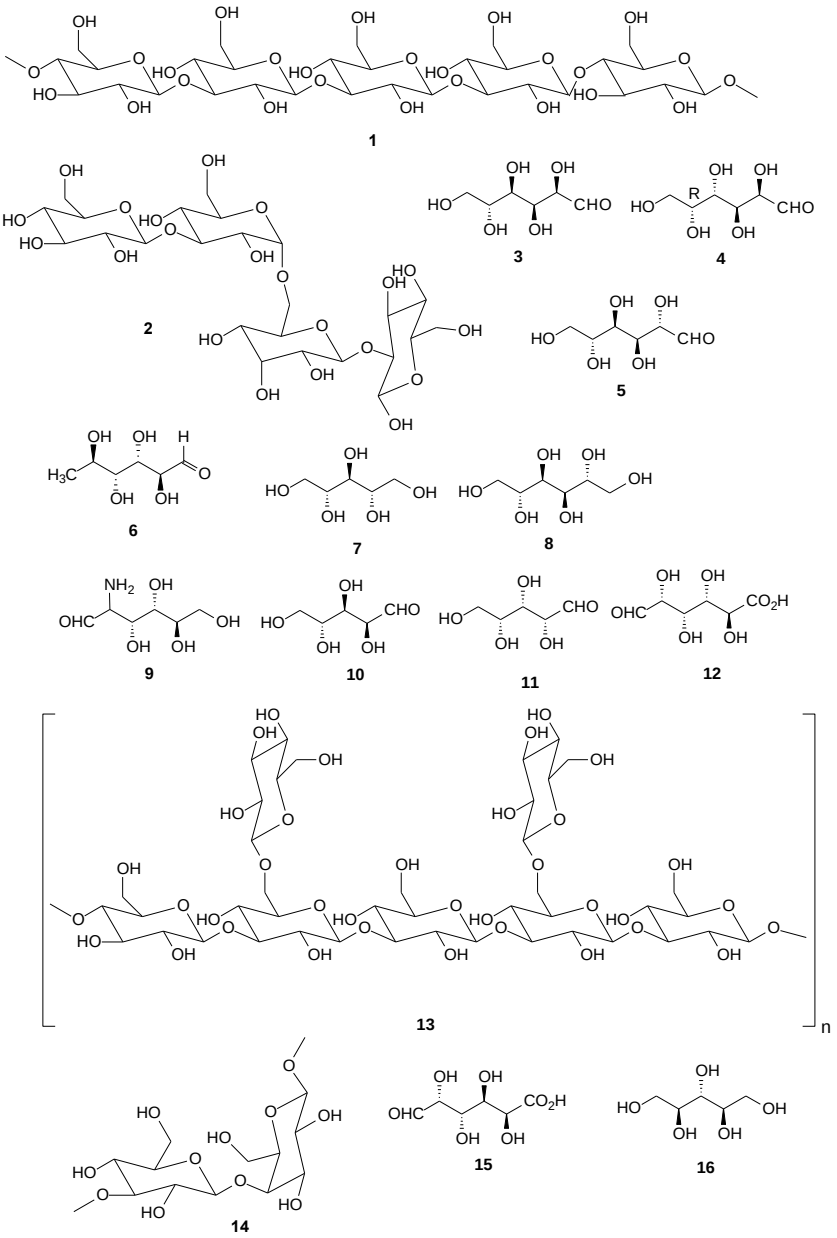


Figure 1. Chemical structures of polysaccharides of *Ramalina* species of lichenized fungi.

2.2. Usnic Acid and Derivatives

One of the most known, isolated and discussed compounds of lichenized fungi is usnic acid (**17**). It was isolated for the first time in the year 1834, by Rochleder *et al.,* from among others, the species *R. calicaris* (L.) Röhl. [3]. However, all species of the genus *Ramalina* contain usnic acid in variable concentration [5,39]. Studies of the isolation and biological activity tests of the compound have presented, almost always, unexpected results, generating numerous publications [5,7,10,12,13,16,21,25–29,34,40–86].

Lee *et al.* [8] isolated from *R. terebrata* Hook. f. & Taylor, a species from the Antarctic, usnic acid derivatives known as usimine A (**18**), B (**19**) and C (**20**), [8,10,11], and the last one presented good anti-proliferation activity results on human dermal fibroblasts [8,11]. González *et al.* (1991) [12] isolated (+)-*iso*-usnic acid (**21**) from the lichen *R. hierrensis* [12], and Asahina and Fukuziro (1932) [13] isolated usninic acid (**22**) from *R. calicaris*, shown in Figure 2 and Table S2 (Supplementary Material).

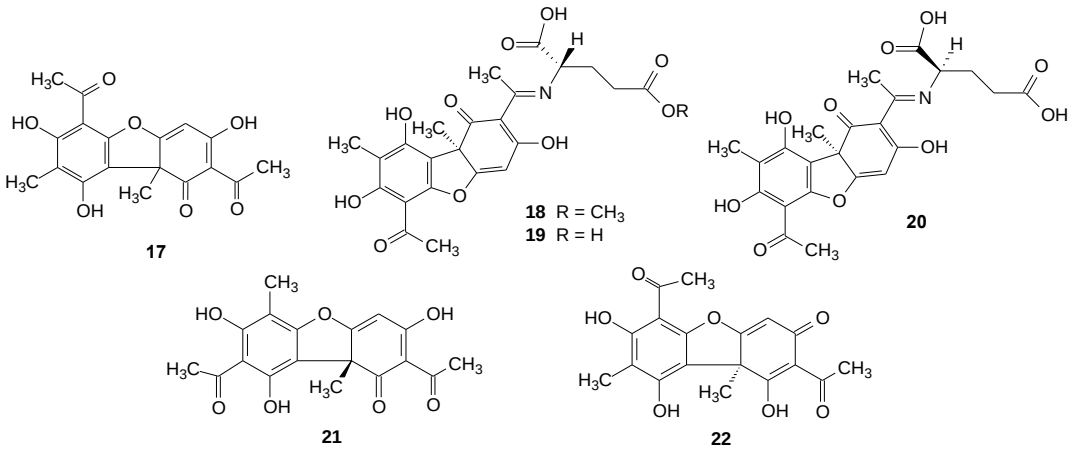


Figure 2. Chemical structures of usnic acid and derivatives from *Ramalina* species of lichenized fungi.

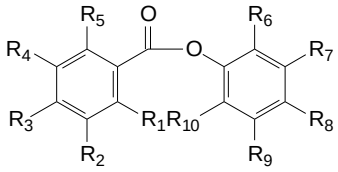
2.3. Depsides

Among the isolated depsides, sekikaic acid (**23**) was found in 26 species of *Ramalina* [1,25,27,40,43,46,50,51,54,55,57,59–63,65,87–96], atranorin (**24**) in 19 species [1,12,28,55,57,59,60,64,66,67,72,83,87,92,95,97], divaricatic acid (**25**) was found in 11 species [12,43,49,57,59,60,98] like homosekikaic acid (**26**) [60,87,89,90,92,96]. Ramalinolic acid (**27**) was found in ten species [1,43,46,60,80,87,88,99], obtusatic acid (**28**) appears in seven species [1,13,25,44,50,68,77,80,88,100] and the depside chlorinated tumidulin (**29**) was found in six species [64,73,101,102], like 4′-*O*-demethylsekikaic (**30**) [1,60,87] and evernic acids (**31**) [1,13,44,50,54,65,68,81,88,100,103]. 4′-*O*-Methylnorhomosekikaic acid (**32**) [60,89,104] was found in five species.′

Some compounds were found in a smaller number of species, usually three, two or one. 4′-*O*-methylnorsekikaic (**33**) [1,40,60], 2′-*O*-methylsekikaic (**34**) [1,60,104,105] acids and chloroatranorin (**35**) [2,55,66] were found in three species. Cryptochlorophaeic (**36**) [55,60], 4′-*O*-demethylhomosekikaic (**37**) [60],

diffractaic (**38**) [46,66], 4-*O*-demethylbarbatic (**39**) [1,106,107], and ramalinaic acids (**40**) [1,108] were found in two species.

In just one species, were found the tridepside gyrophoric (**41**) [46] and trivaric acids (**42**) in *R. americana* Hale [46,109], perlatolic acid (**43**) in *R. stenospora* Müll. Arg. [55], 4-*O*-demethylnorhomosekikaic acid (**44**) [60] in *R. peruviana* [56], 4′-*O*-methylsekikaic (**45**) [60], 4′-*O*-methylpaludosic (**46**) [60,104], 4,4′-di-*O*-methylcryptochlorophaeic (**47**) [104] and boninic (**48**) [76] acids were found in *R. asahinae*, stenosporic acid (**49**) in *R. stenospora* [1,55], 5-hydroxysekikaic
acid (**50**), new hydroquinone depside, in *R. farinacea* [62] and 5-chlorosekikaic acid (**51**) in *R. glaucescens* [78]. From *R. leiodea* [110] was isolated olivetoric acid (**52**), and paludosic acid (**53**) in *R. paludosa* [72]. An orcinol-type meta-depside with an oxidized side chain 4-*O*-methyloxo-cryptochlorophaeic acid (**54**) [111] was isolated from *R. subfraxinea* [111], lecanoric acid (**55**) from *R. lacera* [46] and aliphatic depside bourgeanic acid (**56**) found among other species in *R. bourgeana* [102,112,113]. See Figure 3 below and Table S3 in the Supplementary Material.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
23	OH	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_3H_7	H	OCH_3
24	OH	CHO	OH	H	CH_3	CH_3	OH	$COOCH_3$	CH_3	H
25	OH	H	OCH_3	H	*n*-C_3H_7	H	OH	COOH	*n*-C_3H_7	H
26	OH	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_5H_{11}	H	OCH_3
27	OH	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_5H_{11}	H	OH
28	OH	CH_3	OCH_3	H	CH_3	CH_3	OH	COOH	CH_3	H
29	CH_3	Cl	OH	Cl	OH	H	OH	$COOCH_3$	CH_3	H
30	OH	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_3H_7	H	OH
31	CH_3	H	OCH_3	H	OH	H	OH	COOH	CH_3	H
32	OCH_3	H	OH	H	*n*-C_3H_7	OH	COOH	*n*-C_5H_{11}	H	OCH_3
33	*n*-C_3H_7	H	OH	H	OCH_3	OH	COOH	*n*-C_3H_7	H	OCH_3
34	OCH_3	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_3H_7	H	OCH_3
35	OH	CHO	OH	Cl	CH_3	CH_3	OH	$COOCH_3$	CH_3	H
36	OCH_3	H	OH	H	*n*-C_5H_{11}	OH	COOH	*n*-C_5H_{11}	H	OH
37	OH	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_5H_{11}	H	OH
38	OCH_3	CH_3	OCH_3	H	CH_3	CH_3	OH	COOH	CH_3	H
39	OH	CH_3	OH	H	CH_3	CH_3	OH	COOH	CH_3	H
43	OH	H	OCH_3	H	*n*-C_5H_{11}	H	OH	COOH	*n*-C_5H_{11}	H
44	OCH_3	H	OH	H	*n*-C_3H_7	OH	COOH	*n*-C_5H_{11}	H	OH
45	OH	H	OCH_3	H	*n*-C_3H_7	OH	COOH	*n*-C_3H_7	H	OCH_3
46	*n*-C_3H_7	H	OH	H	OCH_3	OH	COOH	*n*-C_3H_7	H	OCH_3
47	*n*-C_5H_{11}	H	OCH_3	H	OCH_3	OH	COOH	*n*-C_5H_{11}	H	OCH_3
48	*n*-C_3H_7	H	OCH_3	H	OCH_3	OH	COOH	*n*-C_5H_{11}	H	OCH_3
49	OH	H	OCH_3	H	*n*-C_3H_7	H	OH	COOH	*n*-C_5H_{11}	H
50	OH	H	OCH_3	OH	*n*-C_3H_7	OH	COOH	*n*-C_3H_7	H	OCH_3
51	*n*-C_3H_7	Cl	OCH_3	H	OH	OCH_3	H	*n*-C_3H_7	COOH	OH
52	$CH_2COC_5H_{11}$	H	OH	H	OH	H	OH	COOH	*n*-C_5H_{11}	H
53	*n*-C_3H_7	H	OH	H	OCH_3	OH	COOH	*n*-C_5H_{11}	H	OH
54	OH	H	OH	H	CH_3	H	OH	COOH	CH_3	H
55	$CH_2COC_3H_7$	H	OCH_3	H	OCH_3	OH	COOH	*n*-C_5H_{11}	H	OH

Figure 3. *Cont*.

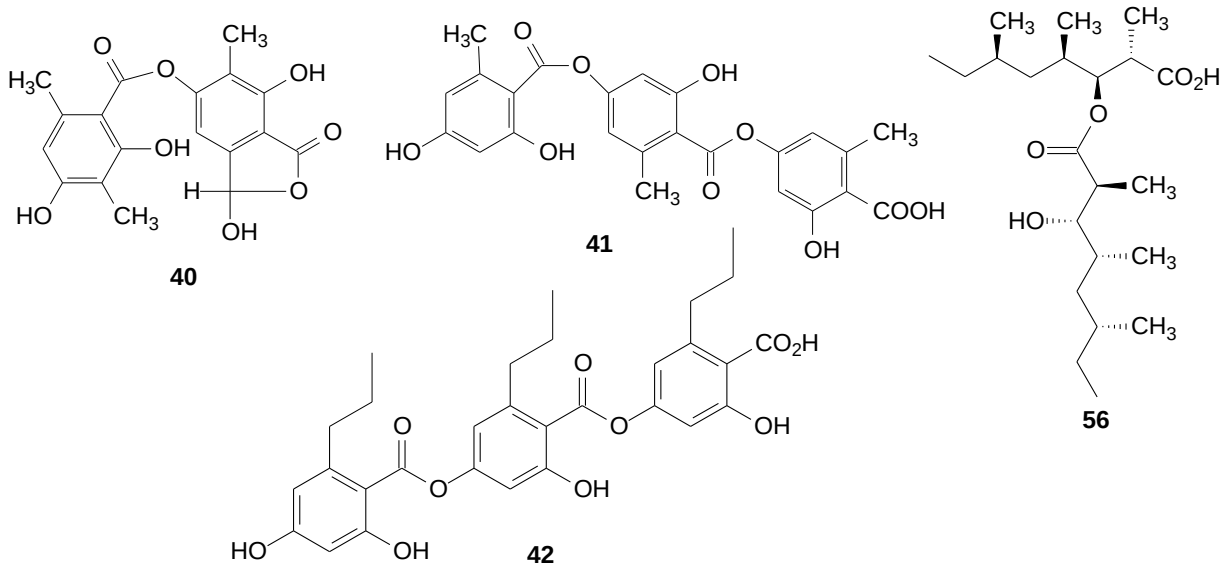


Figure 3. Chemical structures of the depsides and derivatives of the *Ramalina* species of lichenized fungi.

2.4. Depsidones

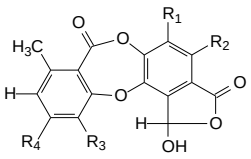
The most common compound of the depsidones class among species of *Ramalina* was salazinic acid (**57**), identified in 27 species [1,28,39–41,46,51,54–56,59–61,70,79,80,88,89,98,106,114,115], followed by norstictic acid (**58**) in 15 species [1,5,39,42,46,50,60,61,70,88,91,115,116]. Hypoprotocetraric acid (**59**) was found in six species [1,5,28,39,115,117], scopuloric acid (or stictic) (**60**), in five species [5,12,28,39,41,57,80,88], and the protocetraric acid (**61**), in four species [1,5,28,39,42,46,50,70,88,106,115].

Some depsidones were found in just one species, such as connorstictic acid (**62**) in *R. anceps* Nyl. [60], cryptostictic acid (**63**) found in *R. cuspidata* in the varieties *cuspidata* and *armorica* [5], peristictic acid (**64**) in *R. cuspidata* var. *armorica* [5], conhypoprotocetraric acid (**65**) in *R. siliquosa* var. x [5], variolaric acid (**66**) and gangaleoidin (**67**) in *R. hierrensis* [12], physodic acid (**68**) in *R. leoidea* [110] and the coquimboic acid (**69**) found in *R. tumidula* [118]. See Figure 4 below and Table S4 in the Supplementary Material.

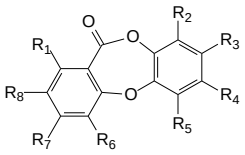
2.5. Fatty Acids

Lichenized fungi contain many of the fatty acids commonly found in higher plants [119] and in marine natural products [120]. Among these fatty acids, oleic

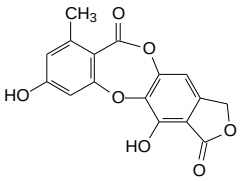
(**70**)**,** palmitic (**71**) and stearic (**72**) acids were found in *R. lacera* [46], *R. yasudae* [119] and in the mycobiont of *R. celastri* [121]. α-Linolenic acid (**73**) was found in *R. yasudae* [119] and *R. lacera* [46]; and linoleic (**74**) and myristic (**75**) [119] acids were found in *R. yasudae* [45]. Arachidonic acid (**76**) [119] was not found in the symbiont of *R. yasudae*, but it was present in small quantity in the photobiont *Trebouxia* and traces of its mycobiont [119]. The long chain γ-lactone acids D-protolichesterinic (**77**) and nephrosterinic (**78**) were obtained from *R. almquistii* Vain. [44,122] and protolichesterinic acid was also found in *R. roesleri* [92]. See Figure 5 below and Table S5 in the Supplementary Material.



	R_1	R_2	R_3	R_4
57	CH_2OH	OH	CHO	OH
58	CH_3	OH	CHO	OH
60	CH_3	OH	CHO	OCH_3
62	CH_3	H	CH_2OH	OH
63	CH_3	OH	CH_2OH	OCH_3
64	CH_3	OH	CO_2H	OCH_3



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
59	CH_3	CH_3	OH	CO_2H	CH_3	CH_3	OH	H
61	CH_3	CH_2OH	OH	CO_2H	CH_3	CHO	OH	H
65	CH_3	CH_2OH	OH	CO_2H	CH_3	CH_3	OH	H
67	CH_3	H	OCH_3	CO_2CH_3	CH_3	Cl	OH	Cl
68	$CH_2COC_5H_{11}$	H	OH	CO_2H	C_5H_{11}	H	OH	H
69	CH_3	CH_3	OH	CO_2H	CH_3	CH_3	OH	H



66

Figure 4. Chemical structures of depsidones from *Ramalina* species of lichenized fungi.

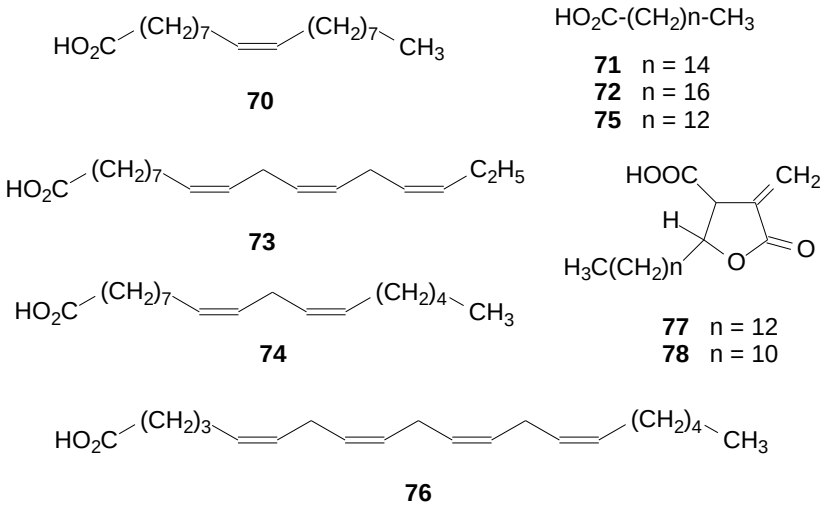


Figure 5. Chemical structures of fatty acids from *Ramalina* species of lichenized fungi.

2.6. Other Compounds

Among other classes of compounds found in lichens of the genus *Ramalina*, the research of Czeczuga and Ferraro (1987) [123] presents isolated carotenoids of lichens from the Argentinian species *R. ecklonii* (a) and *R. usnea* (b) and the review of Dembistsky (1992) [124] features carotenoids of lichens from New Zealand, among them the species *R. celastri* (c), the isolated compounds were β-cryptoxanthin (a,b) (**79**), lutein epoxide (a,b,c) (**80**), violaxanthin (a,c) (**81**), auroxanthin (a,b) (**82**), astaxanthin (b,c) (**83**), mutatoxanthin (b) (**84**), lycoxanthin (a) (**85**), antheroxanthin (a,b) (**86**), ε-carotene (b) (**87**), zeaxanthin (a,c) (**88**), β-carotene (c) (**89**), α-doradexanthin (c) (**90**), lutein (b,c) (**91**), hydroxyechinenone (a) (**92**), diatoxanthin (a) (**93**), neoxanthin (a,b) (**94**) and rhodoxanthin (a,b) (**95**) [123,124]. Several of these carotenoids are also found in higher plants, in algae, yeast and other marine organisms [120]. See Figure 6 below and Table S6 (Supplementary Material).

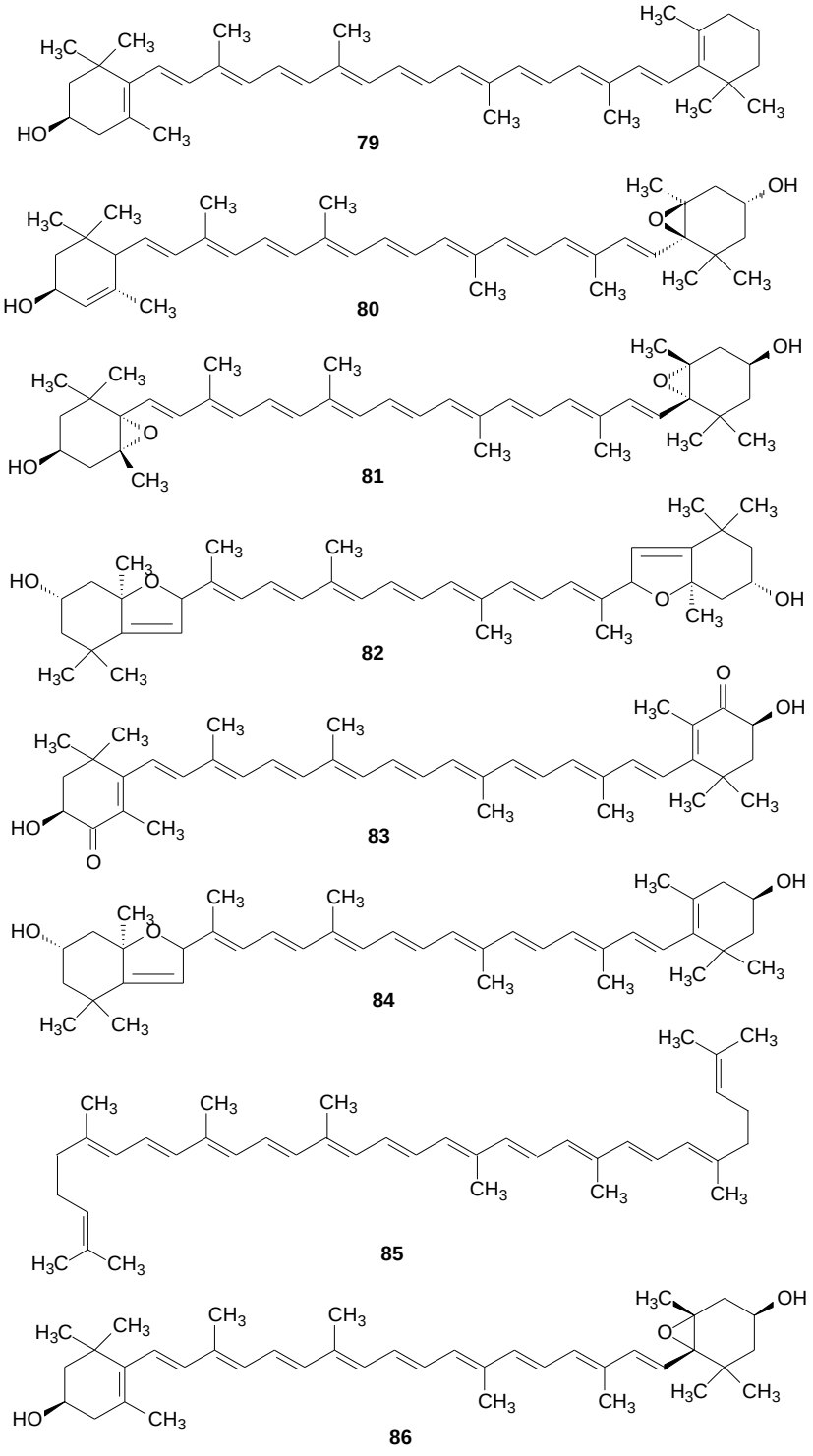


Figure 6. *Cont*.

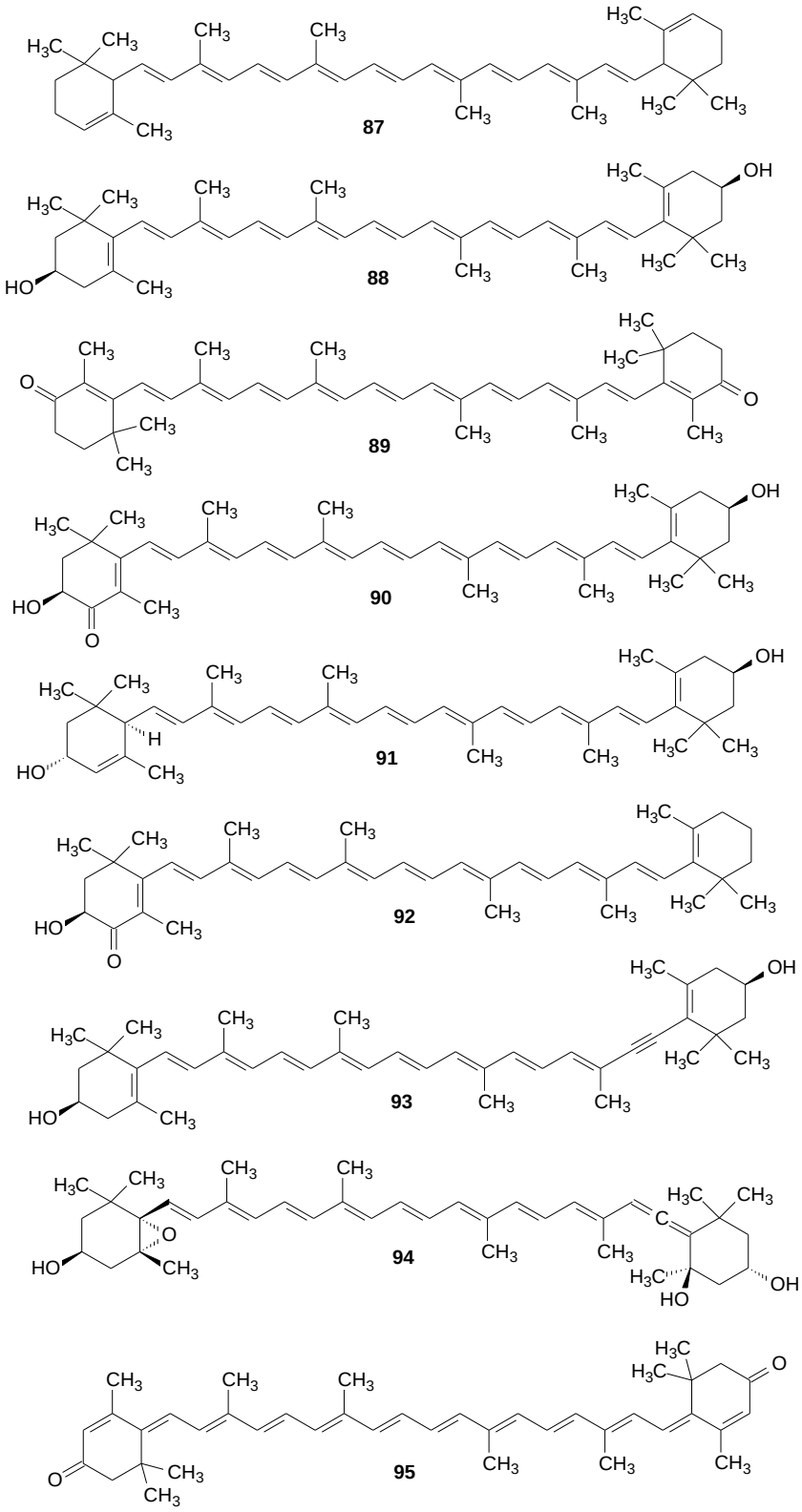


Figure 6. Chemical structures of other compounds: carotenoids of *Ramalina* species of lichenized fungi.

Some steroids isolated from lichens are also found in several marine organisms, including sponges, algae, among others, possessing important biological activities as antitumor agents and against *Mycobacterium tuberculosis*,

such as ergosterol peroxide. β-Sitosterol, a substance widely found in higher plants, has antibacterial and antifungal properties and is an antihypercholesterolaemic, estrogenic and hypolipididemic agent [120].

β-Sitosterol (**96**) was found in *R. africana* [44] and *R. hierrensis* [12], brassicasterol (**97**) in *R. africana* [44] and *R. tingitana* [69], and lichesterol (**98**) [44] in *R. africana* [44]. Ergosterol peroxide (**99**) was isolated from *R. hierrensis* [12] and *R. tingitana* [69] and cerevisterol (**100**) was isolated from *R. hierrensis* [12]. The triterpenes ursolic acid (**101**) and *iso*-arborinol acetate (**102**) were found in *R. hierrensis* [12] and friedelin (**103**) in *R. ecklonii* [83], and the diterpenes (−)-sandaracopimaric acid (**104**) in *R. hierrensis* [12] and ceruchinol (**105**) in *R. tigrina* [125] and *R. ceruchis* var. *tumidula* [126]. Figure 7 is shown below and Table S7 in the Supplementary Material.

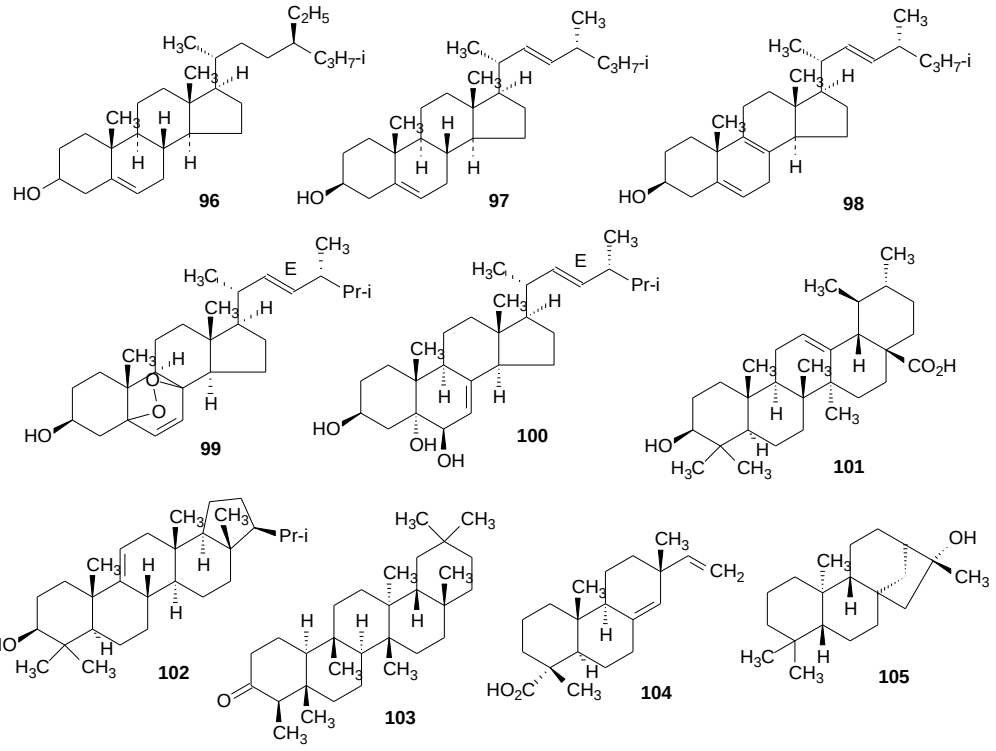


Figure 7. Chemical structures of other compounds: steroids and terpenoids from *Ramalina* species of lichenized fungi.

From *R. lacera* [46] were isolated the polar lipids diacylglyceryl-*N,N,N*-trimethylhomoserine (**106**) (DGTS), diacylglyceryltrimetylalanine (DGTA) (**107**), phosphatidylcholine (PC) (**108**), phosphatidyl-ethanolamine (PE) (**109**), phosphatidylinositol (PI) (**110**), phosphatidic acid (PA) (**111**) and the glycolipid sulfoquinovosyl diacylglycerol (SQDG) (**112**). The glycolipids

monogalactosyldiacylglycerol (MGDG) (**113**) and digalactosyldiacylglycerol (DGDG) (**114**) were isolated both from *R. lacera* [46] and *R. celastri* [127]. From *R. celastri* was obtained a glycosphingolipid, *O*-β-D-galactopyranosyl-(1→1′)-ceramide (**115**) [128], which primary lipids components are (4*E*)-sphingenine, sphinganine and eicosasphinganine, esterified with palmitic, oleic and 2-hydroxypalmitic acids. From the stems of *R. fraxinea* were obtained a fraction containing the amines choline (**116**), betaine (**117**), histamine (**118**), acetylcholine (**119**) and β-phenethylamine (**120**) [25]. In *R. farinacea* the polyamines spermidine (**121**) and diamine putrescine (**122**) were detected [129,130] and in *R. calicaris* the polyamine spermine (**123**) [130]. Figure 8 below and Table S8 (Supplementary Material)

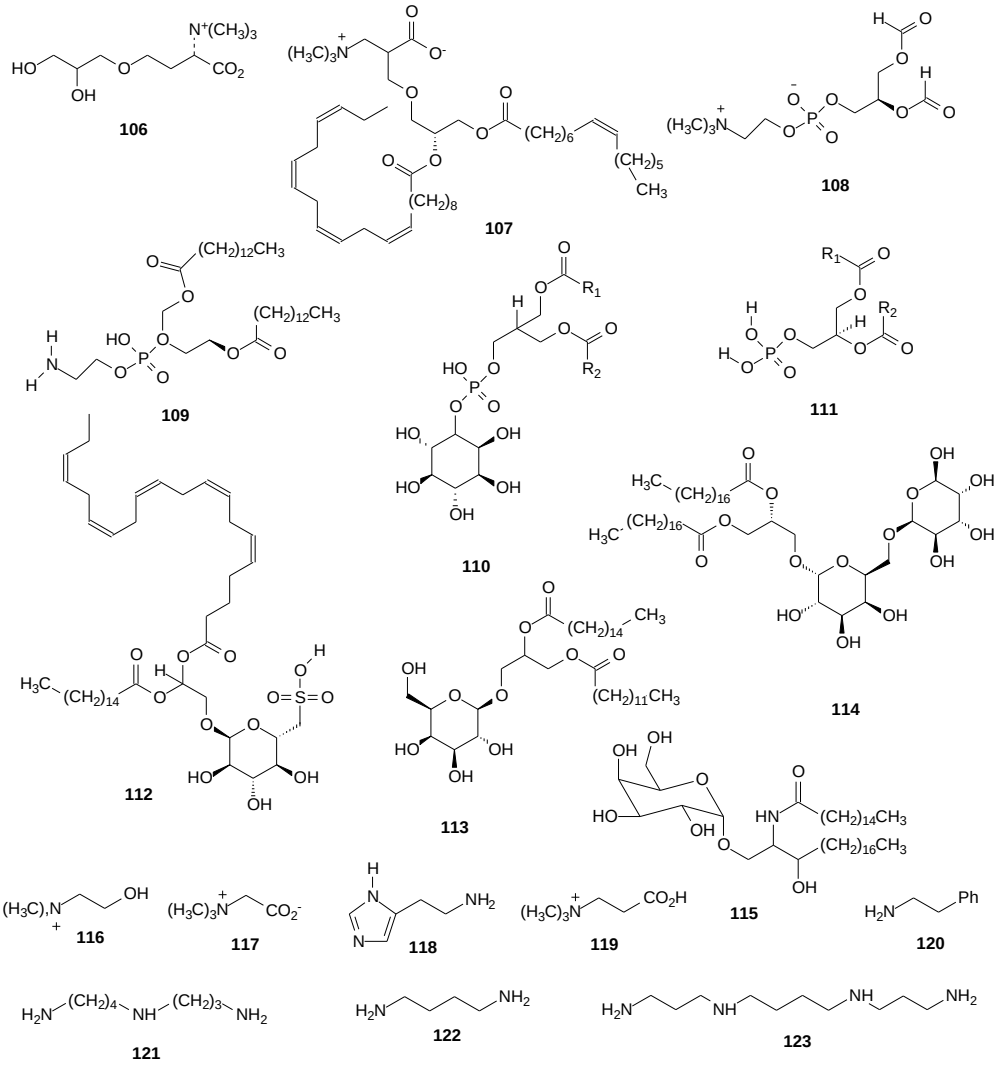


Figure 8. Chemical structures of other compounds: lipids and amines from *Ramalina* species of lichenized fungi.

The amino acids found in lichenized fungi are similar to those found in many marine organisms and higher plants [120]. From *R. siliquosa* [131] and *R. fraxinea* [25], were isolated glutamic acid (**124**), aspartic acid (**125**), alanine (**126**), serine (**127**) and proline (**128**). Alanine was also found in *R. sinensis* [132]. The amino acids arginine (**129**), glycine (**130**), lysine (**131**), leucine (**132**), threonine (**133**), and glucosamine (**134**) were found in *R. siliquosa* [131] and the γ-aminobutyric acid (**135**) was found in *R. fraxinea* [25]. Others amino acids were also found in *R. siliquosa* [131] at very low concentrations. From *R. crassa* the amino acid taurine (**136**) was isolated [133]. See Figure 9 below and Table S9 in Supplementary Material.

From an Antarctic lichen species, *R. terebrata*, were isolated compound ramalin (**137**), a new hydrazide with antioxidant activity [9–11,134–136] and the cyclic depsipeptide stereocalpin A (**138**) [137–139].

Different phenolic compounds have been isolated from the species of the genus *Ramalina*. From *R. farinacea* was isolated 2,3-dihydroxy-4-methoxy-6-pentylphenylmethyl ester (**139**) [62], from *R. africana* were isolated divaric acid (**140**) and ethyl divaricatinate (**141**) [44], from *R. roesleri* 2-hydroxy-4-methoxy-6-propylbenzoic acid (**142**) and 2,4-dihydroxy-3,6-dimethylmethyl ester benzoic acid (**143**) [92] and from *R. dilacerata* was isolated isorhizonic acid (**144**) [140].

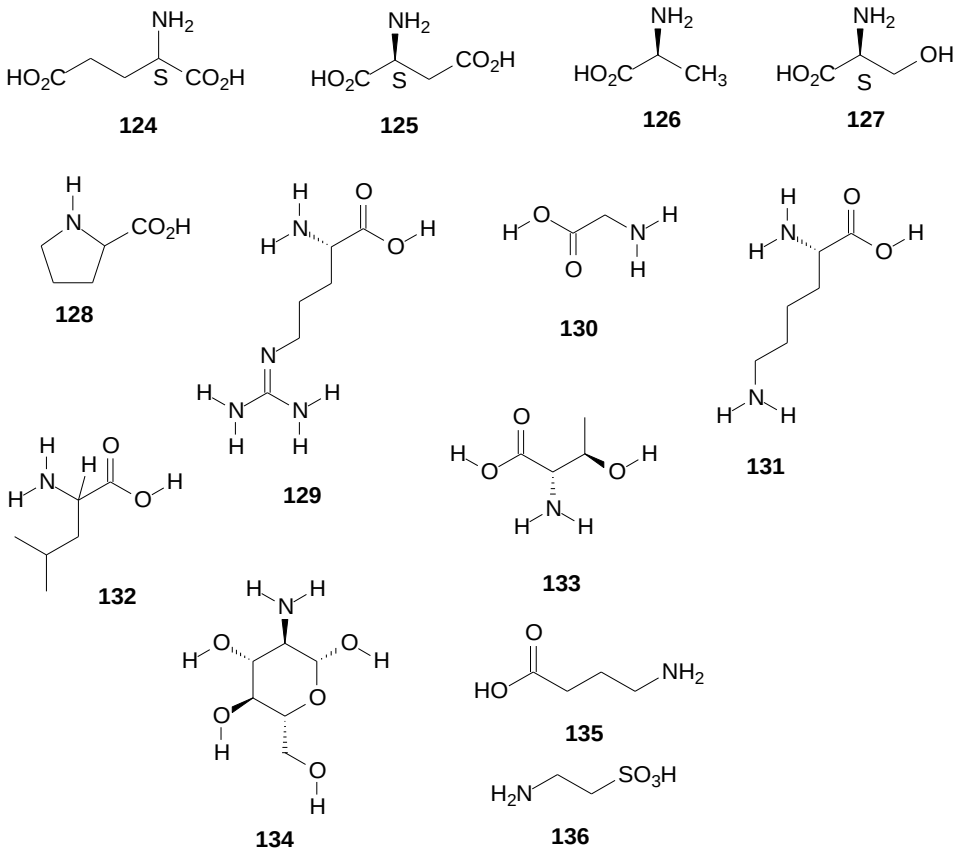


Figure 9. Chemical structures of other compounds: amino acids from *Ramalina* species of the fungi.

Organic acids and derivatives as well as fatty acids esters were found in *Ramalina* species. α-Crotonic acid (**145**) was isolated from *R. reticulata* [26] and abscisic acid (**146**) from *R. farinacea* [129], while from *R. fastigiata* [141] were isolated the esters ethyl caprilate (**147**), ethyl palmitate (**148**) and ethyl stearate (**149**). The compounds benzopyran divaricat acid (**150**) and anthraquinone (**151**) were found in *R. hierrensis* [12].

Aliphatic compounds and cycloaliphatic have been also found in lichens, especially alkanes with C_{13} and C_{17}–C_{40} carbon chains [1]. The research of Zygaldo *et al.* [142] presented a composition of *n*-alkanes from 15 species of lichens belonging to six different families, including the Ramalinaceae family, among the species of this family *R. celastri* and *R. ecklonii* are found. The analyses results by GC/MS from the lichens stems showed the presence of *n*-alkanes with chain ranging from C_{13}–C_{40} and showed that 14 *n*-alkanes with chain C_{20}–C_{33} were common to all studied species. Branched alkanes have not been found. The composition of the *Ramalina n*-alkanes was characterized by a high level of C_{29} and C_{31} (>7%) in both species. Difference between the species were

observed, because *R. ecklonii* presented a higher concentration of C_{27} (11.3%) and C_{31} (19.5%) against 5.6% of C_{27} and 13.2% of C_{29} for *R. celastri* [142]. The compound cycloaliphatic aspicilin (**152**) was isolated from *R. ecklonii* [73] and ethylene (**153**) extract in ethyl ether was found in *R. lacera* [1]. See Figure 10 below and Table S10 in the Supplementary Material.

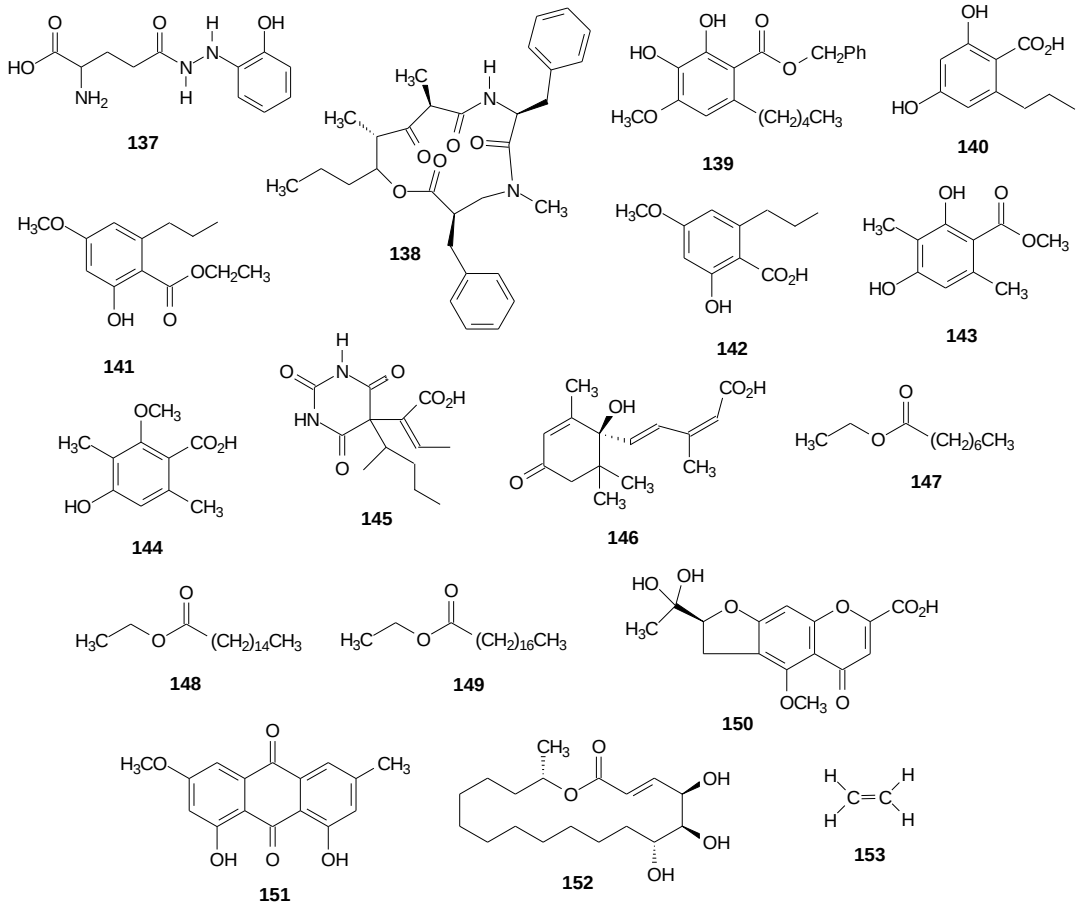


Figure 10. Chemical structures of other compounds from *Ramalina* species of lichenized fungi.

3. Biological Activity

Lichens have been used as a promising biological source of metabolites with different bioactivities [1,2,53]. Huneck and Yoshimura [1] mention in their classic work the monograph of Zopf in 1907 about the pharmacological activities of lichens compounds. The authors also highlighted the following biological activities that have already been researched up to the present time: antibiotic activities, antitumor and antimutagenic activities, activities against human immunodeficiency virus (HIV), allergenic activities, growth inhibitory activities in plants, and enzyme inhibitory activities, among other activities [1]. However, only a very limited number

of lichen compounds were tested for their biological activities and their therapeutic potential in medicine. This happens because of the difficulties in identifying the species, in the small amounts collected for such studies and difficult isolation of pure compounds for structural elucidation and biological activity testing [2].

The cause of the antibiotic activity of many lichens has been assigned to usnic acid [52], although there are authors that mention that the antibiotic activity of lichens is related to the presence of phenolic derivatives [3]. The mechanisms of antibiotic action of lichenic acids, more specifically usnic acid and its derivatives, suggest that these compounds modify the structures of proteins causing irreversible changes, and may even produce apoptosis [3]. This compound presents activity against bacteria, fungi and yeasts [1–3,42,52,53,88,114,143]. Beside the antimicrobial activity against human and plant pathogens, usnic acid (**17**) presents antiprotozoal action [53], analgesic and antipyretic [1,53], anti-inflammatory [53], antitumor and antimutagenic [2,3,53], antiviral [1–3,53] plant growth inhibiting [1] , enzyme activity inhibitory [1,2] and allergenic properties [1].

Another class of compounds, the polysaccharides, which can be from multiple sources (plants, fungi and lichens) have different biological activities, acting as antitumor and anti-inflamatory agents and immunomodulators [31]. Several studies have shown that many polysaccharides can act as biological response modifiers (BRM). This can happen by activation of the immune response involving macrophages, T helpers and natural killer cells (NK cells), T cell differentiation, proliferative response of polymorphnuclear cells, production of interleukins and interferon, as well as increasing the phagocytotic activity [34]. Some biological activities of extracts and compounds isolated from the genus *Ramalina* will be presented next.

3.1. Antimicrobial Activity

The development of new antibacterial compounds is an urgent issue to suppress the evolution of pathogenic bacteria rssistant to the available drugs [123]. Therefore, many studies have been developed in an attempt to discover new substances with this activity.

The usnic acid isolated from *R. reticulata* Kremp. (currently *R. menziesii* Taylor) presented activity against Gram (+) organisms and some acid resistant bacteria, including *Mycobacterium tuberculosis* (Zopf 1883) Lehmann and

Neumann 1896, but not against a range of different Gram (−) organisms [114] in agreement with other performed studies [93].

Tay *et al.* [42] tested the antimicrobial activity of the acetone extract obtained from *R. farinacea* (L.) Ach. and its constituents usnic acid (**17**), norstictic acid (**58**) and protocetraric acid (**61**) against thirteen bacteria, two yeast and ten filamentous fungi. The extract demonstrated activity against six bacteria with concentrations ranging between 3.3–6.6 µg/25 µL and 3.3 µg/25 µL for the two yeasts tested *Candida albicans* and *Candida glabrata* and no activity against filamentous fungi. Regarding the compounds tested, the usnic acid showed the best results, and for the six bacteria on which it had an effect, the minimum inhibitory concentration (MIC) varied between 0.39–3.1 µg/25 µL; the less active was the norstictic acid, with a MIC ranging between 11.7–188 µg/75 µL. Protocetraric acid did not present action against fungi and bacteria. With the yeasts, usnic acid also presented the best results, with a MIC value of 0.05 µg/62.5 µL, while for norstictic acid the MIC value was 2.9 µg/75 µL and for protocetraric acid the value was 3.9 µg/75 µL. The study demonstrated that among the three compounds tested only usnic acid showed any significant activity at low concentration against the Gram (+) and fungi tested.

The antioxidant and antimicrobial properties of methanolic extracts from five lichenized fungi species were tested by Gulluce *et al.* [144], and the results showed that the extracts of *R. polymorpha* and *R. pollinaria* inhibited 10 and 11 bacterial species, respectively, from a total of 35 tested species. Assays were performed using the disk diffusion method and micro-dilution assay to obtain the values of MIC values (µg/mL). The *R. pollinaria* extract presented MICs between 5.62–62.5 $\mu g \cdot \mu L^{-1}$. For both fungi tested, the MIC values of *R. polinaria* were 31.25–62.5 $\mu g \cdot \mu L^{-1}$ for *Trichophyton rubrum* and *Sclerotonia minor,* respectively, while the MIC value of *R. polymorpha* was 62.5 $\mu g \cdot \mu L^{-1}$ for both fungi. Data from this study indicated that there must be antimicrobial compounds in the tested extracts, which include the *Ramalina* genus [144].

Cansaran *et al.* [52] investigated the biological activities of five *Ramalina* species obtained in Turkey: *R. fastigiata* (Pers.) Ach., *R. capitata* (Ach.) Nyl., *R. polymorpha* (Lilj.) Ach., *R. pollinaria* (Westr.) Ach. and *R. fraxinea*. They used the agar disk diffusion method with a tetracycline as control. The study presented that the extracts of lichens showed antimicrobial activity at different rates and that the

greater the concentration of usnic acid in the extract, the greater the inhibition of microorganisms. However, all the extracts inhibited *Bacillus subtilis*, with *R. fastigiata*, which presented a higher concentration of usnic acid among the extracts (about 3.3% by dry weight) showing greater inhibition. Only the *R. fraxinea* extract, with a usnic acid concentration of 0.17% did not inhibit *Bacillus megaterium*. *Enterococcus faecalis* and *Proteus mirabilis* were only inhibited by the extracts of *R. fastigiata*, with 3.3% of usnic acid and *R. capitata*, with 1.25% of usnic acid. Both of this extracts also inhibited *Escherichia coli* and beside them, *Ramalina polymorpha* extract, with 0.27% of usnic acid inhibited this bacteria with a lower inhibition rate. The extracts were especially active against Gram (+) bacteria, although none of them inhibited *S. aureus*. Among tested Gram (−) bacteria, neither of the extracts inhibited *Pseudomonas aeruginosa* and *Escherichia coli*, the bacteria *Proteus mirabilis* and *Escherichia coli* were inhibited by three and two of the extracts, respectively, with higher concentrations of usnic acid [52].

Crude extracts of the species *Ramalina hossei* Vain. produced in methanol, chloroform and petroleum ether solvents, were tested for their antimicrobial activity by the Kirby Bauer method. The results demonstrated that the extracts showed better activity against Gram (+) than against Gram (−) bacteria. Chemical tests of the extracts revealed the presence of usnic acid (**17**) and sekikaic acid (**23**) as a mixture. The extracts were more active against Gram (+) species, confirming the results of other studies, and the methanol extract showed greater inhibition of bacteria than other extracts [93].

In the study conducted by Babita *et al.* in 2008 [145], the antibacterial potential of methanolic extracts from five Antarctic lichens species belonging to four different genres were tested, among them the species *R. terebrata,* and it was shown that considerable antimicrobial activity were obtained against *Bacillus subtilis* (MIC 33.8 ± 0.15 $\mu g \cdot mL^{-1}$ and IC_{50} 16.9 ± 0.1 $\mu g \cdot mL^{-1}$) and *S.* aureus (MIC 85.7 ± 6.7 $\mu g \cdot mL^{-1}$ and IC_{50} 42.9 ± 3.4 $\mu g \cdot mL^{-1}$), but no activity were observed against *Candida albicans*, *Pseudomonas aeruginosa* and *Escherichia coli*; in this case the authors used the methodology of the sterile paper disk described by Bhattarai *et al.* in 2006 [146]. The MIC was determined by the broth dilution method described by Swenson *et al.* in 1982 [147]. The results showed strong antibacterial activity of the extracts against Gram (+) bacteria, indicating that these

species of Antarctic lichens produce compounds with significant antibiotic properties [145].

The research of Paudel *et al.*, conducted in 2010 [134] reported that five compounds isolated from the methanolic extract of the Antarctic lichen *R. terebrata*, namely usnic acid (**17**) and the derivatives, usimine A (**18**), B (**19**), C (**20**) and ramalin (**137**)**,** were tested against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* by the disc diffusion method. All tested samples presented activity against *B. subtilis*, where the values of MIC of the isolated compounds ranged from 1–26 $\mu g \cdot mL^{-1}$ for these bacteria. Only the crude methanolic extract and usnic acid showed activity against *S. aureus* [134].

In 2012, Paudel *et al.* [148] studied the antibacterial activity of twenty-four lichens species of six lichen families from Nepal, among them *Ramalina* spp. Twenty one species were active against *B. subtilis* and seven were active against *S. aureus.* The results showed that *Ramalina* spp. presented MIC values of 8.5 and 15.1 $\mu g \cdot mL^{-1}$ for *B. subtilis* and 65.3 $\mu g \cdot mL^{-1}$ for *S. aureus*, while the MICs of the commercial product ampicilin, used as control, were 0.4 and 0.35 $\mu g \cdot mL^{-1}$ for the respective tested bacteria. These results confirm those of other studies involving lichen species of the same genus [142]. The data showed a strong potential of these extracts as antibacterial agents. The results obtained by Sisodia *et al.* with hexane extract of *R. roesleri* confirmed the high activity against *S. aureus*, and also against *Streptococcus mutans* [75].

3.2. Antioxidant Activity

Several species of lichens from different genres and regions of the World have antioxidant potential [96]. Gulluce *et al.*, in 2006 [144], showed that the methanol extracts of *R. pollinaria* and *R. polymorpha* species did not show antioxidant properties by the diphenylpicrylhydrazyl method (DPPH), however, a low inhibition was exerted on the oxidation of linoleic acid in the linoleic acid/β-carotene oxidation method obtaining a percentage inhibition (I%) of 26 ± 1, 19 ± 2 and 96 ± 1 for *R. pollinaria*, *R. polymorpha,* and for the control compound butylated hydroxytoluene (BHT), respectively, so it was concluded that the extracts of these species showed little antioxidant potential [144].

Kumar *et al.* [94] studied the methanol extracts of two lichens of the Ramalinaceae family, *R. conduplicans* and *R. hossei*, and evaluated their

antioxidant activity by the DPPH method and by the reduction of Fe^{3+} assay. At the concentration of 250 µg/mL an elimination of 56.11% and 48.04% occurred for *R. hossei* and *R. conduplicans,* respectively, and at 500 µg/mL the elimination was 61.53% and 59.01% and with 1000 µg/mL it was 79.05% and 72.63%, below the control values of ascorbic acid, which were 92.52%, 95.12% and 97.33% at the same concentrations. *R. hossei* showed a higher free radical elimination rate than *R. conduplicans.* The substances usnic acid (**17**), sekikaic acid (**23**), salazinic (**57**) acid and tannins were detected in the lichen methanol extracts, which showed promising antioxidant potential results [94].

The research of Luo *et al.* [96] presented data on the antioxidant activity of the lichen *Ramalina conduplicans*. The free radical elimination activity of the lichen methanol extract was tested by the Blois method (1958, [149]), using DPPH and the result was presented as IC_{50}. An assay of the linoleic acid peroxidation activity using the thiocyanate method proposed by Mitsuda *et al.*, in 1996 [150], with some modifications proposed by Luo *et al.* in 2009 [151] was also performed. The percentage of inhibition was found to be 55.8% at the concentration of 330 $\mu g \cdot mL^{-1}$, presenting an IC_{50} of 0.232 $mg \cdot mL^{-1}$, relatively low compared to data from other lichen species tested. The extract exhibited high antioxidant activity against linoleic acid peroxidation, 85.2% at a concentration of 2.0 $mg \cdot mL^{-1}$, which was higher than the inhibition of ascorbic acid used as control at the same concentration. The main compounds detected by bioauthographic thin layer cromatography (TLC), high performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy were sekikaic acid (**23**) and homosekikaic acid (**26**) depsides. The IC_{50} values of the pure compounds were 0.082 $mg \cdot mL^{-1}$ for sekikaic acid and 0.276 $mg \cdot mL^{-1}$ for homosekikaic acid, demonstrating that these compounds are promising antioxidants [96].

Yim [10] has patented a pharmaceutical composition containing ramalin (**137**) as an active ingredient for functional foods used as anti-aging products, cosmetics for skin whitening and as an anti-wrinkle agent since ramalin present better antioxidant effects than conventional commercially available antioxidants [10].

Ramalin (**137**) [9,10,134–136] was isolated from the water-methanol extract of *R. terebrata* by several chromatographic methods by Paudel *et al.* in 2011 [9]. The experimental data showed that this substance was five times more powerful (IC_{50} 0.99 ± 0.08 $\mu g \cdot mL^{-1}$) that the commercial drug BHT (IC_{50} 4.98 ± 0.9

μg·mL^{-1}) in the elimination of free radicals by DPPH method, and twenty-seven times more powerful in eliminating free radicals by the 2,2′-azino-bis(3-ethylbenzothiazoline-6)-sulfonic acid ($ABTS^{+}$) method than the analogous compounds vitamin E and Trolox, and two and a half times more potent than BHT when used for reducing Fe^{3+} ions to Fe^{2+}. Ramalin also proved to be 1.2 times more powerful than ascorbic acid in the elimination of superoxide radicals. The *in vitro* tests of the antioxidant activity showed that 1.0 μg·mL^{-1} significantly reduced nitric oxide (NO) produced and 0.125 μg·mL^{-1} reduced the production of hydrogen peroxide (H_2O_2) in lipopolysaccharides (LPS) stimulated in murine macrophage cells Raw 264.7. Considering the data set, it is verified that ramalin is a therapeutic candidate for the control of the oxidative stress in cells, since the compound has very low or no cytotoxicity on human keratinocytes and fibroblasts cells at its active antioxidant concentrations [9].

Halici *et al.* [152] studied the antioxidant and gastroprotective activities of ethanol-water extracts (1:1), ethanolic, methanolic and aqueous extracts from the lichen *Ramalina capitata*. The results showed that the extracts significantly reduced gastric injuries induced by indomethacin. The most significant gastric protective effect was obtained with ethanol-water extract (1:1) (66.6%) at a dose of 200 mg·kg^{-1}. Indomethacin caused significant decreases in the levels of glutathione peroxidase (GP_x), glutatione S-transferase (GTS), superoxide dismutase (SOD) and reduced glutathione (GSH). However, ethanol–water extract (1:1) showed significant antioxidant activity against oxidative damage in the stomach tissues, increasing the levels of GP_x, GST, SOD and GSH. The catalase and myeloperoxidase levels increased by the indomethacin were lower in the groups administered with ethanol-water (1:1) extract. Furthermore, it can be observed that all tested extracts presented significant antioxidant activity *in vitro*, with 64.9% for the ethanol-water extract, 52.2% for the ethanolic extract, 56.7% for the methanolic extract and 73.7% for the aqueous extract in the assay of linoleic acid peroxidation inhibition. However, the antioxidant activity of these extracts was lower than that of the compound Trolox, used as control, but was higher than that of ascorbic acid. These results indicate that *R. capitata* extract had gastroprotective effects against gastric ulcer [152].

Four lichen genres (*Ramalina*, *Parmotrema*, *Bulbothrix* and *Cladia*) collected in Malaysia were studied by Stanly *et al.* [153] and had their antioxidant

activities compared by the DPPH method. The *R. peruviana* species presented higher activity in the elimination of free radicals (86%) with its extract in acetone at a concentration of 750 $\mu g \cdot mL^{-1}$. *R. peruviana* also gave the lowest effective concentration needed to eliminate 50% (EC_{50}) of free radicals with the extract in acetone presenting and EC_{50} of 60.66 $\mu g \cdot mL^{-1}$ among all four lichens species tested. For the assay using the β-carotene bleaching method, the best activity was obtained by the extract in acetone of *Bulbothix isidiza* (66.7%), followed by the acetone extract of *R. peruviana* (57.3%), yet the level of phenolic compounds found for the *R. peruviana* extract was the lowest among the species tested, demonstrating that there is no correlation between the total phenolic content and antioxidant activity [153].

The study developed in 2012 by Paudel *et al.* [148] presented data on the antioxidant activity of methanol-water extracts (8:2) from 24 Nepal lichen species. All species tested and the commercial product butylated hydroxyanisole (BHA) used as control showed free radical scavenging capacity by the DPPH, $ABTS^{+}$ and Fe^{3+} reduction methods at a dose dependent concentration. For the DPPH method, the inhibitory concentration 50% (IC_{50}) ranged from 5.6–98.6 $\mu g \cdot mL^{-1}$ for the extracts, wherein the NL-17 and NL-18 samples from *Ramalina sp*. presented IC_{50} of 32.9 and 8.7 $\mu g \cdot mL^{-1}$, respectively, while the IC_{50} of the control BHA was 3.5 $\mu g \cdot mL^{-1}$. The results of tests with $ABTS^{+}$ confirmed the DPPH data, with the IC_{50} values ranging from 6.9–99.8 $\mu g \cdot mL^{-1}$, and for the samples NL-17 and NL-18 the IC_{50}s were 52.0 and 33.2 $\mu g \cdot mL^{-1}$ and for the compound Trolox a vitamin E analog used as a control it was 46.4 $\mu g \cdot mL^{-1}$, so the compounds showed strong ability to eliminate free radicals, having great oxidant activity.

The antioxidant activity of extracts and isolated compounds from the lichen *R. roesleri* were assessed by Sisodia *et al.* [92] by the DPPH method. The results showed a range of free radical elimination power in the extracts between 29.42%–87.9%. The compounds atranorin (**24**), protolichesterinic acid (**77**), usnic acid (**17**), 2-hydroxy-4-methoxy-6-propylbenzoic acid (**142**), homosekikaic acid (**26**), sekikaic acid (**23**), 2,4-dihydroxy-6-propylbenzoic acid (**142**) and 2,4-dihydroxy-3,6-dimethylbenzoate (**143**) were isolated from the hexane extract. Among the compounds, the best antioxidant activity was exhibited by sekikaic acid, followed by homosekikaic acid [92].

3.3. Antiviral Activity

Few studies were found on the antiviral effects using extracts or pure compounds isolated from lichens. The research of Fazio *et al.* [48] evaluated the antiviral and cytotoxic activity effects against Vero cells infected with arenavirus Junin (JUNV), causative agent of hemorrhagic fever on human beings in Argentina and against arenavirus Tacaribe (TCRV), a non-pathogenic member of the Arenaviridae family, of two secondary metabolites obtained from mycobiont cultivation of two genera of lichens, *Tlechoschistes chrysophthalmus* and *Ramalina celastri*. The antiviral and virucidal activity of usnic acid, a metabolite isolated from *R. celastri*, the subject focus of this review, will be presented. Parientin, a compound isolated from *T. chrysophthalmus* will not be discussed. Antiviral activity testing was performed using concentrations lower than the 50% cytotoxic concentration (CC_{50}), for usnic acid (65.1 µM). The results demonstrated that usnic acid (**17**) reduced the production of Junin virus in infected Vero cells in a dependent dose manner, and 50% inhibition was obtained at an effective concentration (EC_{50}) of 9.9 µM. Regarding the TCRV arenavirus, the effective concentration was 20.6 µM. The selectivity indexes (CC_{50}/EC_{50}) of usnic acid for JUNV and TCRV arenavirus were 6.8 and 3.2, respectively, indicating a specific antiviral activity against these viruses and not just a general consequence of its action on cellular toxicity. In order to test the viability of virus inactivation by the direct effect on the viral particles, one virucidal assay was performed using the methodology proposed by Garcia *et al.* in 2002 [154]. When suspensions of JUNV or TCRV particles were incubated with usnic acid before cell infection, any remaining difference in infectivity of virus suspensions was detected between treated and untreated cells, so the virus-inhibitory effect observed in the inhibition assay performance was due to a real antiviral activity, exercised during the multiplication of the virus in the host cell [48].

Esimone *et al.* [155] obtained from *R. farinacea* lichen species a fraction soluble in ethyl acetate (ET4) which inhibited infection by adenoviral and lentiviral vectors such as HIV-1 type. Herpes virus type 1 (HSV-1) and respiratory syncytial virus (RSV) inhibition were also evaluated with this fraction. The anti-HIV and anti-HSV activities were quantified by the response of the β-galactosidase expression from the lineages of the indicator cells, while the anti-RSV activity was determined by an immunofluorescence technique. The ET4 effect on enzymatic activity of HIV-

1 reverse transcriptase was also evaluated by chemiluminescence. It was demonstrated that the fraction strongly inhibited HSV-1 and RSV, with IC_{50} values of 6.09 and 3.65 μg·mL^{-1}, respectively. The fraction inhibited reverse transcriptase with an IC_{50} of 0.022 μg·mL^{-1}. Bioassay guided ET4 fractionation lead to a subfraction rf0 that showed activity against lentiviral vector and HIV-1 (RNA virus), but not against HSV-1 (DNA virus) and the rfM subfraction showed activity against HSV-1 but not against the lentivirus vector. Therefore, the study showed that the *R. farinacea* ET4 fraction has antiviral activity against DNA viruses (adenovirus, HSV-1) and against RNA viruses (HIV-1 and RSV) [155].

Recently, Lai *et al.* [62] studied the effect of *R. farinacea* lichen phenolic compounds from Nigeria on respiratory syncytial virus. In a preliminary test, the results showed that the lichen extract inhibited virus development. From the thirteen phenolic compounds isolated and evaluated against the syncytial virus, sekikaic acid (**23**) presented a higher inhibition to the virus RG lineage, with a 50% inhibitory concentration (IC_{50}) of 5.69 μg·mL^{-1}, and for the strain A2 lineage the IC_{50} was 7.73 μg·mL^{-1}. The effect of sekikaic acid on HEp2 cells viability and Vero cell lineages was also investigated and the time addition assay showed that sekikaic acid interferes with viral replication in the virus post-entry stage 4 hours after virus addition, and the compound was 1.3 times more active than ribavirin used as negative control. The study concluded that although other compounds also showed antiviral inhibitory activity, sekikaic acid proved to be a powerful antiviral agent, with a selectivity index (SI) of 5.16 [62].

3.4. Antitumor and Cytotoxic Activity

The action of lichen-derived compounds on tumor cells has been a focus of reviews for a few decades. [156]. Chemoprevention is a pharmacological approach used to prevent or reverse the carcinogenesis process. Natural products are among the agents used in chemoprevention, since many of these primary and secondary metabolite phytochemicals do not present toxicity to normal tissues and are known to have anticancer effects [157].

Hirayama *et al.* [122] tested the antitumor activity of 44 fractions adsorbed on cationic and anionic resins, extracted with hot water, nine lichens and 20 metabolites and their degradation products against ascites, an Ehrlich carcinoma solid type. The results showed that an adsorbed fraction from *R. almquistii,* and

the compounds D-protolichesterinic acid (**77**) and nephrosterinic acid (**78**) were effective against Ehrlich carcinoma [122].

The cytotoxic activity of aqueous, ethanolic, chloroformic and *n*-hexane extracts from the lichen *R. farinacea* was evaluated by Esimone and Adikwu [45] using *Artemia salina*, a specie of saltwater crustacean. The crustacean eggs were incubated in seawater (collected in the Atlantic Ocean beach bar, Lagos, Nigeria), and allowed to incubate for 48 h at 28 °C. After incubation, 10 larvae (nauplii) of *Artemia salina* were introduced into vials containing growing concentrations of lichen extracts in the 10–1000 $\mu g \cdot mL^{-1}$ range. After 24 hours, the number of surviving shrimp in each concentration of the extract was counted and the data were analyzed using the Finney program for determining the lethal concentration 50% (CL_{50}) with a 95% confidence interval. The ethanolic extract showed higher cytotoxicity, with a CL_{50} of 6.0 $\mu g \cdot mL^{-1}$ (IC 0.8–9.3), followed by the hexane extract with CL_{50} 11.3 $\mu g \cdot mL^{-1}$ (IC 6.4–15.2), dichloromethane with a CL_{50} of 16.8 $\mu g \cdot mL^{-1}$ (IC 11.9–26.3) and low cytotoxicity was observed in the aqueous extract, with a CL_{50} of 206.9 $\mu g \cdot mL^{-1}$ (IC 91.5–389.2). The results show that the lichen extracts are promising sources of bioactive substances [45].

Stuelp-Campelo *et al.* [34] observed an effect of α-D-glucan from *R. celastri* on peritoneal exudate cells using a sarcoma-180 cells (S-180) *in vivo* assay. They found that the tumors developed in animals treated with glucan, at a dose of 200 $mg \cdot kg^{-1}$, decreased by 80% in the control group [34]. The research of Leão *et al.* [158] presented a antitumor activity of α-D-glucan polysaccharides with (1→3)(1→4) bonds extracted from *R. celastri* and their sulfated derivatives that had as objective observing morphological alterations in HeLa cells using transmission electron microscopy (TEM). Although the α-D-glucan changed the cell volume, cytoplasmic density and mitosis, the resulting monolayer was similar to the results in the control. Microscopic analysis of cytoplasmic vesicles showed the presence of an eletrodense-free amorphous material in the cytoplasm and inner membranes. However the injury caused by secondary sulfate polysaccharide was evident, causing changes in cell adhesion and causing cells aggregation. Nuclear modifications such as fragmentation and chromatin condensation under the envelope suggest the occurrence of apoptotic cell death [158].

Bézivin *et al.* [47] carried out work on the *in vitro* cytotoxic activity of 24 extracts from five lichens species in two murine cells lineages (L1210-lymphocytic

leukemia and 3LL-Lewis lung carcinoma) and four human cell lineages (K-562-chronic myelogenous leukemia; U251-glioblastoma; DU145-prostate carcinoma and MCF7-breast adenocarcinoma). Some extracts, among them the *R. cuspidata* (Ach.) Nyl. one, show interesting activities, particularly in the cell lineages K-562, U251, DU145 and MCF7 and with a good selectivity index. The (3-[3,4-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) assay indicated significant cytotoxicity to three cell lineages with the hexane, diethyl ether and methanolic extracts of the lichen *R. cuspidata*, with IC_{50} ($\mu g \cdot mL^{-1}$) ± standard deviation (SD) and selectivity index (SI) values for the lineages L1210 of 5.8 ± 1.9 $\mu g \cdot mL^{-1}$ and 8.9; for 3LL 5.7 ± 0.6 $\mu g \cdot mL^{-1}$ and 9.0; for DU145 6.7 ± 2.9 $\mu g \cdot mL^{-1}$ and 7.7; for MCF-7 31.4 ± 11.2 $\mu g \cdot mL^{-1}$ and 1.6; for K562 28.1 ±10.6 $\mu g \cdot mL^{-1}$ and 1.8; and for U251 11.0 ± 7.5 $\mu g \cdot mL^{-1}$ and 4.7 for the hexane extract, being the better results obtained from the three tested extracts. The results showed strong *R. cuspidate* cytotoxic potential, indicating that it must contain promising compounds against human cancer cells lines [47].

Haraldsdóttir *et al.* [159] tested the anti-proliferative effect against twelve human cancer cells lineages, of three lichen-derived substances—protolichesterinic (**77**), lobaric and baeomycesic acids—besides a commercial compound used as a specific inhibitor 5-lipoxygenase (LOX), zileuton. All tested compounds presented 5-lipoxygenase inhibitory activity and the compounds protolichesterinic and lobaric acid also inhibited 12-lipoxygenase. Compound **77** presented great inhibitory effects against all the cell lineages, with EC_{50} values ranging between 2.4–18.1 $\mu g \cdot mL^{-1}$, showing the best result for the pancreatic cancer cell lineages Capan-1 and PANC-1 with EC_{50} 2.4 and 3.1 $\mu g \cdot mL^{-1}$, respectively, and prostate adenocarcinoma PC-53, with EC_{50} 2.6 $\mu g \cdot mL^{-1}$. In all tested lineages, protolichesterinic acid was more active than the control zileuton, showing the potential that this compound has against these human cancer cell lineages [159].

The study of Koparal *et al.* [160] evaluated the *in vitro* cytotoxic activity of (+)-usnic acid and (−)-usnic acid in human lymphocyte cells A549 from lung epithelial carcinoma and V-79 Chinese hamster lung fibroblast cells lineages. The tests were performed using the MTT methodology and the cytokinesis blocked micronucleus (CBMN) assay. The results showed that both enantiomers are not genotoxic, demonstrated by the lack of micronucleus induction and have

significant cytotoxic and apoptotic effects in both cell lineages. Even at low doses, (+)-usnic acid showed high cytotoxic activity against cells. The results of MTT and the values of cell proliferation index (CPI), based on the results of the CBMN test obtained good agreement [160].

Einarsdóttir *et al.* [161] studied the mechanism of action of the usnic acid enantiomers against the breast cancer cell lineages T-47D and pancreatic cancer Capan-2 cells and also the proliferation, growth, and cell death effects. Both enantiomers exhibited similar anti-proliferative effects against both cell lineages. The IC_{50} values were 4.2 $\mu g \cdot mL^{-1}$ and 4.0 $\mu g \cdot mL^{-1}$ for (+)- and (−)-usnic acid against T-47D, and 5.3 $\mu g \cdot mL^{-1}$ and 5.0 $\mu g \cdot mL^{-1}$ against Capan-2, respectively. The other tests were performed only with the (+)-usnic acid. Proliferation assays showed that usnic acid at the concentration of 5.0 $\mu g \cdot mL^{-1}$ caused a reduction in both cell lineages. The inhibitory effect on cell cycle was also confirmed by cytometric flow analysis, where the cells exposed to usnic acid showed a reduction in S-phase and few cells entering the G2/M phase, so the tests indicated that usnic acid affects the growth of the cells and their proliferation [161].

Bačkorová *et al.* [162] studied the cytotoxic/proliferative effect of four lichen secondary metabolites—parietin (**151**), atranorin (**24**), usnic acid (**17**) and gyrophoric acid (**41**)—against nine human cancer cell lineages. The best results of the MTT assay were obtained by usnic acid with HL-60 (promyelocytic leukemia), A2780 (ovarian carcinoma) and Jurkat (T cell lymphoblastic leukemia) lineages, presenting IC_{50} values of 48.5, 75.9 and 76.3 $\mu g \cdot mL^{-1}$, respectively, being the most effective compound against all tested cells. Parientin showed cytotoxicity against HL-60 cells, Jurkat, HCT-116 $p53^{-/-}$ (colon carcinoma submanifold without p53) and A2780, with IC_{50} values of 93.5, 181.6, 197.5 and 197.9 $\mu g \cdot mL^{-1}$, respectively, and gyrophoric acid showed effect against HL-60 and A2780, with IC_{50} values of 146.7 and 198.3 $\mu g \cdot mL^{-1}$. In a clonogenic assay usnic acid and atranorin in concentrations of 100 and 200 µM were the most effective compounds, significantly inhibiting the cloning capacity of all tested tumor cells. Regarding viability assays and quantifying the number of floating cells, usnic acid at a 50 µM concentration completely damaged A2780 cells and seriously affected HL-60 cells. Atranorin and gyrophoric acid also showed high cytotoxicity at tested concentrations of 100 and 200 µM. The research has also shown the preferential effect on the cell distribution and accumulation in S-phase of usnic acid, atranorin

and gyophoric acid through a cell cycle assay with four cell lines. Usnic acid and atranorin were also the most effective compounds in the apoptosis induction in the four cell lines, followed by gyrophoric acid, and parientin was effective only in the HCT-116 $p53^{-/-}$ lineage [162].

In the research carried out by Bačkorová *et al.* in 2012 [163] the cytotoxic mechanism results from the same metabolites parientin, atranorin, usnic acid and gyrophoric acid in the indiction of apoptosis in the human cancer cell lines A2780 and HT-29 were presented using five different methods from those used in 2011. The test results of the metabolites' effect on the mitochondrial membrane potential (MMP) showed that usnic acid produced the greatest inhibition in both cell lines, at both concentrations of 50 and 100 µM. Atranorin produced high inhibition in A2780 at both concentrations (100 and 200 µM), but only produced a significant inhibition in the HT-29 lineage at a concentration of 200 µM. Gyrophoric acid gave good inhibition of A2780 at a concentration of 200 µM and parietin did not cause any cell lineage inhibition. In a phosphatidylserine externalization test, atranorin and usnic acid were effective at both concentrations tested, 50 and 100 µM, in HT-29 and A2780 cells. Gyrophoric acid was effective against A2780 at 200 µM and parietin caused no effect in both cells. In general, the A2780 cells were more sensitive than HT-29 cells. Regarding the reactive oxygen species (ROS) and nitrogen (RNS) assays, atranorin produced no increase of ROS in either cell line, but produced a significant increase in the production of RNS in both cells. Gyrophoric acid produced a ROS and RNS increase in both cells, but was more effective against HT-29. Caspase-3 activation had usnic acid as the most powerful inducer in HT-29 cell lineage, followed by atranorin and gyrophoric acid. Parietin did not cause significant activation changes of these proteins. Based on the detection of protein expression, it was shown that usnic acid and atranorin are programmed cell death activators in A2780 and HT-29 cells, probably by the mitochondrial pathway. In general, it can be concluded that usnic acid and atranorin are more effective at inhibiting cell proliferation and induce cells death more effectively compared to parietin and gyrophoric acid. The study demonstrated specific programmed cell death mechanisms induced by lichen secondary metabolites [163].

Brandão *et al.* [156] evaluated the cytotoxic activity of nine compounds isolated from seven different lichen species, including the *Ramalina* genus, among

which seven of the nine compounds have been isolated in the genus *Ramalina*, namely atranorin (**24**) and usnic (**17**), diffractaic (**38**), divaricatic (**25**), perlatolic (**43**), protocetraric (**61**) and norstitic (**58**) acids and the also tested psoromic acid and lichexanthone. The compounds were evaluated against murine melanoma B16-F10, human melanoma UACC-62 and fibroblast cells NIH/3T3. The test was performed with sulforhodamine B (SRB) and the anticancer drug doxorubicin was used as positive control. The results from SRB assays were expressed as growth inhibition 50% rate (GI_{50}) and lethal growth 50% (LC_{50}), according to Holbeck [164] and also in terms of selectivity index (SI), when a value greater than three indicates that the neoplastic cells atr more sensitive to a certain compound than normal cells [133].

The test with SRB revealed a significant cytotoxic activity in UACC-62 cells with protocetraric acid (GI_{50} 0.52 $\mu g \cdot mL^{-1}$ and SI 93.3), with an inhibitory concentration very close to the control doxorubicin (GI_{50} 0.47 $\mu g \cdot mL^{-1}$ and SI 1.2), but with a significantly better selectivity index than the control. Other compounds such as divaricatic acid (GI_{50} 2.7 $\mu g \cdot mL^{-1}$ andSI 5.4) and perlatolic acid (GI_{50} 3.3 $\mu g \cdot mL^{-1}$ and SI 7.9) also had a good response to this cell line. Difractaic, usnic, norstitic and psoromic acids had intermediate sensitivity, with GI_{50} values ranging from 24.7–36.6 $\mu g \cdot mL^{-1}$. Atranorin presented low sensitivity with a GI_{50} of 147.2 $\mu g \cdot mL^{-1}$ and SI of 1.7 and lichexanthone was inactive against this lineage. For B16-F10 cells the best result was the divaricatic acid, showing high sensitivity and selectivity with GI_{50} 4.4 $\mu g \cdot mL^{-1}$ and SI 3.3, followed by perlatolic, protocetraric, diffractaic acids with average sensitivity and usnic and nortictic acids with low sensitivity. Atranorin and lichexantone demonstrated no sensitivity to this lineage. For the 3T3 lineage, divaricatic acid showed the best result, with a GI_{50} of 14.5 $\mu g \cdot mL^{-1}$. The remaining compounds presented GI_{50} values ranging from 26.0–248.6 $\mu g \cdot mL^{-1}$. Atranorin and lichexantone also had no sensitivity against this lineage. The CL_{50} values only showed satisfactory results for the UACC-62 lineage for divaricatic (CL_{50} 19.5 $\mu g \cdot mL^{-1}$) and perlatolic (CL_{50} 27.6 $\mu g \cdot mL^{-1}$) acids as the other compounds showed values close to 250 $\mu g \cdot mL^{-1}$, presenting low cytotoxicity, as with the other cell lineage where all the compounds demonstrated CL_{50} values close to or higher than 250 $\mu g \cdot mL^{-1}$.

Singh *et al.* [157] studied the effect of usnic acid on the growth inhibition, cell induction cycle control and apoptosis in A549 human lung carcinoma cells

using the MTT method. Treatment with usnic acid at the concentration of 25–100 µM for 24 and 48 h decreased the number of cells from 39%–67% and 68%–89%, respectively, and increased cell death two- to eight-fold, respectively. Usnic acid at the concentration of 1–10 µM also significantly suppressed the formation of A549 cell colonies. Inhibition of cell growth was associated with the control phase G0/G1. Usnic acid decreased the protein expression of cyclin dependent kinase CDK4, CDK6 and cyclin D1 and elevated the expression of inhibitory protein (CDK1) p21/cip1. When examined, cell death associated with molecular changes was observed whereby usnic acid induces mitochondrial membrane depolarization and leads to an increase in the cells apoptosis by more than twice. The effect of usnic acid on apoptosis was accompanied by increased poly(ADP-ribose)polymerase cleavage. The study thus showed that usnic acid inhibits cell growth involving the phase cell cycle G0/G_1 control and induces cell death by the mitochondrial membrane depolarization and apoptosis of human lung carcinoma cells [157].

3.5. Anti-Inflammatory Activity

A galactofuranose heteropolysaccharide with predominant (1→5)-Gal*f* bonds and side chains in position 6, isolated from *Trebouxia* sp., a photobiont from *R. gracilis,* extracted by Cordeiro *et al.* [31] presented induction properties in the *in vitro* activation ofperitoneal macrophages at all tested concentrations (1–150 µg·mL^{-1}). At the concentration of 150 µg·mL^{-1}, there was a 60% increase of the macrophage activation compared to the control group, confirmed by scanning electron microscopy (SEM) [31].

A pharmaceutical composition containing ramalin with circulation anti-inflammatory effects was presented and patented by Yim *et al.* [11]. The action is manifested as a result of of iNOS expression suppression in the transcription stage and also by suppressing the creation of NO, which is a nuclear substance mediator in the inflammatory reactions, and the activation suppression of the nuclear transcription factor kappa B (NF-kB) which is a precursor of the inflammatory mediation by suppressing the signal transmission route from protein kinases p38 MAPK, ERK ½ and JNK and also suppressing the expression of Toll-like 4 (TLR4) which is a lipopolysaccharide receptor (LPS) [11].

Byeon *et al.* [138] presented data on the *in vitro* effects of stereocalpin-A (**138**) concerning the compound's ability to suppress the expression of vascular cell adhesion molecules (VCAM-1), induced by TNF-α in vascular smooth muscle cells (VSMCs). The pretreatment of VSMCs for 2 hours with the substance at non-toxic concentrations of 0.1–10 $\mu g \cdot mL^{-1}$ inhibited TNF-α, inducing the adhesion of monocytic THP-1 cells and the expression of vascular cell adhesion molecules (VCAM-1) and inner cell adhesion molecule (ICAM-1). The compound also reduced the phosphorylation of P38, ERK, JNK and Akt. Stereocalpin-A demonstrated anti-inflammatory activity due to the negative regulation of induced adhesion molecules by TNF-α and the expression of MCP-1, the adhesion of monocytes and production of reactive oxygen species (ROS) in vascular smooth muscle cells (VSMCs) exerting a protective effect by inflammation modulation inside the atherosclerotic lesion. Previous studies have mentioned the participation of VSCMs in the initiation of atherosclerosis [138].

Another invention developed by Yim *et al.* [139] presents a pharmaceutical composition containing stereocalpin-A which can inhibit the expression of cell adhesion molecules mediated by TNF-α, and therefore can be used to prevent or effectively treat arteriosclerosis [139].

3.6. Other Activities

Besides the biological activities presented above for the lichens from the genus *Ramalina*, other activitiesfound are presented in this section. Lichen metabolites collected in Mato Grosso do Sul State*,* Brazil, such as difractaic acid, atranorin, chloroatranorin, usnic acid and the artifact ethyl orsenilate were tested against the phytopathogenic fungus *Cladosporium sphaerospermum* using the bioautographic test by Honda *et al.* [165]. The results showed that these compounds effective inhibited the growth of the fungus [165].

Different species of Koreans and Chinese lichens were evaluated for their activity against the phytopathogenic fungus *Colletotrichum acutatum*, the causing agent of anthracnose in pepper, by Wei *et al.* [166]. Among the tested species, *R. conduplicans,* obtained 59.5% inhibition, the second highest rate of mycelial growth inhibition of the tested fungus, showing that lichens can be useful as new fungicidal natural sources [166].

The methanolic extracts from the lichens *Ramalina hossei* and *Ramalina conduplicans* had their anthelmintic efficacy assessed by Kumar *et al.* [94]. The results showed that both lichens exhibited anti-helminth activity in dose-dependent form, as revealed by paralysis and death of tested Indian adult worms [94].

The antimycobacterial activity of twenty-six compounds derived from lichens of four different families, included the family Ramalinaceae was tested. Most of the compounds already had been isolated from species of the genus *Ramalina*. The results showed that the diffractaic acid (**38**) was the most active compound, with MIC value 15.6 $\mu g \cdot mL^{-1}$, followed by norstitic acid (**58**), 62.5 $\mu g \cdot mL^{-1}$ and usnic acid (**17**) 62.5 $\mu g \cdot mL^{-1}$. The hypostictic and protocetraric (**61**) acids showed moderate inhibitory activity, with MIC 94.0 $\mu g \cdot mL^{-1}$ and 125 $\mu g \cdot mL^{-1}$ respectively. The other compounds showed low inhibitory activity on the growth of *Mycobacterium tuberculosis*, with MIC values > 250 $\mu g \cdot mL^{-1}$ [167].

Research by Lee *et al.* [8] showed that usimine C (**20**), from *R. terebrata*, induced proliferation of human dermal fibroblast cells CCD-986SK up to 1.6 times after treatment with 90 $\mu g \cdot mL^{-1}$ during48 h. The type I procollagen synthesis was significantly increased 1.3 times, 3 times and 5 times after treatment with 0.14, 0.72 and 3.6 µg of usimine-C/mL/24 h, respectively, while no significant increase was observed after treatment with usimine-A or -B [8]. The invention of a pharmaceutical composition containing usimine-C was presented and patented by Lim *et al.* [168] for proliferation of dermal epithelium fibroblast acting in collagen production, preventing, in this way, the formation of wrinkles [168].

The anti-schistosoma activity of the sulfated polysaccharide α-D-glucan ($Glu.SO_4$) extracted from *R. celastri* were evaluated, after encapsulation in liposomes ($Glu.SO_4$-LIPO), in mice infected with *Schistosoma mansoni*. The effect of treatment with $Glu.SO_4$ and $Glu.SO_4$-LIPO (10 $mg \cdot kg^{-1}$) on the disposal of eggs, parasite load and liver granuloma formation was evaluated using Swiss albino female mice, between the ages of 35–40 days, weighing about 25 ± 2 g, infected with 150 cercariae/animal (*Biomphalaria glabrata*, BH strain). Four groups were studied containing 10 samples each (N = 10), two controls (empty liposomes and NaCl) and two treatment groups with $Glu.SO_4$ and $Glu.SO_4$-LIPO, using a single dose. The results of the parasitological analysis revealed that $Glu.SO_4$-LIPO was as efficient as $Glu.SO_4$ in the reduction of egg disposal and parasite load.

Treatment with free Glu.SO_4 and Glu.SO_4-LIPO produced a statistically significant reduction in the number of granulomas, by 62% and 63%, respectively [169].

The larvicidal activity of some lichen metabolites like (+)-usnic acid, atranorin and gyrophoric acid found in species from the genus *Ramalina*, and also of 3-hydroxyphysodic acid, were tested against second and third stage larvae from the *Culiseta longiareolata* mosquito by Cetin *et al.* [170]. All metabolites presented high larvicidal properties. The LC_{50} values were 0.41 $\mu g \cdot mL^{-1}$ for gyrophoric acid, 0.48 $\mu g \cdot mL^{-1}$ for (+)-usnic acid, 0.52 $\mu g \cdot mL^{-1}$ for atranorin and 0.97 $\mu g \cdot mL^{-1}$ for 3-hydroxyphysodic acid. However, when LC_{90} values were compared, the best result was from the (+)-usnic acid with 1.54 $\mu g \cdot mL^{-1}$, followed by gyrophoric acid with 1.93 $\mu g \cdot mL^{-1}$, then 3-hydroxy-physodic acid with 4.33 $\mu g \cdot mL^{-1}$ and finally the compound atranorin with 5.63 $\mu g \cdot mL^{-1}$. Thus it was revealed that some lichen secondary metabolites have a promising role as larvicides [170].

Lim *et al.* [135] patented a pharmaceutical composition containing ramalin or one of its salts, that can be used as functional food for treating liver disease, which may inhibit liver fibrosis and reduce liver cirrhosis levels. When compared with silymarin (a protective drug in liver cells) in animal experiments significant results were achieved without liver cell cytotoxicity. In this way, the composition can be effectively used to prevent or treat liver fibrosis and liver cirrhosis [135].

Kosugi *et al.* [24] demonstrated that sugar arabitol, extracted from the green algae *Trebouxia* sp. lichen photobiont *R. yasudae* has the ability to increase the expression of drought-induced non-photochemical (NPQ-d), dissipating light energy excess and protecting the photobiont of photoinhibition [24].

4. Concluding Remarks

From the 246 lichens species that compose the genus *Ramalina* mentioned in the literature, about 47%, or about 118 species with studied chemistry and biological activity were described in this review, which covers a total of 153 isolated and identified compounds. As for biological activity, the percentage of species studied is even smaller, because if the total of 13 species is considered, only about 5% had some biological activity study. The vast majority of studies were with crude lichens extracts, and only 27 compounds, or about 18% of the 153 identified compounds, underwent biological tests.

However, the results are promising, besides the diversity of biological activities presented by crude extracts and the few compounds whose activities were tested, some of these activities have great importance for medicine, as is the case of the observed antitumor, cytotoxic, and anti-inflammatory properties, in some cases leading to patented pharmaceutical formulations containing lichen substances with medical purposes.

In this regard, due to the good results demonstrated by various extracts and some isolated compounds from the genus *Ramalina* that have shown promising potential, especially with antimicrobial, antioxidant, antitumor, cytotoxic, antiviral, anti-inflammatory properties, and for the prevention and treatment of liver disease, among other benefits, it can be concluded that other species of this genus that have been little or not studied deserve special attention with research involving both the chemical part and the biological one to increase the contribution to the discovery of new compounds that may serve as models for new drugs with therapeutic properties.

Acknowledgments

The authors are grateful to Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for grants and a research fellowship, to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for research fellowships.

Author Contributions

All authors contributed equally to the work.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Huneck, S.; Yoshimura, I. *Identification of Lichen Substances*; Springer-Verlag: Berlin, Germany, 1996.
2. Boustie, J.; Grube, M. Lichens—A promising source of bioactive secondary metabolites. *Plant Genet. Resour.* **2005**, *3*, 273–287.
3. Honda, N.K.; Vilegas, W. A química dos liquens. *Quim. Nova* **1998**, *21*, 110–125.
4. Feige, G.B.; Lumbsch, H.T. Identification of lichen substances by a standardized high-performance liquid chromatographic method. *J. Chromatogr.* **1993**, *647*, 417–427.
5. Parrot, D.; Jan, S.; Baert, N.; Guyot, S.; Tomasi, S. Comparative metabolite profiling and chemical study of *Ramalina siliquosa* complex using LC-ESI-MS/MS approach. *Phytochemistry* **2013**, *89*, 114–124.
6. Oksanen, I. Ecological and biotechnological aspects of lichens. *Appl. Microbiol. Biotechnol.* **2006**, *73*, 723–734.
7. Kirk, P.M.; Cannon, P.F.; Minter, D.W.; Stalpers, J.A. *Dictionary of the Fungi*; CABI—Europe-UK, Cromwell Press: Trowbridge, UK, 2008.
8. Lee, S.G.; Koh, H.Y.; Oh, H.; Han, S.J.; Kim, I.C.; Lee, H.K.; Yim, J.H. Human dermal fibroblast proliferation activity of usimine-C from Antarctic lichen *Ramalina terebrata. Biotechnol. Lett.* **2010**, *32*, 471–475.
9. Paudel, B.; Bhattarai, H.D.; Koh, H.Y.; Lee, S.G.; Han, S.J.; Lee, H.K.; Oh, H.; Shin, H.W.; Yim, J.H. Ramalin, a novel nontoxic antioxidant compound from the Antarctic lichen *Ramalina terebrata*. *Phytomedicine* **2011**, *18*, 1285–1290.
10. Yim, J.H.; Lee, H.K.; Bhattarai, H.D.; Babita, P.; Kim, I.C.; Hong, S.G.; Kim, D.K.; Lee, Y.K.; Gu, S.; Oh, H.C. Antioxidant Activities of Ramalin Isolated from *Ramalina terebrata* Extracts. WO 2010053327 A2, 14 May 2010.
11. Yim, J.H.; Kim, I.C.; Lee, S.G.; Kim, D.K.; Han, S.J.; Lee, H.S.; Seok, K.H.; Kim, S.J.; Kim, T.K.; Kang, P.S.; *et al.* Pharmaceutical Composition for the Treatment of Inflammatory Disease or Immune Disease Containing Ramalin. WO 2011152671 A2, 8 December 2011.
12. Gonzalez, A.G.; Barrera, J.B.; Rodriguez, E.M.P.; Padron, C.E.H. Chemical constituents of the lichen *Ramalina hierrensis*. *Planta Med.* **1992**, *58*, 214–218.
13. Asahina, Y.; Fukuziro, F. Lichen substances.III. Constitution of obtusatic acid. *Ber. Dtsch. Chem. Ges*. B **1932**, *65B*, 580–583.
14. Miceno, A.M.; Gorin, P.A.J.; Iacomini, M. Galactomannan and isolichenan componentes of the carbohydrate-rich lichen *Ramalina ecklonii* (Spreng.) Mey. & Flot. *Agr. Biol. Chem.* **1991**, *55*, 1391–1392.

15. Teixeira, A.Z.A.; Iacomini, M.; Gorin, A. J. An unusual glucomannan from *Tornabenia intricata*. *Phytochemistry* **1992**, *31*, 3467–3470.
16. Stuelp, P.M.; Carneiro Leão, A.M.A.; Gorin, P.A.J.; Iacomini, M. The glucans of *Ramalina celastri*; relation with chemotypes of other lichens. *Carbohyd. Polym.* **1999**, *40*, 101–106.
17. Cordeiro, L.M.C.; Reis, R.A.; Tisher, C.A.; Gorin, P.A.J.; Ferreira, J.C.; Iacomini, M. Linear β-mannose containig polysaccharide, β-xylan, and amylose from the cultured photobiont Trebouxia sp. of the ascolichen *Ramalina celastri*. *FEMS Microbiol. Lett.* **2003**, *220*, 89–94.
18. Cordeiro, L.M.C.; Stocker-Wörgötter, E.; Gorin, P.A.J.; Iacomini, M. Comparative studies of the polysaccharides from species of the genus Ramalina-lichenized fungi-of three distinct habitats. *Phytochemistry* **2003**, *63*, 967–975.
19. Cordeiro, L.M.C.; Stocker-Wörgötter, E.; Gorin, P.A.J.; Iacomini, M. Elucidation of polysaccharide origin in *Ramalina peruviana* symbiosis. *FEMS Microbiol. Lett.* **2004**, *238*, 79–84.
20. Cordeiro, L.M.C.; Carbonero, E.R.; Sassaki, G.L.; Reis, R.A.; Stocker-Wörgötter, E.; Gorin, P.A.J.; Iacomini, M. A fungus-type β-galactofuranan in the cultivated *Trebouxia* photobiont of the lichen *Ramalina gracilis*. *FEMS Microbiol. Lett.* **2005**, *244*, 193–198.
21. Gorin, P.A.J.; Iacomini, M. Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. *Carbohyd. Res.* **1984**, *128*, 119–132.
22. Takahashi, K.; Takeda, T.; Shibata, S. Polysaccharides of lichen symbionts. *Chem. Pharm. Bull.* **1979**, *27*, 238–241.
23. Mamut, R.; Tursun, T.; Abbas, A. Analysis monosaccharide of the polysaccharide in *Ramalina sinensis* with HPLC. *Shipin Gongye* **2013**, *34*, 209–211.
24. Kosugi, M.; Miyake, H.; Yamakawa, H.; Shibata, Y.; Miyazawa, A.; Sugimura, T.; Satoh, K.; Itoh, S.; Kashino, Y. Arabitol provided by lichenous fungi enhances ability to dissipate excess light energy in a symbiotic green alga under desiccation. *Plant Cell Physiol.* **2013**, *54*, 1316–1325.
25. Krzacek, T.; Smolarz, H. Chemical composition of *Ramalina fraxinea* (L.) Ach. Thallus. *Acta Soc. Bot. Pol.* **1979**, *48*, 27–34.
26. Stark, J.B.; Walter, E.D.; Owens, H.S. Method of isolation of usnic acid from *Ramalina reticulata*. *J. Am. Chem. Soc.* **1950**, *72*, 1819–1820.
27. Mittal, O.P.; Neelakantan, S.; Seshaadri, T.R. Chemical investigation of Indian lichens. XIV. Chemical components of *Ramalina calicaris* and *Ramalina sinensis*. *J. Sci. Ind. Res. India* **1952**, *11B*, 386–387.
28. Culberson, C.F. Some constituents of the lichen *Ramalina siliquosa*. *Phytochemistry* **1965**, *4*, 951–961.

29. Seshadri, T.R.; Subramanian, S.S. Indian lichens. VIII. Lichens growing on sandal trees. *Proc.-Indian Acad. Sci., Sect. A* **1949**, *30A*, 15–22.
30. Ashina, Y.; Yanagita, M. Lichen substances. XXXIX. A new lichen acid, norstictinic acid, and the occurrence of D-arabitol in lichens. *Ber. Dtsch. Chem. Ges. B* **1934**, *67B*, 799–803.
31. Cordeiro, L.M.C.; de Oliveira, S.M.; Buchi, D.F.; Iacomini, M. Galactofuranose-rich heteropolysaccharide from Trebouxia sp., photobiont of the lichen *Ramalina gracilis* and its effect on macrophage activation. *Int. J. Biol. Macromol.* **2008**, *42*, 436–440.
32. Gorin, P.A.J.; Iacomini, M. Structural diversity of D-galacto-D-mannan components isolated from lichens having ascomycetous mycobionts. C*arbohyd*. *Res.* **1985**, *142*, 253–267.
33. Teixeira, A.Z.A.; Iacomini, M.; Gorin, P.A.J. Chemotypes of mannose-containing polysaccharides of lichens mycobionts: A possible aid in classification and identification. *Carbohyd. Res.* **1995**, *266*, 309–314.
34. Stuelp-Campelo, P.Ma.; de Oliveira, M.B.M.; Leão, A.M.A.C.; Carbonero, E.R.; Gorin, P.A.J.; Iacomini, M. Effect of a soluble α-D-glucan from the lichenized fungus *Ramalina celastri* on macrophage activity. *Int. Immunopharmacol.* **2002**, *2*, 691–698.
35. Cordeiro, L.M.C.; Messias, D.; Sassaki, G.L.; Gorin, P.A.J.; Iacomini, M. Does apossymbiotically cultivated fungus *Ramalina* produce isolichenan? *FEMS Microbiol. Lett.* **2011**, *321*, 50–57.
36. Peveling, E. Occurrence of starch in *Chlorophyceae* phycobionts. *Planta* **1970**, *93*, 82–85.
37. McAvoy, K.; Igartuburu, J.M.; Romgni-Colvin, J. Polysaccharide composition of *Ramalina terebrata*. *Abstr. Pap. Am. Chem. Soc.* **2012**, *1*, 25–29.
38. Komiya, T.; Shibata, S. Polyols produced by the cultured phyco- and mycobionts of some *Ramalina* species. *Phytochemistry* **1971**, *10*, 695–699.
39. Culberson, W.L. Analysis of chemical and morphological variation in the *Ramalina siliquosa* species complex. *Brittonia* **1967**, *19*, 333–352.
40. Molho, L.; Bodo, B.; Molho, D. L'acide *O*-méthyl-4′ norsékikaïque, nouveau metadepside isole d'um lichen du genre *Ramalina*. *Phytochemistry* **1979**, *18*, 2049–2051.
41. Curd, F.H.; Robertson, A. Lichen acids. Part VI. Constituensts of *Ramalina scopulorum*. *J. Chem. Soc.* **1935**, *329*, 1379–1381.
42. Tay, T.; Türk, A.Ö.; Yilmaz, M.; Türk, H.; Kivanç, M. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstitic acid, and protocetraric acid constituents. *Z. Naturforsch. C* **2004**, *59*, 384–388.
43. Imshaug, H.A. Typification of *Ramalina usnea* (L.) R. H. Howe. *Lichenologist* **1972**, *5*, 317–318.

44. Shukla, V.; Negi, S.; Rawat, M.S.M.; Pant, G.; Nagatsu, A. Chemical study of *Ramalina africana* (Ramalinaceae) from the Garhwal Himalayas. *Biochem. Syst. Ecol.* **2004**, *32*, 449–453.
45. Esimone, C.O.; Adikwu, M.U. Antimicrobial activity and cytotoxicity of *Ramalina farinacea*. *Fitoterapia* **1999**, *70*, 428–431.
46. Hanus, L.O.; Temina, M.; Dembitsky, V. Biodiversity of the chemical constituents in the epiphytic lichenized ascomycete *Ramalina* lacera grown on difference substrates *Crataegus sinaicus*, *Pinus halepensis*, and *Quercus calliprinos*. *Biomed. Pap.* **2008**, *152*, 203–208.
47. Bézivin, C.; Tomasi, S.; Lohézic-Le, F.; Boustie, J. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine* **2003**, *10*, 499–503.
48. Fazio, A.T.; Adler, M.T.; Bertoni, M.D.; Sepúlveda, C.S.; Damonte, E.B.; Maier, M.S. Lichen secondary metabolites from the cultured lichen mycobionts of *Teloschistes chrysophthalmus* and *Ramalina celastri* and their antiviral activities. *Z. Naturforsch*. C **2007**, *62*, 543–549.
49. Hauck, M.; Willenbruch, K.; Leuschner, C. Lichen substances prevent lichens from nutrient deficiency. *J. Chem. Ecol.* **2009**, *35*, 71–73.
50. Solberg, Y.J. Dyeing of wool with lichens and lichen substances. *Acta Chem. Scand.* **1956**, *10*, 1116–1123.
51. Verma, N.; Behera, B.C.; Joshi, A. Studies on nutritional requirement for the culture of lichen *Ramalina nervulosa* and *Ramalina pacifica* to enhance the production of antioxidant metabolites. *Folia Microbiol.* **2012**, *57*, 107–114.
52. Cansaran, D.; Atakol, O.; Halici, M.G.; Aksoy, A. HPLC analysis of usnic acid in some *Ramalina* species from Anatolia and investigation of their antimicrobial activities. *Pharm. Biol.* **2007**, *45*, 77–81.
53. Ingólfsdóttir, K. Usnic acid. *Phytochemistry* **2002**, *61*, 729–736.
54. Yamamoto, Y.; Mizuguchi, R.; Yamada, Y. Tissue cultures of *Usnea rubescens* and *Ramalina yasudae* and production of usnic acid in their cultures. *Agr. Biol. Chem.* **1985**, *49*, 3347–3348.
55. Culberson, C.F. Stenosporic acid, a new depside in *Ramalina stenospora*. *Phytochemistry* **1970**, *9*, 841–844.
56. Komiya, T.; Shibata, S. Formation of lichen substances by mycobionts of lichens. Isolation of (+)-usnic acid and salazinic acid from mybobionts of *Ramalina* sp. *Chem. Pharm. Bull.* **1969**, *17*, 1305–1306.
57. Bendz, G.; Santesson, J.; Wachtmeister, C.A. Studies on the chemistry of lichens. *Acta Chem. Scand.* **1965**, *19*, 1185–1187.
58. Urdapilleta, A.A.A. Atividade de Substâncias Isoladas de Líquens Sobre Formas Promastigotas de *Leishmania* (L.) *amazonensis* e *Leishmania* (V.) *braziliensis*. Master's Thesis, Universidade de Brasília, Brasilia-DF, 3 March **2006**.

59. Aptroot, A.; Bungartz, F. The lichen genus *Ramalina* on the Galapagos. *Lichenologist* **2007**, *39*, 519–542.
60. Kashiwadani, H.; Kalb, K. The genus *Ramalina* in Brazil. *Lichenologist* **1993**, *25*, 1–31.
61. Rundel, P.W. Evolution relationships in the *Ramalina usnea* complex. *Lichenologist* **1978**, *10*, 141–156.
62. Lai, D.; Odimewu, D.; Esimone, C.; Grunwald, T.; Proksch, P. Phenolic compounds with *in vitro* activity against respiratory syncytial from the Nigerian lichen *Ramalina farinacea*. *Planta Med.* **2013**, *79*, 1440–1446.
63. Huneck, S.; Troted, G. Lichen constituents. XXXIV. Structure and composition of *Ramalina boulhautiana*. *Z. Naturforsch. B* **1966**, *21*, 904 –904.
64. Huneck, S.; Troted, G. Lichen constituents. XX. Chemistry of Chilean lichens. 8. Componets of *Ramalina chilensis*. *Z. Naturforsch. B* **1966**, *21*, 90–91.
65. Asahina, Y.; Nonomura, S. Lichen substances. XVI. Constituents of *Ramalina* species with special reference to sekikaic acid. *Ber. Dtsch. Chem. Ges. B* **1933**, *66B*, 30–35.
66. Kumar, K.C.S.; Banskota, A.H.; Manandhar, M.D. Chemical constituents and antibacterial activity of *Ramalina subcomplanata*. *J. Nepal Chem. Soc.* **1995**, *14*, 28–35.
67. Morozova, O.V.; Dargaeva, T.D.; Brutko, L.I. Components of *Ramalina pollinaria*. *Khim. Prirod. Soed.* **1984**, *20*, 252-253.
68. Rao, P.S.; Shripathy, V. Chemical constituents of *Umbilicaria indica* Frey and *Ramalina farinacea* L. Ach. *Curr*. *Sci.* **1976**, *45*, 517–518.
69. Gonzalez, A.G.; Martin, J.D.; Martin, M.M. Chemical components of lichens. II. *Ramalina tingitana*. *Anal. Quim.* **1973**, *69*, 807–809.
70. Hawksworth, D.L. Typification of lichen farinaceous. *Bryologist* **1969**, *72*, 254–255.
71. Popiolek, Z. Content of lichen substances in some species of *Ramalina*. *Ann. Univ. Mariae Curie–Sklodowska, Sect. C* **1967**, *22*, 165–168.
72. Culberson C.F. Chemical constituents of *Ramalina paludosa*. *Bryologist* **1967**, *70*, 397–405.
73. Huneck, S.; Follmann, G. Lichen constituents. XXXVI. Chemistry of Chilean lichens. 15. Constituents of *Ramalina cactacearum*, var. ambigua, and *Medusulina chilena*. *Z. Naturforsch. B* **1967**, *22*, 110–111.
74. Huneck, S.; Follmann, G. The presence of usnic acid in *Lecanora melanophthalma* and *Ramalina terebrata*. *Naturwissenschaften* **1964**, *51*, 291–292.
75. Briner, G.P.; Gream, G.E.; Riggs, N.V. Chemistry of Australian lichens. I. Some constituents of *Parmelia conspersa*, *Ramalina fraxinea*, *Usnea barbata*, and *Usnea ceratina* from the New England region. *Aust. J. Chem*. **1960**, *13*, 277–284.

76. Asahina, Y.; Kusaka, T. Lichen substances. LXXXIII. A new depside, boninic acid; synthesis of boninic acid and of homosekikaic acid. *Ber. Dtsch. Chem. Ges*. *B* **1937**, *70B*, 1815–1821.
77. Nakao, M. Chemical constituents of a Chinese drug “shi-hoa” and their constitutions. *Yakuga. Zasshi* **1923**, *496*, 423–497.
78. Ryan, H.; O’Riordan, W.M. Tinctorial constituents of some lichens, which are used as dyes in Irland. *P. Roy. Irish Acad*. *B* **1917**, *33*, 91–104.
79. Salkowski, H. Circular polarization of usninic acids and other lichen substances. *Liebigs Ann. Chem.* **1911**, *377*, 123–126.
80. Zopf, W. A contribution to the knowledge of the lichen constituents. *Liebigs Ann. Chem.* **1907**, *352*, 1–44.
81. Zopf, W. To the knowledge of the lichen material. *Liebigs Ann*. **1897**, *297*, 271–312.
82. Tugunay, T.; Reyim, M.; Adilijiang, A.A. Content determination and antioxidant activity evaluation of usnic acid in *Ramalina sinensis* Jatta. *Shipin Kexue* **2010**, *31*, 150–152.
83. Reyes, Q.A.; Vicuna, L.P.; Almonacid, V.J. Usnic acid and atranorin in some regional lichens. *Rev. Latinoam. Quim.* **1981**, *12*, 130–131.
84. Wade, A.E. The Genus *Ramalina* in the British Isles. *Lichenologist* **1961**, *5*, 226–241.
85. Marshak, A.G. Extracting Usnic Acid. US 2557987 A, 26 June 1951.
86. Zopf, W. To the knowledge of the lichen substances. *Liebigs Ann. Chem.* **1905**, *340*, 276–309.
87. Cordeiro, L.M.C.; Iacomini, M.; Stocker-Wörgötter, E. Culture studies and secondary compounds of six *Ramalina* species. *Mycol. Res.* **2004**, *108*, 489–497.
88. Asahina, Y.; Shibata, S. *Chemistry of Lichen Substances, A*; Asher & Co. Ltd: Vaals–Amsterdam, The Netherlands, 1971.
89. Hamada, N. Individual variation in the contents of lichen substances in *Ramalina nervulosa* and *Ramalina subcomplanata*. *Bryologist* **1988**, *91*, 95–97.
90. Molho, D.; Bodo, B.; Culberson, W.L. A chemically distinctive new *Ramalina* from Fiji. *Bryologist* **1981**, *84*, 396–398.
91. Rangaswami, S.; Rao, V.S. Chemical components of *Ramalina farinacea*. *Indian J. Pharm.* **1954**, *16*, 197–199.
92. Sisodia, R.; Geol, M.; Verma, S.; Rani, A.; Dureja, P. Antibacterial and antioxidante activity of lichen species *Ramalina roesleri*. *Nat. Prod. Res.* **2013**, *27*, 2235–2239.
93. Vinayaka, K.S.; Kekuda, T.R.P.; Swathi, D.; Praveen Kumar, S.V. Studies on chemical composition and *in vitro* antibacterial activity of solvent extracts of the lichen *Ramalina hossei* Vain. *BioTechnol. Indian J.* **2009**, *3*, 309–311.

94. Praven Kumar, S.V.; Kekuda, T.R.P.; Vinayaka, K.S.; Sudharshan, S.J. Anthelmintic and antioxidant efficacy of two macrolichens of Ramalinaceae. *Pharmacogn. J*. **2009**, *1*, 238–242.
95. Dias, D.A.; Urban, S. Phytochemical investigation of the Australian lichens *Ramalina glaucescens* and *Xanthoria parietina*. *Nat. Prod. Commun.* **2009**, *4*, 959–964.
96. Luo, H.; Wei, X.; Yamamoto, Y.; Liu, Y.; Wang, L.; Jung, J.S.; Koh, Y.J.; Hur, J.-S. Antioxidant activities of edible lichen *Ramalina conduplicans* and its free radical scavenging constituents. *Mycoscience* **2010**, *51*, 391–395.
97. Ramesh, P.; Baig, E.S.A. Chemical investigation of south Indian lichens: *Ramalina* species. *Indian Drugs* **1994**, *31*, 445–446.
98. Hamada, N. The effect of temperature on lichen substances in *Ramalina subbreviuscula* (Lichens). *Bot. Mag.* **1983**, *96*, 121–126.
99. Asahina, Y.; Kusaka, T. Lichen substances. LXV. A new depside, ramalinolic acid. *Ber. Dtsch. Chem. Ges*. B **1936**, *69B*, 450–455.
100. Huneck, S. Lichen constituents. LXXXIX. Secondary substances of some lichens. *Phytochemistry* **1972**, *11*, 1493–1495.
101. Huneck, S. Lichen constituents. XXXIV. Structure of tumidulin, a new chlorine containing depside. *Chem. Ber.* **1966**, *99*, 1106–1110.
102. Bendz, G.; Santesson, J.; Wachtmeister, C.A. Chemistry of lichens. XXI. Isolation and synthesis of methyl 3,5-dichlorolecanorate, a new depside from *Ramalina* spp. *Acta Chem. Scand.* **1965**, *19*, 1188–1190.
103. Koller, G. Ramalic acid. *Monatsh. Chem.* **1932**, *61*, 286–292.
104. Chester, D.O.; Elix, J.A. The identification of four new meta-depsides in the lichen *Ramalina asahinae*. *Aust. J. Chem.* **1978**, *31*, 2745–2749.
105. Islas, N.V.; Zambrano, A.; Rojas, J.L. Ozone Reactivity and Free Radical Scavenging Behavior of Phenolic Secondary Metabolites in Lichens Exposed to Chronic Oxidant Air Pollution from Mexico City. *J. Chem. Ecol*. **2007**, *33*, 1619–1634.
106. Hamada, N.; Ueno, T. Depside from an isolated lichen mycobiont. *Agr. Biol. Chem.* **1987**, *51*, 1705–1706.
107. Huneck, S.; Follmann, G.; Santesson, J. Lichen components. XLIX. 4-*O*-demethylbarbatinic acid, a new depside from *Ramalina subdecipiens*. *Z. Naturforsch. B* **1968**, *23*, 856–860.
108. Hesse, O. Contribution for the knowledge of the lichens and their characteristic components. *J. Prakt. Chem.* **1903**, *68*, 1–71.
109. Culberson, C.F.; LaGreca, S.; Johnson, A.; Culberson, W.L. Trivaric acid, a new tridepside in the *Ramalina americana* chemotype complex (lichenized Ascomycota: Ramalinaceae). *Bryologist* **2000**, *102*, 595–601.

110. Elix, J.A.; Peculiarities of the Australiasian lichen flora: Accessory metabolites, chemical and hybrid strains. *J. Hattori Bot. Lab.* **1982**, *52*, 407–415.
111. Culberson, C.F.; Culberson, W.L.; Johnson, A. A novel meta-depside from the *Ramalina subfraxinea* complex (Ascomycotina, Ramalinaceae). *Bryologist* **1990**, *93* 193–196.
112. Bodo, B.; Hebrard, P.; Molho; L.; Molho, D. Desmaziera evernioides and *Ramalina bourgeana*. *Tetraedron Lett.* **1973**, *18*, 1631–1634.
113. Bodo, B. Bourgeanic acid: A new metabolite of lichens. Structure, synthesis and biosynthesis. *Bull. Mus. Natl. Hist. Nat., Sci. Phys.-Chim.* **1975**, *3*, 23–97.
114. Marshak, A.; Barry, G.T.; Craig, L.C. Antibiotic compound isolated from the lichen *Ramalina reticulate. Science* **1947**, *24*, 394–395.
115. Stocker-Worgotter E.; Elix, J.A.; Grube, M. Secondary chemistry of lichen forming fungi: Chemosyndromic variation and DNA analyses of cultures and chemotypes in the *Ramalina farinacea*. *Bryologist* **2004**, *107*, 152–162.
116. Hawksworth, D.L. Chemical strains of the lichen *Ramalina subfariancacea*. *Bot. Notiser* **1968**, *121*, 317–320.
117. Culberson, C.F. Microchemical tests for the lichen depsidone hypoprotocetraric acid. *Bryologist* **1965**, *68*, 301–304.
118. Huneck, S.; Lehn, J.M. Lichen constituents. XXVII. Identity between coquimboic acid and hypoprotocetraric acid. *Z. Naturforsch.B* **1966**, *21*, 299–299.
119. Yamamoto Y.; Watanabe, A. Fatty acid composition of lichens and their phyco and mycobionts. *J. Gen. Appl. Microbiol.* **1974**, *20*, 83–86.
120. Blunt, J.W.; Munro, M.H.G. *Dictionary of Marine Natural Products*; Chapman & Hall–Taylor & Francis Group: New York, USA, 2008.
121. Fazio, A.T.; Adler, M.; Maier, M.S. Usnic acid and triacylglycerides production by the cultured lichen mycobiont of *Ramalina celastri*. *Nat. Prod. Commun.* **2014**, *9*, 213–214.
122. Hirayama, T.; Fujikawa, F.; Kasahara, T.; Otsuka, M.; Nishida, N.; Mizuno, D. Anti-tumor activities of some lichen products and their degradation products. *Yakugaku Zasshi* **1980**, *100*, 755–759.
123. Czeczuga, B.; de Corona, L.F. Carotenoids in lichens from Argentina. *Biochem. Syst. Ecol.* **1987**, *15*, 519–522.
124. Dembitsky, V.M. Lipids of lichens. *Prog. Lipid Res.* **1992**, *31*, 373–397.
125. Huneck, S.; Follmann, G. Lichen constituents. XXVIII. Chemistry of Chilean lichen. Compounds from *Ramalina tigrina*, and *Ramalina inanis*. *Z. Naturforsch. B* **1966**, *21*, 713–714.
126. Lehn, J.M.; Huneck, S. Lichen constituents. XVIII. First separation of the diterpene (−)-16-α-hydroxykaurane from a lichen. *Z. Naturforsch. B* **1965**, *20*, 1013-1013.

127. Sassaki, G.L.; Cruz, L.M.; Gorin, P.A.J.; Iacomini, M. Fatty acid composition of lipids present in selected lichenized fungi: A chemotyping study. *Lipids* **2001**, *36*, 167–174.
128. Machado, M.J.; Guerrini, M.; Gorin, P.A.J.; Torri, G.; Iacomoni, M. A galactosphingolipid from the lichen, *Ramalina celastri*. *Phytochemistry* **1997**, *45*, 651–653.
129. Unal, D.; Senkardesler, A. Abscisic acid and polyamine contents in the lichens *Pseudevernia furfuracea* and *Ramalina farinacea*. A. Sukatar, *Russ. J. Plant Physiol.* **2008**, *55*, 115–118.
130. Mateos, J.L.; Legaz, M.E. Presence and Identification of Polyamines and their Conjugation to Phenolics in Some Epiphytic Lichens. *Eukaryotism and Symbiosis*; Springer-Verlag: Berlin, Germany, 1997; pp. 491–498.
131. Solberg, Y.J. Studies on the chemistry of lichens. IX Quantitative determination of monosaccharides and amino acids in hydrolysates of several Norwegian lichen species. *Lichenologist* **1970**, *4*, 283–288.
132. Ramakrishnan, S.; Subrmanian, S.S. Amino acids of Lobaria subisidiosa, Umbilicaria pustulata, Parmelia nepalensis, and Ramalina sinensis. *Curr. Sci. Indian* **1966**, 35, 124–125.
133. Fujikawa, F.; Hirai, K.; Hirayama, T.; Toyota, T.; Urasaki, M.; Takagawa, M.; Fukuda, M.; Moritani, K.; Sanae, T. Free amino acids in lichens of Japan. *Yakugaku Zasshi* **1972**, *92*, 823–827.
134. Paudel, B.; Bhattarai, H.D.; Lee, H.K.; Oh, H.; Shin, H.W.; Han, J.; Yim, J.H. Antibacterial activities of ramalin, usnic acid and its tree derivatives isolated from the Antarctic lichen *Ramalina terebrata. Z. fuer Naturforsch. C* **2010**, *65*, 34–38.
135. Yim, J.H.; Kim, I.C.; Han, S.J.; Lee, H.S.; Battarai, H.D.; Yang, S-G.; Lee, D.H. Ramalin for Preveting or Treating Hepatic Fibrosis and Liver Cirrhosis. WO 2013129714 A1, 6 September 2013.
136. Yim, J.H.; Kim, I.C.; Lee, S.G.; Kim, D.G.; Han, S.J.; Lee, H.S.; Kim, S.J.; Kim, T.G.; Kang, P.S.; Park, H.Y. Ramalin for Preveting and Treating Inflammatory Diseases and Immune Diseases. KR 1290745 B1, 29 September 2013. Please double check the patent number.
137. Seo, C.; Yim, J.H.; Lee, H.K.; Park, S.M.; Sohn, J.H.; Oh, H. Stereocalpin A, a bioactive cyclic depsipeptide from the Antarctic lichen *Stereocaulon alpinum*. *Tetrahedron Lett.* **2008**, *49*, 29–31.
138. Byeon, H.E.; Park, B.K.; Yim, J.H.; Lee, H.K.; Moon, E.Y.; Rhee, D.K.; Pyo, S. Stereocalpin A inhibits the expression of adhesion molecules in activated vascular smooth muscle cells. *Int. Immunopharmacol*. **2012**, *12*, 315–325.
139. Yim, J.H.; Kim, I.C.; Kim, D.G.; Han, S.J.; Lee, H.S.; Oh, H.C.; Pyo, S.N. Stereoclapin-A for preveting or treating arteriosclerosis. KR 20130043995 A, 02 May 2013.

140. Pfau, A.S. Constituents of lichens. II. Constitution of barbatinic acid. *Helv. Chim Acta* **1928**, *11*, 864–876.
141. Huang, W.; Dai, W. The constituents of absolute from *Ramalina fastigiata* (Pors) Ach. The main ethyl ester of fatty acids. *Nat. Prod. Res. Dev.* **1989**, *1*, 27–28.
142. Zygadlo, J.A.; Pignata, M.L.; Gonzalez, C.M.; Levin, A. Alkanes in lichens. *Phytochemistry* **1993**, *32*, 1453–1456.
143. Rundel, P.W. The ecological role of secondary lichen substances. *Biochem. Syst. Ecol.* **1978**, *6*, 157–170.
144. Gulluce, M.; Aslan, A.; Sokmen, M.; Sahin, F.; Adiguzel, A.; Agar, G.; Sokmen, A. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderiana*. *Phytomedicine* **2006**, *13*, 515–521.
145. Paudel, B.; Bhattarai, H.D.; Lee, J.S.; Hong, S.G.; Shin, H.W.; Yim, J.H. Antibacterial potential of Antarctic lichens against human pathogenic Gram-positive bacteria. *Phytother. Res.* **2008**, *22*, 1269–1271.
146. Bhattarai, H.D.; Lee, Y.K.; Cho, K.H.; Shin, H.V. The study of antagonistic interactions among pelagic bacteria: a promising way to coin environmentally friendly antiflouling compounds. *Hydrobiologia* **2006**, *568*, 417–423.
147. Swenson, J.M.; Thornsberry, C.; Silcox, V.A. Rapidly growing mycobacteria: testing of susceptibility to 34 antimicrobial agentes by microdilution. *Antimiob Agent Chemother.* **1982**, *22*, 186–192.
148. Paudel, B.; Bhattarai, H.D.; Pandey, D.P.; Hur, J.S.; Hong, S.G.; Kim, I.-C.; Yim, J.H. Antioxidant, antibacterial activity and brine shrimp toxicity test of some mountainous lichens from Nepal. *Biol. Res.* **2012**, *45*, 387–391.
149. Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *26*, 1199–1200.
150. Mitsuda, H.; Yuasumoto, K.; Iwami, K. Antioxidation action of indole compounds during the autoxidation of linoleic acid. *Nihon Eiyo Shokuryo Gakkai Shi* **1996**, *19*, 210–214.
151. Luo, H.; Yamamota, Y.; Kim, J.A.; Jung, J.S.; Koh, Y.J.; Hur, J.S. Lecanoric acid, a secondary lichen substance with antioxidant properties from *Umbilicaria Antarctica* in maritime Antarctica (King George Island). *Polar. Biol.* **2009**, *32*, 1033–1040.
152. Halici, M.; Kufrevioglu; O.I.; Odabasoglu, F.; Halici, Z.; Cakir, A.; Aslan, A. The ethanol-water extract of *Ramalina capitata* has gastroprotective and antioxidative properties: an experimental study in rats with indomethacin-induced gastric injuries. *J. Food Biochem*. **2011**, *35*, 11–26.

153. Stanly, C.; Hag, D.M.; Keng, C.L.; Boey, P.-L.; Bhatt, A. Comparative evaluation of antioxidant activity and total phenolic content of selected species from Malaysia. *J. Pharm. Res*. **2011**, *4*, 2824–2827.
154. García, C.C.; Rosso, M.L.; Bertoni, M.D.; Maier, M.S., Damonte, E.B. Evaluation of the antiviral activity against Junin vírus of macrocyclic trichothecenes produced by the hypocrealean epibiont of *Baccharis corififolia*. *Planta Med.* **2002**, 68, 209–212.
155. Esimone, C.O.; Grunwald, T.; Nworu, C.S.; Kuate, S.; Proksch, P.; Ueberla, K. Broad spectrum antiviral fractions from the lichen *Ramalina farinacea* (L.) Ach. *Chemotherapy* **2009**, *55*, 119–126.
156. Brandão, L.F.G.; Alcantara, G.B.; Matos, M.F.C.; Bogo, D.; Freitas, D.S.; Oyama, N.M.; Honda, N.K. Cytotoxic evaluation of phenolic compounds from lichens against melanona cells. *Chem. Pharm. Bull.* **2013**, *61*, 176–183.
157. Singh, N.; Nambiar, D.; Kale, R.K.; Singh, R.P. Usnic acid inhibits and induces cell cycle arrest and apoptosis in human lung carcinoma A549 cells. *Nutr. Cancer* **2013**, *65*, 36–43.
158. Carneiro-Leão, A.M.A.; Buchi, D.F.; Iacomini, M.; Gorin, P.A.J.; Oliveira, M.B.M. Cytotoxic effect againts HeLa cells of polysaccharides from the lichen *Ramalina celastri*. *J. Sub*micr*. Cytol. Path.***1997**, *29*, 503–509.
159. Haraldsdóttir, S.; Guölaugsdóttir, E.; Ingólfsdóttir, K.; Ögmundsdóttir, H.M. Anti-proliferative effects of lichen-derived lipoxygenase inhibitors on twelve human cancer cell lines of different tissue origin *in vitro*. *Planta Med*. **2004**, *70*, 1098–1100.
160. Koparal, A.T.; Tuylu, B.A.; Turk, H. In vitro cytotoxic activities of (+)-usnic acid and (−)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. *Nat. Prod. Res.* **2006**, *20*, 1300–1307.
161. Einarsdóttir, E.; Groeneweg, J.; Björnsdóttir, G.G.; Haröardottir, G.; Omarsdóttir, S.; Ingólfsdóttir, K.; Ögmundsdóttir, H.M. Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. *Planta Med.* **2010**, *76*, 969–974.
162. Bačkorová, M.; Bačkor, M.; Mikeš, J.; Jendželovský, R.; Fedoročko, P. Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. *Toxicol. in Vitro* **2011**, 25, 37–44.
163. Bačkorová, M.; Jendželovský, R.; Kello, M.; Bačkor, M.; Mikeš, J.; Fedoročko, P. Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. *Toxicol. in Vitro* **2012**, *26*, 462–468.
164. Holbeck, SL. Update on NCI *invitro* drug screen utilities. *E*ur. *J. Cancer* **2004**, *40*, 785–793.

165. Honda, N.K.; Brum, R.L.; Marques, M.R.; Marcelli, M.P.; Fleig, M.; Vilegas, W. Fungitoxic activity of compounds isolated from lichens. *South. Braz. J. Chem.* **1999**, *7*, 61–66.
166. Wei, X.; Xinli, J.; Jeon, H.S.; Han, K.S.; Koh, Y.J.; Hur, J.S. Antifungal activity of lichen forming fungi against *Colletotrichum acutatum* on hot pepper. *Plant Pathology J.* **2008**, *24*, 202–206.
167. Honda, N.K.; Pavan, F.R.; Coelho, R.G.; Andrade Leite, S.R.; Micheletti, A.C.; Lopes, T.I.B.; Misutsu, M.Y.; Beatriz, A.; Brum, R.L.; Leite, C.Q.F. Antimycobacterial activity of lichen substances. *Phytomedicine* **2010**, *17*, 328–332.
168. Lim, J.H.; Lee, H.G.; Kim, I.C.; Hong, S.G.; Kim; D.G.; Lee, Y.G.; Lee, S.G.; Han, S.J.; Lee, H.S.; Lee, Y.M. Cosmetics Containing Usimine-C for Promoting Collagen Production. KR 20110084753 A, 26 July 2011.
169. Araojo, R.V.S.; Melo-Junior, M.R.; Beltrão, E.I.C.; Mello, L.A.; Iacomini, M.; Carneiro-Leao, A.M.A.; Carvalho, L.B.; Santos-Magalhães, N.S. Evaluation of the antischistosomal activity of sulfated α-D-glucan from the lichen Ramalina celastri free and encapsulated into liposomes. *Braz. J. Med. Biol. Res.* **2011**, *44*, 311–318.
170. Cetin, H.; Tufan-Cetin, O.; Turk, A.O.; Tay, T.; Candan, M.; Yanikoglu, A.; Sumbul, H. Insecticidal activity of major lichen compounds, (−)- and (+)-usnic acid, against the larvae of house mosquito, *Culex pipiens* L. *Parasitol. Res.* **2008**, *102*, 1277–1279.

Supplementary Information

Table S1. Polysaccharides reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names Of Species *
1	Carbohydrates	α-Glucan	*R. celastri*	[1–6]	*R. celastri*
			R. dendriscoides	[7]	*R. dendriscoides*
			R. fraxinea	[7]	*R. fraxinea*
			R. gracilis	[7]	*R. gracilis*
			R. gracilis (photobiont)	[8]	*R. gracilis*
			R. peruviana	[7]	*R. peruviana*
			R. peruviana (mycobiont)	[9]	*R. peruviana*
			R. usnea	[10]	*R. australiensis*
2		Galactomannan	*R. celastri*	[3]	*R. celastri*
			R. dendriscoides	[7]	*R. dendriscoides*
			R. ecklonii	[1]	*R. ecklonii*
			R. fraxinea	[7]	*R. fraxinea*
			R. gracilis	[7]	*R. gracilis*
			R. gracilis (photobiont)	[8]	*R. gracilis*
			R. peruviana	[7]	*R. peruviana*
			R. peruvina (mycobiont)	[5]	*R. peruviana*
			R. usnea	[10,11]	*R. australiensis*
3		Glucose	*R. crassa* (mycobiont)	[12]	*R. siliquosa*
			R. sinensis	[13]	*R. sinensis*
			R. fraxinea	[14]	*R. fraxinea*
4		Galactose	*R. crassa* (phycobiont)	[12]	*R. siliquosa*
			R. fraxinea	[14]	*R. fraxinea*
5		Mannose	*R. sinensis*	[13]	*R. sinensis*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names Of Species *
6		Rhamnose	*R. sinensis*	[13]	*R. sinensis*
			R. fraxinea	[14]	*R. fraxinea*
7		D-Arabitol	*R. reticulata*	[15]	*R. menziessi*
			R. siliquosa	[16]	*R. siliquosa*
			R. calicaris	[17]	*R. calicaris*
			R. sinensis	[17]	*R. sinensis*
			R. tayloriana	[18]	*R. luciae*
			R. yasudae (photobiont)	[19]	*R. yasudae*
			R. geniculata	[20]	*R. inflata subsp. inflata*
			R. scopulorum	[20]	*R. siliquosa*
8		Mannitol	*R. fraxinea*	[14]	*R. fraxinea*
9		Glucosamine	*R. fraxinea*	[14]	*R. fraxinea*
10		Arabinose	*R. fraxinea*	[14]	*R. fraxinea*
11		Xylose	*R. fraxinea*	[14]	*R. fraxinea*
12		Glucoronic acid	*R. fraxinea*	[14]	*R. fraxinea*
13		Lentinan	*R. complanata*	[21]	*R. complanata*
14		Amylose	*R. celastri*	[3]	*R. celastri*
			R. celastri (photobiont)	[4]	*R. celastri*
			R. gracilis (photobiont)	[8]	*R. gracilis*
15		Galacturonic acid	*R. terebrata*	[22]	*R. terebrata*
16		Ribitol	*R. crassa* (phycobiont)	[23]	*R. siliquosa*
			R. subbreviuscula (phycobiont)	[23]	*R. subbreviuscula*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014

Table S2. Usnic acid and derivatives reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source	References	Current Names of Species *
17	Usnic acid and derivatives	Usnic acid	*R. africana*	[24]	*R. africana*
			R. asahinae	[25]	*R. asahinae*
			R. boninensis	[26,27]	*R. boninensis*
			R. boulhautina	[28]	*R. boulhautiana*
			R. anceps	[29–31]	*R. anceps*
			R. atlantica	[32]	*R. cuspidata*
			R. breviuscula	[33]	*R. breviuscula*
			R. cactacearum	[34]	*R. cactacearum*
			R. calcarata	[30]	*R. calcarata*
			R. calicaris	[9,17,35,36]	*R. calicaris*
			R. camptospora	[29,30]	*R. camptospora*
			R. capitata	[36–38]	*R. capitata*
			R. celastri	[30,39–41]	*R. celastri*
			R. celastri (mycobiont)	[39]	*R. celastri*
			R. ceruchis	[42,34]	*R. ceruchis*
			R. ceruchoides	[42]	*R. ceruchoides*
			R. chilensis	[31,34,43,44]	*R. chilensis*
			R. cochlearis	[30]	*R. cochlearis*
			R. combeoides	[42]	*R. combeoides*
			R. complanata	[29,30]	*R. complanata*
			R. conduplicans	[45]	*R. conduplicans*
			R. crassa	[32,46]	*R. siliquosa*
			R. crassa (mycobiont)	[46,47]	*R. siliquosa*
			R. curnowii	[32,36]	*R. cuspidata*
			R. cuspidata	[9,48–50]	*R. cuspidata*

No.	Class of Compounds	Compounds Name	Source	References	Current Names of Species *
			R. cuspidata var. *armorica*	[51]	*R. cuspidata*
			R. cuspidata var. *cuspidata*	[51]	*R. cuspidata*
			R. cuspidate var. *stenoclada*	[51]	*R. cuspidata*
			R. darwiniana	[29]	*R. darwiniana*
			R. dendriscoides	[30]	*R. dendriscoides*
			R. dendroides	[30]	*R. dendroides*
			R. diracerata	[33,52]	*R. diracerata*
			R. druidarum	[32]	*R. siliquosa*
			R. ecklonii	[34,53]	*R. ecklonii*
			R. evernioides	[33]	*R. evernioides*
			R. farinacea	[9,24,33,43,50,54–63]	*R. farinacea*
			R. fastigiata	[37,36]	*R. fastigiata*
			R. flaccescens	[34,42]	*R. flaccescens*
			R. fragilis	[29]	*R. fragilis*
			R. fraxinea	[14,27,36–38,64]	*R. fraxinea*
			R. furcellangulida	[29]	*R. furcellangulida*
			R. geniculata	[65,66]	*R. inflate subsp. inflata*
			R. glaucescens	[67]	*R. glaucescens*
			R. gracilis	[30]	*R. gracilis*
			R. grumosa	[29,30]	*R. grumosa*
			R. hierrensis	[68]	*R. hierrensis*
			R. homalea	[42]	*Niebla homalea*
			R. hossei	[42]	*R. hossei*
			R. inanis	[34]	*R. inanis*
			R. inflata	[40]	*R. inflata*
			R. intermedia	[33]	*R. intermedia*

No.	Class of Compounds	Compounds Name	Source	References	Current Names of Species *
			R. kullensis	[69]	*R. kullensis*
			R. lacera	[43]	*R. lacera*
			R. landröensis	[50,69]	*R. landröensis*
			R. menziensii	[70]	*R. menziensii*
			R. minuscula	[50,69]	*R. dilacerata*
			R. montagnei	[29]	*R. montagnei*
			R. nervulosa	[71]	*R. nervulosa*
			R. obtusata	[27,33,50,69]	*R. obtusata*
			R. pacifica	[40,71]	*R. pacifica*
			R. paludosa	[72]	*R. paludosa*
			R. peranceps	[31,73]	*R. peranceps*
			R. peruviana	[30,34]	*R. peruviana*
			R. pollinaria	[27,37,59,66,74,75]	*R. pollinaria*
			R. polyforma	[29]	*R. polyforma*
			R. polymorpha	[36,37,59]	*R. polymorpha*
			R. prolifera	[30]	*R. prolifera*
			R. puiggarii	[30]	*R. puiggarii*
			R. pusilla	[30]	*R. inflate subsp. australis*
			R. rectangularis	[30]	*R. rectangularis*
			R. reticulata	[15,27,33,76,77]	*R. menziesii*
			R. rigida	[30]	*R. rigida*
			R. roesleri	[78]	*R. roesleri*
			R. scorpulorum	[16,49,50,69,79]	*R. siliquosa*
			R. sekika	[27]	*R. sekika*
			R. sharpii	[31]	*R. sharpii*

No.	Class of Compounds	Compounds Name	Source	References	Current Names of Species *
			R. sideriza	[29]	*R. sideriza*
			R. siliquosa	[9,16,32,47]	*R. siliquosa*
			R. siliquosa var. *crassa*	[51]	*R. siliquosa*
			R. siliquosa var. *siliquosa*	[51]	*R. siliquosa*
			R. siliquosa var. *x*	[51]	*R. siliquosa*
			R. siliquosa var. zopfii	[51]	*R. siliquosa*
			R. sinensis	[17,80]	*R. sinensis*
			R. sorediantha	[29]	*R. sorediantha*
			R. sorediosa	[29,30]	*R. sorediosa*
			R. stenoclada	[32]	*R. cuspidata*
			R. stenospora	[81]	*R. stenospora*
			R. subcomplanata	[43,82]	*R. subcomplanata*
			R. subfarinacea	[9,36,50,69]	*R. subfarinacea*
			R. subpollinaria	[30]	*R. subpollinaria*
			R. terebrata	[83–85]	*R. terebrata*
			R. tingitana	[86]	*R. tingitana*
			R. tayloriana	[18]	*R. luciae*
			R. thrausta	[50]	*R. thrausta*
			R. tumidula	[34]	*R. tumidula*
			R. usnea	[29–31,87]	*R. australiensis*
			R. yasudae	[43,65]	*R. yasudae*
			R. yasudae (mycobiont)	[46]	*R. yasudae*
18		Usimine A	*R. terebrata*	[83,85,88,89]	*R. terebrata*
19		Usimine B	*R. terebrata*	[83,85,88,89]	*R. terebrata*
20		Usimine C	*R. terebrata*	[83,85,88,89]	*R. terebrata*
21		*Iso*-usnic acid	*R. hierrensis*	[68]	*R. hierrensis*

No.	Class of Compounds	Compounds Name	Source	References	Current Names of Species *
22		Usninic acid	*R. bornholmiensis*	[50]	*R. bornholmiensis*
			R. calicaris	[35]	*R. calicaris*
			R. cuspidata	[50]	*R. cuspidata*
			R. farinacea	[50]	*R. farinacea*
			R. landroënsis	[50]	*R. landroënsis*
			R. minuscula	[50]	*R. dilacerata*
			R. obtusata	[50]	*R. obtusata*
			R. scopulorum	[50]	*R. siliquosa*
			R. subfarinacea	[50]	*R. subfarinacea*
			R. thrausta	[50]	*R. thrausta*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S3. Depsides reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
23	Depsides	Sekikaic acid	*R. boulhautina*	[28,47]	*R. boulhautiana*
			R. calicaris	[17,27]	*R. calicaris*
			R. conduplicans	[45,90]	*R. conduplicans*
			R. chilensis	[31,44]	*R. chilensis*
			R. cochlearis	[30]	*R. cochlearis*
			R. darwiniana	[29]	*R. darwiniana*
			R. farinacea	[9,54,60,66,91]	*R. farinacea*
			R. farinacea var. nervulosa	[27]	*R. farinacea*
			R. fragilis	[29]	*R. fragilis*
			R. fraxinea	[14]	*R. fraxinea*
			R. furcellangulida	[29]	*R. furcellangulida*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. geniculata	[27,43,66]	*R. inflata subsp. inflata*
			R. glaucescens	[43,67]	*R. glaucescens*
			R. hossei	[45,92]	*R. hossei*
			R. luciae	[93]	*R. luciae*
			R. montagnei	[29]	*R. montagnei*
			R. nervulosa	[71,94]	*R. nervulosa*
			R. peruviana	[30,43,95]	*R. peruviana*
			R. polyforma	[29]	*R. polyforma*
			R. pusiola	[30]	*R. pusiola*
			R. roesleri	[78]	*R. roesleri*
			R. sekika	[27]	*R. sekika*
			R. sorediosa (race II)	[30]	*R. sorediosa*
			R. subcomplanata	[94]	*R. subcomplanata*
			R. subpollinaria (race I)	[30]	*R. subpollinaria*
			R. tayloriana	[18,43]	*R. luciae*
			R. usnea	[30,31,87]	*R. australiensis*
24		Atranorim	*R. celastri*	[30]	*R. celastri*
			R. combeoides	[42]	*R. combeoides*
			R. complanata	[30]	*R. complanata*
			R. darwiniana	[29]	*R. darwiniana*
			R. dendriscoides	[30]	*R. dendriscoides*
			R. ecklonii	[53]	*R. ecklonii*
			R. fragilis	[29]	*R. fragilis*
			R. furcellangulida	[29]	*R. furcellangulida*
			R. glaucescens	[67]	*R. glaucescens*
			R. hierrensis	[68]	*R. hierrensis*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. pacifica	[40]	*R. pacifica*
			R. paludosa	[72]	*R. paludosa*
			R. peruviana (mycobiont)	[95]	*R. peruviana*
			R. roesleri	[78]	*R. roesleri*
			R. siliquosa	[16,47]	*R. siliquosa*
			R. stenospora	[81]	*R. stenospora*
			R. subcomplanata	[82]	*R. subcomplanata*
			R. subpllinaria	[30]	*R. subpollinaria*
25		Divaricatic acid	*R. aspera* (race I)	[30]	*R. aspera*
			R. calcarata	[30]	*R. calcarata*
			R. complanata (race I)	[30]	*R. complanata*
			R. darwiniana	[29]	*R. darwiniana*
			R. furcellangulida	[29]	*R. furcellangulida*
			R. hierrensis	[68]	*R. hierrensis*
			R. homalea	[42]	*Niebla homalea*
			R. menziesii	[70]	*R. menziessi*
			R. polyforma	[29]	*R. polyforma*
			R. subbreviuscula	[96]	*R. subbreviuscula*
			R. usnea	[87]	*R. australiensis*
			R. usnea (race II)	[30]	*R. australiensis*
26		Homosekikaic acid	*R.cochlearis*	[30]	*R. cochlearis*
			R. conduplicans	[90]	*R. conduplicans*
			R. luciae	[93]	*R. luciae*
			R. nervulosa	[94]	*R. nervulosa*
			R. peruviana	[30,95]	*R. peruviana*
			R. pusiola	[30]	*R. pusiola*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. roesleri	[78]	*R. roesleri*
			R. sorediosa	[30]	*R. sorediosa*
			R. subcomplanata	[94]	*R. subcomplanata*
			R. subpollinaria	[30]	*R. subpollinaria*
			R. usnea	[30]	*R. australiensis*
27		Ramalinoic acid	*R. calicaris*	[27,47]	*R. calicaris*
			R. cochlearis	[30]	*R. cochlearis*
			R. farinacea	[27,43,97]	*R. farinacea*
			R. geniculata	[27]	*R. inflata subsp. Inflate*
			R. intermediella	[27,97]	*R. intermediella*
			R. nervulosa	[47]	*R. nervulosa*
			R. obtusata	[69]	*R. obtusata*
			R. peruviana	[43,95]	*R. peruviana*
			R. usnea	[87]	*R. australiensis*
			R. usneoides	[27]	*R. australiensis*
28		Obtusatic acid	*R. calicaris*	[9,35]	*R. calicaris*
			R. farinacea	[24,57]	*R. farinacea*
			R. fraxinea	[14]	*R. fraxinea*
			R. mediterranea	[98]	*R. mediterranea*
			R. obtusata	[27,47,52,69]	*R. obtusata*
			R. pollinaria	[27]	*R. pollinaria*
			R. sekika	[27]	*R. sekika*
29		Tumidulin	*R. ceruchis*	[34,99]	*R. ceruchis*
			R. chilensis	[34,44]	*R. chilensis*
			R. flaccescens	[34,99]	*R. flaccescens*
			R. inanis	[34]	*R. inanis*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. peruviana	[34]	*R. peruviana*
			R. tumidula	[34,100]	*R. tumidula*
30		4’-*O*-demethylsekikaic acid	*R. americana*	[47]	*R. Americana*
			R. cochlearis	[30]	*R. cochlearis*
			R. farinacea	[47]	*R. farinacea*
			R. peruviana	[30,95]	*R. peruviana*
			R. sorediosa	[30]	*R. sorediosa*
			R. usnea (races I and II)	[30]	*R. australiensis*
31		Evernic acid	*R. calicaris*	[9,35]	*R. calicaris*
			R. commixta	[27]	*R. commixta*
			R. farinacea	[24,57]	*R. farinacea*
			R. mediterranea	[98]	*R. mediterranea*
			R. pollinaria	[27,59,66,74,75]	*R. pollinaria*
			R. yasudae	[47,101]	*R. yasudae*
32		4’-*O*-methylnorhomosekikaic acid	*R. cochlearis*	[30]	*R. cochlearis*
			R. farinacea	[54]	*R. farinacea*
			R. peruviana	[30]	*R. peruviana*
			R. pusiola	[30]	*R. pusiola*
			R. subcomplanata	[94]	*R. subcomplanata*
			R. usnea (races I and II)	[30]	*R. australiensis*
33		4’-*O*-methylnorsekikaic acid	*R. cochlearis*	[30]	*R. cochlearis*
			R. farinacea	[47,54]	*R. farinacea*
			R. usnea (races I and II)	[30]	*R. australiensis*
34		2’-*O*-methylsekikaic acid	*R. asahinae*	[25,30,47,102]	*R. asahinae*
			R. grumosa	[30]	*R. grumosa*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. sorediosa	[30]	*R. sorediosa*
35		Chloroatranorin	*R. siliquosa*	[16]	*R. siliquosa*
			R. stenospora	[81]	*R. stenospora*
			R. subcomplanata	[82]	*R. complanata*
36		Chryptochorophaeic acid	*R. aspera* (race II)	[30]	*R. aspera*
			R. paludosa	[72,81]	*R. paludosa*
37		4'-*O*-demethylhomosekikaic acid	*R. peruviana*	[30]	*R. peruviana*
			R. usnea (races I and II)	[30]	*R. australiensis*
38		Diffractaic acid	*R. lacera*	[43]	*R. lacera*
			R. subcomplanata	[43,82]	*R. subcomplanata*
39		4-*O*-demethylbarbatic acid	*R. siliquosa* (mycobiont)	[103]	*R. siliquosa*
			R. siliquosa var. *Zopfii*	[47,51]	*R. siliquosa*
			R. subdecipiens	[47]	*R. subdecipiens*
40		Ramalinaic acid	*R. americana*	[47]	*R. americana*
			R. farinacea	[61]	*R. farinacea*
41		Gyrophoric acid	*R. americana*	[43]	*R. americana*
42		Trivaric acid	*R. americana*	[43,104]	*R. americana*
43		Perlatolic acid	*R. stenospora*	[81]	*R. stenospora*
44		4-*O*-demethylnorhomosekikaic acid	*R. peruvina*	[30]	*R. peruviana*
45		4'-*O*-methylsekikaic acid	*R. asahinae*	[30]	*R. asahinae*
46		4'-*O*-methylpaludosic acid	*R. asahinae*	[30,102]	*R. asahinae*
47		4,4'-di-*O*- methylcryptochlorophaeic acid	*R. asahinae*	[102]	*R. asahinae*
48		Boninic acid	*R. asahinae*	[25,26]	*R. asahinae*
49		Stenosporic acid	*R. stenospora*	[47,81]	*R. stenospora*
50		5-Hydroxysekikaic acid	*R. farinacea*	[91]	*R. farinacea*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
51		5-Chlorosekikaic acid	*R. glaucescens*	[67]	*R. glaucescens*
52		Olivetoric acid	*R. leiodea*	[105]	*R. leiodea*
53		Paludosic acid	*R. paludosa*	[72]	*R. paludosa*
54		4-*O*-methyl-oxocryptochlorophaeic acid	*R. subfraxinea*	[106]	*R. subfraxinea*
55		Lecanoric acid	*R. lacera*	[43]	*R. lacera*
56		Bourgeanic acid	*R. bourgeana*	[107,108]	*R. bourgeana*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S4. Depsidones reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
57	Depsidones	Salazinic acid	*R. anceps*	[31]	*R. anceps*
			R. angustissima	[27]	*R. subfarinacea*
			R. calcarata	[30]	*R. calcarata*
			R. chilensis	[31,43]	*R. chilensis*
			R. complanata	[29]	*R. complanata*
			R. complanata (races I and II)	[30]	*R. complanata*
			R. crassa	[32,47]	*R. siliquosa*
			R. crassa (mycobiont)	[46]	*R. siliquosa*
			R. darwiniana	[29]	*R. darwiniana*
			R. dendriscoides	[30]	*R. dendriscoides*
			R. farinacea	[43,54,109]	*R. farinacea*
			R. furcellangulida	[29]	*R. furcellangulida*
			R. nervulosa	[47]	*R. nervulosa*
			R. pacifica	[71]	*R. pacifica*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. peranceps	[31]	*R. peranceps*
			R. polyforma	[29]	*R. polyforma*
			R. rectangularis	[30]	*R. rectangularis*
			R. rigida	[30]	*R. rigida*
			R. scopulorum	[16,79]	*R. siliquosa*
			R. sharpie	[31]	*R. sharpie*
			R. siliquosa	[47]	*R. siliquosa*
			R. siliquosa var. *crassa*	[47,51]	*R. siliquosa*
			R. siliquosa (mycobiont)	[103]	*R. siliquosa*
			R. subbreviuscula	[96]	*R. subbreviuscula*
			R. subcomplanata	[94]	*R. subcomplanata*
			R. subfarinacea var. *reagens*	[58]	*R. subfarinacea*
			R. subfarinacea var. *salazinic*	[58]	*R. subfarinacea*
			R. sorediosa (races I, II and III)	[30]	*R. sorediosa*
			R. subfarinacea	[9,33,43,50,69]	*R. subfarinacea*
			R. subpollinaria (races I, II and III)	[30]	*R. subpollinaria*
			R. yasudae	[47,65]	*R. yasudae*
			R. yasudae (mycobiont)	[46]	*R. yasudae*
58		Norstictic acid	*R. anceps*	[30,31]	*R. anceps*
			R. angustissima	[27]	*R. subfarinacea*
			R. arabum	[43]	*R. arabum*
			R. chilensis	[31,43,44]	*R. chilensis*
			R. curnowii	[32]	*R. cuspidata*
			R. cuspidata var. *cuspidata*	[51]	*R. cuspidata*
			R. cuspidata var. *stenoclada*	[51]	*R. cuspidata*
			R. farinacea	[43,55,58,60,109]	*R. farinacea*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. gracilis (race II)	[30]	*R. gracilis*
			R. lacera	[43]	*R. lacera*
			R. peranceps	[47]	*R. peranceps*
			R. pusiola	[30]	*R. pusiola*
			R. sharpii	[31]	*R. sharpii*
			R. siliquosa	[9]	*R. siliquosa*
			R. stenoclada	[32]	*R. cuspidata*
			R. subfarinacea	[43]	*R. subfarinacea*
			R. subfarinacea var. *subfarinacea*	[110]	*R. subfarinacea*
			*R. subfarina*cea var. *reagens*	[110]	*R. subfarinacea*
59		Hypoprotocetraric acid	*R. druidarum*	[32]	*R. siliquosa*
			R. cuspidata var. *armorica*	[51]	*R. cuspidata*
			R. cuspidata var. cuspidata	[51]	*R. cuspidata*
			R. farinacea	[109]	*R. farinacea*
			R. hypoprotocetraric	[47]	*R. farinacea*
			R. siliquosa	[16,111]	*R. siliquosa*
			R. siliquosa var. *druidarum*	[51]	*R. siliquosa*
			R. tumidula	[47]	*R. tumidula*
60		Scopuloric acid (or stictic)	*R. combeoides*	[42]	*R. combeoides*
			R. curnowii	[32]	*R. cuspidata*
			R. cuspidata var. *armorica*	[51]	*R. cuspidata*
			R. cuspidata var. *cuspidata*	[51]	*R. cuspidata*
			R. hierrensis	[68]	*R. hierrensis*
			R. scopulorum	[16,27,69,79]	*R. siliquosa*
61		Protocetraric acid	*R. farinacea*	[9,27,55,58,109]	*R. farinacea*
			R. lacera	[112]	*R. lacera*

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
			R. pacifica	[112]	*R. pacifica*
			R. siliquosa	[16,32]	*R. siliquosa*
			R. siliquosa (mycobiont)	[3]	*R. siliquosa*
			R. siliquosa var. *siliquosa*	[30,31]	*R. siliquosa*
62		Connorstictic acid	*R. anceps*	[30]	*R. anceps*
63		Criptostictic acid	*R. cuspidata* var. *armorica*	[51]	*R. cuspidata*
			R. cuspidata var. *cuspidata*	[51]	*R. cuspidata*
64		Peristictic acid	*R. cuspidata* var. *armorica*	[51]	*R. cuspidata*
65		Conhypoprotocetraric acid	*R. siliquosa* var. x	[51]	*R. siliquosa*
66		Variolaric acid	*R. hierrensis*	[68]	*R. hierrensis*
67		Gangaleodin	*R. hierrensis*	[68]	*R. hierrrensis*
68		Physodic acid	*R. leiodea*	[105]	*R. leiodea*
69		Coquimboic acid	*R. tumidula*	[113]	*R. tumidula*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S5. Fatty acids reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina species*)	References	Current Names of Species *
70	Fatty acids	Oleic acid	*R. lacera*	[43]	*R. lacera*
			R. celastri (mycobiont)	[41]	*R. celastri*
			R. yasudae	[114]	*R. yasudae*
71		Palmitic acid	*R. lacera*	[114]	*R. lacera*
			R. celastri (mycobiont)	[41]	*R. celastri*
72		Stearic acid	*R. lacera*	[43]	*R. lacera*
			R. celastri (mycobiont)	[41]	*R. celastri*
			R. yasudae	[114]	*R. yasudae*
73		α-Linolenic	*R. celastri* (mycobiont)	[43]	*R. celastri*
			R. yasudae	[114]	*R. yasudae*

74	Linoleic acid	*R. yasudae*	[114]	*R. yasudae*
75	Myristic acid	*R. yasudae*	[114]	*R. yasudae*
76	Arachidonic acid	*R. yasudae*	[114]	*R. yasudae*
77	D-Protolichesterinic acid	*R. almquistii*	[24,115]	*R. almquistii*
		R. roesleri	[78]	*R. roesleri*
78	Nephrosterinic acid	*R. almquistii*	[24,115]	*R. almquistii*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S6. Other compounds—Carotenoids reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina* species)	References	Current Names of Species *
79	Carotenoids	β-Cryptoxanthin	*R. ecklonii*	[116]	*R. ecklonii*
			R. usnea	[116]	*R. australiensis*
80		Lutein epoxide	*R. ecklonii*	[116]	*R. ecklonii*
			R. usnea	[116]	*R. australiensis*
81		Violaxanthin	*R. ecklonii*	[116]	*R. ecklonii*
82		Auroxanthin	*R. ecklonii*	[116]	*R. ecklonii*
			R. usnea	[116]	*R. australiensis*
83		Astaxanthin	*R. celastri*	[117]	*R. celastri*
			R. usnea	[116]	*R. australiensis*
84		Mutatoxanthin	*R. usnea*	[116]	*R. australiensis*
85		Lycoxanthin	*R. ecklonii*	[116]	*R. ecklonii*
86		Antheroxanthin	*R. ecklonii*	[116]	*R. ecklonii*
			R. usnea	[116]	*R. australiensis*
87		Ɛ-Caroten	*R. usnea*	[116]	*R. australiensis*
88		Zeaxanthin	*R. celastri*	[117]	*R. celastri*
89		β-Caroten	*R. celastri*	[117]	*R. celastri*
90		α-Doradexanthin	*R. celastri*	[117]	*R. celastri*
91		Lutein	*R. celastri*	[117]	*R. celastri*
			R. usnea	[116]	*R. australiensis*
92		Hydroxyechinenone	*R. ecklonii*	[116]	*R. ecklonii*
93		Diatoxanthin	*R. ecklonii*	[116]	*R. ecklonii*
94		Neoxanthin	*R. ecklonii*	[116]	*R. ecklonii*
			R. usnea	[116]	*R. australiensis*
95		Rhodoxanthin	*R. ecklonii*	[116]	*R. ecklonii*
			R. usnea	[116]	*R. australiensis*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S7. Other compounds—Steroids and terpenoids reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina* species)	Refences	Current Names of Species *
96	Steroids	α-Sitosterol	*R. africana*	[24]	*R. africana*
			R. hierrensis	[68]	*R. hierrrensis*
97		Brassicasterol	*R. africana*	[24]	*R. africana*
			R. tingitana	[86]	*R. tingitana*
98		Lichesterol	*R. africana*	[24]	*R. africana*
99		Ergosterol peroxide	*R. hierrensis*	[68]	*R. hierrensis*
			R. tingitana	[86]	*R. tingitana*
100		Cerevisterol	*R. hierrensis*	[68]	*R. hierrensis*
101	Terpenoids	Ursolic acid	*R. hierrensis*	[68]	*R. hierrensis*
102		*Iso*-arborinol acetate	*R. hierrensis*	[68]	*R. hierrensis*
103		Friedelin	*R. ecklonii*	[53]	*R. ecklonii*
104		(−)-Sandaracopimaric acid	*R. hierrensis*	[68]	*R. hierrensis*
105		Ceruchinol	*R. ceruchis* var. *tumidula*	[118]	*R. ceruchis*
			R. tigrina	[119]	*R. tigrina*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S8. Other compounds—Lipids and amines reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina* species)	References	Current Names of Species *
106	Lipids	Diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine	*R. lacera*	[43]	*R. lacera*
107		Diacylglyceryltrimetylalanine	*R. lacera*	[43]	*R. lacera*
108		Phosphatidylcholine	*R. lacera*	[43]	*R. lacera*
109		Phosphatidyletanolamine	*R. lacera*	[43]	*R. lacera*
110		Phosphatidylinositol	*R. lacera*	[43]	*R. lacera*
111		Phosphatidic acid	*R. lacera*	[43]	*R. lacera*
112		Sulfoquinovosyldiacylglycerol	*R. lacera*	[43]	*R. lacera*
113		Monogalactosyldiacylglycerol	*R. celastri*	[120]	*R. celastri*
			R. lacera	[43]	*R. lacera*
114		Gigalactosyldiacylglycerol	*R. celastri*	[120]	*R. celastri*
			R. lacera	[43]	*R. lacera*
115		*O*-β-D-Galactopyranosyl-(1→1')-ceramide	*R. celastri*	[121]	*R. celastri*
116	Amines	Choline	*R. fraxinea*	[14]	*R. fraxinea*
117		Betaine	*R. fraxinea*	[14]	*R. fraxinea*
118		Histamine	*R. fraxinea*	[14]	*R. fraxinea*
119		Acetylcholine	*R. fraxinea*	[14]	*R. fraxinea*
120		β-Fenethylamine	*R. fraxinea*	[14]	*R. fraxinea*
121		Spermidine	*R. farinacea*	[122]	*R. farinacea*
122		Putrescine	*R. farinacea*	[123,124]	*R. farinacea*
123		Spermine	*R. calicaris*	[124]	*R. calicaris*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S9. Other compounds—Amino acids reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina* species)	References	Current Names of Species *
124	Amino acids	Glutamic acid	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. fraxinea	[14]	*R. fraxinea*
			R. siliquosa	[125]	*R. siliquosa*
125		Aspartic acid	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. fraxinea	[14]	*R. fraxinea*
			R. siliquosa	[125]	*R. siliquosa*
126		Alanine	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. fraxinea	[14]	*R. fraxinea*
			R. siliquosa	[125]	*R. siliquosa*
			R. sinensis	[126]	*R. sinensis*
127		Serine	*R. fraxinea*	[14]	*R. fraxinea*
			R. siliquosa	[125]	*R. siliquosa*
128		Proline	*R. fraxinea*	[14]	*R. fraxinea*
			R. siliquosa	[125]	*R. siliquosa*
129		Arginine	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. siliquosa	[125]	*R. siliquosa*
130		Glycine	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. siliquosa	[125]	*R. siliquosa*
131		Lysine	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. siliquosa	[125]	*R. siliquosa*
132		Leucine	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. siliquosa	[125]	*R. siliquosa*
133		Threonine	*R. celastri* (photobiont)	[4]	*R. celastri*
			R. siliquosa	[125]	*R. siliquosa*
134		Glucosamine	*R. siliquosa*	[125]	*R. siliquosa*
135		γ-Aminobutiric acid	*R. fraxinea*	[14]	*R. fraxinea*
136		Taurine	*R. crassa*	[127]	*R. siliquosa*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

Table S10. Other compounds reported in the *Ramalina* species of lichenized fungi (Fungi: Ascomycota).

No.	Class of Compounds	Compounds Name	Source (*Ramalina* species)	References	Current Names of Species *
137	Hidrazide	Ramalin	*R. terebrata*	[83,85,128–130]	*R. terebrata*
138	Cyclic peptide	Stereocalpin A	*R. terebrata*	[131–133]	*R. terebrata*
139	Phenolics compounds	2,3-Dihydroxy-4-methoxy-6-pentyl-phenylmethyl ester	*R. farinacea*	[91]	*R. farinacea*
140		Divaric acid	*R. africana*	[24]	*R. africana*
141		Ethyl divaricatinate	*R. africana*	[24]	*R. africana*
142		2-Hydroxy-4-methoxy-6-propyl benzoic acid	*R. roesleri*	[78]	*R. roesleri*
143		2,4-Dihydroxy-3,6-dimethyl-methyl ester benzoic acid	*R. roesleri*	[78]	*R. roesleri*
144		Isorhizonic acid	*R. dilacerata*	[134]	*R. dilacerate*
145	Organics acids and derivatives	α-Crotonic acid	*R. reticulata*	[15]	*R. menziesii*
146		Abscisic acid	*R. farinacea*	[123]	*R. farinacea*
147		Ethyl caprilate	*R. fastigiata*	[135]	*R. fastigiata*
148		Ethyl palmitate	*R. fastigiata*	[135]	*R. fastigiata*
149		Ethyl stearate	*R. fastigiata*	[135]	*R. fastigiata*
150	Benzopyran	Divaricat acid	*R. hierrensis*	[68]	*R. hierrensis*
151	Anthraquinone	Parientin	*R. hierrensis*	[68]	*R. hierrensis*
152	Hydrocarbon	Aspicilin	*R. ecklonii*	[34]	*R. ecklonii*
153		Ethylene	*R. lacera*	[47]	*R. lacera*

* Updating scientific names validated by Index Fungorum, available at http://www.indexfungorum.org/names/Names.asp in May 2014.

References

Miceno, A.M.; Gorin, P.A.J.; Iacomini, M. Galactomannan and isolichenan componentes of the carbohydrate-rich lichen *Ramalina ecklonii* (Spreng.) Mey. & Flot. *Agr. Biol. Chem.* **1991**, *55*, 1391–1392.

Teixeira, A.Z.A.; Iacomini, M.; Gorin, A. J. An unusual glucomannan from *Tornabenia intricata*. *Phytochemistry* **1992**, *31*, 3467–3470.

Stuelp, P.M.; Carneiro Leão, A.M.A.; Gorin, P.A.J.; Iacomini, M. The glucans of *Ramalina celastri*; relation with chemotypes of other lichens. *Carbohyd. Polym.* **1999**, *40*, 101–106.

Cordeiro, L.M.C.; Reis, R.A.; Tisher, C.A.; Gorin, P.A.J.; Ferreira, J.C.; Iacomini, M. Linear β-mannose containig polysaccharide, β-xylan, and amylose from the cultured photobiont Trebouxia sp. of the ascolichen *Ramalina celastri*. *FEMS Microbiol. Lett.* **2003**, *220*, 89–94.

Cordeiro, L.M.C.; Stocker-Wörgötter, E.; Gorin, P.A.J.; Iacomini, M. Elucidation of polysaccharide origin in *Ramalina peruviana* symbiosis. *FEMS Microbiol. Lett.* **2004**, *238*, 79–84.

Teixeira, A.Z.A.; Iacomini, M.; Gorin, P.A.J. Chemotypes of mannose-containing polysaccharides of lichens mycobionts: A possible aid in classification and identification. *Carbohyd. Res.* **1995**, *266*, 309–314.

Cordeiro, L.M.C.; Stocker-Wörgötter, E.; Gorin, P.A.J.; Iacomini, M. Comparative studies of the polysaccharides from species of the genus Ramalina-lichenized fungi-of three distinct habitats. *Phytochemistry* **2003**, *63*, 967–975.

Cordeiro, L.M.C.; Carbonero, E.R.; Sassaki, G.L.; Reis, R.A.; Stocker-Wörgötter, E.; Gorin, P.A.J.; Iacomini, M. A fungus-type β-galactofuranan in the cultivated *Trebouxia* photobiont of the lichen *Ramalina gracilis*. *FEMS Microbiol. Lett.* **2005**, *244*, 193–198.

Solberg, Y.J. Dyeing of wool with lichens and lichen substances. *Acta Chem. Scand.* **1956**, *10*, 1116–1123.

Gorin, P.A.J.; Iacomini, M. Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. *Carbohyd. Res.* **1984**, *128*, 119–132.

Gorin, P.A.J.; Iacomini, M. Structural diversity of D-galacto-D-mannan components isolated from lichens having ascomycetous mycobionts. C*arbohyd. Res.* **1985**, *142*, 253–267.

Takahashi, K.; Takeda, T.; Shibata, S. Polysaccharides of lichen symbionts. *Chem. Pharm. Bull.* **1979**, *27*, 238–241.

Mamut, R.; Tursun, T.; Abbas, A. Analysis monosaccharide of the polysaccharide in *Ramalina sinensis* with HPLC. *Shipin Gongye* **2013**, *34*, 209–211.

Krzacek, T.; Smolarz, H. Chemical composition of *Ramalina fraxinea* (L.) Ach. Thallus. *Acta Soc. Bot. Pol.* **1979**, *48*, 27–34.

Stark, J.B.; Walter, E.D.; Owens, H.S. Method of isolation of usnic acid from *Ramalina reticulata*. *J. Am. Chem. Soc.* **1950**, *72*, 1819–1820.

Culberson, C.F. Some constituents of the lichen *Ramalina siliquosa*. *Phytochemistry* **1965**, *4*, 951–961.

Mittal, O.P.; Neelakantan, S.; Seshaadri, T.R. Chemical investigation of Indian lichens. XIV. Chemical components of *Ramalina calicaris* and *Ramalina sinensis*. *J. Sci. Ind. Res. India* **1952**, *11B*, 386–387.

Seshadri, T.R.; Subramanian, S.S. Indian lichens. VIII. Lichens growing on sandal trees. Proc.-*Indian Acad. Sci., Sect. A* **1949**, *30A*, 15–22.

Kosugi, M.; Miyake, H.; Yamakawa, H.; Shibata, Y.; Miyazawa, A.; Sugimura, T.; Satoh, K.; Itoh, S.; Kashino, Y. Arabitol provided by lichenous fungi enhances ability to dissipate excess light energy in a symbiotic green alga under desiccation. *Plant Cell Physiol.* **2013**, *54*, 1316–1325.

Ashina, Y.; Yanagita, M. Lichen substances. XXXIX. A new lichen acid, norstictinic acid, and the occurrence of D-arabitol in lichens. *Ber. Dtsch. Chem. Ges. B* **1934**, *67B*, 799–803.

Cordeiro, L.M.C.; Messias, D.; Sassaki, G.L.; Gorin, P.A.J.; Iacomini, M. Does apossymbiotically cultivated fungus *Ramalina* produce isolichenan? *FEMS Microbiol. Lett.* **2011**, *321*, 50–57.

McAvoy, K.; Igartuburu, J.M.; Romgni-Colvin, J. Polysaccharide composition of *Ramalina terebrata*. *Abstr. Pap. Am. Chem. Soc.* **2012**, *1*, 25–29.

Komiya, T.; Shibata, S. Polyols produced by the cultured phyco- and mycobionts of some *Ramalina* species. *Phytochemistry* **1971**, *10*, 695–699.

Shukla, V.; Negi, S.; Rawat, M.S.M.; Pant, G.; Nagatsu, A. Chemical study of *Ramalina africana* (Ramalinaceae) from the Garhwal Himalayas. *Biochem. Syst. Ecol.* **2004**, *32*, 449–453.

Islas, N.V.; Zambrano, A.; Rojas, J.L. Ozone Reactivity and Free Radical Scavenging Behavior of Phenolic Secondary Metabolites in Lichens Exposed to Chronic Oxidant Air Pollution from Mexico City. *J. Chem. Ecol*. **2007**, *33*, 1619–1634.

Asahina, Y.; Kusaka, T. Lichen substances. LXXXIII. A new depside, boninic acid; synthesis of boninic acid and of homosekikaic acid. *Ber. Dtsch. Chem. Ges. B* **1937**, *70B*, 1815–1821.

Asahina, Y.; Shibata, S. *Chemistry of Lichen Substances, A*; Asher & Co. Ltd: Vaals–Amsterdam, The Netherlands, 1971.

Huneck, S.; Troted, G. Lichen constituents. XXXIV. Structure and composition of *Ramalina boulhautiana*. *Z. Naturforsch. B* **1966**, *21*, 904–904.

Aptroot, A.; Bungartz, F. The lichen genus *Ramalina* on the Galapagos. *Lichenologist* **2007**, *39*, 519–542.

Kashiwadani, H.; Kalb, K. The genus *Ramalina* in Brazil. *Lichenologist* **1993**, *25*, 1–31.

Rundel, P.W. Evolution relationships in the *Ramalina usnea* complex. *Lichenologist* **1978**, *10*, 141–156.

Culberson, W.L. Analysis of chemical and morphological variation in the *Ramalina siliquosa* species complex. *Brittonia* **1967**, *19*, 333–352.

Marshak, A.; Barry, G.T.; Craig, L.C. Antibiotic compound isolated from the lichen *Ramalina reticulate*. *Science* **1947**, *24*, 394–395.

Huneck, S.; Follmann, G. Lichen constituents. XXXVI. Chemistry of Chilean lichens. 15. Constituents of *Ramalina cactacearum*, var. ambigua, and *Medusulina chilena*. *Z. Naturforsch. B* **1967**, *22*, 110–111.

Asahina, Y.; Fukuziro, F. Lichen substances.III. Constitution of obtusatic acid. *Ber. Dtsch. Chem. Ges*. B **1932**, *65B*, 580–583.

Wade, A.E. The Genus *Ramalina* in the British Isles. *Lichenologist* **1961**, *5*, 226–241.

Cansaran, D.; Atakol, O.; Halici, M.G.; Aksoy, A. HPLC analysis of usnic acid in some *Ramalina* species from Anatolia and investigation of their antimicrobial activities. *Pharm. Biol.* **2007**, *45*, 77–81.

Popiolek, Z. Content of lichen substances in some species of *Ramalina*. *Ann. Univ. Mariae Curie–Sklodowska, Sect. C* **1967**, *22*, 165–168.

Fazio, A.T.; Adler, M.T.; Bertoni, M.D.; Sepúlveda, C.S.; Damonte, E.B.; Maier, M.S. Lichen secondary metabolites from the cultured lichen mycobionts of *Teloschistes chrysophthalmus* and *Ramalina celastri* and their antiviral activities. *Z. Naturforsch*. C **2007**, *62*, 543–549.

Ramesh, P.; Baig, E.S.A. Chemical investigation of south Indian lichens: *Ramalina* species. *Indian Drugs* **1994**, *31*, 445–446.

Fazio, A.T.; Adler, M.; Maier, M.S. Usnic acid and triacylglycerides production by the cultured lichen mycobiont of *Ramalina celastri*. *Nat. Prod. Commun.* **2014**, *9*, 213–214.

Bendz, G.; Santesson, J.; Wachtmeister, C.A. Studies on the chemistry of lichens. *Acta Chem. Scand.* **1965**, *19*, 1185–1187.

Hanus, L.O.; Temina, M.; Dembitsky, V. Biodiversity of the chemical constituents in the epiphytic lichenized ascomycete *Ramalina* lacera grown on difference substrates *Crataegus sinaicus*, *Pinus halepensis*, and *Quercus calliprinos*. *Biomed. Pap.* **2008**, *152*, 203–208.

Huneck, S.; Troted, G. Lichen constituents. XX. Chemistry of Chilean lichens. 8. Componets of *Ramalina chilensis*. *Z. Naturforsch. B* **1966**, *21*, 90–91.

Praven Kumar, S.V.; Kekuda, T.R.P.; Vinayaka, K.S.; Sudharshan, S.J. Anthelmintic and antioxidant efficacy of two macrolichens of Ramalinaceae. *Pharmacogn. J.* **2009**, *1*, 238–242.

Komiya, T.; Shibata, S. Formation of lichen substances by mycobionts of lichens. Isolation of (+)-usnic acid and salazinic acid from mybobionts of *Ramalina* sp. *Chem. Pharm. Bull.* **1969**, *17*, 1305–1306.

Huneck, S.; Yoshimura, I. *Identification of Lichen Substances*; Springer-Verlag: Berlin, Germany, 1996.

Bézivin, C.; Tomasi, S.; Lohézic-Le, F.; Boustie, J. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine* **2003**, *10*, 499–503.

Ryan, H.; O'Riordan, W.M. Tinctorial constituents of some lichens, which are used as dyes in Irland. *P. Roy. Irish Acad. B* **1917**, *33*, 91–104.

Salkowski, H. Circular polarization of usninic acids and other lichen substances. *Liebigs Ann. Chem.* **1911**, *377*, 123–126.

Parrot, D.; Jan, S.; Baert, N.; Guyot, S.; Tomasi, S. Comparative metabolite profiling and chemical study of *Ramalina siliquosa* complex using LC-ESI-MS/MS approach. *Phytochemistry* **2013**, *89*, 114–124.

Nakao, M. Chemical constituents of a Chinese drug "shi-hoa" and their constitutions. *Yakuga. Zasshi* **1923**, *496*, 423–497.

Reyes, Q.A.; Vicuna, L.P.; Almonacid, V.J. Usnic acid and atranorin in some regional lichens. *Rev. Latinoam. Quim.* **1981**, *12*, 130–131.

Molho, L.; Bodo, B.; Molho, D. L'acide *O*-méthyl-4' norsékikaïque, nouveau metadepside isole d'um lichen du genre *Ramalina*. *Phytochemistry* **1979**, *18*, 2049–2051.

Tay, T.; Türk, A.Ö.; Yilmaz, M.; Türk, H.; Kivanç, M. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstitic acid, and protocetraric acid constituents. *Z. Naturforsch. C* **2004**, *59*, 384–388.

Esimone, C.O.; Adikwu, M.U. Antimicrobial activity and cytotoxicity of *Ramalina farinacea*. *Fitoterapia* **1999**, *70*, 428–431.

Rao, P.S.; Shripathy, V. Chemical constituents of *Umbilicaria indica* Frey and *Ramalina farinacea* L. Ach. *Curr. Sci.* **1976**, *45*, 517–518.

Hawksworth, D.L. Typification of lichen farinaceous. *Bryologist* **1969**, *72*, 254–255.

Zopf, W. To the knowledge of the lichen material. *Liebigs Ann.* **1897**, *297*, 271–312.

Rangaswami, S.; Rao, V.S. Chemical components of *Ramalina farinacea*. *Indian J. Pharm.* **1954**, *16*, 197–199.

Hesse, O. Contribution for the knowledge of the lichens and their characteristic components. *J. Prakt. Chem.* **1903**, *68*, 1–71.

Koparal, A.T.; Tuylu, B.A.; Turk, H. In vitro cytotoxic activities of (+)-usnic acid and (−)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. *Nat. Prod. Res.* **2006**, *20*, 1300–1307.

Zopf, W. To the knowledge of the lichen substances. *Liebigs Ann. Chem.* **1905**, *340*, 276–309.

Briner, G.P.; Gream, G.E.; Riggs, N.V. Chemistry of Australian lichens. I. Some constituents of *Parmelia conspersa*, *Ramalina fraxinea*, *Usnea barbata*, and *Usnea ceratina* from the New England region. *Aust. J. Chem.* **1960**, *13*, 277–284.

Yamamoto, Y.; Mizuguchi, R.; Yamada, Y. Tissue cultures of *Usnea rubescens* and *Ramalina yasudae* and production of usnic acid in their cultures. *Agr. Biol. Chem.* **1985**, *49*, 3347–3348.

Asahina, Y.; Nonomura, S. Lichen substances. XVI. Constituents of *Ramalina* species with special reference to sekikaic acid. *Ber. Dtsch. Chem. Ges. B* **1933**, *66B*, 30–35.

Dias, D.A.; Urban, S. Phytochemical investigation of the Australian lichens *Ramalina glaucescens* and *Xanthoria parietina*. *Nat. Prod. Commun.* **2009**, *4*, 959–964.

Gonzalez, A.G.; Barrera, J.B.; Rodriguez, E.M.P.; Padron, C.E.H. Chemical constituents of the lichen *Ramalina hierrensis*. *Planta Med.* **1992**, *58*, 214–218.

Zopf, W. A contribution to the knowledge of the lichen constituents. *Liebigs Ann. Chem.* **1907**, *352*, 1–44.

Hauck, M.; Willenbruch, K.; Leuschner, C. Lichen substances prevent lichens from nutrient deficiency. *J. Chem. Ecol.* **2009**, *35*, 71–73.

Verma, N.; Behera, B.C.; Joshi, A. Studies on nutritional requirement for the culture of lichen *Ramalina nervulosa* and *Ramalina pacifica* to enhance the production of antioxidant metabolites. *Folia Microbiol.* **2012**, *57*, 107–114.

Culberson C.F. Chemical constituents of *Ramalina paludosa*. *Bryologist* **1967**, *70*, 397–405.

Urdapilleta, A.A.A. Atividade de Substâncias Isoladas de Líquens Sobre Formas Promastigotas de *Leishmania* (L.) *amazonensis* e *Leishmania* (V.) *braziliensis*. Master's Thesis, Universidade de Brasília, Brasilia-DF, 3 March **2006**.

Morozova, O.V.; Dargaeva, T.D.; Brutko, L.I. Components of *Ramalina pollinaria*. *Khim. Prirod. Soed.* **1984**, *20*, 252-253.

Koller, G. Ramalic acid. *Monatsh. Chem.* **1932**, *61*, 286–292.

Kirk, P.M.; Cannon, P.F.; Minter, D.W.; Stalpers, J.A. *Dictionary of the Fungi*; CABI - Europe-UK, Cromwell Press, Trowbridge, 2008.

Marshak, A.G. Extracting Usnic Acid. US 2557987 A, 26 June 1951.

Sisodia, R.; Geol, M.; Verma, S.; Rani, A.; Dureja, P. Antibacterial and antioxidante activity of lichen species *Ramalina roesleri*. *Nat. Prod. Res.* **2013**, *27*, 2235–2239.

Curd, F.H.; Robertson, A. Lichen acids. Part VI. Constituensts of *Ramalina scopulorum*. *J. Chem. Soc.* **1935**, *329*, 1379–1381.

Tugunay, T.; Reyim, M.; Adilijiang, A.A. Content determination and antioxidant activity evaluation of usnic acid in *Ramalina sinensis* Jatta. *Shipin Kexue* **2010**, *31*, 150–152.

Culberson, C.F. Stenosporic acid, a new depside in *Ramalina stenospora*. *Phytochemistry* **1970**, *9*, 841–844.

Kumar, K.C.S.; Banskota, A.H.; Manandhar, M.D. Chemical constituents and antibacterial activity of *Ramalina subcomplanata*. *J. Nepal Chem. Soc.* **1995**, *14*, 28–35.

Yim, J.H.; Lee, H.K.; Bhattarai, H.D.; Babita, P.; Kim, I.C.; Hong, S.G.; Kim, D.K.; Lee, Y.K.; Gu, S.; Oh, H.C. Antioxidant Activities of Ramalin Isolated from *Ramalina terebrata* Extracts. WO 2010053327 A2, 14 May 2010.

Huneck, S.; Follmann, G. The presence of usnic acid in *Lecanora melanophthalma* and *Ramalina terebrata*. *Naturwissenschaften* **1964**, *51*, 291–292.

Paudel, B.; Bhattarai, H.D.; Lee, H.K.; Oh, H.; Shin, H.W.; Han, J.; Yim, J.H. Antibacterial activities of ramalin, usnic acid and its tree derivatives isolated from the Antarctic lichen *Ramalina terebrata. Z. fuer Naturforsch. C* **2010**, *65*, 34–38.

Gonzalez, A.G.; Martin, J.D.; Martin, M.M. Chemical components of lichens. II. *Ramalina tingitana*. *Anal. Quim.* **1973**, *69*, 807–809.

Imshaug, H.A. Typification of *Ramalina usnea* (L.) R. H. Howe. *Lichenologist* **1972**, *5*, 317–318.

Lee, S.G.; Koh, H.Y.; Oh, H.; Han, S.J.; Kim, I.C.; Lee, H.K.; Yim, J.H. Human dermal fibroblast proliferation activity of usimine-C from Antarctic lichen *Ramalina terebrata*. *Biotechnol. Lett.* **2010**, *32*, 471–475.

Yim, J.H.; Kim, I.C.; Lee, S.G.; Kim, D.K.; Han, S.J.; Lee, H.S.; Seok, K.H.; Kim, S.J.; Kim, T.K.; Kang, P.S.; *et al.* Pharmaceutical Composition for the Treatment of Inflammatory Disease or Immune Disease Containing Ramalin. WO 2011152671 A2, 8 December 2011.

Luo, H.; Wei, X.; Yamamoto, Y.; Liu, Y.; Wang, L.; Jung, J.S.; Koh, Y.J.; Hur, J.-S. Antioxidant activities of edible lichen *Ramalina conduplicans* and its free radical scavenging constituents. *Mycoscience* **2010**, *51*, 391–395.

Lai, D.; Odimewu, D.; Esimone, C.; Grunwald, T.; Proksch, P. Phenolic compounds with *in vitro* activity against respiratory syncytial from the Nigerian lichen *Ramalina farinacea*. *Planta Med.* **2013**, *79*, 1440–1446.

Vinayaka, K.S.; Kekuda, T.R.P.; Swathi, D.; Praveen Kumar, S.V. Studies on chemical composition and *in vitro* antibacterial activity of solvent extracts of the lichen *Ramalina hossei* Vain. *BioTechnol. Indian J.* **2009**, *3*, 309–311.

Molho, D.; Bodo, B.; Culberson, W.L. A chemically distinctive new *Ramalina* from Fiji. *Bryologist* **1981**, *84*, 396–398.

Hamada, N. Individual variation in the contents of lichen substances in *Ramalina nervulosa* and *Ramalina subcomplanata*. *Bryologist* **1988**, *91*, 95–97.

Cordeiro, L.M.C.; Iacomini, M.; Stocker-Wörgötter, E. Culture studies and secondary compounds of six *Ramalina* species. *Mycol. Res.* **2004**, *108*, 489–497.

Hamada, N. The effect of temperature on lichen substances in *Ramalina subbreviuscula* (Lichens). *Bot. Mag.* **1983**, *96*, 121–126.

Asahina, Y.; Kusaka, T. Lichen substances. LXV. A new depside, ramalinolic acid. *Ber. Dtsch. Chem. Ges*. B **1936**, *69B*, 450–455.

Huneck, S. Lichen constituents. LXXXIX. Secondary substances of some lichens. *Phytochemistry* **1972**, *11*, 1493–1495.

Bendz, G.; Santesson, J.; Wachtmeister, C.A. Chemistry of lichens. XXI. Isolation and synthesis of methyl 3,5-dichlorolecanorate, a new depside from *Ramalina* spp. *Acta Chem. Scand.* **1965**, *19*, 1188–1190.

Huneck, S. Lichen constituents. XXXIV. Structure of tumidulin, a new chlorine containing depside. *Chem. Ber.* **1966**, *99*, 1106–1110.

Ingólfsdóttir, K. Usnic acid. *Phytochemistry* **2002**, *61*, 729–736.

Chester, D.O.; Elix, J.A. The identification of four new meta-depsides in the lichen *Ramalina asahinae*. *Aust. J. Chem.* **1978**, *31*, 2745–2749.

Hamada, N.; Ueno, T. Depside from an isolated lichen mycobiont. *Agr. Biol. Chem.* **1987**, *51*, 1705–1706.

Culberson, C.F.; LaGreca, S.; Johnson, A.; Culberson, W.L. Trivaric acid, a new tridepside in the *Ramalina americana* chemotype complex (lichenized Ascomycota: Ramalinaceae). *Bryologist* **2000**, *102*, 595–601.

Elix, J.A.; Peculiarities of the Australiasian lichen flora: Accessory metabolites, chemical and hybrid strains. *J. Hattori Bot. Lab.* **1982**, *52*, 407–415.

Culberson, C.F.; Culberson, W.L.; Johnson, A. A novel meta-depside from the *Ramalina subfraxinea* complex (Ascomycotina, Ramalinaceae). *Bryologist* **1990**, *93* 193–196.

Bodo, B.; Hebrard, P.; Molho; L.; Molho, D. Desmaziera evernioides and *Ramalina bourgeana*. *Tetraedron Lett.* **1973**, *18*, 1631–1634.

Bodo, B. Bourgeanic acid: A new metabolite of lichens. Structure, synthesis and biosynthesis. *Bull. Mus. Natl. Hist. Nat., Sci. Phys.-Chim.* **1975**, *3*, 23–97.

Stocker-Worgotter E.; Elix, J.A.; Grube, M. Secondary chemistry of lichen forming fungi: Chemosyndromic variation and DNA analyses of cultures and chemotypes in the *Ramalina farinacea*. *Bryologist* **2004**, *107*, 152–162.

Hawksworth, D.L. Chemical strains of the lichen *Ramalina subfariancacea*. *Bot. Notiser* **1968**, *121*, 317–320.

Culberson, C.F. Microchemical tests for the lichen depsidone hypoprotocetraric acid. *Bryologist* **1965**, *68*, 301–304.

Stuelp-Campelo, P.Ma.; de Oliveira, M.B.M.; Leão, A.M.A.C.; Carbonero, E.R.; Gorin, P.A.J.; Iacomini, M. Effect of a soluble α-D-glucan from the lichenized fungus *Ramalina celastri* on macrophage activity. *Int. Immunopharmacol.* **2002**, *2*, 691–698.

Huneck, S.; Lehn, J.M. Lichen constituents. XXVII. Identity between coquimboic acid and hypoprotocetraric acid. *Z. Naturforsch.B* **1966**, *21*, 299–299.

Yamamoto Y.; Watanabe, A. Fatty acid composition of lichens and their phyco and mycobionts. *J. Gen. Appl. Microbiol.* **1974**, *20*, 83–86.

Hirayama, T.; Fujikawa, F.; Kasahara, T.; Otsuka, M.; Nishida, N.; Mizuno, D. Anti-tumor activities of some lichen products and their degradation products. *Yakugaku Zasshi* **1980**, *100*, 755–759.

Czeczuga, B.; de Corona, L.F. Carotenoids in lichens from Argentina. *Biochem. Syst. Ecol.* **1987**, *15*, 519–522.

Dembitsky, V.M. Lipids of lichens. *Prog. Lipid Res.* **1992**, *31*, 373–397.

Lehn, J.M.; Huneck, S. Lichen constituents. XVIII. First separation of the diterpene (−)-16-α-hydroxykaurane from a lichen. *Z. Naturforsch. B* **1965**, *20*, 1013-1013.

Huneck, S.; Follmann, G. Lichen constituents. XXVIII. Chemistry of Chilean lichen. Compounds from *Ramalina tigrina*, and *Ramalina inanis*. *Z. Naturforsch. B* **1966**, *21*, 713–714.

Sassaki, G.L.; Cruz, L.M.; Gorin, P.A.J.; Iacomini, M. Fatty acid composition of lipids present in selected lichenized fungi: A chemotyping study. *Lipids* **2001**, *36*, 167–174.

Machado, M.J.; Guerrini, M.; Gorin, P.A.J.; Torri, G.; Iacomoni, M. A galactosphingolipid from the lichen, *Ramalina celastri*. *Phytochemistry* **1997**, *45*, 651–653.

Cetin, H.; Tufan-Cetin, O.; Turk, A.O.; Tay, T.; Candan, M.; Yanikoglu, A.; Sumbul, H. Insecticidal activity of major lichen compounds, (−)- and (+)-usnic acid, against the larvae of house mosquito, *Culex pipiens* L. *Parasitol. Res.* **2008**, *102*, 1277–1279.

Unal, D.; Senkardesler, A. Abscisic acid and polyamine contents in the lichens *Pseudevernia furfuracea* and *Ramalina farinacea*. A. Sukatar, *Russ. J. Plant Physiol*. **2008**, *55*, 115–118.

Mateos, J.L.; Legaz, M.E. Presence and Identification of Polyamines and their Conjugation to Phenolics in Some Epiphytic Lichens. *Eukaryotism and Symbiosis*; Springer-Verlag: Berlin, Germany, 1997; pp. 491–498.

Solberg, Y.J. Studies on the chemistry of lichens. IX Quantitative determination of monosaccharides and amino acids in hydrolysates of several Norwegian lichen species. *Lichenologist* **1970**, *4*, 283–288.

Ramakrishnan, S.; Subrmanian, S.S. Amino acids of Lobaria subisidiosa, Umbilicaria pustulata, Parmelia nepalensis, and Ramalina sinensis. *Curr. Sci. Indian* **1966**, 35, 124–125.

Fujikawa, F.; Hirai, K.; Hirayama, T.; Toyota, T.; Urasaki, M.; Takagawa, M.; Fukuda, M.; Moritani, K.; Sanae, T. Free amino acids in lichens of Japan. *Yakugaku Zasshi* **1972**, *92*, 823–827.

Paudel, B.; Bhattarai, H.D.; Koh, H.Y.; Lee, S.G.; Han, S.J.; Lee, H.K.; Oh, H.; Shin, H.W.; Yim, J.H. Ramalin, a novel nontoxic antioxidant compound from the Antarctic lichen *Ramalina terebrata*. *Phytomedicine* **2011**, *18*, 1285–1290.

Yim, J.H.; Kim, I.C.; Han, S.J.; Lee, H.S.; Battarai, H.D.; Yang, S-G.; Lee, D.H. Ramalin for Preveting or Treating Hepatic Fibrosis and Liver Cirrhosis. WO 2013129714 A1, 6 September 2013.

Yim, J.H.; Kim, I.C.; Lee, S.G.; Kim, D.G.; Han, S.J.; Lee, H.S.; Kim, S.J.; Kim, T.G.; Kang, P.S.; Park, H.Y. Ramalin for Preveting and Treating Inflammatory Diseases and Immune Diseases. KR 1290745 B1, 29 September 2013.

Seo, C.; Yim, J.H.; Lee, H.K.; Park, S.M.; Sohn, J.H.; Oh, H. Stereocalpin A, a bioactive cyclic depsipeptide from the Antarctic lichen *Stereocaulon alpinum*. *Tetrahedron Lett.* **2008**, *49*, 29–31.

Byeon, H.E.; Park, B.K.; Yim, J.H.; Lee, H.K.; Moon, E.Y.; Rhee, D.K.; Pyo, S. Stereocalpin A inhibits the expression of adhesion molecules in activated vascular smooth muscle cells. *Int. Immunopharmacol*. **2012**, *12*, 315–325.

Yim, J.H.; Kim, I.C.; Kim, D.G.; Han, S.J.; Lee, H.S.; Oh, H.C.; Pyo, S.N. Stereoclapin-A for preveting or treating arteriosclerosis. KR 20130043995 A, 02 May 2013.

Pfau, A.S. Constituents of lichens. II. Constitution of barbatinic acid. *Helv. Chim Acta* **1928**, *11*, 864–876.

Huang, W.; Dai, W. The constituents of absolute from *Ramalina fastigiata* (Pors) Ach. The main ethyl ester of fatty acids. *Nat. Prod. Res. Dev.* **1989**, *1*, 27–28.

Larvicidal activity of *Ramalina usnea* lichenized fungi against *Aedes aegypti*

Antônio Sérgio Nascimento Moreira • Roberta Oliveira Salvador Fernandes • Francisco José Alves Lemos • Raimundo Braz-Filho • Ivo José Curcino Vieira

Abstract

The larvicidal activity of the methanol extract, fractions and from compounds 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) and (+)- usnic acid (**2**) identified from the *Ramalina usnea* lichen was tested against the third instar larvae of the *Aedes aegypti* mosquito. The mortality was observed after 24 hour of larvae exposure. The methanol extract and three fractions showed considerable activity, killing 100% of the larvae at a concentration of 150 µg/mL. The isolated compounds, 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) and the (+)- usnic acid (**2**) showed high larvicidal activity, presenting LC_{50} values of 4.850 and 4.475 µg/mL, respectively. This is the first study of this kind of lichen reporting the larvicidal activity against the *Aedes aegypti* mosquito with compound **(1)**.

1. Introduction

Mosquitoes are vectors that transmit many diseases, such as malaria, encephalitis, dengue fever among others. Dengue fever is a neglected tropical disease, currently ranked as the most important viral disease transmitted by mosquitoes worldwide (WHO 2012). The mosquito that transmits dengue, the Aedes aegypti, is also responsible for the transmission of other diseases, such as yellow fever, chikungunya fever (WHO 2015) and the fever caused by zika virus (BRASIL- MS 2015).

The chikungunya fever is caused by a virus of the *Alphavirus* genus, transmitted by mosquitoes of the *Aedes* genus, being the *Aedes aegypti* and *Aedes albopictus* the main vectors. Since 2004, the virus has been identified in 60 countries, and in 2013 it was recorded autochthonous transmission in several Caribbean countries and in March 2014 appeared in the Dominican Republic and Haiti, because until then, its circulation was limited to Africa and

Asia. However, there were already outbreaks also in Europe and in regions of the Indian and Pacific Oceans (WHO 2015).

Brazil is currently living a dengue epidemic in several states, according to the Epidemic Update of Health Surveillance Secretariat of Ministry of Health, which tracks cases of dengue and chikungunya fever, until the 18th of last April were registered 745,957 reported cases of dengue in the country with about 230 deaths, while for chikungunya fever were reported 3,135 autochthonous suspected cases, from these, 1,688 were confirmed (portal.saude.gov.br /imagens/pdf/2015/maio/04/2015). Already the Pan American Health Organization report presenting the situation in the Americas for chikungunya fever shows a total of 1,379,788 suspected cases, with 31,024 confirmed cases and a total of 191 deaths until the present moment (PAHO/WHO 2015).

As there is no specific antiviral drug for the treatment of these diseases, a strategy used to control the transmission is by the transmission of vectors (WHO 2015; Polson et al. 2011). For this, many insecticides may be used to control mosquitoes, but many of them are not selective and can harm beneficial insects (Cetin et al. 2008; Cetin et al. 2012; Góis et al. 2013), increase the resistance of these insects (Benli et al. 2009; Polson et al. 2011) and produce environmental contamination (Geris et al. 2008; Santos et al. 2011; Góis et al. 2013). In the search for alternative control methods less or non-toxic to the population and the environment, several studies have searched plants as alternative control agents (Viegas Júnior, 2003; Simas et al. 2004; Mendonça et al. 2005; Santiago et al. 2005; Trevisan et al. 2006; Anees 2008; Rahuman; Venkatesan 2008; Rahuman et al. 2008; Geris et al. 2008; Feitosa et al. 2009; Sreelatha et al. 2010; Murugan et al. 2012; Góis et al. 2013; Sharma et al. 2014; Govindarajan; Sivakumar 2014) among many others, or other natural sources such as lichens (Cetin et al. 2008; Cetin et al. 2012; Yildirim et al. 2012) and marine natural products (Samiduari; Saravanakumar 2011), as these natural sources have a rich variety of bioactive chemical compounds.

Lichenized fungi (lichens) consist in a form of symbiosis between a fungus and an algae or cyanobacteria which contains a large number of organic compounds, some of them are specific to those organisms (Huneck; Yoshimura 1996; Oksanen 2006; Parrot et al. 2013) and constitute a stable, systematic and independent unit that belongs to the Fungi Kingdom (Filo Ascomycota/Basidiomycota) (Boustin; Grube 2005). Lichens produce a variety of secondary metabolites such as, aliphatic and cycloaliphatic compounds, aromatic compounds, meta and para Depsides, depsidones, dibenzofurans, diphenyl ether, naphthopyrans, xanthones, quinones, naphthoquinones, usnic acid among other classes (Feige; Lumsch 1993; Huneck; Yoshimura 1996; Oksanen 2006).

Lichens have been used as sources of many metabolites with different biological activities (Huneck; Yoshimura 1996; Ingólfsdóttir 2002; Boustin; Grube 2005) such as antimicrobial (Tay et al. 2004; Cansaran et al. 2007), antibiotic (Honda; Vilegas 1998; Ingólfsdóttir 2002; Paudel et al. 2008; Paudel et al. 2010; Paudel et al. 2012), antioxidant (Gulluce et al. 2006; Kumar et al. 2009; Luo et al. 2010; Yim et al. 2010; Halice et al. 2011; Paudel et al. 2012; Sisodia et al. 2013), antiviral (Fazio et al. 2007; Esimone et al. 2009; Lai et al. 2013), antitumor (Hirayama et al. 1980; Stuelp-Campelo et al. 2002; Haraldsdóttir et al. 2004; Koparal et al. 2006; Einarsdóttir et al. 2010; Bačkorová et al. 2011; Bačkorová et al. 2012; Singh et al. 2013; Brandão et al. 2013), anti-inflammatory (Cordeiro et al. 2008; Yim et al. 2011; Byeon et al. 2012), larvicidal (Cetin et al. 2012) among others.

In the present study was evaluated the larvicidal activity of two identified compounds from species *Ramalina usnea*, 2-Hydroxy-4-methoxy-6-propyl-methyl beazoate (**1**) and the (+)- usnic acid (**2**). This study is the first report in the literature with this kind of lichen for larvicidal activity against *Aedes aegypti* with the compound 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate **(1)**.

2. Materials and methods

General Experimental Procedures

For structural determination of compounds, were used spectrometers Bruker operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C on the nuclear magnetic resonance analysis (NMR) in single and two-dimensional experiments (1D and 2D) with chemical shifts (δ_H e δ_C) obtained in parts per million (ppm) and the coupling constants (*J*) in hertz. All spectra were acquired using deuterated chloroform ($CDCl_3$) as solvent, using tetramethylsilane (TMS) as internal standard. Chemical shifts were measured on the scale δ, and they were referred by the non-deuterated solvent for the proton signal at δ_H 7.27 ppm, and the carbon signal at δ_C 75.96 ppm. The infrared spectroscopy analyses were made with equipment Shimadzu IRAffinity-1 using KBr pellets. The gas chromatography analyzes coupled with mass spectrometry were performed using equipment Shimadzu QP-5050 A. For the isolation of the compounds was used Merck silica gel (0.063 – 0.2 mm) in the column chromatography processes and Merck silica gel 60 F_{254} was used in the procedures of preparative chromatography in analytical thin layer. The identification points of the compounds in sheets of thin layer chromatography (TLC) were detected by ultraviolet light at 254 nm and revelation was performed with vanillin sulfuric acid solution.

Lichenized fungus

The lichen *Ramalina usnea* was collected on the Restinga of Iquipari, coastline of Grussaí in the São João da Barra municipality, Rio de Janeiro state. The voucher specimen was analyzed and identified by Dr. Michel Navarro Benatti, herbarium curator of the Botanical Institute of São Paulo and it was deposited in the herbarium of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), in Biosciences and Biotechnology Center, CBB, with identification number LI 0001.

Extract preparation and isolation of the compounds

The lichen stems were dried at room temperature and powdered. About 550 g of the dried material were macerated with methanol in three replications. After filtration, the solvent was removed under reduced pressure and it produced approximately 60 g of crude extract. A sample was taken to perform preliminary biological tests and 35 g were fractionated by column chromatography on ambient pressure using silica gel, and eluted with hexane: dichloromethane with gradient of polarity, obtaining 8 fractions (LM1 to LM8). The fraction LM6 (2.82 g) was chromatographed by methods similar to those described, and eluted with hexane: dichloromethane with polarity gradient, obtaining 6 new fractions (LM6-1 a LM6-6). The fraction LM6-2 (689.6 mg) was chromatographed through a similar process to that of LM6 producing 10 new fractions (LM6-2-1 a LM6-2-10). The fraction LM6-2-3 (89.5 mg) was analyzed by spectroscopic methods and identified as 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) (Figure 1). The fraction LM6-2-7 (149 mg), after analysis by spectroscopic methods has been identified as usnic acid (**2**) (Figure 1).

2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**): dark yellow amorphous solid; pf 122,5-123,8 °C; IR (KBr, cm^{-1}): 3435, 2924, 2852, 1726, 1624, 1462, 1373, 1288, 1192, 1159, 960, 741. $[M^{+\cdot}]$ = *m/z* 224 (calc. for $C_{12}H_{16}O_4$).

Usnic acid **(2)**: yellow crystalline solid; pf 202,5-204 °C; IR (KBr, cm^{-1}): 3662, 3088, 1695, 1631, 1560, 1452, 1373, 1357, 1286, 1190, 1141, 1118, 1066, 1037, 956, 841, 817. $[M^{+\cdot}]$ = *m/z* 344 (calc. for $C_{18}H_{16}O_7$, 344.089)

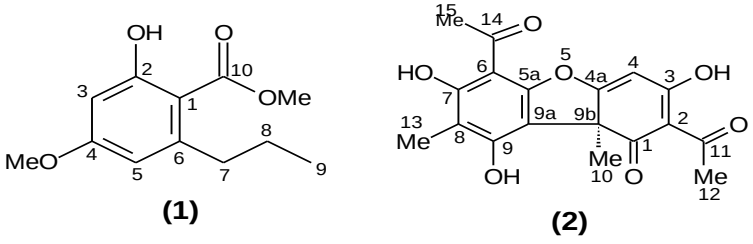


Figure 1. Compounds identified fungus lichenized *Ramalina usnea*

The chemical shifts values from nuclear magnetic resonance analysis of ^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) of the two compounds shown in Tables 3 and 4 confirm those found in the literature (Huneck; Yoshimura, 1996; König; Wrigth 1999; Rashid et al. 1999; Bézivin et al. 2004; Shmeda-Hirschmann et al. 2008).

Larvicidal bioassay

The mosquitoes *A. aegypti* of the Rockefeller are kept in the insectary of Biotechnology Laboratory from the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF).

The larvicidal toxicity assays were developed according to standard methodology of World Health Organization (WHO, 2005) with adaptation. The concentration of the stock solution and the solutions tested was chosen based on preliminary tests. The compounds were solubilized in DMSO/H_2O or DMSO pure for usnic acid (**2**). Fifteen larvae of third instar were added to the pots containing distilled water and added to the test solutions with concentrations that were between 1 and 30 µg/mL, in the total of five concentrations, at room temperature, 27°C. The tests were performed in triplicate and in two replicates. The negative control was pure water, pure DMSO and a DMSO/H_2O (2.5%) solution. For positive control was used Imidacloprid compound, with concentrations between 0.01 µg/mL and 1.0 µg/mL. Assessment of mortality was made 24 hours after exposure of the larvae to the solutions.

3. Statistical analysis

Data were analyzed using the analysis program Probit/EPA version 1.5, to calculate the lethal concentration 50% (LC_{50}) and 90% (LC_{90}) and the confidence limit for each treatment in the confidence level of 95% (http//www.epa.gov/nerleerd/stat2.htm).

4. Results

Bioassays

In preliminary bioassays performed with crude methanol extract and fractions, were used a solution with a concentration of 150 µg/mL, in triplicate. The crude extract (LM) showed 100% of mortality in the larvae of the third instar of *A. aegypti* after 24 h, the fraction LM6 57%, the LM7 96.6%, and 100% for all fractions LM6-1 (97.8 mg) and LM6-2 (689.6 mg) in the same concentration, the other fractions of this column had low mortality rates. However, the results of the fractions LM6-2-3 (**1**) and LM6-2-7 (**2**) were expressive (Table 1). The analysis of the percentage mortality of the larvae to the fractions matching of the compounds (**1**) and (**2**) through dose curve *versus* response allowed the

determination of the LC_{50} and LC_{90} values, shown in Table 2, together with those of controls in µg/mL.

Table 1. Larval mortality (in percent) of the methanol extract of *Ramalina usnea* lichen, fractions and control against third- instar of *Aedes aegypti* mosquito larvae after 24 hours.

Concentration Sample	150 (µg/mL)	30 (µg/mL)	15 (µg/mL)	10 (µg/mL)	5 (µg/mL)	1 (µg/mL)
Extract MeOH	100	-	-	-	-	-
LM6	57	-	-	-	-	-
LM7	96.6	-	-	-	-	-
LM6-1	100	-	-	-	-	-
LM6-2	100	-	-	-	-	-
LM6-3	0	-	-	-	-	
LM6-4	0	-	-	-	-	-
LM6-5	10	-	-	-	-	-
LM6-2-3 **(1)**	-	100	88.67	78	35.33	11.33
LM6-2-7 **(2)**	-	100	93.33	66.67	33.33	20
DMSO	0	0	0	0	0	0
DMSO/H_2O	0	0	0	0	0	0

- not tested concentration

Table 2. The LC_{50} and LC_{90} values (µg/mL) compounds 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate **(1)**, usnic acid **(2)** and control Imidaclopride against third-instar of *Aedes aegypti* mosquito larvae after 24 hours.

Compound	LC_{50} (limits)	LC_{90} (limits)	Slope (±SE)(limits)
(1)	4.850 (2.929-6.955)	17.485 (11.689-36.333)	2.301 (0.461) (1.397-3.205)
(2)	4.475 (2.508-6.731)	20.735 (12.846-51.468)	1.925 (0.398) (1.145-2.704)
Control	0,0412 (0.0199-0.062)	0.0947 (0.0639-0.2609)	0.0363 (0.0113) (0.0141 - 0.0585)

The compound 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) was evaluated for larvicidal activity against third instar of the *Aedes aegypti* mosquito larvae and were found the values of 11.3, 35.3, 78.0, 88.7 and 100% of mortality, while for usnic acid (**2**) were obtained 20.0, 33.3, 66.7, 93.3 and 100 %, at concentrations of 1.0, 5.0, 10.0, 15.0 and 30.0 µg/mL, respectively, after 24 hours (**Table 1**). The data from the compounds (**1**) and (**2**), as well as

the positive control, the Imidacloprid insecticide, were processed in the analysis program EPA-Probit to obtain the lethal concentrations 50% (LC_{50}) and 90% (LC_{90}). The values for LC_{50} were 4.850, 4.475 and 0.0412 µg/mL and for the LC_{90} were 17.485, 20.735 and 0.0947 µg/mL for (**1**), (**2**) and Imidacloprid, respectively (**Table 2**).

Identification of compounds **1** and **2**.

The IR spectrum of compound (**1**) revealed a broadband in ν_{max} = 3435 cm^{-1} typical of grouping OH phenolic, in ν_{max} = 2924 an acute intense broadband characteristic of axial stretching C–H from aromatic, a broadband in ν_{max} = 1726 typical of axial deformation from carbonyl group C=O and in ν_{max} = 1624 a broadband characteristic of axial deformation from linkages C–C of the aromatic ring. The NMR spectrum ^{13}C-DEPTQ showed signals to 12 carbon atoms, being one of carbonyl (C-10; δ_C 171.9), two methinic sp^2 (CH-3 and CH-5, δ_C 98.7 and 110.7), two methoxyl groups (MeO-4 and MeO-10, δ_C 55.2 and 51.9), two methylene sp^3 and one methyl group relating to a n-propyl group (CH_2-7, CH_2-8 and CH_3-9, δ_C 38.8, 24.9 and 14.2) and four non-hydrogenated sp^2 (C-1, C-2, C-4 and C-6, δ_C 104.6, 163.8, 165.5, 147.7, respectively). The spectrum of ^{1}H NMR showed signals of two singlets for δ_H 6.34 and 6.29 suggesting the presence of tetra-substituted aromatic ring. The signals at 3.80 (s) and 3.92 (s) suggest the presence of two methoxyl groups in the molecule. The signals relating to the 2D magnetic resonance spectrum, HSQC and HMBC (Table 3) corroborate to the structure of compound (**1**).

Table 3. ^{1}H-^{13}C NMR ($^nJ_{CH}$, n=1, 2 and 3) spectroscopic data of compound 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) in $CDCl_3$. Chemical shifts in δ (ppm) and *J* (Hertz).

	LM6-2-3: 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate			
	HSQC		HMBC	
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
1	104.6	-		HO-3; H-4; H-6; 2H-7
2	163.8	-	HO-3; H-6	
4	165.5	-	H-4; H-6	MeO-5
6	147.7	-	2H-7	2H-8
10	171.9	-		MeO-10
CH				
3	98.7	6.34 (s)		HO-3; H-6
5	110.7	6.29 (s)		H-4; 2H-7
CH_2				
7	38.8	2.82 (t, 7.0)	2H-8	H-6; 3H-9
8	24.9	1.54 (m)	2H-7; 3H-9	
CH_3				
9	14.2	0.95 (t, 7.0)	2H-8	2H-7
MeO-4	55.2	3.80 (s)		
MeO-10	51.8	3.92 (s)		
HO-2	-	11.76 (s)		

The IR spectrum of usnic acid (**2**) revealed broadband at ν_{max} = 3663 cm^{-1} to an -OH group, to ν_{max} = 3088 cm^{-1} for aromatic C-H stretch, ν_{max} = 1695 cm^{-1} carbonyl group characteristically, C=O, and an intense broadband at ν_{max} = 1631 cm^{-1} to a double bond stretch suggesting the presence of an aromatic ring. The analysis of NMR spectrum ^{13}C-DEPTQ of the compound showed the presence of signals for 18 carbon atoms, corresponding to four methyl groups (CH_3-10, CH_3-12, CH_3-13 and CH_3-15, δ_C 31.9, 27.9, 7.9 and 29.4, respectively), one methinic carbon (CH-4, δ_C 98.4) and thirteen carbon un-hydrogenated, all sp^2. The signal δ_C 7,89 suggests the presence of the methyl group attached to an aromatic carbon. The spectrum ^{1}H-RMN confirmed the presence of the four methyl groups (δ_H 1.79, 2.08, 2.68 and 2.71) all singlets. The spectrum data of RMN ^{1}H, ^{13}C-DEPTQ, HSQC and HMBC are shown in Table 4. The data from Tables 3 and 4 are in agreement with previously published data and confirm the proposals for compounds (**1**) (Huneck; Yoshimura 1996; Shmeda-Hirschmann et al. 2008) and (**2**) (Huneck; Yoshimura 1996; Rashid et al. 1999; Bézivin et al. 2004; König; Wrigt 1999). The NMR, IR spectra and chromatograms of compounds 1 and 2 are shown in the supplementary material.

Table 4. ^{1}H-^{13}C NMR ($^nJ_{CH}$, n=1, 2, and 3) spectroscopic data of compound usnic acid (**2**) in $CDCl_3$. Chemical shifts in δ (ppm) and *J* (Hertz)

LM6-2-7: Usnic acid				
	HSQC		HMBC	
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
1	198.1	-		3H-10
2	105.2	-		H-4; 3H-12
3	191.8	-	H-4	
4a	179.3	-	H-4	3H-10
5a	155.2	-		
6	101.5	-		HO-7; 3H-15
7	163.9	-	HO-7	3H-13
8	109.3	-	3H-13	HO-7; HO-9
9	157.5	-	HO-9	3H-13
9a	104.0	-		HO-9; 3H-10
9b	59.1	-	3H-10	H-4
11	201.8	-	3H-12	
14	200.4	-	3H-15	
CH				
4	98.4	6.05 (s)		
CH_3				
10	31.9	1.79 (s)		
12	27.9	2.68 (s)		
13	7.9	2.08 (s)		
15	29.4	2.71 (s)		
HO				
7	-	13.34 (s)		
9	-	11.06 (s)		

5. Argument

The larvicidal activity results presented in Table 1 show that the methanol extract and some fractions showed high activity against larvae of *Aedes aegypti* third instar. The bioassays also showed that the mortality for the compounds 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) and usnic acid (**2**) isolated from lichen *Ramalina usnea,* occurred in a dose dependent manner, and the results of lethal concentrations 50%, LC_{50} showed values of 4.850 and 4.475 µg/mL for (**1**) and (**2**), respectively, showing that these compounds have a strong potential larvicidal. The literature data on larvicidal activity of lichen compounds are scarce, especially for *Aedes aegypti*. The only study found about this genre presented the data of larvicidal activity of the methanol extract of the *Ramalina conduplicans* Vain. species with *Aedes aegypti* second instar larvae, which presented a DL_{50} around 10 mg/mL and with a concentration of 25 mg/ml (or 25,000 µg/mL) killed 100% of the larvae (Vinayaka et al, 2009). A high value compared to the aforementioned in our research. For the compound (**1**) was not found any reports in the larvicidal activity literature, and for the compound (**2**) was found reports with other insects. Cetin et al. 2008, tested the activity of the isomers (-) and (+)-usnic acid (**2**) against mosquito larvae *Culex pipiens* L., and the isomers showed intense activity, with DL_{50} of 0.8 and 0.9 µg/mL for the (-) and (+)-usnic acid (**2**). Cetin et al. 2012, have also obtained excellent results on lichen metabolites, among them the (+)-usnic acid (**2**) against mosquito larvae of *Culiseta longiareolata* Macquart (Dipterous: Culicidae), that showed high larvicidal activity, with DL_{50} values of 0.48 µg/mL.

Regarding the research with larvicidal tests carried out with plants, using essential oils, extracts and or isolated compounds, there is a large number of publications and some compounds showed such impressive results as the lichens compounds.

Sharma et al. (2014) tested the potential larvicidal extract in *Artemisia annua* chloroform against larvae of *Anopheles stephensi* Liston (malaria) and *Aedes aegypti* L. (dengue) and obtained LC_{50} values of 0.84 and 0.67 µg/mL, respectively. Sreelatha et al. (2010) presented data from mosquitocidal activity of isoshinanolone and plumbagin compounds isolated from rhizomes of *Plumbago capensis* Thumb, presenting high toxicity against *A. aegypti* larvae with LC_{50} values of 1.26 and 5.43 µg/mL respectively. Edinilza et al. (2009) tested the larvicidal activity against *A. aegypti* from essential oil of the methanol extract roots and an oxoaporphine *alkaloids*, liriodenine, isolated from *Rollinia leptopetala* Annonaceae). The results obtained from LC_{50} were 104.7 and 34.7 µg/mL for the essential oils from the leaves and branches, 64.6 µg/mL for the methanolic extract and 3.6 µg/mL to liriodenine. Geris et al. (2008) also obtained excellent results of larvicidal activity against *A. aegypti* from diterpenoids 3-β-acetoxylabdano-8(17)-13-diene-15-oic acid and alepterolic acid, isolated from the *Copaifera reticulata* essential oil. The compounds

showed LC_{50} of 0.8 and 87.3 µg/mL, respectively, showing that the first compound has strong larvicidal activity.

6. Conclusion

From the study performed, was found a high larvicidal activity of the compounds 2-Hydroxy-4-methoxy-6-propyl-methyl benzoate (**1**) and usnic acid (**2**) isolated from the lichen *Ramalina usnea,* suggesting to them the insecticidal activity, and also as a template for the development of synthesis of new molecules with larvicidal activity in the control of *Aedes aegypti*, corroborating with the larvicidal compounds line of research from natural sources.

Acknowledgement

The authors are grateful to Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for grants and a research fellowship, to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for research fellowships. We also thank Dr. Michell Navarro Benatti, herbarium curator of the Instituto de Botânica de São Paulo for the identification of lichen.

References

Anees AM (2008) Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Parasitol Res 103:1451–1453

Bačkorová M, Bačkor M, Mikeš J, Jendželovský R, Fedoročko P (2011) Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. Toxicol in Vitro 25:37–44

Bačkorová M, Jendželovský R, Kello M, Bačkor M, Mikeš J, Fedoročko P (2012) Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. Toxicol in Vitro 26:462–468

Bézivin C, Tomasi S, Rouaud I, Delcios J-G, Boustin J (2004) Cytotoxic activity of compounds from the lichen: *Cladonia convoluta*. Planta Med 70:877-880

Benli ACK, Selvi M, Sepici-Dincel A, Sarikaya R, Yildirim MZ, Ozkul A (2009) Acute toxicity of beta-cypermethrim on Nile tilapia (*Oreochromis niloticus* L.) finderlings. J Environ Prot Ecol 10:104-109

Boustie J. Grube M (2005) Lichens - A promising source of bioactive secondary metabolites. Plant Genet Resour 3: 273–287

Brandão LFG, Alcantara GB, Matos MFC, Bogo D, Freitas DS, Oyama NM, Honda NK (2013) Cytotoxic evaluation of phenolic compounds from lichens against melanona cells. Chem. Pharm. Bull 61:176–183

Brasil - Ministério da Saúde (MS) (2015) A febre do zika virus. Acesso em: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal

Byeon HE, Park BK, Yim JH, Lee HK, Moon EY, Rhee DK, Pyo S (2012) Stereocalpin A inhibits the expression of adhesion molecules in activated vascular smooth muscle cells. Int Immunopharmacol 12:315–325

Cansaran D, Atakol O, Halici MG Aksoy A (2007) HPLC analysis of usnic acid in some *Ramalina* species from Anatolia and investigation of their antimicrobial activities. Pharm Biol 45:77–81

Cetin H, Tufan-Cetin O, Turk AO, Tay T, Candan M, Yanikoglu A, Sumbul H (2008) Insecticidal activity of major lichen compounds, (-)- and (+)- usnic acid, against the larvae of house mosquito, *Culex pipiens* L. Parasitol Res 102:1277-1279

Cetin H, Tufan-Cetin O, Turk AO, Tay T, Candan M, Yanikoglu A, Sumbul H (2012) Larvicidal activity of some secondary lichen metabolites agains the mosquito *Culiseta longiareolata* Macquart (Diptera: Culicidae). Nat Prod Res 26(4):350-355

Cordeiro LMC, de Oliveira SM, Buchi DF, Iacomini M (2008) Galactofuranose-rich heteropolysaccharide from *Trebouxia* sp., photobiont of the lichen *Ramalina gracilis* and its effect on macrophage activation. Int J Biol Macromol 42:436–440

Einarsdóttir E, Groeneweg J, Björnsdóttir GG, Haröardottir G, Omarsdóttir S, Ingólfsdóttir K, Ögmundsdóttir HM (2010) Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. Planta Med 76:969–974

Fazio AT, Adler MT, Bertoni MD, Sepúlveda CS, Damonte EB, Maier MS (2007) Lichen secondary metabolites from the cultured lichen mycobionts of

Teloschistes chrysophthalmus and *Ramalina celastri* and their antiviral activities. Z Naturforsch C 62:543–549

Feitosa EMA, Arriaga AMC, Santiago GMP, Lemos TLG, Oliveira MCF, Vasconcelos JN, Lima JQ, Malcher GT, Nascimento RF, Braz-Filho R (2009) Chemical composition and larvicidal activity of *Rollinia leptopetala* (Annonaceae). J Braz Chem Soc 20(2):375-378

Lai D, Odimewu D, Esimone C, Grunwald T, Proksch P (2013) Phenolic compounds with *in vitro* activity against respiratory syncytial from the Nigerian lichen *Ramalina farinacea*. Planta Med 79:1440–1446

Geris R, Silva IG, Silva HHG, Barison A, Rodrigues-Filho E, Ferreira AG (2008) Diterpenoids from *Copaifera reticulata* Ducke with larvicidal activity against *Aedes aegypti* (L.) (Diptera, Culicidae). Rev Inst Med trop S Paulo 50(1):25-28

Góis RWS, Souza LM, Santiago GMP, Romero NR, Lemos TLG, Arriaga, AMC, Braz-Filho R (2013) Larvicidal activity against *Aedes aegypti* of pacharin from *Bauhinia acuruana*. Parasitol Res 112:2753-2757

Govindarajan M, Sivakumar R (2014) Larvicidal, ovicidal, and adulticidal efficacy of *Erythrina indica* (Lam.) (Family: Fabaceae) against *Anopheles stephensi* , *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 113:777–791

Gulluce M, Aslan A, Sokmen M, Sahin F, Adiguzel A, Agar G, Sokmen A (2006) Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderiana*. Phytomedicine 13:515–521

Honda NK, Vilegas W (1998) A química dos liquens. Quim Nova 21:110–125

Esimone CO, Grunwald T, Nworu CS, Kuate S, Proksch P, Ueberla K (2009) Broad spectrum antiviral fractions from the lichen *Ramalina farinacea* (L.) Ach Chemotherapy 55:119–126

Feige GB, Lumbsch HT (1993) Identification of lichen substances by a standardized high-performance liquid chromatographic method. J Chromatogr 647:417–427

Halici M, Kufrevioglu OI, Odabasoglu F, Halici Z, Cakir A, Aslan A (2011) The ethanol-water extract of *Ramalina capitata* has gastroprotective and

antioxidative properties: an experimental study in rats with indomethacin-induced gastric injuries. J Food Biochem 35:11–26

Haraldsdóttir S, Guölaugsdóttir E, Ingólfsdóttir K, Ögmundsdóttir HM (2004) Anti-proliferative effects of lichen-derived lipoxygenase inhibitors on twelve human cancer cell lines of different tissue origin *in vitro*. Planta Med 70: 1098–1100

Hirayama T, Fujikawa F, Kasahara T, Otsuka M, Nishida N, Mizuno D (1980) Anti-tumor activities of some lichen products and their degradation products. Yakugaku Zasshi, 100:755–759

Huneck S, Yoshimura I (1996) Identification of Lichen Substances. Springer-Verlag: Berlin, Germany

Ingólfsdóttir K (2002) Usnic acid. Phytochemistry 61:729–736

König GM, Wright AD (1999) ^{1}H and ^{13}C-NMR and biological activity investigations of four lichen-derived compounds. Phytochem Anal 10:279-284

Koparal AT, Tuylu BA, Turk H (2006) In vitro cytotoxic activities of (+)-usnic acid and (−)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. Nat Prod Res 20:1300–1307

Kumar SVP, Kekuda TRP, Vinayaka KS, Sudharshan SJ (2009) Anthelmintic and antioxidant efficacy of two macrolichens of Ramalinaceae. Pharmacogn J 1:238–242

Luo H, Wei X, Yamamoto Y, Liu Y, Wang L, Jung JS, Koh YJ, Hur J.-S (2010) Antioxidant activities of edible lichen *Ramalina conduplicans* and its free radical scavenging constituents. Mycoscience 51:391–395

Mendonça FAC, Silva KFS, Santos KK, Ribeiro Júnior KAL, Sant'Ana AEG (2005) Activities of some Brazilian plants against larve of the mosquito *Aedes aegypti*. Fitoterapia 76:629-636

Murugan K, Kumar PM, Kovendan K, Amerasan D, Subrmaniam J, Hwang J-S (2012) Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 111:1757–1769

Oksanen I (2006) Ecological and biotechnological aspects of lichens. Appl Microbiol Biotechnol 73:723–734

PAHO/WHO (2015) Number of reported cases of chikungunya fever in the Americas, by country or territory. Pan American Health Organization/World Health Organization. Epidemiological week/EW 17, update as of 1 may 2015. Available at http://www.paho.org/hq/index.php

Parrot D, Jan S, Baert N, Guyot S, Tomasi S (2013) Comparative metabolite profiling and chemical study of *Ramalina siliquosa* complex using LC-ESI-MS/MS approach. Phytochemistry 89:114–124

Paudel B, Bhattarai HD, Lee JS, Hong SG, Shin HW, Yim JH (2008) Antibacterial potential of Antarctic lichens against human pathogenic Gram-positive bacteria. Phytother Res 22:1269–1271

Paudel B, Bhattarai HD, Lee HK, Oh H, Shin HW, Han J, Yim JH (2010) Antibacterial activities of ramalin, usnic acid and its tree derivatives isolated from the Antarctic lichen *Ramalina terebrata.* Z fuer Naturforsch C 65: 34–38

Paudel B, Bhattarai HD, Pandey DP, Hur, JS, Hong SG, Kim I-C, Yim JH (2012) Antioxidant, antibacterial activity and brine shrimp toxicity test of some mountainous lichens from Nepal. Biol Res 45:387–391

Polson KA, Brogdon WG, Rawlins SC, Chadee DD (2011) Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes. Acta Tropica 117:31-38

Rahuman A A, Venkatesan P (2008) Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. Parasitol Res 103:133-139

Rahuman A A, Venkatesan P, Gopalakrishnan G (2008) Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. Parasitol Res 103:1383-1390

Rashid MA, Majid MA, Quader MA (1999) Complete NMR assignments of (+)-usnic acid. Fitoterapia 70:113-115

Samidurai K, Saravanakumar A (2001) Mosquitocidal properties of nereistoxin against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res (2011) 109:1107–1112

Santiago GMP, Viana FA, Pessoa ODL, Pouliquen YBM, Arriaga AMC, Andrade-Neto M, Braz-Filho R (2005) Avaliação da atividade larvicida de saponinas triterpênicas isoladas de *Pentaclethra macroloba* (Willd.)

Kuntze (Fabaceae) e *Cordia piauhiensis* (Boraginaceae) sobre *Aedes aegypti*. Braz J Pharmacog 15(3):187-190

Santos SRL, Melo MA, Cardoso AV, Santos RLC, De Sousa DP, Cavalcanti SCH (2011) Structure–activity relationships of larvicidal monoterpenes and derivatives against *Aedes aegypti* Linn. Chemosphere 84:150–153

Schmeda-Hischmann G, Tapia A, Lima B, Pertino M, Sortino M, Zacchino S, Arias AR, Feresin EG (2008) A new antifungal and antiprotozoal depside from the andean lichen *Protousnea poeppigii*. Phytother Res 22:349-355

Sharma G, Kapoor H, Chopra M, Kumar k, Agrawal V (2014) Strong larvicidal potencial of *Artemisia annua* leaf extract against malaria (*Anopheles stephensi* Liston) and dengue (*Aedes aegypti* L.) vectors and bioassay-driven isolation of the marker compounds. Parasitol Res 113:197:209

Simas NK, Lima EC, Conceição SR, Kuster RM, Oliveira Filho AM (2004) Produtos naturais para o controle da transmissão da dengue – atividade larvicida de *Myroxylon balsamum* (Óleo Vermelho) e de terpenóides e fenilpropanódies. Quim. Nova 27(1): 46-49

Singh N, Nambiar D, Kale RK, Singh RP (2013) Usnic acid inhibits and induces cell cycle arrest and apoptosis in human lung carcinoma A549 cells. Nutr Cancer 65:36–43

Sisodia R, Geol M, Verma S, Rani A, Dureja P (2013) Antibacterial and antioxidante activity of lichen species *Ramalina roesleri*. Nat Prod Res 27: 2235–2239

Sreelatha T, Hymavathi A, Murthy JM, Rani PU, Rao JM, Babu KS (2010) Bioactivity-guided isolation of mosquitocidal constituents from the rhizomes of *Plumbago capensis* Thunb. Bioorg Med Chem Lett 20:2974-2977

Stuelp-Campelo PMa, de Oliveira MBM, Leão AMAC, Carbonero ER, Gorin PAJ, Iacomini M (2002) Effect of a soluble α-D-glucan from the lichenized fungus *Ramalina celastri* on macrophage activity. Int Immunopharmacol 2: 691–698

Tay T, Türk AÖ, Yilmaz M, Türk H, Kivanç M (2004) Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstitic acid, and protocetraric acid constituents. Z Naturforsch C 59:384–388

Trevisan MTS, Bezerra MZB, Santiago GMP, Feitosa CM, Verpoorte R, Braz-Filho R (2006) Atividade larvicida e anticolinesterásica de plantas do gênero *Kalanchoe*. Quim Nova 29(3):415-418

Viegas Jr C (2003) Terpenes with insecticidal activity: an alternative to chemical control of insects. Quím Nova 26:390-400

Yildirim E, Aslan A, Emsen B, Cakir A, Ercisli S (2012) Insecticidal effect of *Usnea longissima* (Parmeliaceae) extract against *Sitophilus granarius* (Coleoptera: Curculionidae). Int. J. Agric. Biol 14:303–306

Yim JH, Lee HK, Bhattarai HD, Babita P, Kim IC, Hong SG, Kim DK, Lee YK, Gu S, Oh HC (2010) Antioxidant Activities of Ramalin Isolated from *Ramalina terebrata* Extracts. WO 2010053327 A2, 14 May 2010

Yim JH, Kim IC, Lee SG, Kim DK, Han SJ, Lee HS, Seok KH, Kim SJ, Kim TK, Kang PS (2011) Pharmaceutical Composition for the Treatment of Inflammatory Disease or Immune Disease Containing Ramalin. WO 2011152671 A2, 8 December 2011

WHO (2012) Global strategy for dengue prevention and control 2012-2020. World Health Organization. Available at http://www.who.int/about/licensing/copyright_form/en/index.html

WHO (2005) Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13.

WHO (2015) Chikungunya. World Health Organization, Media Centre, Fact sheet n. 327, update February 2015. Available at: http://www.who.int/mediacentre/factsheets/es

SUPPLEMENTARY MATERIAL

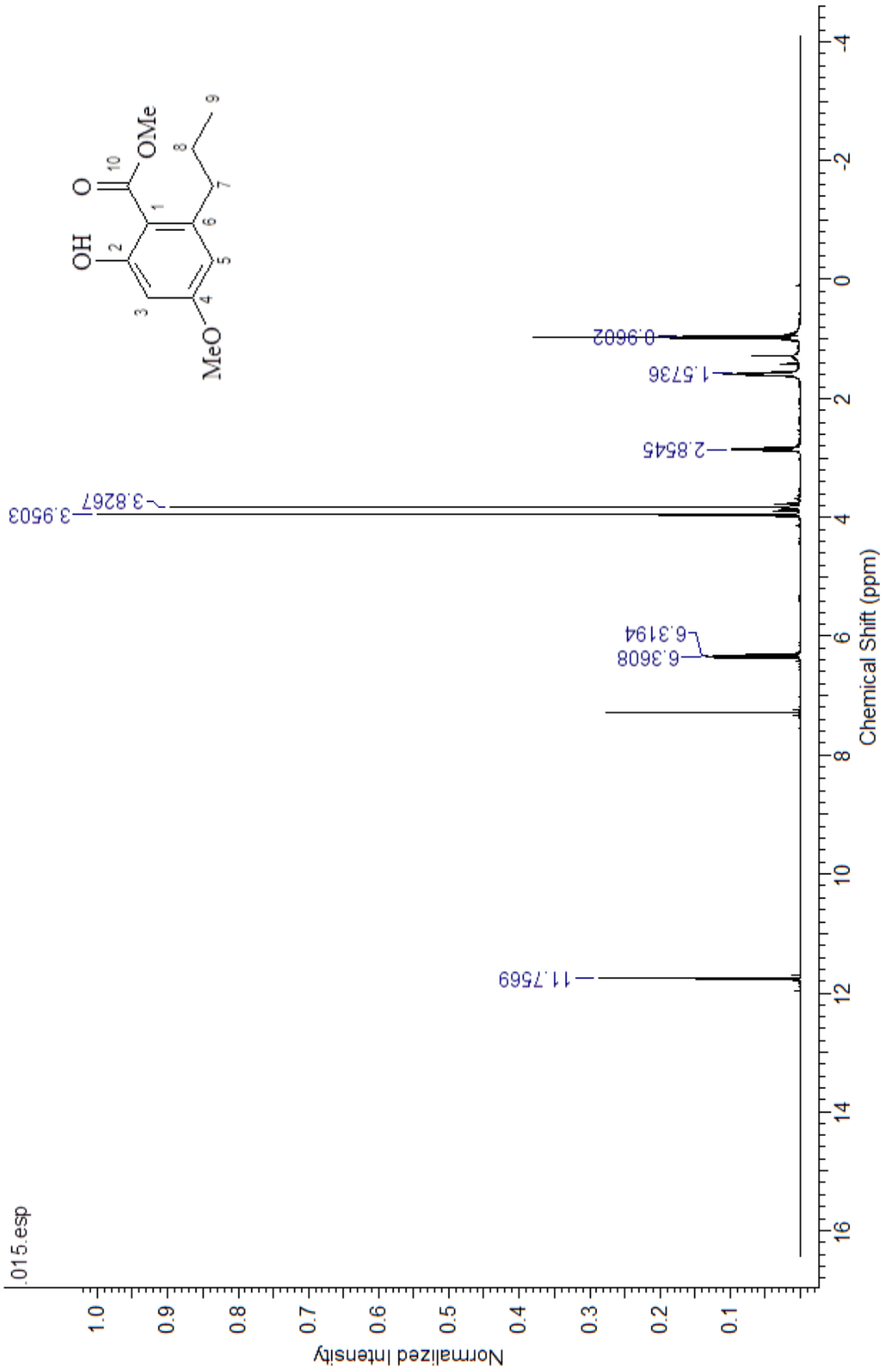


Figure 1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the compound 2-hydroxy-4-methoxy-6-propyl- methyl benzoate.

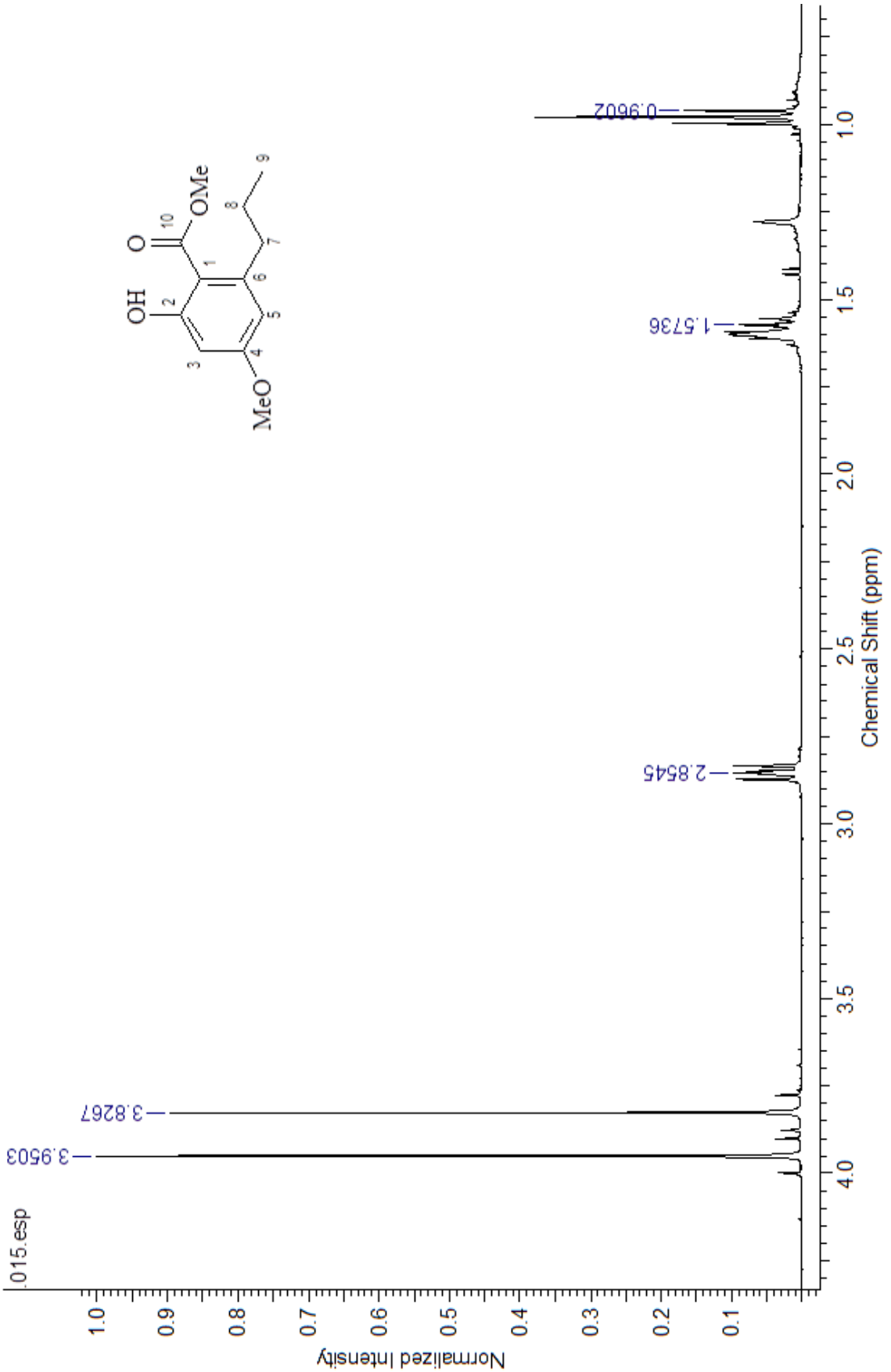


Figure 2. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of the compound 2-hydroxy-4-methoxy-6-propyl- benzoate Methyl. Expansion of the region 0.5 – 4.5 ppm.

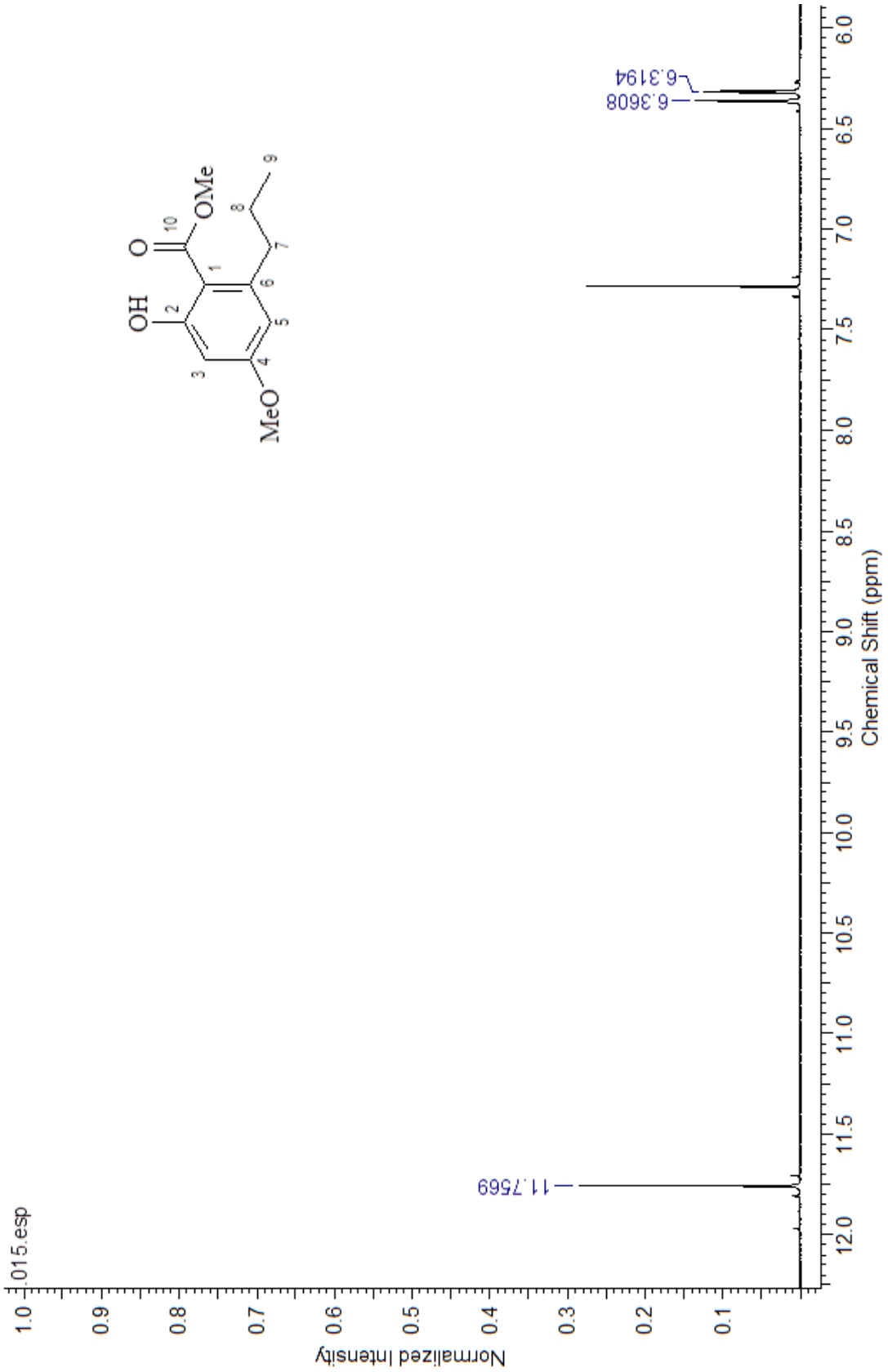


Figure 3. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of the compound 2-hydroxy-4-methoxy-6-propyl- methyl benzoate. Expansion of the region 6.0 – 12.5 ppm.

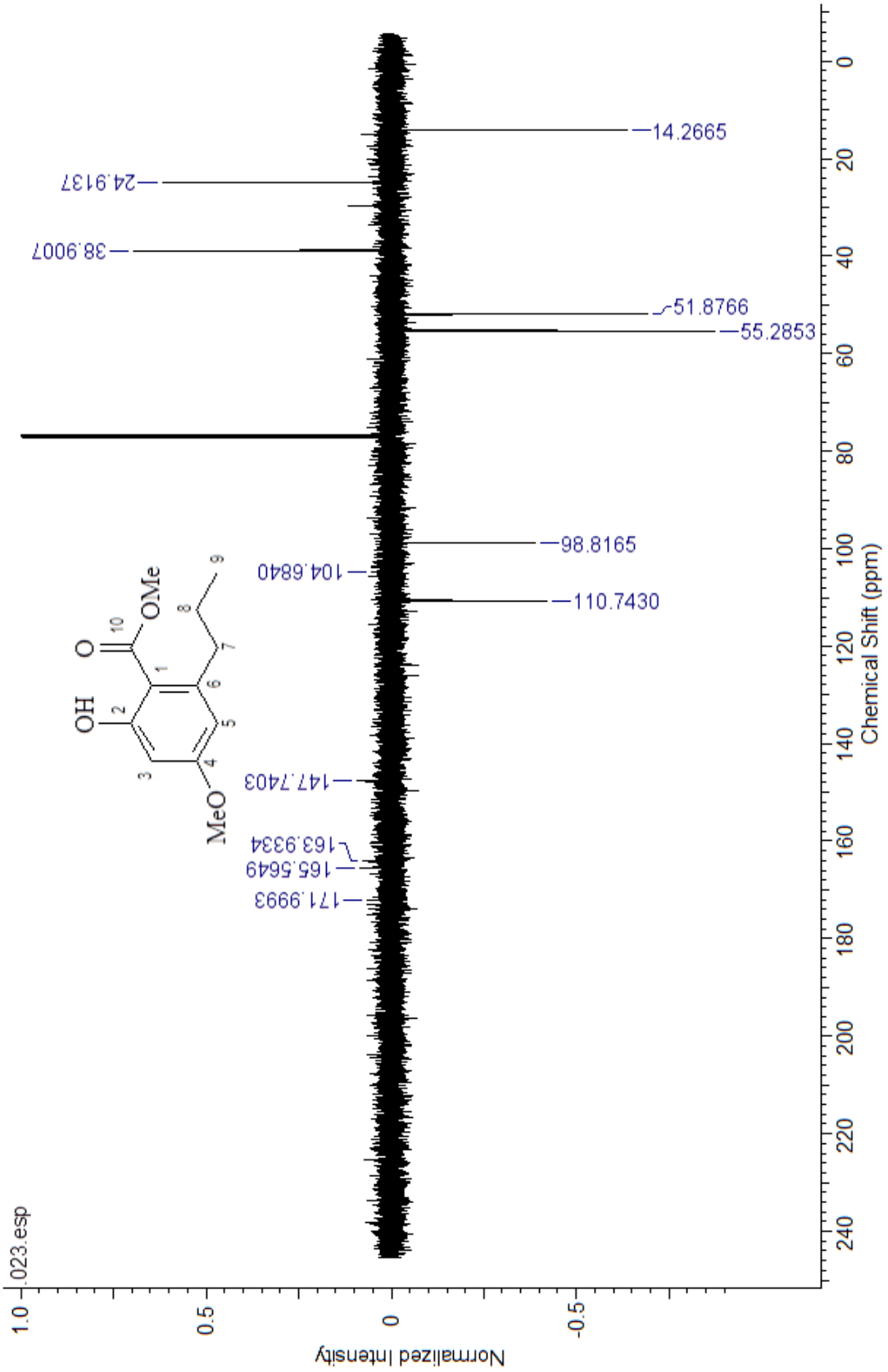


Figure 4. ^{13}C-NMR spectrum (DEPTQ, 125 MHz, $CDCl_3$) of the compound 2-hydroxy-4-methoxy-6-propyl-methyl benzoate.

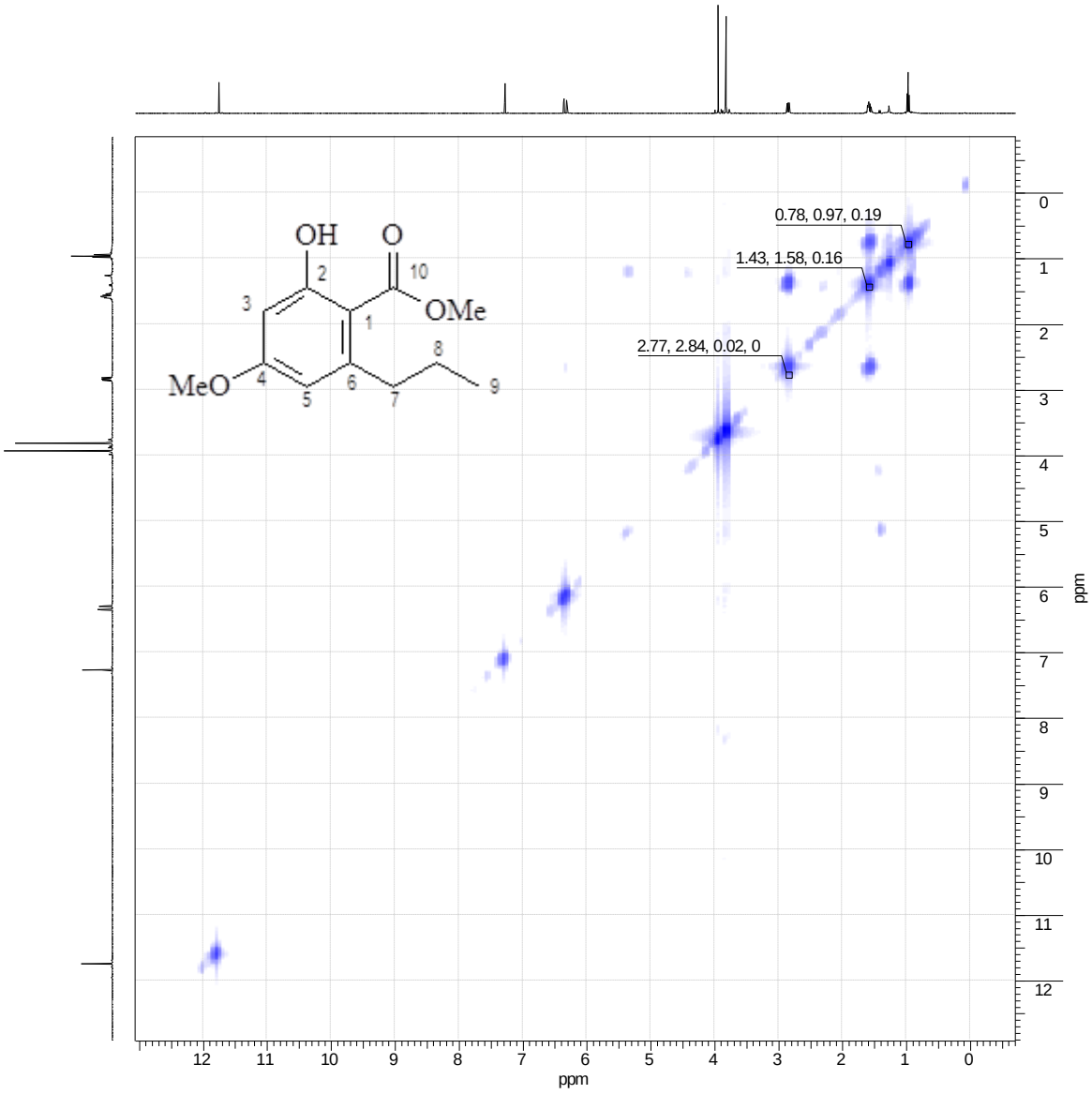


Figure 5. Correlation map homonuclear ^{1}H-^{1}H two-dimensional (COSY, 500 MHz, $CDCl_3$) of the compound 2-hydroxy-4-methoxy-6-propyl- methyl benzoate.

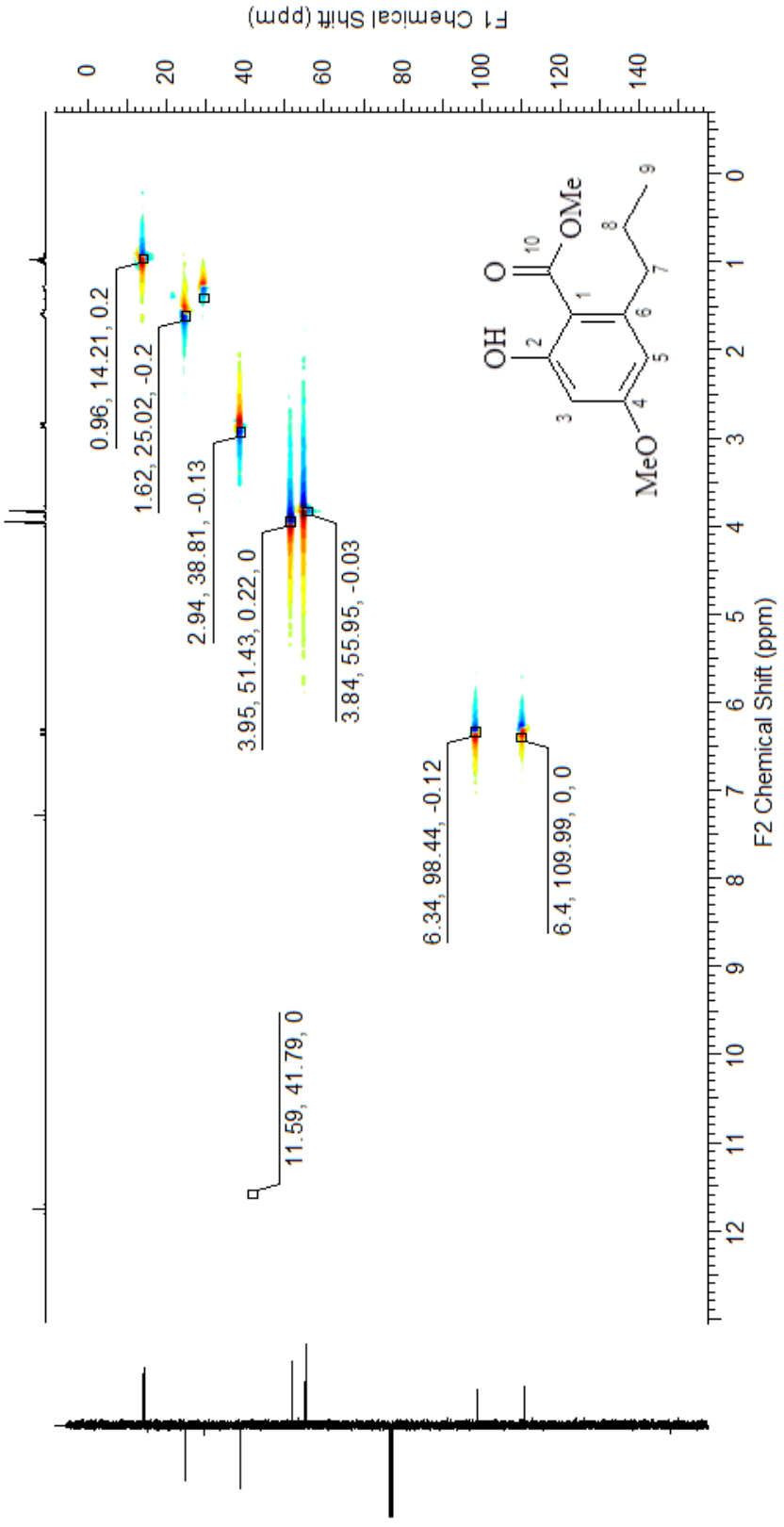


Figure 6. Two-dimensional heteronuclear correlation ^{1}H-^{13}C (HSQC, 500 MHz, $CDCl_3$) map of the compound 2-hydroxy-4-methoxy-6-propyl- methyl benzoate.

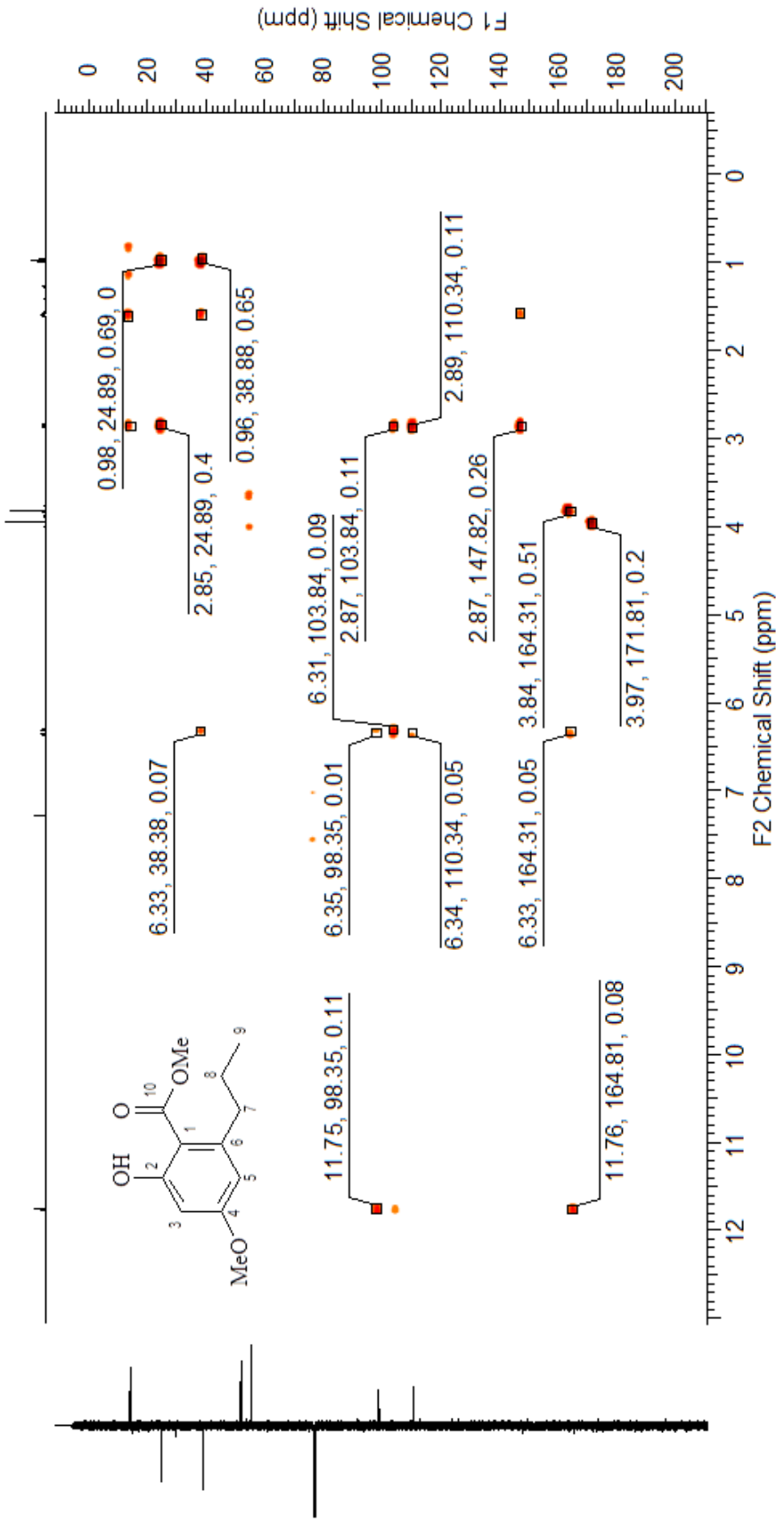


Figure 7. Two-dimensional heteronuclear correlation ^{1}H-^{13}C (HMBC, 500 MHz, $CDCl_3$) map of the compound 2-hydroxy-4-methoxy-6-propyl- methyl benzoate.

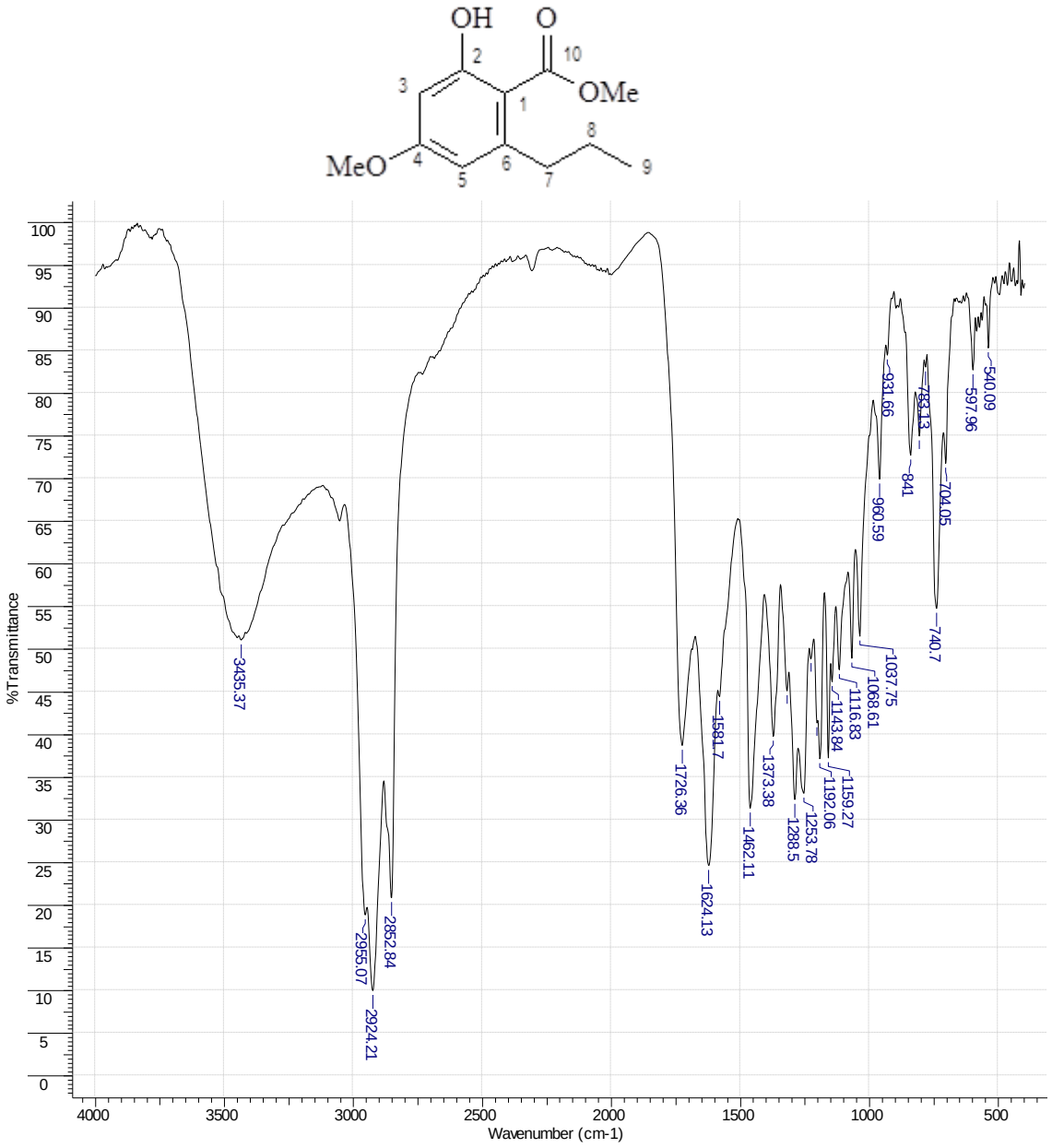


Figure 8. Infra-red spectrum of the compound 2-hydroxy-4-methoxy-6-propyl- methyl benzoate.

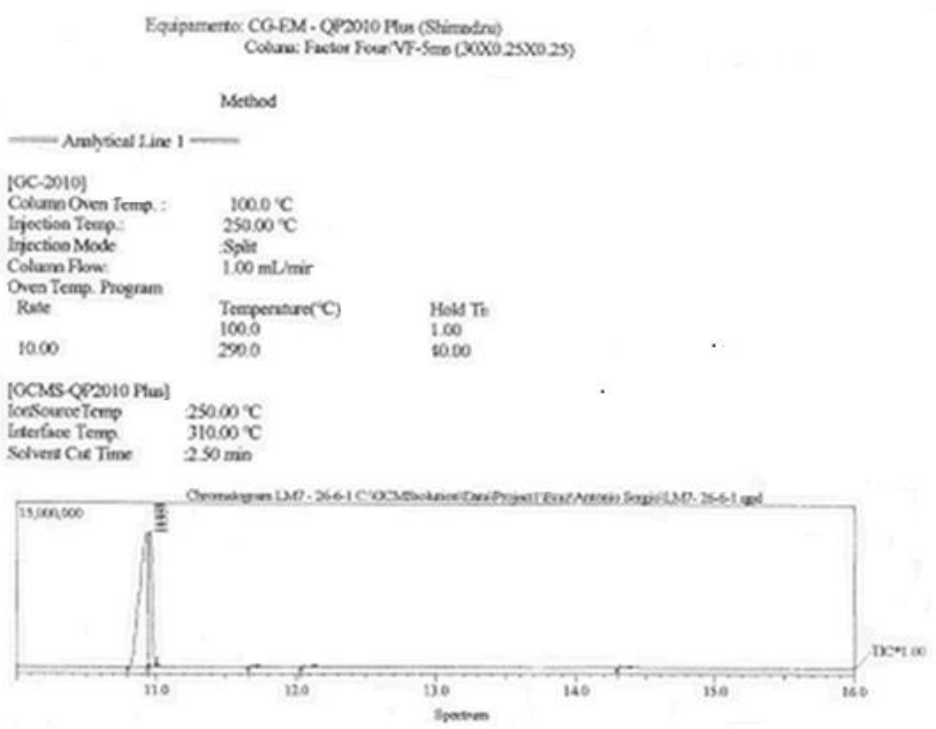


Figure 9. Chromatogram of the compound 2-hydroxy-4-methoxy-6- propyl methyl benzoate.

R. Time 10.92

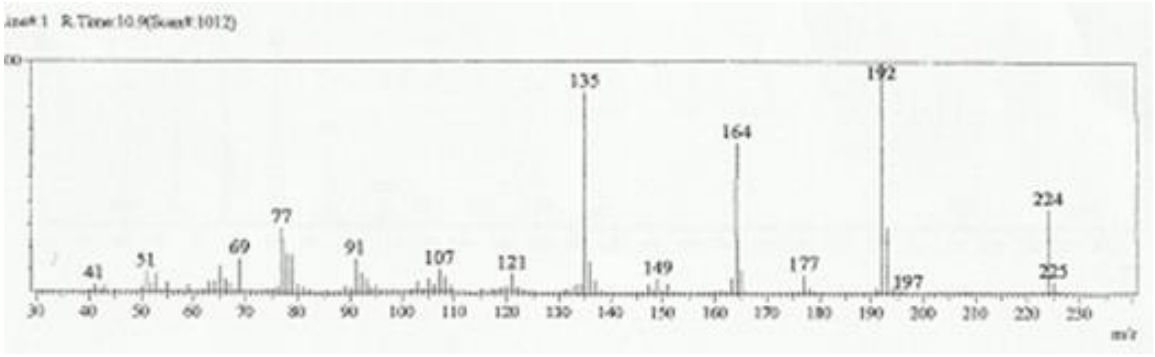


Figure 10. Mass spectrum of 2-hydroxy-4-methoxy-6- propyl- methyl benzoate.

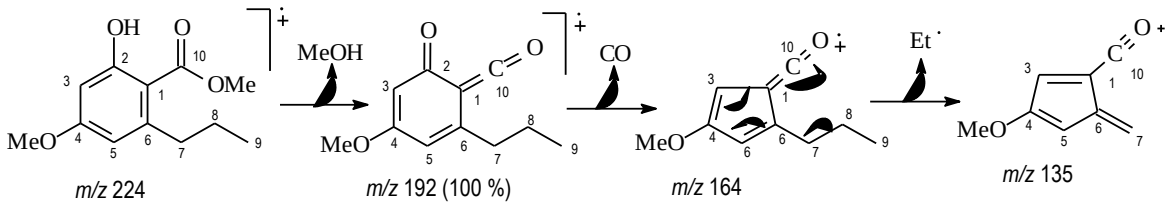


Figure 11. Proposal of the 2-hydroxy-4-methoxy-6- propyl- methyl benzoate compound main fragments mechanism.

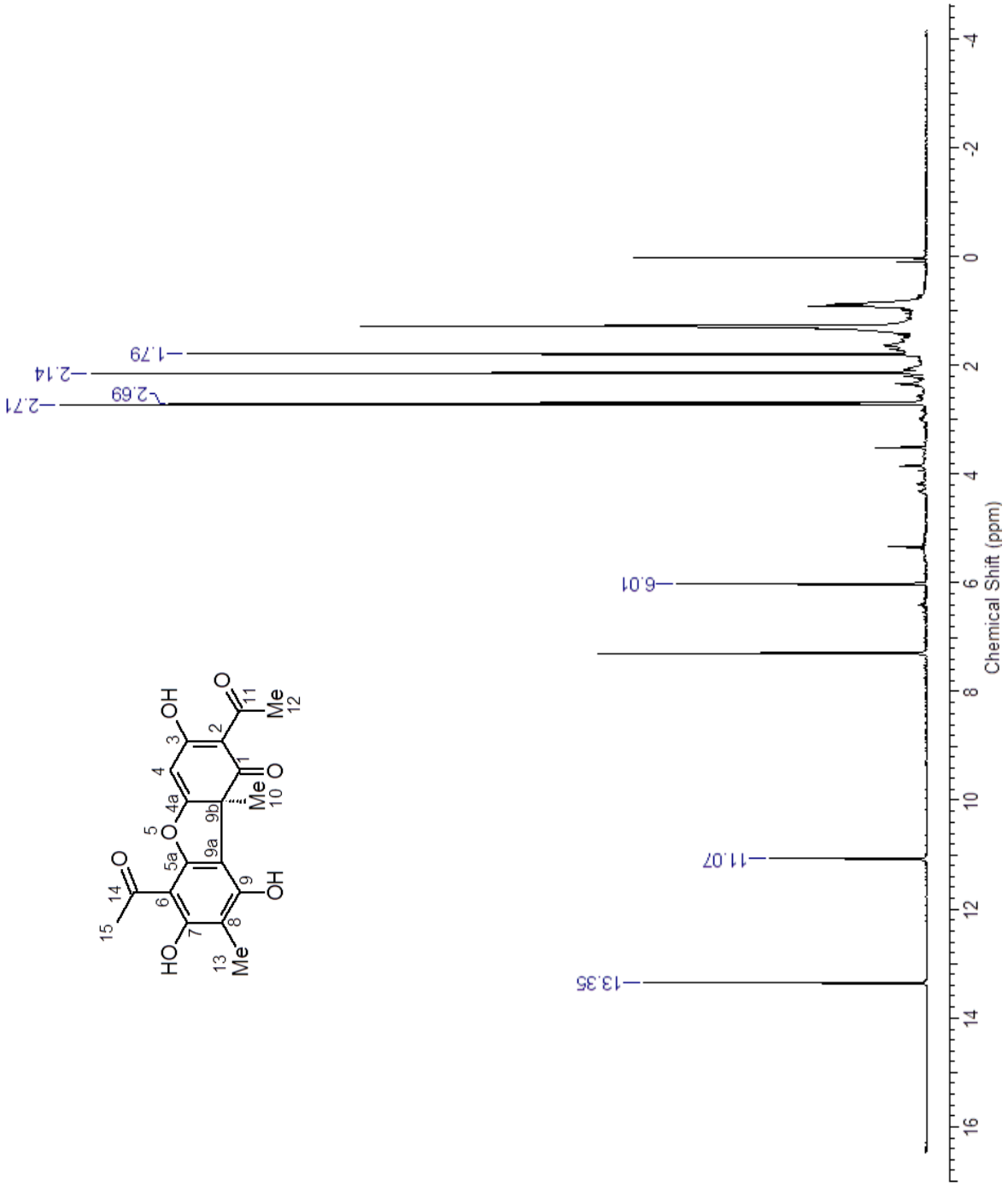


Figure 12. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the compound usnic acid.

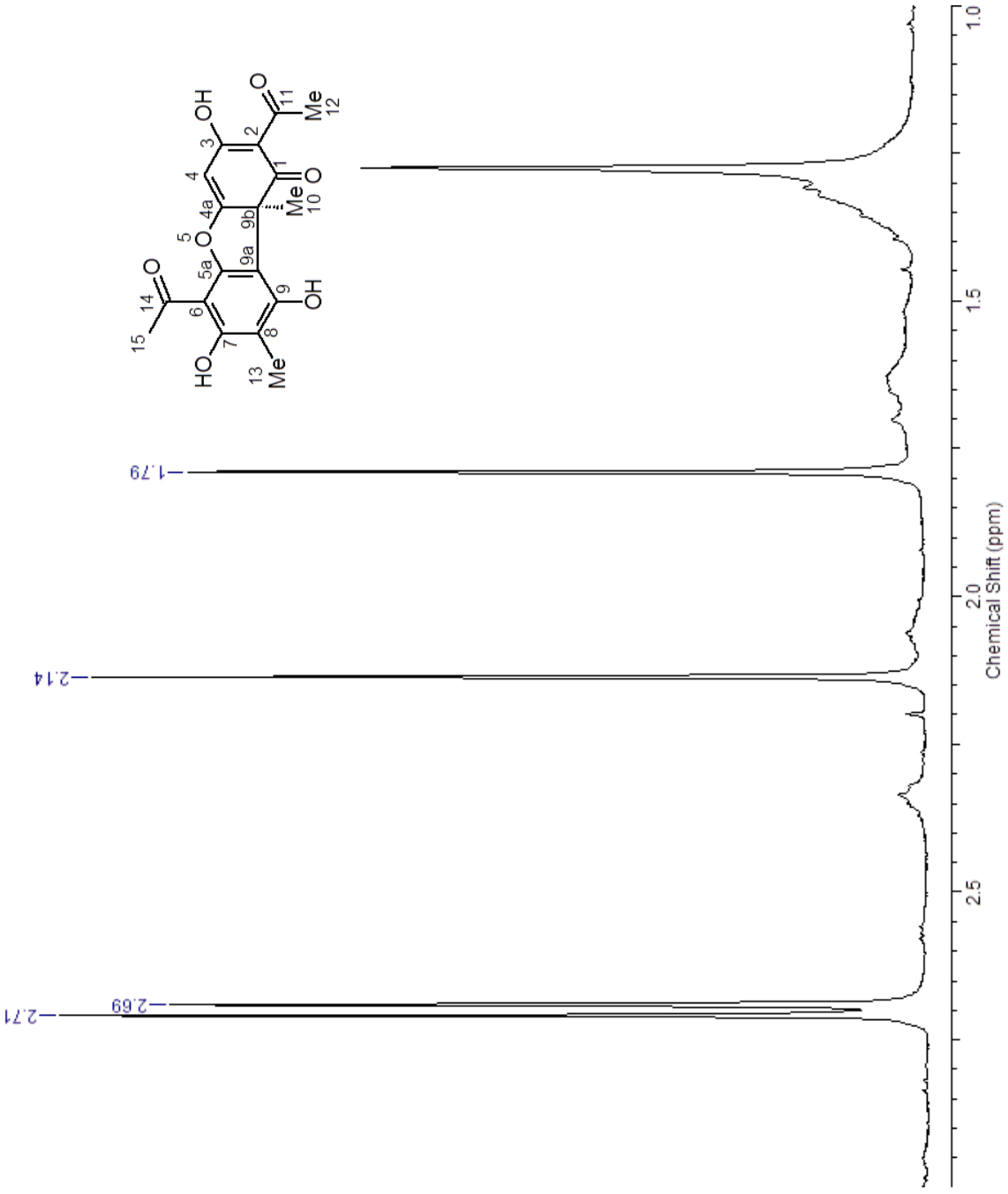


Figure 13. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the compound usnic acid. Expansion of the region 1.0 - 2.95 ppm

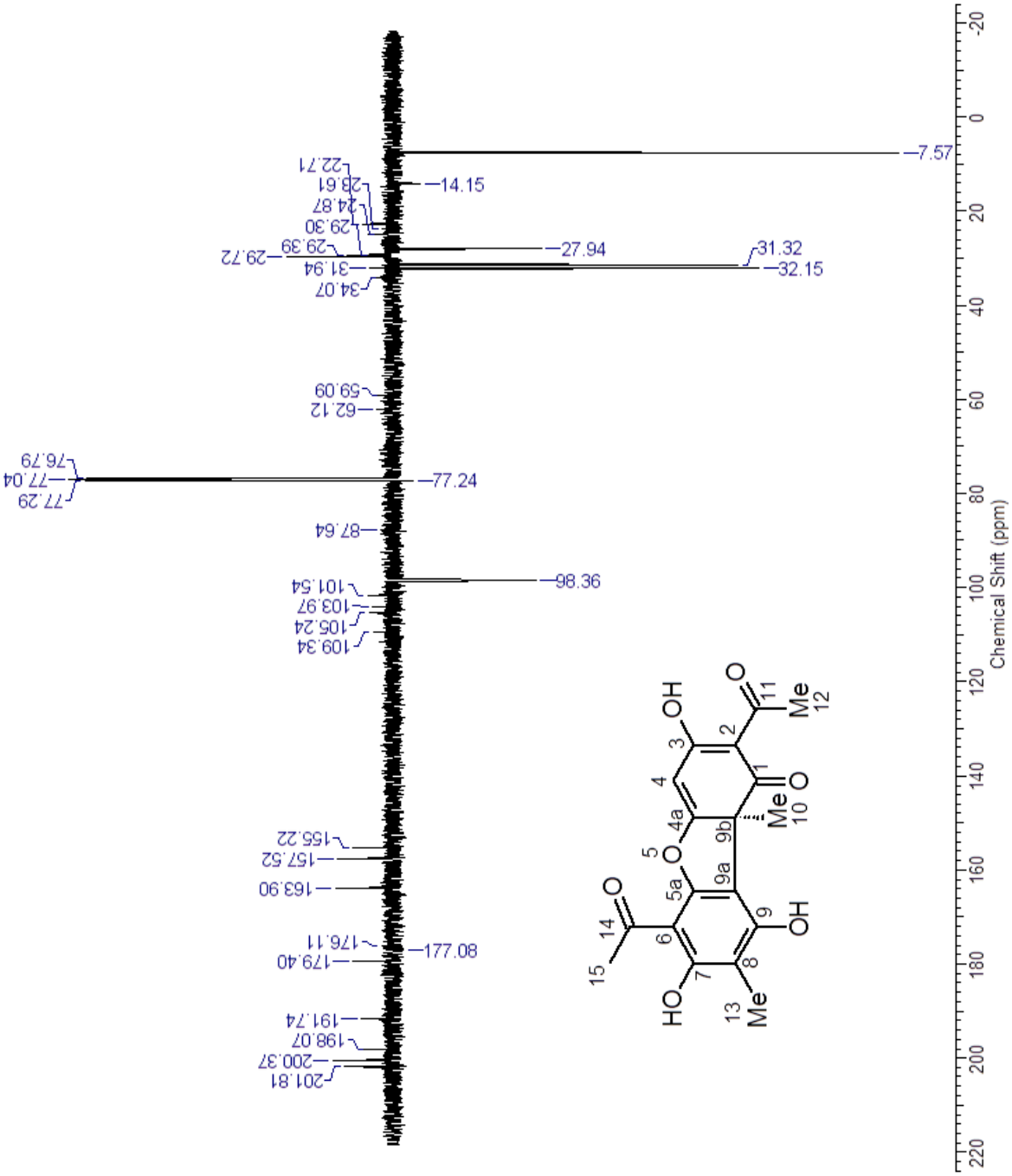


Figure 14. ^{13}C-NMR spectrum (DEPTQ, 125 MHz, $CDCl_3$) of the compound usnic acid.

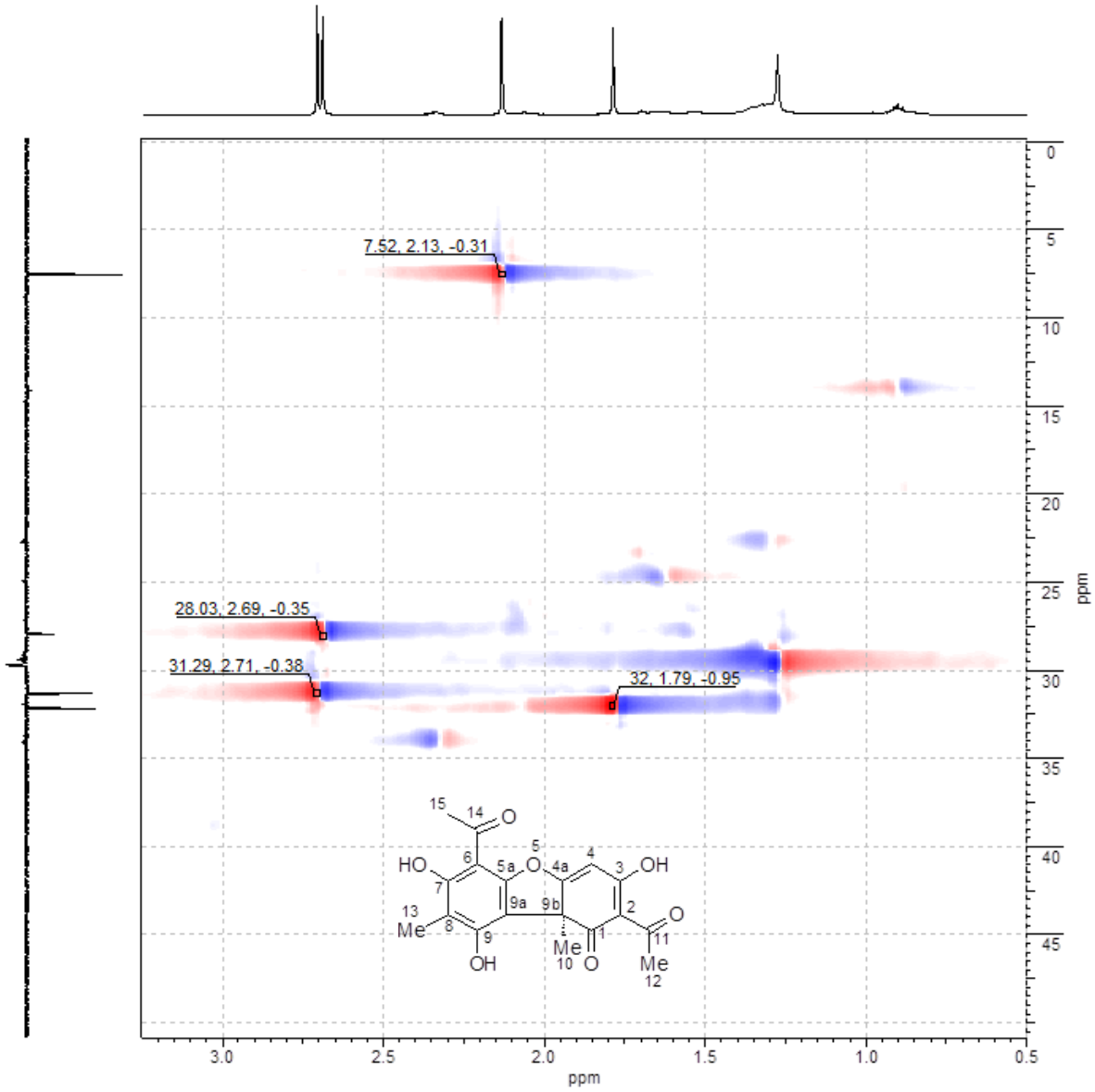


Figure 15. Two-dimensional heteronuclear correlation ^{1}H-^{13}C (HSQC, 500 MHz, $CDCl_3$) map of the compound usnic acid.

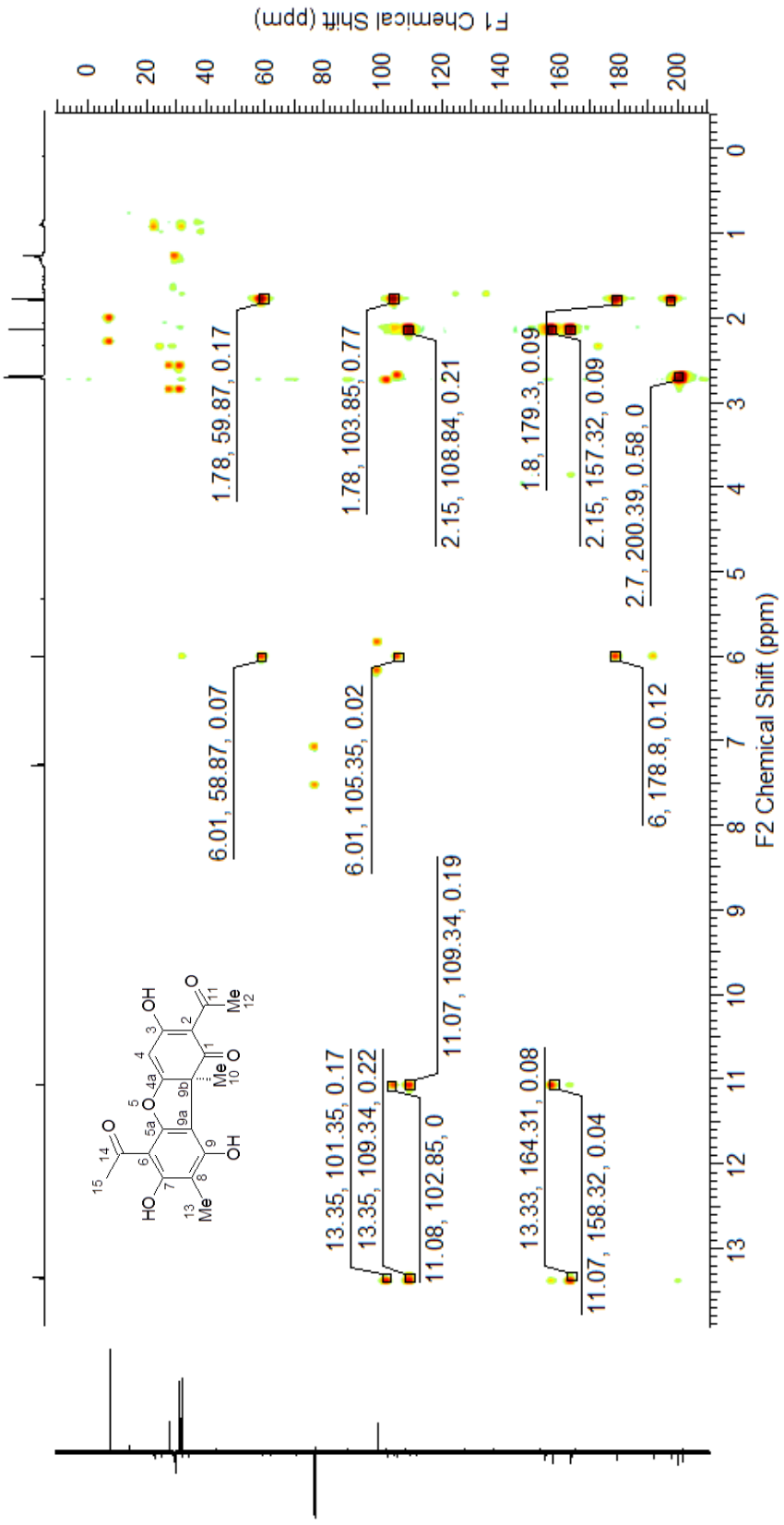


Figure 16. Two-dimensional heteronuclear correlation ^{1}H-^{13}C (HMBC, 500 MHz, $CDCl_3$) map of the compound usnic acid.

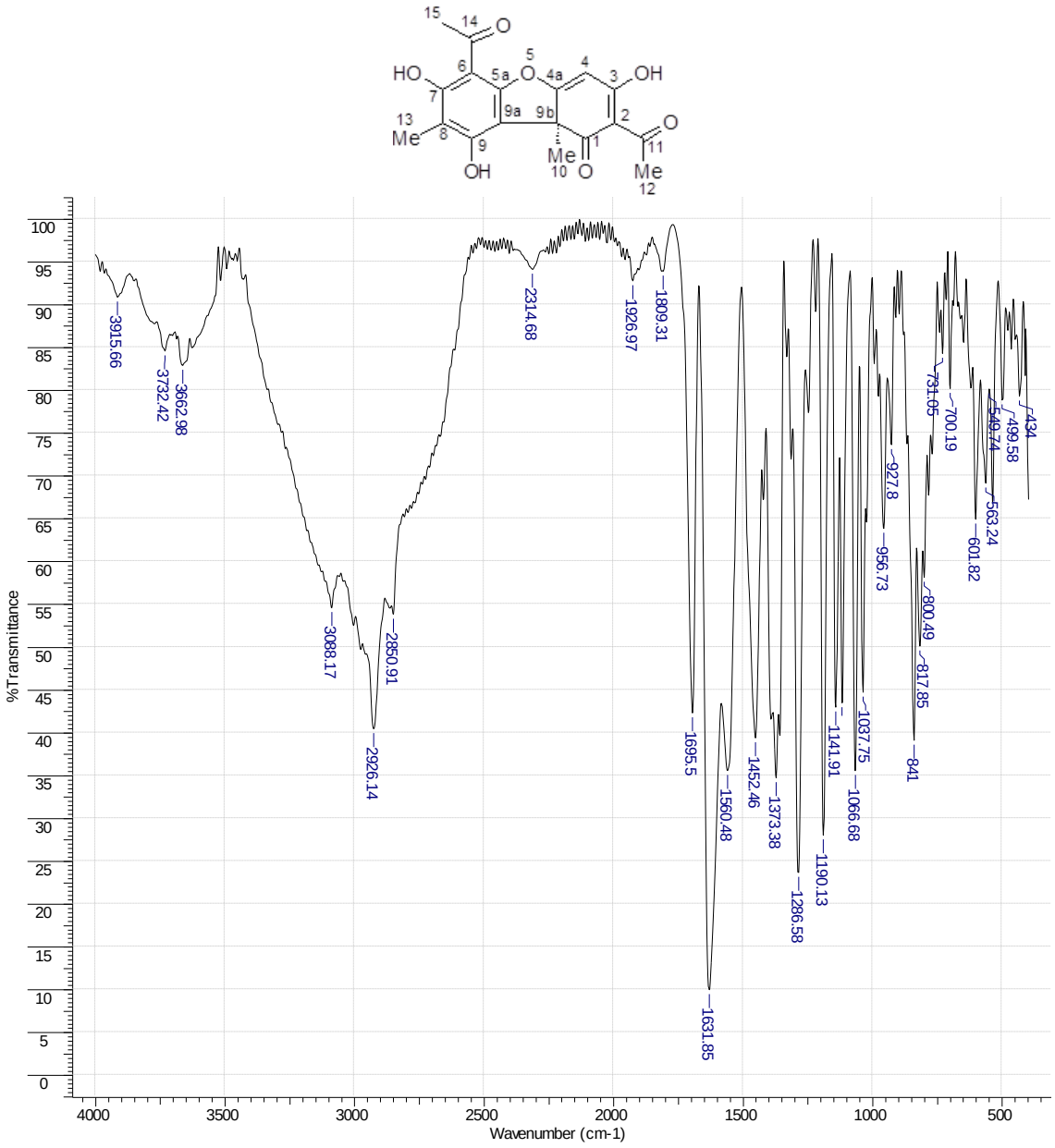


Figure 17. Infra-red spectrum of the compound usnic acid.

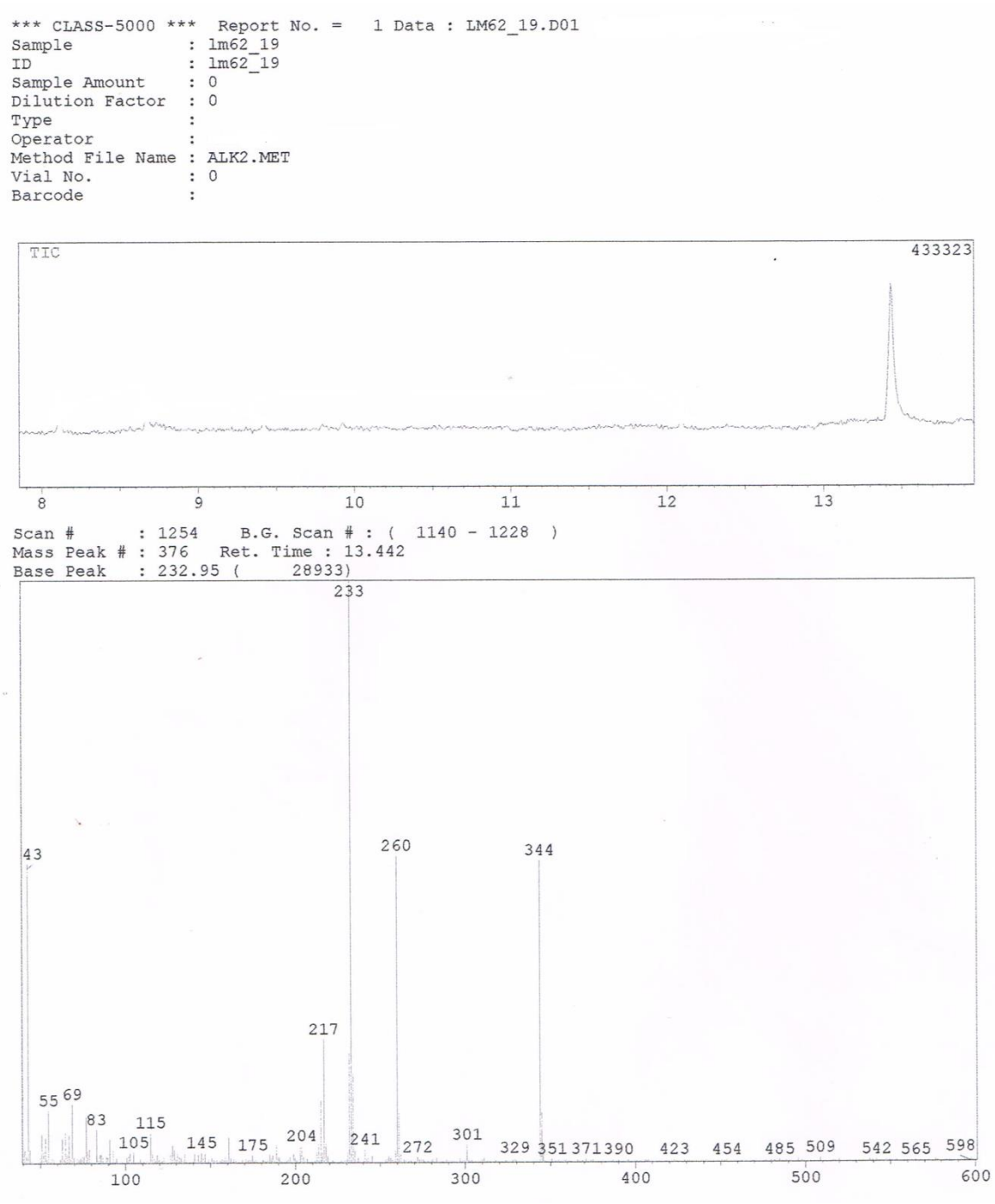


Figure 18. Chromatogram of the compound usnic acid.

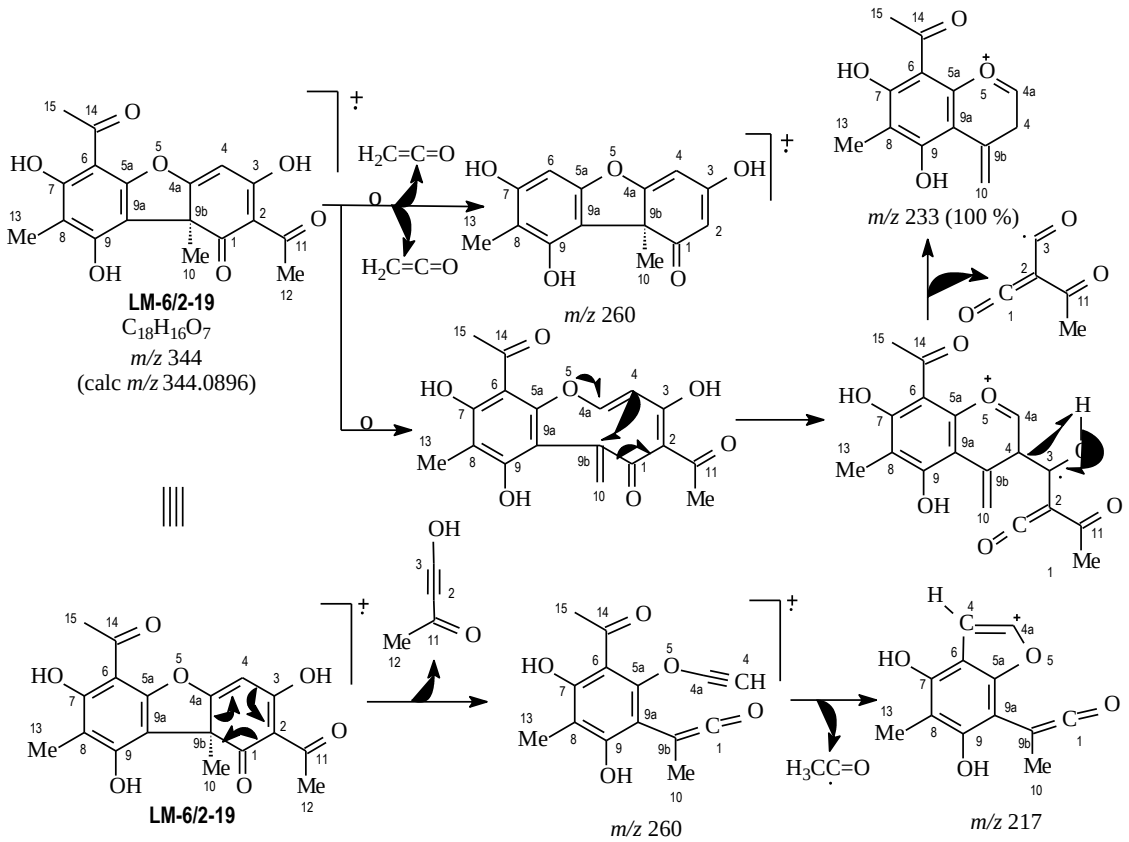


Figure 19. Proposal of the usnic acid compound main fragments mechanism

Potencial Antimicrobiano de *Ramalina usnea*

Moreira, A. S. N. *; Araújo, M. F. de; Motta, O. V.; Samarão, S. S.; Braz-Filho, R.; Vieira, I. J. C.

Rev. Virtual Quim., **Ano**, n. (v.), Data de publicação na Web: ?? Dia/Mês/Ano

http://www.uff.br/rvq

Resumo: Líquens são organismos simbióticos entre fungos e algas, os quais possuem uma alta diversidade de compostos químicos. Estes possuem diferentes e importantes atividades biológicas, entre as quais, antimicrobiana. Sendo assim, o presente trabalho teve como objetivo avaliar o potencial antimicrobiano do extrato em metanol, frações e da substância isolada, ácido úsnico (1), frente a três linhagens de *Staphylococcus aureus* ATCC e *Burkhalderia cepacea* ATCC 25416 e três espécies de fungos do gênero *Candida*. O ácido úsnico apresentou atividade sobre os fungos testados, mas não sobre as bactérias. O extrato bruto e algumas frações apresentaram atividade sobre as bactérias, mas não sobre os fungos testados e três frações apresentaram ótimos resultados contra duas linhagens de *Staphylococcus*.
Palavras-chave: *Ramalina usnea*, Ácido úsnico, Atividade antibacteriana, Atividade antifúngica.

Abstract: Lichens are symbiotic organisms of fungi and algae, which have a high diversity of chemical compounds. These have various important biological activities, among which antimicrobial. Therefore, this study aimed to evaluate the antimicrobial potential of methanol extract, fractions and isolated the compound usnic acid (**1**) against three strains of *Staphylococcus aureus* ATCC 25416 and ATCC *Burkhalderia cepacia* and three species of fungi of the genus *Candida*. The usnic acid was active against the fungi tested, but not on bacteria. The crude extract and some fractions showed activity against bacteria, but not tested on fungi and and three fractions showed excellent results against two strains of *staphylococcus*.
Keywords: *Ramalina usnea*, Usnic Acid, Antibacterial Active, Antifungal Active.

*****Universidade Estadual do Note Fluminense Darcy Ribeiro, Laboratório de Ciências Químicas, Departamento de Química de Produtos Naturais, 28013-602, Campos dos Goytacazes, RJ, Brasil. Este artigo fará parte da tese de doutorado do autor.

ansernam@iff.edu.br

Potencial Antimicrobiano de *Ramalina usnea*

Antônio Sérgio N. Moreira,[a,b]* Marcelo F. de Araújo,[b] Olney V. da Motta,[c] Solange S. Samarão,[c] Raimundo Braz-Filho[d], Ivo J. Curcino Vieira,[d]

[a]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Programa de Pós-Graduação em Ciências Naturais, Campos dos Goytacazes-RJ, Brasil.

[b]Instituto Federal Fluminense, campus Guarus, Campos dos Goytacazes-RJ, Brasil.

[c]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias-CCTA-Laboratório de Sanidade Animal.

[d]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciência e Tecnologia – CCT –Laboratório de Ciências Químicas III - Av. Alberto Lamego, 2000 – Pq. Califórnia – Campos dos Goytacazes – RJ – CEP: 28013-602

*ansernam@iff.edu.br

Recebido em .... Aceito para publicação em...

1. Introdução

2. Experimental

2.1 Equipamentos e Reagentes

2.2 Coleta e identificação do líquen *R. usnea.*

2.3 Extração e isolamento

2.4 Avaliação do Potencial antimicrobiano

2.4.1 Material biológico

2.4.2 Método de difusão em ágar

2.4.3 Método em meio líquido

3. Resultados e Discussão

3.1 Ensaios em meio sólido

3.2 Ensaios em meio líquido

4. Conclusões

Agradecimentos

Referências

1. Introdução

Fungos liquenizados constituem uma forma de mutualismo simbiótico entre um fungo (micobionte) e uma alga ou uma cianobactéria (fotobionte) que, geralmente apresenta um grande número de substâncias orgânicas.[1]

Os liquens são bem conhecidos pela diversidade de metabólitos secundários que produzem.[1-2] Esta diversidade despertou o interesse pelo estudo da composição química desses organismos[1], sendo vários desses metabólitos típicos deste grupo e, dentre as diferentes classes, encontram-se substâncias contendo nitrogênio, fósforo e enxofre, polióis, monossacarídeos, ácidos e éteres graxos e hidrocarbonetos alifáticos, *meta*- e *para*- depsideos, depsidonas, dibenzofuranos, difenileter, naftopiranos, bifenis, difenilmetanos, xantonas, quinonas, naftoquinonas e derivados do ácido úsnico. Também ocorrem terpenos, esteroides, terfenilquinonas e derivados do ácido pulvínico.[1,3-4]

O gênero *Ramalina* Ach., família Ramalinaceae, atualmente engloba 246 espécies distribuídas pelo mundo.[4-5] Nas espécies já estudadas desse gênero foi encontrada alta diversidade de substâncias, tanto metabólitos primários quanto secundários. Dentre os metabólitos primários, os carboidratos são os mais presentes, detectando-se também aminoácidos, glicolipídeos, polióis e glicoesfingolipídeos. Dentre os metabólitos secundários o ácido úsnico merece atenção pela alta frequência de citação. Derivados desse ácido (usimine) também foram encontrados,[6-9] além de depsídeos, depsidonas, ácidos graxos e esteróis.

Os metabólicos secundários isolados de línquens apresentam uma gama de propriedades biológicas e farmacológicas, destacando atividade fotoprotetora,[10] antitumoral,[11-16] antibacteriana,[13-14,17-20] anti-Leishmania,[10] anti-inflamatória,[21,23] antifungica,[18-20] antioxidante,[13-14,22,24-25] antiviral,[26-27] inseticida,[28] e antibiótica.[29] O trabalho de isolamento e purificação de metabólitos secundários vem contribuindo de forma significativa para a sistemática e quimiotaxonomia dos fungos liquenizados.

Uma das substâncias mais conhecidas, isolada e trabalhada de fungos liquenizados é o ácido úsnico (Figura 1), isolado pela primeira vez, em 1834 por Rochleder e colaboradores, proveniente da espécie *R. calicaris* (L.) Röhl.[30-

[31] No entanto, todas as espécies do gênero *Ramalina* contêm ácido úsnico em concentração variável.[4,32] Estudos de isolamento e de testes de atividade biológica dessa substância apresentam, quase sempre, resultados surpreendentes, gerando inúmeras publicações.[5,8,19,27,31,33-40] Segundo Francolini *et. al.* (2004) os líquens produzem antibióticos, inclusive, o ácido úsnico, para se protegerem de bactérias.[41]

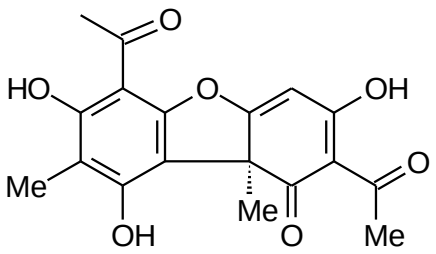


Figura1. Estrutura química da substância ácido úsnico

A atividade antibiótica de muitos líquens tem sido atribuída ao ácido úsnico,[20] embora existam autores que mencionem que a atividade antibiótica dos líquens está relacionada à presença de derivados fenólicos.[30] Os mecanismos da ação antibiótica de ácidos liquênicos, mais precisamente do ácido úsnico e seus derivados, sugerem que essas substâncias modifiquem a biogênese de proteínas causando alterações irreversíveis, podendo até mesmo produzir apoptose.[30] Essa substância apresenta atividade contra bactérias, fungos e leveduras.[1,2,19,27,30,42-44]

O fenômeno de resistência bacteriana aos fármacos é um fato e motivo de grande preocupação em todo o mundo. Os antibióticos utilizados no tratamento de várias doenças, entre eles os β-lactâmicos, que atuam sobre a parede de bactérias, entre elas *Staphylococcus aureus* e outras espécies do gênero, de forma inesperada podem acarretar o surgimento de indivíduos resistentes. Em *Escherichia coli*, um dos mecanismos alternativos que esta bactéria utiliza para dar origem a clones resistentes é o sistema de resposta SOS. Os antibióticos que interferem na replicação de DNA e na viabilidade da célula bacteriana ativam o sistema de resposta SOS.[45] Em *S. aureus*, a resposta induzida por SOS promove a replicação e a transferência horizontal em alta frequência, de fatores de virulência, codificados por ilhas de patogeniciadade. Este fenômeno envolve a ativação das proteínas RecA e

LexA, os principais reguladores do sistema de reposta SOS. Drogas comumente utilizadas, como os antibióticos fluoroquinolonas e β-lactâmicos podem induzir a replicação de ilhas de patogenicidade estafilocócicas mostrando que estes antibióticos podem ter uma consequência indesejada de promover o surgimento e disseminação de fatores de virulência nestas bactérias.[46]

Estudos da química, estrutura e atividade biológica sobre as comunidades liquênicas brasileiras são escassos, além de terem sido realizados a cerca de vinte anos, como é o caso de *R. usnea* realizado por Kashiwadani e Kalb[47].

Desta forma, o presente trabalho teve como objetivo a avaliação da atividade antimicrobiana do extrato metanólico, suas frações e do ácido úsnico, isolado do líquen *R. usnea* sobre o crescimento *in vitro* de bactérias Gram-positivas, Gram-negativas e fungos do gênero *Candida*.

2. Experimental

2.1. Equipamentos e Reagentes

Os procedimentos cromatográficos foram conduzidos em colunas de vidro utilizando gel de sílica 60 (0.063–0.200 mm).

Os experimentos de Ressonância Magnética Nuclear de ^{1}H e ^{13}C-RMN foram realizados em um espectrômetro Bruker Utrashield 500 Plus, com janela espectral de 500 (^{1}H) e 125 (^{13}C) MHz. $CDCl_3$ foi utilizado como solvente deuterado com TMS como padrão interno. Os deslocamentos químicos (δ) foram medidos em (ppm) e as constantes de acoplamento (J) em Hz.

Para análise do potencial antimicrobiano foram utilizados meios de cultura Agar e caldo Müeller-Hinton para bactérias e Agar e caldo Sabouraud para fungos ambos da Himedia®.

2.2. Coleta e identificação de *Ramalina usnea.*

O líquen *R. usnea* foi coletado na restinga de Iquipari, litoral de Gruçaí no município de São João da Barra - RJ. A espécie foi identificada pelo Dr. Michel Navarro Benatti do Instituto de Botânica de São Paulo. A exsicata

analisada encontra-se depositada no herbário da Universidade Estadual do Norte Fluminense Darcy Ribeiro com registro LI-0001.

2.3. Extração, obtenção das frações e isolamento dos compostos

O material referente ao fungo liquenizados foi seco, triturado (550 g) e submetido à maceração exaustiva com metanol. O extrato foi concentrado em um destilador sob pressão reduzida fornecendo 60 g de extrato.

O extrato metanólico (35 g) foi submetido a uma cromatografia em coluna com gel de sílica, eluida com hexano e diclorometano em modo gradiente de polaridade até diclorometano 100%, e resultou em 8 frações após união das frações por TLC, que foram denominadas como LM-1 a LM-8. A fração LM-6 (2,82 g) após ser submetida a processos cromatográficos e reunidas as frações por TLC produziu 6 frações. A fração LM6-2 foi cromatografada por processo semelhante e gerou 10 frações após mistura, e a fração LM6-2-7 forneceu 149 mg do ácido úsnico. O ácido úsnico foi identificado através das análises espectroscópicas de RMN de ^{1}H e ^{13}C, espectrometria de Massas de Baixa Resolução-EMBR, e com comparação com dados da literatura. A fração LM-7 foi submetida à cromatografia em coluna com gel de sílica usando como eluentes C_6H_6, C_6H_6 + CH_2Cl_2 e CH_2Cl_2 em modo gradiente de polaridade até diclorometano puro, que após submetidas a CCDA para análise do perfil cromatográfico foram agrupadas gerando 8 frações. A fração LM7-5 (21 a 25) foi cromatografada por processo semelhante produzindo 197 frações que após união por TLC geraram 24 frações. As frações que apresentaram massa superior a 350 mg foram submetidas a avaliação do potencial antimicrobiano (Tabelas 1, 2 e 3). Os espectros de RMN de ^{1}H 3 ^{13}C mono e bi- dimensionais, o espectro de massas e o cromatograma (CG/EM) serão apresentados no material suplementar no final do artigo.

2.4. Avaliação do Potencial antimicrobiano

2.4.1. Material biológico

As bactérias utilizadas no experimento foram *Staphylococcus aureus* ATCC 33591, ATCC 25923, 66 (cepa clínica) e *Burkholderia cepacia* ATCC 25416. Os fungos foram *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 28707 e *C. kefyr* ATCC 4135.

2.4.2. Método de difusão em ágar

O potencial antimicrobiano do extrato metanólico, das frações LM7-18, LM7-26, LM7-21-1, LM7a-107, LM7-21-39, LM7a-14, e do ácido úsnico foi avaliado em diferentes concentrações (Tabela 1) utilizando o protocolo baseado no método de Hufford et al (1975).[48]

O inóculo foi preparado a partir de colônias de bactérias e fungos crescidos em placa de Petri por 12 horas em estufa modelo V-100R (Visomes Plus) a 37 °C para bactérias e 30 °C para fungos.

As suspensões bacterianas foram padronizadas em solução salina (0,85 %) estéril lidas em um espectrofotômetro com leitura em densidade óptica a 550 nm (Densimat, BioMérieux) obtendo-se a padronização de 0,1 % para uma concentração de 10^6 CFU/mL (unidades formadoras de colônias), utilizando a escala de McFarland. Em seguida, 100 µL destas suspensões foram depositadas com auxílio de swab (Venturi, Transystem, Copan Innovation) estéril sobre as superfícies das placas de Petri de 10 cm, contendo 20 mL de meio Müeller-Hinton (Himedia®) para bactérias e ágar Sabouraud para fungos (Himedia®). Após 5 minutos para secagem dos inóculos bacteriano e das leveduras, foram feitos orifícios de 6 mm de diâmetro, com auxílio de um perfurador cilíndrico de aço inox. Em um dos orifícios foi colocado 50 µL do antibiótico gentamicina na concentração de 200 µg/mL para as bactérias e do antifúngico miconazol na concentração de 1000 µg/mL para os fungos leveduriformes como controles positivos. Nos demais orifícios foram depositados 50 µL das amostras testadas, frações, extrato bruto e ácido úsnico nas concentrações apresentadas na Tabela 1, e 50 µL de DMSO como controle negativo para as bactérias. Após a incubação das placas, por 12 h, em estufa a 37 °C, o resultado da susceptibilidade aos produtos foi expresso em termos de tamanho de diâmetro, em milímetros, dos halos de inibição do crescimento dos microorganismos medidos com auxílio de um paquímetro e transformado em taxa de mortalidade expressos em porcentagem. Os resultados do teste de susceptibilidade foram interpretados de acordo com a tabela de zonas de inibição baseada em *Performance Standards** for Antimicrobial Disck Suscetibility Tests* (Laborclin, Paraná, Brasil).

2.4.3. Método em meio líquido

Este ensaio seguiu a metodologia proposta por Balaban e colaboradores.[49] A análise foi realizada utilizando-se 170 µL do meio de cultura, 20 µL do inóculo e 10 µL de amostra (extrato, frações e do ácido úsnico) e o tratamento controle negativo com 180 µL de meio de cultura e 20 µL de inoculo. O inóculo foi preparado como descrito anteriormente. Em seguida uma colônia foi retirada da placa e colocada em 2 mL de solução salina (0,85 %0 e padronizado em densimat. Os tratamentos ficaram em estufa a 37 ºC e 30 ºC para bactérias e fungos, respectivamente. Após o período de incubação, 50 µL das suspensões das suspensões dos inóculos microbianos cultivados com as amostras e o controle foram semeados na superfície do meio de cultura, com o auxílio de swabs estéreis. Em seguida foram incubados por 12 horas. Todos os experimentos foram realizados em triplicata. Após 24 horas foi avaliado o crescimento das colônias bacterianas e das leveduras, observando-se a concentração mínima inibitória (CMI), ou seja, o crescimento das colônias na faixa de 30 a 300 colônias e avaliado como efeito de inibição do crescimento das colônias. Nas placas com crescimento microbiano, utilizou-se um contador de colônias CP 600 modelo Phoenix. Este procedimento conduziu as amostras testadas a concentrações finais apresentadas nas tabelas 2 e 3.

3. Resultados e Discussão

3.1 Ensaios em meio sólido

Nos ensaios realizados com as amostras de *Ramalina usnea* em meio sólido com três linhagens ATCC de bactérias *Staphylococcus aureus* e *Burkholderia cepacia* ATCC 25416, houve presença de inibição semelhante ao do controle gentamicina com o extrato metanólico para *S. aureus* ATCC-33591, mas numa concentração bem superior. As frações testadas para essa linhagem LM7-18 e LM7-26 apresentaram boa inibição. Para as linhagens ATCC 25923 e 66- cepa clínica, entre as frações, a melhor inibição foi da fração LM7-21-39. O mesmo ocorreu frente à bactéria *B. cepacia,* em que todas as amostras testadas tiveram inibição menor que o do controle, mas a fração LM7-21-39

apresentou melhor inibição. O ácido úsnico, não produziu inibição para nenhuma bactéria, por isso não foram realizados testes em meio líquido.

Tabela 1: Porcentagem de inibição (%) com as bactérias *S. aureus* ATCC (33591, 25923 e 66 – cepa clínica) e *B. cepacia* ATCC 25416 utilizando extrato metanólico, frações e ácido úsnico obtidos do fungo liquenizado *Ramalina usnea* e controle.

Nome	*S. aureus* ATCC			*B. cepacea* ATCC	Concentração
	33591	25923	66-Cepa clínica	25416	(µg/mL)
Ext. MeOH	100	74	86	79	12.000
LM7-18	90	*	*	*	4.000
LM7-26	90	64	65	76	11.000
LM7-21-39	*	66	68	77	800
LM7-21-1	*	55	58	67	800
LM7a-107	*	59	59	65	1.700
Ác. úsnico	0,0	0,0	0,0	0,0	1.200, 1.500, 1.600
DMSO	0,0	0,0	0,0	0,0	P.A
Gentamicina	100	100	100	100	200

*Amostras não testadas

3.2 Ensaios em meio líquido

Na tabela 2 são apresentados os dados de inibição dos ensaios realizados em meio líquido com as bactérias *S. aureus* ATCC 33591 e *B. cepacia* ATCC 25416 com o extrato MeOH e frações. Observa-se que entre as amostras testadas com *S. aureus* a melhor inibição ocorreu com a fração LM7-21-39 com concentração de 40 µg/mL, inibiu 100% das colônias. Com a bactéria *B. cepacea*, as duas amostras testadas inibiram 100% das colônias, mas a fração LM7-21-1 inibiu com a metade da concentração da fração LM7a-107.

Tabela 2: Dados do efeito de inibição em meio líquido com as bactérias *S. aureus* ATCC 33591 e *B. cepacea* ATCC 25416 com o extrato metanólico e frações do fungo liquenizado *Ramalina usnea* e controles.

Nome	*S. aureus* ATCC 33591	*B. cepacea* ATCC 25416	Conc. (µg/mL)
Ext. MeOH	100%	*	570
Ext. MeOH	100%	*	120
Ext. MeOH	+ 300 colônias	*	60
LM7-26	100%	*	570
LM7-26	100%	*	170
LM7-26	+ 300 colônias	*	60
LM7-21-39	100%	*	40
LM7-21-39	Não inibiu	*	20
LM7a-107	*	100%	80
LM7-21-1	*	100%	40
DMSO	Não inibiu	Não inibiu	P.A
Gentamicina	100%	100%	10

*Amostras não testadas

É importante destacar que as amostras que apresentaram resultado como +300 colônias, significa que na contagem do número de colônias houve crescimento de um número maior que 300 colônias, tendo a amostra com esse tipo de resultado pouca inibição nessa concentração testada. O ideal é que fique entre 30 e 300 colônias para se obter o valor da concentração mínima inibitória (CMI).

Os experimentos realizados com os fungos, *Candida albicans* ATCC 10231 e *Candida glabrata* foram testados com cinco frações: LM7-21-39, LM7-26, LM7-18, LM7-21-1 e LM7a-107 e o extrato MeOH, mas não houve inibição com nenhuma dessas amostras. Enquanto que com *Candida tropicalis* ATCC 28707 e *Candida kefyr* ATCC 4135, foram testadas somente com o ácido úsnico em diferentes concentrações. Observou-se que essas leveduras foram bastante sensíveis ao ácido úsnico, já que *C. tropicalis* teve inibição total até 15 µg/mL de concentração. É possível notar que a concentração mínima inibitória para esta bactéria deve encontrar-se entre 10 e 15 µg/mL, enquanto que para *C. kefyr* a CMI deve encontrar-se entre 40 e 60 µg/mL (Tabela 3). Os experimentos já realizados para encontrar a CMI de *C. kefyr* encontrou o valor de 38,5 µg/mL.

Tabela 3: Dados de inibição dos fungos *C. albicans* ATCC 10231, *C. tropicalis* ATCC 28707 e *C. kefyr* ATCC 4135 com o extrato, frações e o ácido úsnico do fungo liquenizados *Ramalina usnea*

Nome	*C. albicans* ATCC 10231	*C. tropicalis* ATCC 28707	*C. kefyr* ATCC 4135	Conc. (µg/mL)
Ext. MeOH	Não inibiu	*	*	570
LM7-26	Não inibiu	*	*	570
LM7-21-39	Não inibiu	*	*	40
LM7-18	Não inibiu	*	*	200
LM7a-107	Não inibiu	*	*	80
LM7-21-1	Não inibiu	*	*	40
Ác. úsnico	*	100%	100%	80
Ác. úsnico	*	100%	100%	75
Ác. úsnico	*	100%	100%	60
Ác. úsnico	*	*	+300 colônias	40
Ác. úsnico	*	100%		15
Ác. úsnico	*	+ 300 colônias		10
DMSO	Não inibiu	Não inibiu	Não inibiu	P.A
Miconazol	100%	100%	100%	10

*Amostra não testada

O trabalho realizado por Eisimone e Adikwu[37] mostrou a atividade antimicrobiana de três extratos orgânicos (EtOH, $CHCl_3$ e *n*-hexano) do líquen *R. farinacea* contra quatro bactérias, duas Gram (+) *S. aureus* e *Bacillus subtilis* e duas Gram (-) *Pseudomonas aeruginosa* e *Salmonella typhimurium* e três fungos, *C. albicans*, *Trychophyton rubrum* e *Trychophyton mentagrophytes* (C.P.Robin) Sabour. Todos os extratos inibiram todos os microrganismos. A concentração mínima inibitória dos extratos variou entre 159 e 641,81 µg/mL para as bactérias e entre 161,64 e 190,66 µg/mL para os fungos, ambos para o extrato etanólico.

Falcão e colaboradores[17] estudaram o efeito antimicrobiano do líquen *Heterodermia leucomelo* (L.) Poelt obtendo inibição contra as bactérias Gram (+) *S. aureus* e *B. subtilis* e duas espécies de fungos leveduriformes do gênero *Candida*, *C. parapsilosis* (Ashford) Langeron & Talice e *C. albicans*, porém não encontraram efeito de inibição nas Gram (-) *E. coli*, *Klebsiella pneumoniae* e *P. aeruginosa.*

Tay e colaboradores[19] avaliaram a atividade antimicrobiana do extrato em acetona e de três substâncias, os ácidos (+)- úsnico, norstítico e protocetrárico do líquen *R. farinacea* contra 13 bactérias, sendo oito Gram (-) e cinco Gram (+) e dois fungos, *C. albicans* e *C. glabrata*. Das bactérias Gram (-) *Yersinia enterocolitica* e *Proteus vulgaris* foram inibidas pelo extrato e pelo ácido úsnico, enquanto o ácido norstítico inibiu *Aeromonas hydrophila* e *P.*

vulgaris. Entre as bactérias Gram (+) *B. subtilis*, *Listeria monocytogenes*, *S. aureus* e *Streptococcus faecalis* foram inibidas pelo extrato e pelos ácidos úsnico e norstitico. Somente *B. cereus* não foi inibida por nenhuma amostra testada. Os fungos foram inibidos por todas as amostras testadas. O ácido protocetrárico não inibiu nenhuma bactéria.

Cansaran e colaboradores[20] analisaram o ácido úsnico de cinco espécies de líquens do gênero *Ramalina* por HPLC e investigaram a atividade antimicrobiana dos extratos em acetona dessas espécies contra três bactérias Gram (-), *Proteus mirabilis, P. aeruginosa* e *E. coli* e quatro Gram (+), *Enterococcus faecalis*, *S. aureus*, *B. subtilis* e *B. megaterium*. Os dados mostraram que entre as Gram (-), *P. aeruginosa* não foi inibida por nenhum dos extratos testados, por outro lado *P. mirabilis* e *E. coli* foram inibidas por extratos de duas e três espécies de *Ramalina*, respectivamente. Entre as bactérias Gram (+), *S. aureus* não foi inibida por nenhum extrato, *B. subtilis* foi inibida por todos os extratos, *B. megaterium* só não foi inibida pelo extrato de *R. fraxinea* e *E. faecalis* não foi inibida pelas espécies *R. polymorpha*, *R. fraxinea* e *R. pollinaria*.

Os dados descritos na literatura sustentam e corroboram alguns dos resultados obtidos nessa pesquisa. Da análise do extrato MeOH, frações e do ácido úsnico frente aos microrganismos testados, verifica-se que o extrato MeOH e as frações inibiram as linhagens das bactérias *S. aureus* e também *B. cepacea* nos testes em meio sólido. Entretanto o ácido úsnico não inibiu nenhuma das bactérias testadas em nenhuma das concentrações, diferente dos resultados obtidos por Tay,[19] em que o ácido úsnico inibiu *S. aureus* numa concentração de 50 µg/mL. Lauterwein[50] também menciona que o ácido úsnico não inibe fungos, enquanto os resultados obtidos nessa pesquisa e de outros estudos, como Tay *et al*,[19] Esimone e Adikwu[37] e Falcão e colaboradores[17] mostram o contrário. Logo, observa-se que não existe uma conformidade nos resultados de todas as pesquisas tanto para os testes com bactérias quanto com fungos.

4. Conclusões

Os resultados obtidos em meio líquido foram significativamente melhores do que os do meio sólido. Para *S. aureus* observa-se que a concentração mínima inibitória (CMI) do extrato metanólico deve encontra-se entre 60 e 120 µg/mL, a da fração LM7-26 entre 60 e 170 µg/mL e a da fração LM7-21-39 entre 20 e 40 µg/mL, apresentando boa atividade para esse microorganismo.

Para a bactéria *B. cepacea* as frações testadas LM7a-107 e LM7-21-1 também obtiveram um ótimo resultado, com 100% de inibição em baixa concentração, 80 e 40 µg/mL, respectivamente .

Com o fungo *C. albicans* aconteceu o contrário, nenhuma fração e nem o extrato bruto produziu inibição. O ácido úsnico inibiu totalmente *C. tropicalis* a 15 µg/mL e *C. kefyr* a 60 µg/mL, onde a concentração mínima inibitória (CMI) encontra-se entre 40-60 µg/mL para *C. kefyr* e 10-15 µg/mL para *C. tropicalis*. Algumas das frações que apresentaram elevado potencial antibacteriano como a LM7-21-39 já foi trabalhada para promover o isolamento e identificação dos metabólitos responsáveis pela atividade. Os resultados serão apresentados em artigos que serão publicados posteriormente. O extrato do líquen *Ramalina usnea*, as frações testadas e o composto isolado ácido úsnico, como outras substâncias isoladas e espécies do gênero *Ramalina* mostraram ter um ótimo potencial como agente antimicrobiano.

Agradecimentos

Somos muito grato ao Dr Michell Navarro Benatti, curador do herbário do Instituto de Botânica de São Paulo, pela identificação do líquen, o que tornou essa pesquisa viável.

Os autores também agradecem à Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) pela subvenção e uma bolsa de pesquisa, e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) pelas bolsas de pesquisa.

Referências

[1] Huneck, S., Yoshimura, I. Identification of Lichen Substances, Springer-Verlag Berlin Heidelberg, 1996.

[2] Feige, G. B.; Lumbsch, H. T. Identification of lichen substances by a standardized higt-performance liquid chromatographic method. *Journal of Chromatography* **1993**, *647*, 417.[CrossRef]

[3] Oksanen, I. Ecological and biotechnological aspects of lichens. *Applied Microbiology Biotechnology.* **2006**, *73*, 723. [CrossRef]

[4] Parrot, D.; Jan, S.; Baert, N.; Guyot, S.; Tomasi, S. Comparative metabolite profiling and chemical study of Ramalina siliquosa complex using LC-ESI-MS/MS approach. *Phytochemistry* **2013**, *89*, 114. [CrossRef]

[5] Kirk, P. M.; Cannon, P. F.; Minter, D. W.; Stalpers, J. A. Dictionary of the fungi, Europe-UK , 2008.

[6] Lee, S. G.; Koh, H. Y.; Oh, H.; Han, S. J.; Kim, I. C.; Lee, H. K.; Yim, J. H. Human dermal fibroblast proliferation activity of usimine-C from Antarctic lichen *Ramalina terebrata*. *Biotecnology Letters.* **2010**, *32*, 471. [CrossRef]

[7] Paudel, B.; Bhattarai, H. D.; Koh, H. Y.; Lee, S. G.; Han, S. J.; Lee, H. K.; Oh, H.; Shin, H. W.; Yim, J. H. Ramalin, a novel nontoxic antioxidant compound from the Antarctic lichen *Ramalina terebrata*. *Phytomedicine* **2011**, *18*, 1285. [CrossRef]

[8] Yim, J. H.; Lee, H. K.; Bhattarai, H. D.; Babita, P.; Kim, I. C.; Hong, S. G.; Kim, D. K.; Lee, Y. K.; Gu, S.; Oh, H. C. Antioxidant activities of ramalin isolated from *Ramalina terebrata* extracts. PCT Int. Appl. WO 20100533327 A2 20100514 **2010**.

[9] Yim, J. H.; Kim, I. C.; Lee, S. G.; Kim, D. K.; Han, S. J.; Lee, H. S.; Seok, K. H.; Kim, S. J.; Kim, T. K.; Kang, P. S.; Park, H. Pharmaceutical composition for the treatment of inflammatory disease or immune disease containing ramalin. PCT Int. Appl WO *2011152671 A2 20111208* **2011.**

[10] Urdapilleta, A. A. A. *Dissertação de mestrado*, Universidade de Brasília 2006.[CrossRef]

[11] Shibata, S. *et al*. Polysaccharides in lichens and fungi. I. Antitumor active polysaccharides of *Gyrophora esculenta* Miyoshi and *Lasallia papulosa* (Ach.) Lano. *Chemical and Pharmaceutical Bulletin* **1968**, *16*, 2362. [CrossRef]

[12] Nishikawa, Y. *et al*. Polysaccarides of Lichens and Fungi. IV. Antitumor active *O*-Acetylated Pustulan – Type Glucans from the Lichens of *Umbilicaria* Species. *Chemical and Pharmaceutical Bulletin* **1970**, *18*, 1431. [CrossRef]

[13] Rankovic, B. *et al*. Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata*, *Lecanora atra* and *Lecanora muralis*. *BCM Complementary & Alternative Medicine* Oct 20, 11:97, 2011.

[14] Kosanic, M. *et al*. Antioxidant, antimicrobial and anticancer activities of three *Parmelia species*. *Journal of Science Food Agriculture* **2012**, *92*, 1909.[CrossRef]

[15] Singh, N.; Nambiar, D.; Kale, K. K.; Singh, R. P. Usnic acid inhibits and induces cell cycle arrest and apoptosis in human lung carcinoma A549 cells. *Nutrition Cancer* **2013**, *65*, 36. [CrossRef]

[16] Brandão, L. F. G.; Alcantara, G. B.; Matos, M. F. C.; Bogo, D.; Freitas, D. S.; Oyama, N. M.; Honda, N. K. Cytotoxic evaluation of phenolic compounds from lichens against melanoma cells. *Chemical and Pharmaceutical Bulletin* **2013**, *61*, 176. [CrossRef]

[17] Falcão, E. P. S; Silva, N. H.; Gusmão, N. B; Ribeiro,S. M.; Honda, N. K.; Pereira, E. C. Atividade Antimicrobiana de Compostos Fenólicos do Líquen *Heterodermia leucomela* (L.) Poelt. *Acta Farmeutica Bonaerense* **2002**, *21* 43.

[18] Piovano, P. *et al*. Evaluation of antifungal and antibacterial of aromaatic metabolites from lichens. *Boletín de la Sociedad Chilena de Química* **2002**, *47*, 235.[CrossRef]

[19] Tay, T.; Türk, A. Ö.; Yilmaz, M.; Türk, H.; Kivanç, M. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)- usnic acid, norstictic acid, and protocetraric acid constituents. Zeitschrift für Naturforschung C **2004**, *59*, 384. [CrossRef]

[20] Cansaran, D.; Atakol, O.; Halici, M. G.; Aksoy, A. HPLC analysis of usnic acid in some *Ramalina* species from Anatolia and investigation of their antimicrobial activities. *Pharmaceutical Biology* **2007**, *45*, 77.[CrossRef]

[21] Pereira, E. C. *et al*. Determination of *Teloschites flavicans* (SW) Norm. anti-inflamatory activity. *Pharmacognosy Research*, **2010**, *2*, 205. [CrossRef]

[22] Mitrovic, T. *et al*. Antioxidant, antimicrobial and antiproliferative of five lichen species. *International Journal of Molecular Sciences* **2011**, *12*, 5428. [CrossRef]

[23] Cordeiro, L. M. C.; Oliveira, S. M.; Buchi, D. F.; Iacomini, M. Galactofuranose rich heteropolysaccaride from *Trebouxia* sp., photobiont of the lichen *Ramalina gracilis* and its effect on macrophage activation. *International Journal of Biological Macromolecules* **2008**, *42*, 436. [CrossRef]

[24] Luo, H.; Wei, X.; Yamamoto, Y.; Liu, Y.; Wang, L.; Jung, J. S.; Koh, Y. J.; Hur, J. S. Antioxidant activities of edible lichen *Ramalina conduplicans* and its free radical-scavenging constituents. *Mycoscience* **2010**, *51*, 391. [CrossRef]

[25] Paudel, B.; Bhattarai, H. D.; Koh, H. Y.; Lee, S. G.; Han, S. J.; Lee, H. K.; Oh, H.; Shin, W. Y.; Yim, J. H. Ramalin, a novel nontoxic antioxidant compound from the Antarctic lichen *Ramalina terebrata. Phytomedicine* **2011**, 18, 1285. [CrossRef]

[26] Lai, D., Odimewu, D. C.; Esimone, C.; Grunwald, T.; Proksch, P. Phenolic compounds with *in vitro* activity against respiratory Syncytial virus from the Nigerian lichen *Ramalina farinacea*. *Planta Medica* **2013**, *79*, 1440. [CrossRef]

[27] Fazio, A. T.; Adler, M. T.; Bertoni, M. D.; Sepúlveda, C. S.; Damonte, E. B.; Maier, M. S. Lichen secondary metabolites from the cultured lichen mycobionts of *Teloschistes chrysophthalmus* and *Ramalina celastri* and their antiviral activities. *Zeitschrift für Naturforschung C* **2007**, *62*, 543. [CrossRef]

[28] Cetin, H.; Tufan-Cetin, O.; Turk, A. O.; Tay, T.; Candan, M.; Yanikoglu, A.; Sumbul, H. Insecticidal activity of major lichen compounds, (-)- and (+)- usnic

acid, against the larvae of house mosquito, *Culex pipiens* L. *Parasitology Research* **2012,** *26*, 350.

[29] Boustie, J.; Grube, M. Lichens – a promising source of bioactive secondary metabolites. *Plant Genetic Resources* **2005,** *3*, 273.[CrossRef]

[30] Honda, N. K. e Vilegas, W. A química dos Liquens. *Química Nova* **1999**, *22*, 110. [CrossRef]

[31] Ingólfsdóttir, K. Usnic acid. *Phytochemistry* **2002**, *61*, 729. [CrossRef]

[32] Culberson, W. L. Analysis of chemical and morphological variation in the *Ramalina siliquosa* species complex. *Brittonia* **1967**, *19*, 333. [CrossRef]

[33] Molho, L.; Bodo, B.; Molho, D. L'acide *O*-méthyl-4' norsékikaïque, nouveau meta-depside isole d'um lichen du genre *Ramalina*., *Phytochemistry* **1979**, *18*, 2049. [CrossRef]

[34] Curd, F. H.; Robertson, A. Lichens acids. Part VI. Constituents of *Ramalina scopulorum*. *Journal of the Chemical Society* (Resumed) **1935**, 1379.[CrossRef]

[35] Imshaug, H. A. Typification of *Ramalina usnea* (L.) R. H. Howe. *Lichenologist* **1972**, *5*, 317. [CrossRef]

[36] Shukla, V.; Negi, S.; Rawat, M. S. M.; G. Pant, G.; Nagatsu, A. Chemical study of *Ramalina africana* (Ramalinaceae) from the Garhwal Himalayas. *Biochemical Systematics and Ecology* **2004**, *32*, 449. [CrossRef]

[37] Esimone, C. O.; Adikwu, M. U. Antimicrobial activity and cytotoxicity of *Ramalina farinacea*. *Fitoterapia* **1999**, *70*, 428. [CrossRef]

[38] Yamamoto, Y.; Mizuguchi, R.; Yamada, Y. Tissue cultures of *Usnea rubescens* and *Ramalina yasudae* and production of usnic acid in their cultures. *Agricultural and Biological Chemistry* **1985**, *49*, 3348. [CrossRef]

[39] Bézivin, C.; Tomasi, S.; Lohézic-Le, F.; Boustie, J. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine* **2003**, *10*, 499. [CrossRef]

[40] Hauck, M.; Willenbruch, K.; Leuschner, C. Lichen substances prevent lichens from nutrient deficiency. *Journal of Chemical Ecology* **2009**, *35*, 71. [CrossRef]

[41] Francolini, I.; Norris, P.; Piozzi, A.; Donelli, G.; Stoodley, P. Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. *Antimicrobial Agents and Chemotherapy* **2004**, *48*(11), 4360.

[42] Rundel, P. W. The ecological role of secondary lichen substances. *Biochemical Systematic Ecology* **1978**, *6*, 157.[CrossRef]

[43] Marshak, A.; Barry, G. T.; Craig, L. C. Antibiotic compound isolated from the lichen *Ramalina reticulate*. *Science* (Washington, DC, U.S.) **1947**, *24*, 394. [CrossRef]

[44] Asahina, Y.; Shibata, S. Chemistry of Lichen Substances, A. Asher & CO LTD Vaals – Amsterdam 1971.

[45] Miller, C., Thonsen, L. E., Gaggero, C., Mosseri, R., Ingmer, H., Cohen, S.N. SOS response induction by β-lactams and bacterial defense against antibiotic lethality. Science **2004**, *305*, 1629.

[46] Maiques, E., Ubeda, C., Campoy, S., Salvador, N., Lasa, I., Novick, R.P., Barbe, J., Penades, J.R. β-Lactam antibiotics induce the SOS response and horizontal transfer of virulence factors in *Staphylococcus aureus. J. Bacteriol*. **2006**, *188*(7), 2726.

[47] Kashiwadani, H.; Kalb, K. The genus *Ramalina* in Brazil. *The Lichenologist* **1993,** *25*, 1. [CrossRef] [CrossRef]

[48] Hufford, C. D., Funderbuck, M. J., Morgan, J. M., and Robertson, L. W. (1975). Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera* L. *J. Pharm. Sci.* **1975**, *64*, 789.

[49] Balaban, N., Collins, L. V., Cullor, J. S., Hume, E. B., Medina-Acosta, E., Vieira da Motta, O., O Callaghan, R., Rossitto, P. V., Shirtliff, M. E., Serafim da Silveira, L., Tarkowski, A., Torres, J. V ;. Prevention of diseases caused by Staphylococcus aureus using the peptide RIP. *Peptides* **2000**, *21*(9) 1301.

[50] Lauterwein, M.; Oethinger, M.; Belsner, K.; Peters, T.; Marre, R. In vitro activities of the lichen secondary metabolites vulpinic acid, (+)- usnic acid, and (-)- usnic acid against aerobic and anaerobic microorganisms. *Antimicrobial Agents and Chemotherapy*, **1995**, *39,* 2541. [CrossRef]

MATERIAL SUPLEMENTAR

Table 1. Dados das análises espectroscópicas de RMN - ^{1}H e ^{13}C mono e bi-dimensionais ($^{n}J_{CH}$, n=1, 2, and 3) do ácido úsnico em $CDCl_3$, com deslocamentos químicos (δ) em ppm e cosntantes de acoplamentos (J) em Hertz

LM6-2-7: Usnic acid				
	HSQC		HMBC	
C	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$
1	198.1	-		3H-10
2	105.2	-		H-4; 3H-12
3	191.8	-	H-4	
4a	179.3	-	H-4	3H-10
5[a]	155.2	-		
6	101.5	-		HO-7; 3H-15
7	163.9	-	HO-7	3H-13
8	109.3	-	3H-13	HO-7; HO-9
9	157.5	-	HO-9	3H-13
9[a]	104.0	-		HO-9; 3H-10
9b	59.1	-	3H-10	H-4
11	201.8	-	3H-12	
14	200.4	-	3H-15	
CH				
4	98.4	6.05 (s)		
CH_3				
10	31.9	1.79 (s)		
12	27.9	2.68 (s)		
13	7.9	2.08 (s)		
15	29.4	2.71 (s)		
HO				
7	-	13.34 (s)		
9	-	11.06 (s)		

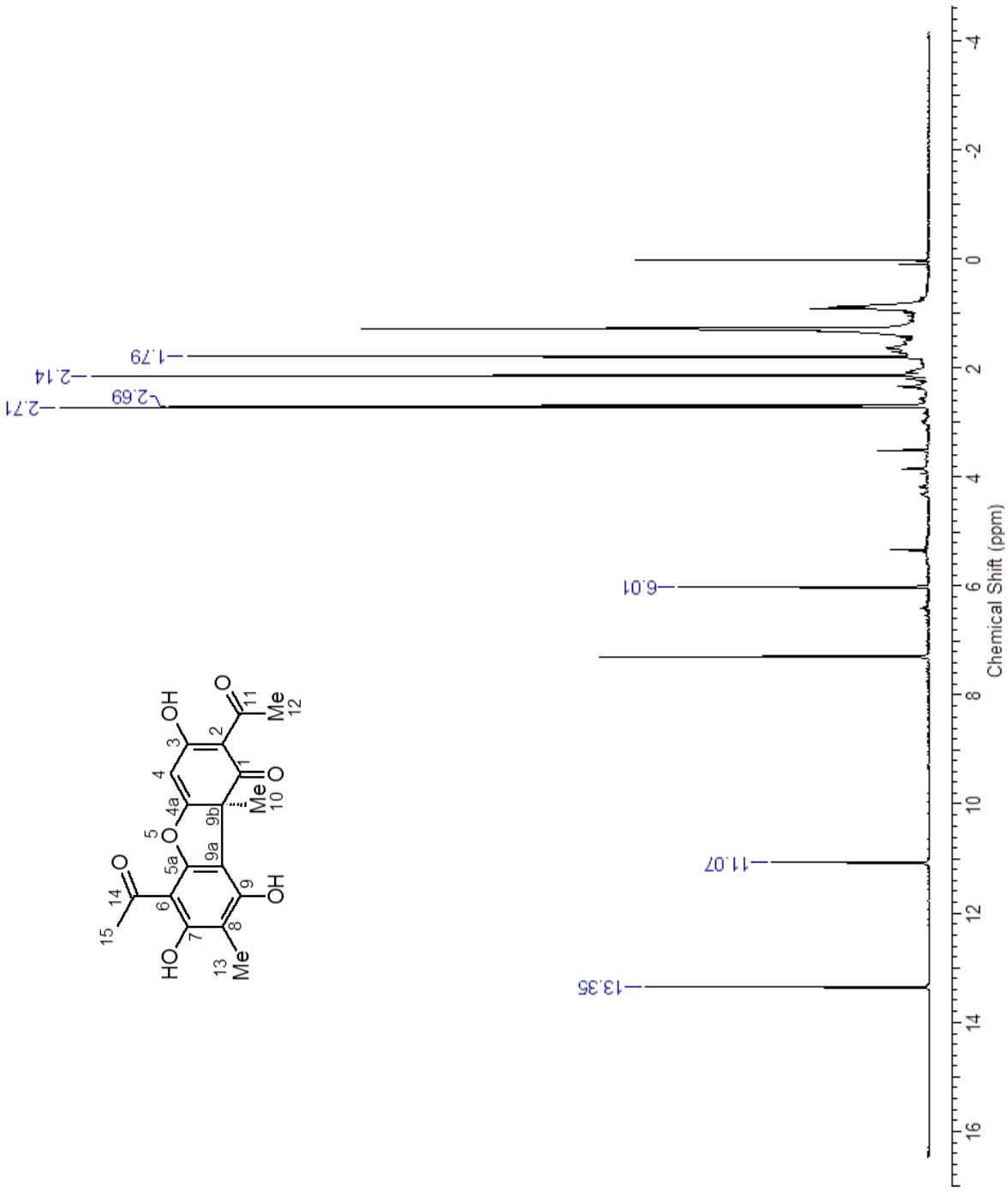


Figura 1. Espectro de RMN de ^{1}H (500 MHz, $CDCl_3$) do composto ácido úsnico.

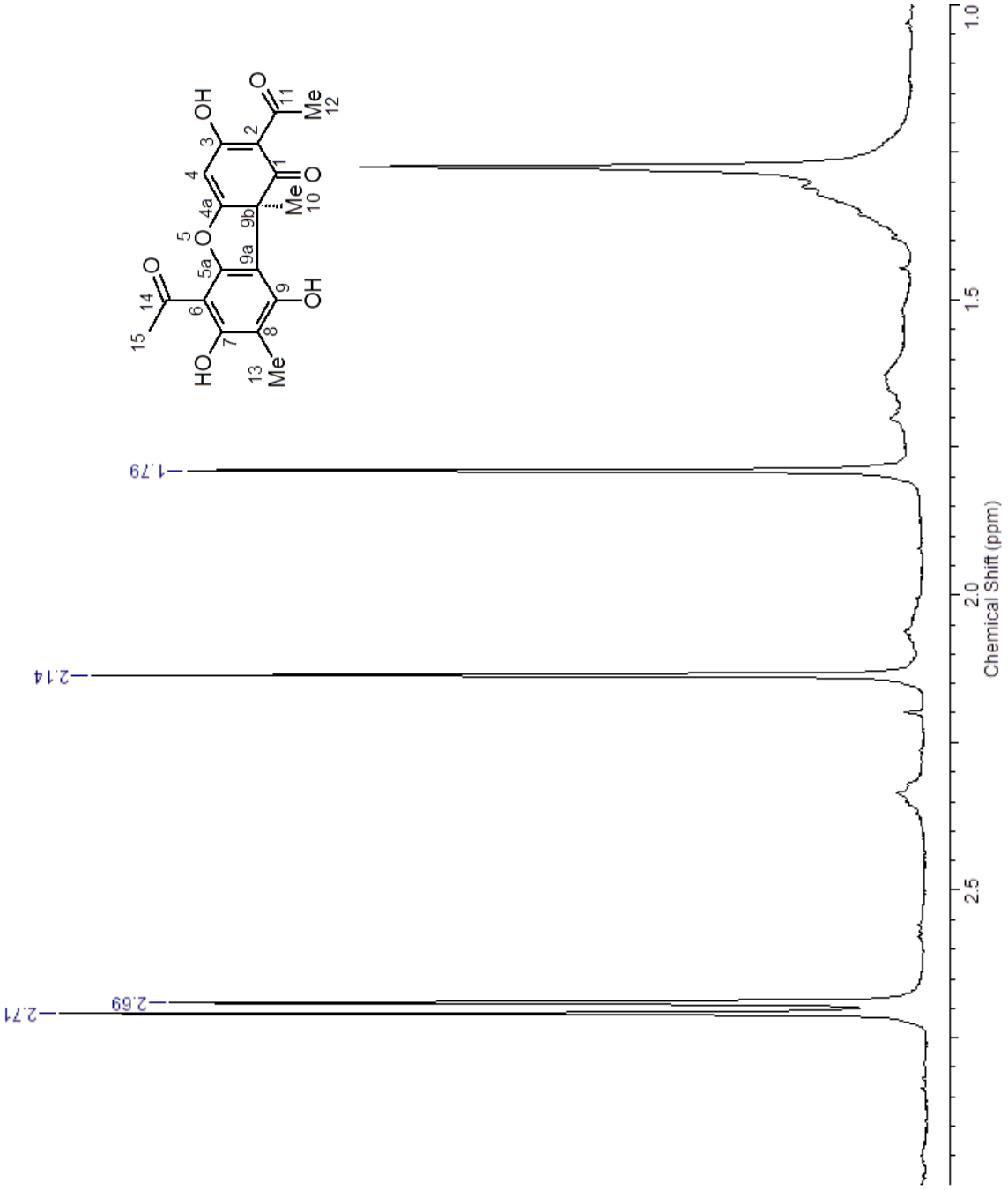


Figura 2. Espectro de RMN de ^{1}H (500 MHz, $CDCl_3$) do composto ácido úsnico. Expansão da região entre 1.0 - 2.95 ppm

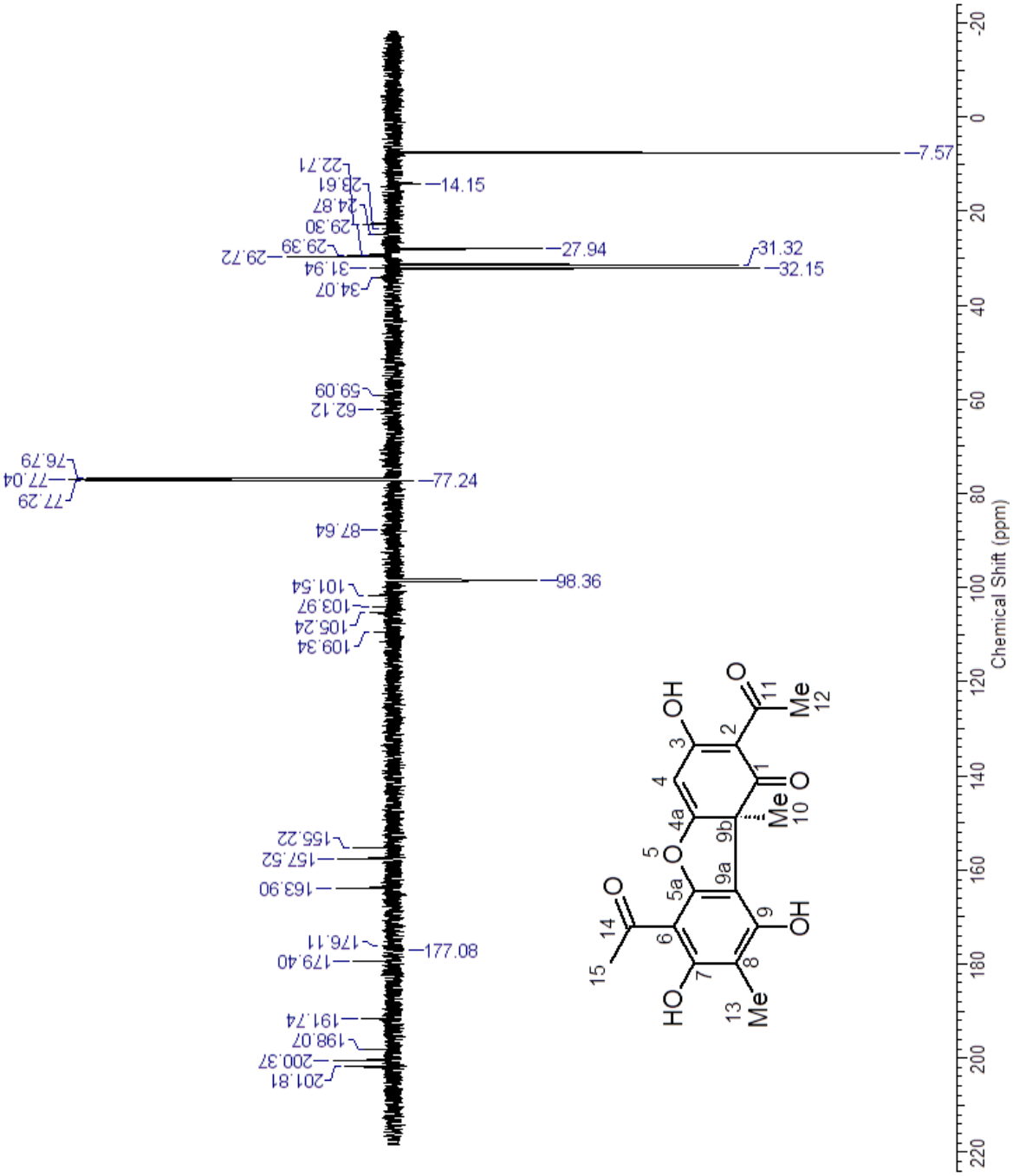


Figura 3. Espetro de RMN de ^{13}C (DEPTQ, 125 MHz, $CDCl_3$) do composto ácido úsnico.

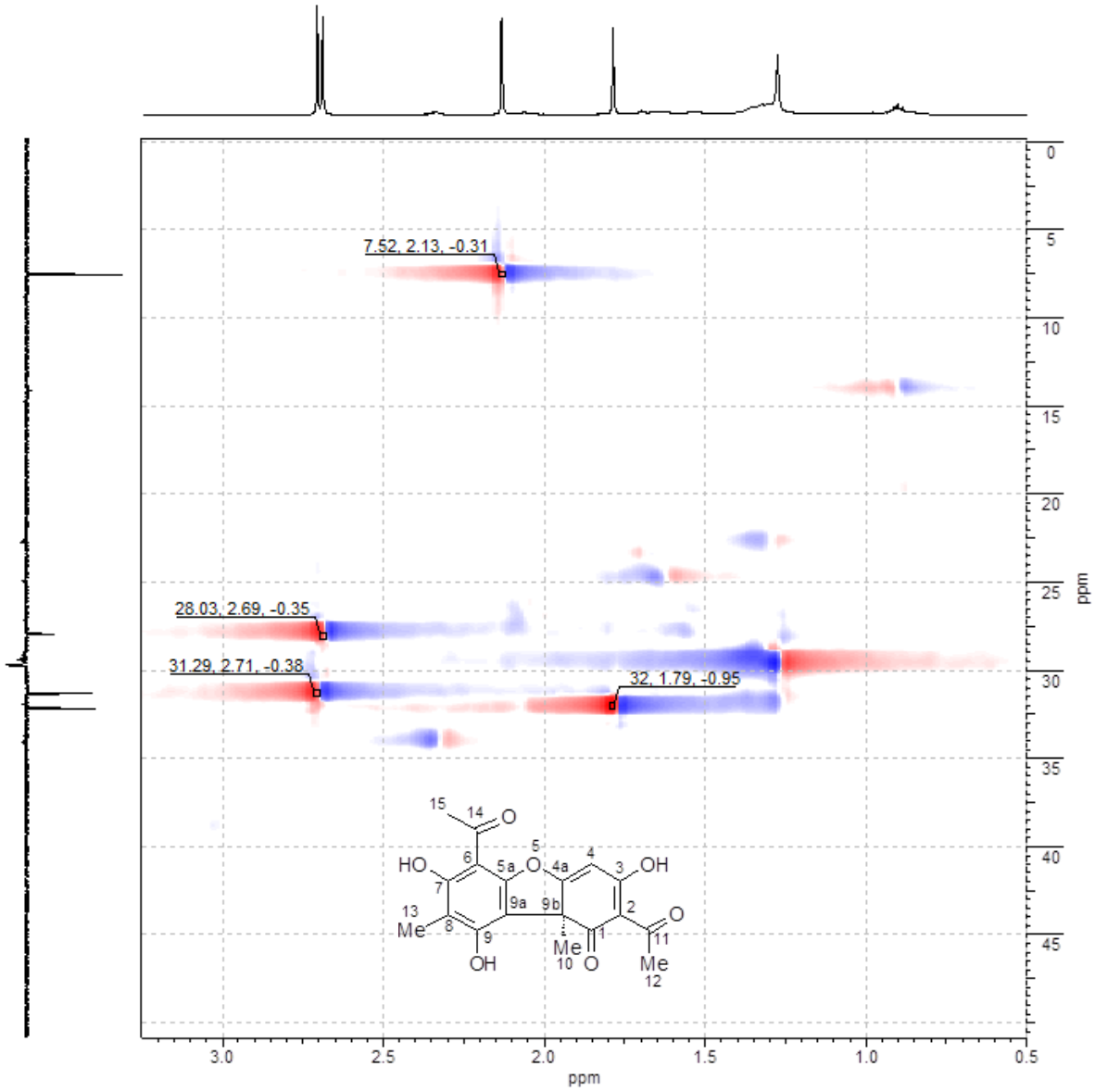


Figura 4. Mapa de correlação heteronuclear bidimensional ^{1}H-^{13}C (HSQC, 500 MHz, $CDCl_3$) do compost ácido úsnico.

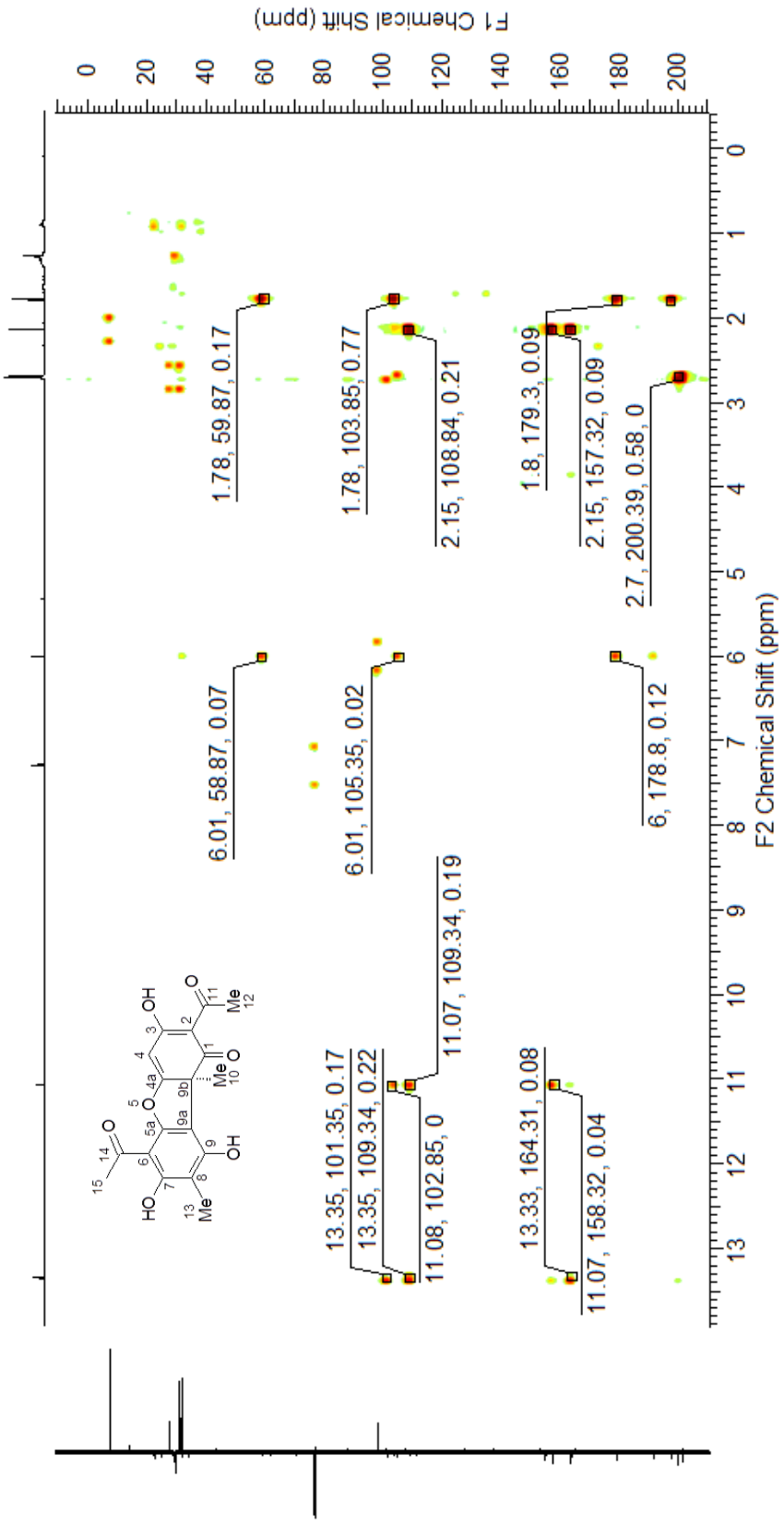


Figura 5. Mapa de correlação heteronuclear bidimensional ^{1}H-^{13}C (HMBC, 500 MHz, $CDCl_3$) co compost ácido úsnico.

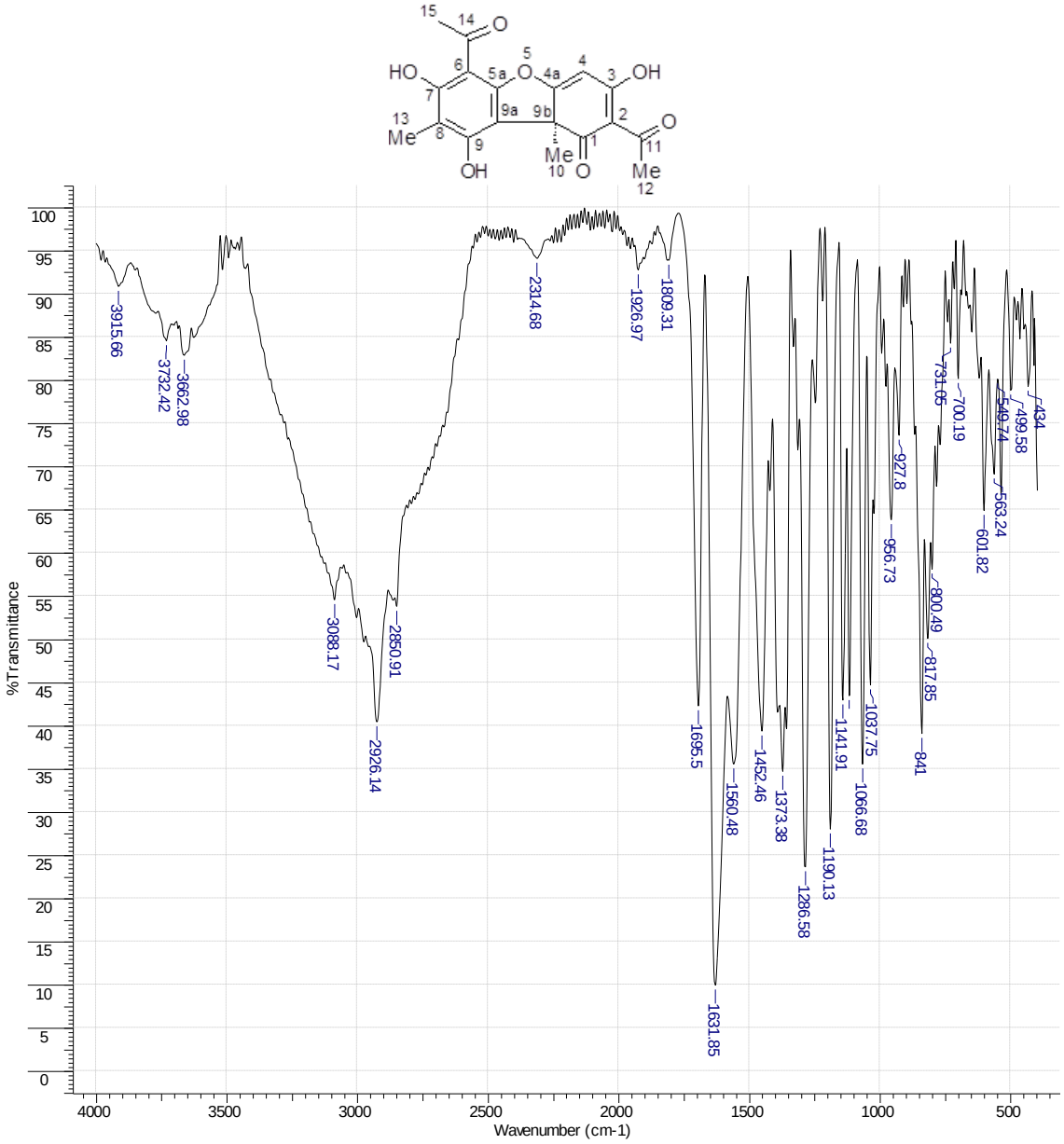


Figura 6. Espectro de infravermelho do composto ácido úsnico.

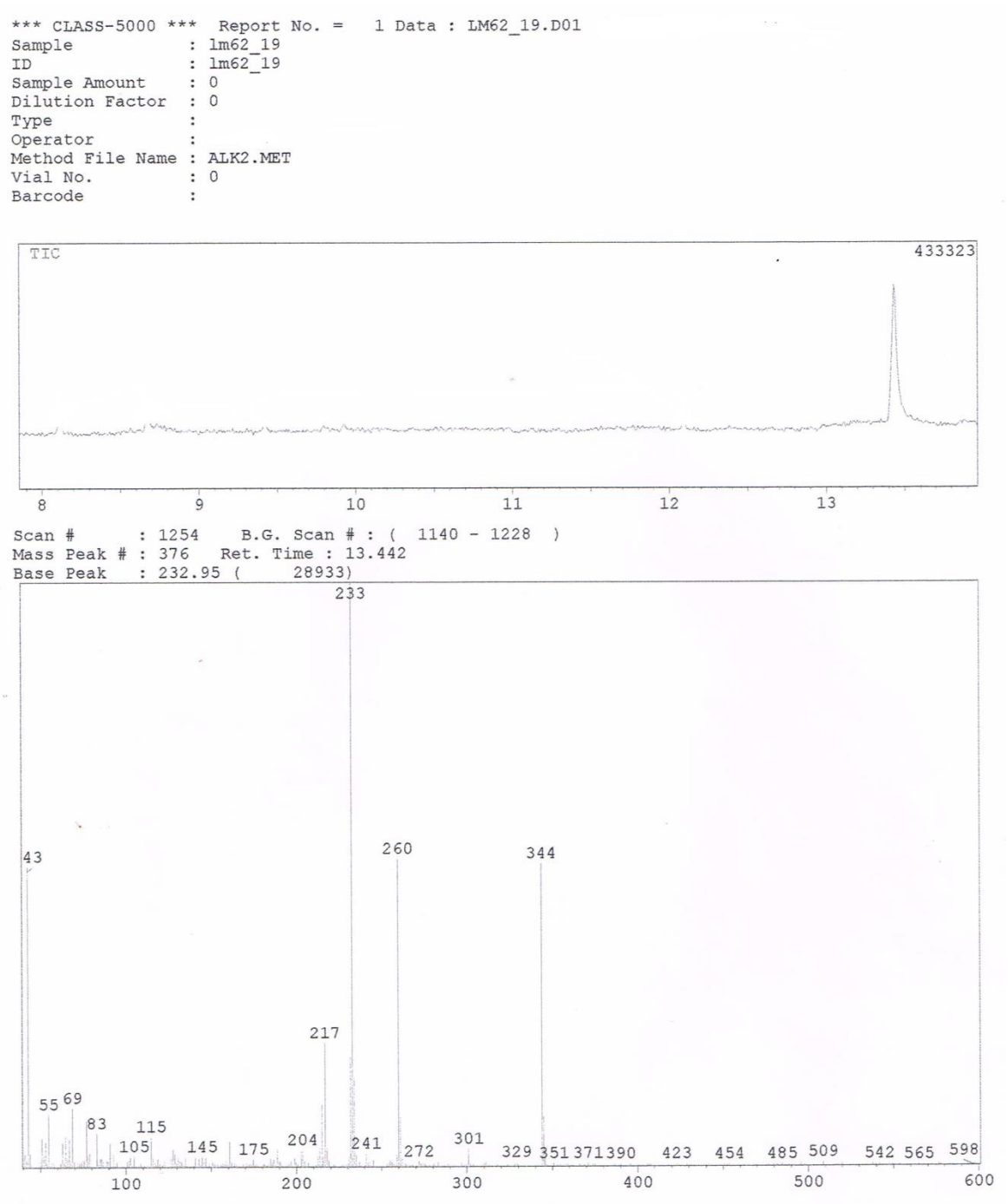


Figura 7. Cromatograma e espectro de massas do composto ácido úsnico.

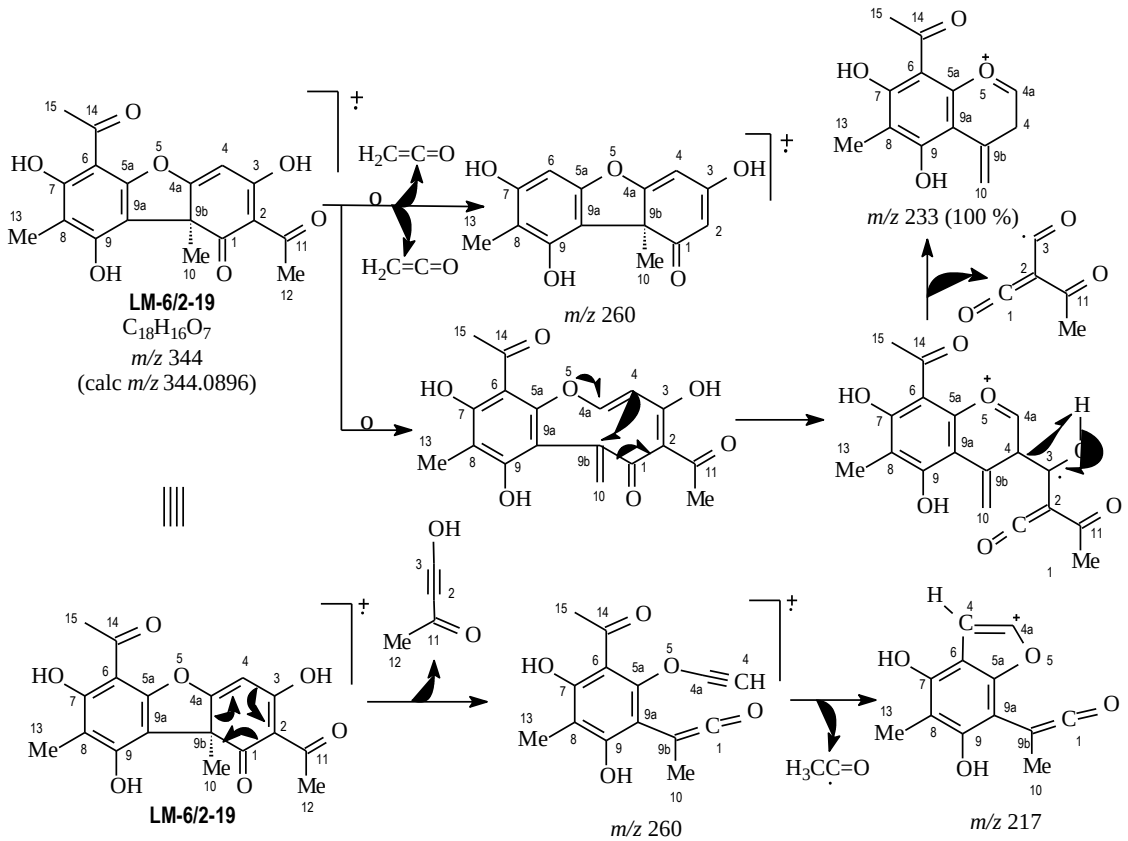


Figura 8. Proposta do mecanismo de formação dos prinipais fragmentos de massa do composto ácido úsnico.

4. RESUMO E CONCLUSÕES

O estudo da espécie *Ramalina usnea* possibilitou comprovar que esta espécie apresenta propriedades biológicas semelhantes as demais espécies do gênero relatadas na literatura.

Foi possível verificar que o gênero *Ramlina* é composto por 246 espécies contudo somente cerca de 118 espécies, 47% do total, teve algum estudo químico ou de atividade biológica. Foram identificados 153 compostos, mas somente 27 deles, cerca de 18% tiveram algum estudo de atividade biológica.

O ácido úsnico foi o composto com a maior quantidade de pesquisas, e nas pesquisas envolvendo atividade biológica ou farmacológica, quase sempre ele apresentou excelentes resultados nas atividades testadas.

Apesar de poucas espécies deste gênero terem sido testadas para suas propriedades biológicas, cerca de 13 espécies, os resultados obtidos dos extratos, frações e compostos isolados apresentaram resultados satisfatórios nas atividades antitumoral, anti-inflamatória, antimicrobiana, inseticida, antiviral entre outras.

Desta forma verifou-se que o gênero *Ramalina* e suas espécies são fontes promissoras de compostos bioativos que precisam de mais pesquisas, principalmente daquelas espécies pouco ou não estudadas, bem como ampliar a gama de atividades farmacológicas ainda não testadas.

Os resultados dos testes de atividade larvicida contra as larvas de terceiro instar do mosquito *Aedes aegypti* apresentaram boa atividade do extrato e de frações, no entanto, os compostos testados tiveram atividade larvicida mais expressiva, com valores baixos das concentrações letais 50%, de 4,850 e 4,475 µg/mL para os compostos 2-hidróxi-4-metoxi-6-propil-benzoato de metila e ácido úsnico, respectivamente. Apesar dos baixos valores encontrados, a literatura mostra que os compostos liquênicos possuem alta atividade larvicida, já que valores menores foram apresentados para atividade com outros insetos que não o *A. aegypti*.

Este é o primeiro trabalho realizado sobre atividade larvicida com o composto 2-hidróxi-4-metoxi-6-propil- benzoato de metila até o presente momento. Esse composto também não foi descrito em nenhuma espécie do gênero *Ramalina* até o momento.

Os resultados dos testes da atividade antimicrobiana do líquen *Ramalina usnea* mostraram que o ácido úsnico não inibiu o crescimento de nenhumas das basctérias testadas em nenhuma das concentrações utilizadas, mas inibiu 100% o crescimento dos fungos *Candida tropicalis* e *Candida kefyr* em concnentrações baixas. Ao contrário do ácido úsnico, as frações não inibiram os fungos mas inibiram as bactérias, em alguns casos 100% de inibição em baixa concentração. A fração LM7-21-39 foi a que apresentou melhor inibição de *Staphylococcus aureus* e *Burkholderia cepacia*.

Esta espécie de líquen apresenta um forte potencial antimicrobiano, possuindo compostos em suas frações que podem inibir fungos e bactérias em baixas concentrações.

REFERÊNCIAS BIBLIOGRÁFICAS

Boustie, J. Grube, M. (2005) Lichens – a promising source of bioactive secondary metabolites. *Plant Genetic Resources*, 3 (2): 273-287.

Bustinza, F.Y López, C. (1948) Contribución al studio de los antibióticos procedentes de líquenes (1948). http://www.rjb.csic.es/pdfs/Anales 07(1)511-548.pdf. Acesso em 20/4/2012.

Cansaran, D., Kahya, D., Yurdakulola, E., Atakol, O. (2006) Identification and quantitation of usnic acid from the lichen Usnea species of Anatolia and antimicrobial activity. *Z. Naturforsc. C*, 61:773-776.

Esimone, C.O., Grunwald, T., Nworu, C.S., Kuate, S., Proksch, P., Ueberla, K. (2009) Broad spectrum antiviral fractions from the lichen *Ramalina farinacea* (L.) Ach. *Chemotherapy*, 55: 119–126.

Falcão, E.P. da S., Silva, N.H. da, Gusmão, N.B. de, Ribeiro, S.M., Honda, N.K., Pereira, E.C. (2002) Atividade antimicrobiana de compostos fenólicos do líquen *Heterodermia leucomela* (L.) Poelt. *Acta Farm. Bonaerense* 21(1):43-49.

Fazio, A.T., Adler, M.T., Bertoni, M.D., Sepúlveda, C.S., Damonte, E.B., Maier, M.S. (2007) Lichen secondary metabolites from the cultured lichen mycobionts of *Teloschistes chrysophthalmus* and *Ramalina celastri* and their antiviral activities. *Z. Naturforsch*. C, 62, 543–549.

Gewandsznajder, F., Linhares, S. (2000) Biologia Hoje. 7. ed. São Paulo`: Ática, 408p.

Honda, N.K., Vilegas, W. (1998) A Química dos Líquens. Químí. Nova, 21 (6), 110-125.

Imshaug, H.A. (1972) Typification of Ramalina usnea (L.) R. H. Howe. *The Lichenologist*, 5: 317-318.

Joly, A.B. (2002) Introdução à taxonomia vegetal. 13. ed. São Paulo: Companhia Editora Nacional, 777p.

Kashiwadani, H., Kalb, K. (1993) The genus Ramalina in Brazil. *The Lichenologist*, 25 (1): 1-31.

Kosanić, M.M., Ranković, B.R., Stanojković, T.P. (2012) Antioxidant, antimicrobial and anticancer activities of three *Parmelia species*. *J. Sci. Food Agric*, jul. 92 (9). 1909-1916

Kosanić, M.M., Ranković, B.R., Stanojković, T.P. (2012) Antioxidant, antimicrobial and anticancer activity of three *Umbilicaria species*. *J. Food. Sci*, jan. 77 (1): 20-25.

Manojlovic, N.T., Solujic, S., Sukdolak, S. (2002) Antimicrobial activity of an extract and anthraquinones from *Caloplaca schaereri*. *The Lichenologist*, 34: 83-85.

Marcelli, M.P. (1997) Estudo da diversidade de espécies de fungos liquenizados do Estado de São Paulo. Versão preliminar. Instituto de Botânica – Seção de Micologia e Liquenologia – São Paulo, junho. http://www.biota-fapesp.net/revisoes/liquens.pdf Acesso em 23/11/2013

Molnár, K., Farkas, E. (2010) Current results on biological activies of lichen secondary metabolites: a review. *Z. Naturforsch C*., mar-apr, 65 (3): 157-173.

Mitrović, T., Stamenković, S., Cvetković, V., Tošić, S., Stanković, M., Radojević, I., Stefanović, O., Comić, L., Dačić, D., Curčić, M., Marković, S. (2011) Antioxidant, antimicrobial and antiproliferative of five lichen species. *Int. J. Mol. Sci*, 12 (8): 5428-5448.

Nishikawa, Y., Tanaka, M., Shibata, S., Fukuoka, F. (1970) Polysaccarides of Lichens and Fungi. IV. Antitumor active O-Acetylated Pustulan – Type Glucans from the Lichens of *Umbilicaria* Species. *Chem. Pharm. Bull*., 18(7)1431-1434.

Nultsch, W. (2000) Botânica Geral. Trad. Paulo Luiz Oliveira – 10. ed. Revisada e atualizada – Porto Alegre: Artes Médicas Sul, 489p.

Piovano, P., Garbarino, J.A., Giannini, F.A., Correche, E.R., Feresin, G., Tapia, A., Zacchino, S., Enriz, R.D (2002) Evaluation of antifungal and antibacterial of aromaatic metabolites from lichens. *Bol. Soc. Chil. Quim.* 47 (3): 235-240.

Pereira, E.C., da Silva, N.H., Santos, R.A., Sudário A.P.P., Rodrigues e Silva, A.A., Maia, M.B. de S. (2010) Determination of Teloschites flavicans (SW) Norm. anti-inflamatory activity. *Pharmacognosy Res.*, 2 (4), 1: 205-210.

Ranković, B.R., Kosanić, M.M., Stanojković, T.P. (2011) Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata*, *Lecanora atra* and *Lecanora muralis*. *BMC Complement Altern. Med.* 20, 11: 97.

Rundel, P. W. (1978) Evolutionary Relationships in the *Ramalina Usnea* Complex. *The Lichenologist*, 10:141-156.

Shibata, S., Nishikawa, Y., Tanaka, M. (1968) Polysaccharides in lichens and fungi. I. Antitumor active polysaccharides of *Gyrophora esculenta* Miyoshi and *Lasallia papulosa* (Ach.) Lano. *Chem. Pharm. Bull.* 16(12) 2362-2369.

Tay, T.; Türk, A.Ö.; Yilmaz, M.; Türk, H.; Kivanç, M. (2004) Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstitic acid, and protocetraric acid constituents. *Z. Naturforsch. C*, 59, 384–388.

Urdapilleta, A. A. A. (2006) Atividade de substâncias isoladas de líquens sobre formas promastigotas de *Leishmaniose* (L.) *amazonenses* e *Leishmaniose* (V.) *braziliensis*. Dissertação (Mestrado em Ciências da Saúde). Universidade de Brasília – UnB, 87p.

Werth, S. (2012) Fungal – algal interactions in *Ramalina menziessi* and its associated epiphytic lichen community. *The Lichenologist,* 44 (4), 543 -560.

PESPECTIVAS FUTURAS

Resultados dos Testes Antineoplásicos

Foram realizados os ensaios de citotoxicidade celular através do método de MTT ([3-(4,5- dimetiltiazol-2-il)-2,5-difenil brometo de tetrazólio]) com as células leucêmicas humanas (THP-1, U937, MOLT-4 e JURKAT), pulmão (H460), melanoma humano (SK-mel5) e cólon (COLO205), adquiridas da American Type Culture Collection (ATCC), para as amostras S1(ácido úsnico) e S2 (fração LM7-21-39) e o controle negativo, o composto cis-platina, usado comercialmente para diversos tipos de câncer. Os resultados obtidos são representados pelo índice de citotoxicidade 50% (IC_{50}), ou seja, a concentração capaz de matar ou reduzir a viabilidade celular em 50%. A IC_{50} foi determinada a partir das curvas dose resposta obtidas utilizando o programa GraphPad Prism versão 5.0 e a Tabela 1 apresenta os resultados de IC_{50} obtidos.

Tabela 1: Índice de citoxicidade 50% (IC_{50}) em µmol/L dos compostos S1 e S2 e da cis-platina

	Colo205	**H460**	**U937**	**THP-1**	**Molt-4**	**SK-MEL-5**
S1	65	31	10	20	>100	13
S2	>100	> 100	35	> 100	90	74
Cis-platina	16	38	16	12	14	39

O composto ácido úsnico (amostra S1), apresentou ótimo índice de citotoxicidade frente a três linhagens de células, sendo melhor que o próprio controle, e muito boa citotoxidade frente a outras duas células. Já a fração LM7-21-39 (amostra S2) apresentou boa citotoxicidade frente a uma linhagem de célula e razoável citotoxicidade frente a duas outras linhagens de células, mostrando que essas amostras são promissoras na busca por novos agentes antineoplásicos.