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Dissertação

Macroalgas da Antártica:

Propriedades Químicas e Avaliação Biológica

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MACROALGAS DA ANTÁRTICA

Propriedades Químicas e Avaliação Biológica

Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências (Área do conhecimento: Bioquímica e Bioprospecção).

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Priscila Oliveira de Souza

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Isaac Newton

Resumo

SOUZA, Priscila Oliveira de. **Macroalgas da Antártica:** Propriedades Químicas e Avaliação Biológica. 2014. 187f. Dissertação (Mestrado) – Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas.

O continente Antártico atingiu sua atual posição há 45 milhões de anos (Ma) e tem sido isolado geograficamente dos outros continentes desde a separação da Península Antártica da América do Sul (há 30 Ma). Devido ao elevado grau de endemismo existente na Antártica, decorrente de tal isolamento, e às condições ambientais extremas às quais as espécies, particularmente de macroalgas, são expostas, elas evolutivamente necessitaram desenvolver mecanismos de adaptação e, consequentemente, são capazes de produzir uma diversidade de metabólitos secundários. A busca por potenciais substâncias biologicamente ativas com aplicação farmacológica tem atraído o interesse para o ambiente marinho, em especial na terapêutica do câncer, visto ser uma doença que apresenta muitos desafios para a cura, além de acometer grande parcela da população mundial. Em relação à Península Antártica, particularmente devido ao limitado estudo da diversidade local, busca-se caracterizar os materiais biológicos provenientes da região. Nessa perspectiva, o objetivo do presente estudo foi caracterizar quimicamente cinco macroalgas endêmicas do continente Antártico e da ilhas subantárticas e avaliar suas potenciais atividades antitumorais. Extratos com diferentes graus de polaridade (hexano, clorofórmio e etanol) foram preparados, a fim de extrair diferentes classes de moléculas das algas e testados nas células de linhagens tumorais de glioma de rato (C6), glioblastoma multiforme humano (U87) e adenocarcinoma de pulmão (A549). A fim de avaliar a citotoxicidade dos extratos foram avaliados os efeitos dos extratos nas linhagens não tumorais de astrócitos de rato e fibroblastos de pulmão humano. As macroalgas, com exceção da *Palmaria decipiens*, apresentaram considerável atividade antitumoral, sendo que os extratos mais promissores foram os apolares, demonstrando efeito seletivo. Dentre os melhores resultados obtidos no tratamento de A549, os extratos clorofórmicos das algas *Pyropia endiviifolia, Desmarestia anceps* e *Iridaea cordata* apresentaram as respectivas concentrações que inibem 50% das células (IC50) 45,66µg.mL^{-1}; 61,16µg.mL^{-1} e 67,54µg.mL^{-1}; enquanto o IC_{50} do extrato hexânico da clorófita *Prasiola crispa* foi 93,02µg.mL^{-1}. Já em relação ao tratamento com os gliomas, os quais são células mais agressivas e malignas, a alga parda *D. anceps* apresentou inibição de crescimento em todos os extratos analisados, sendo que os efeitos foram intensificados após 48 horas de exposição atingindo em 250µg.mL^{-1} 50% de inibição (hexano) e 45% (clorofórmio) e no extrato etanólico 50% de inibição em 500µg.mL^{-1}. O extrato clorofórmico bruto da alga vermelha *I. cordata* inibiu 40% do crescimento do glioma em 250µg.mL^{-1}, enquanto os extratos hexânico e etanólico da *Pyropia endiviifolia* inibiram o crescimento em 10µg.mL^{-1}. Dessa forma, o isolamento das moléculas apolares biologicamente ativas presentes nesses extratos com potencial efeito antineoplásico torna-se um próximo alvo de estudo do nosso grupo de pesquisa.

Palavras-chave: Algas. Atividade Antitumoral. Câncer de Pulmão. Continente Antártico. Endemismo. Glioblastoma.

Abstract

SOUZA, Priscila Oliveira de. **Seaweeds from Antarctica:** Chemistry Properties and Biological Evaluations. 2014. 187f. Thesis (Master) - Graduate Program in Biochemistry and Bioprospecting. Federal University of Pelotas, Pelotas.

The Antarctic continent reached its current position for 45 million years (Ma) and has been geographically isolated from other continents since the breakup of the Antarctic Peninsula from South America (30 Ma ago). Due to the high degree of endemism in Antarctica, resulting from such isolation, and extreme environmental conditions to which the species, particularly macroalgae, are exposed, they needed to develop evolutionary adaptation mechanisms and therefore are able to produce a variety of secondary metabolites. The search for biologically active substances with potential pharmacological application has attracted the interest to the marine environment, particularly in the treatment of cancer, as a disease that presents many challenges to healing, besides affecting large portion of the world population. Regarding the Antarctic Peninsula, particularly given the limited study of local diversity, we seek to characterize biological materials from the region. In this perspective, the objective of this study was to characterize chemically five endemic seaweeds of the Antarctic continent and the subantarctic islands and assess their potential antitumor activities. Extract with different degrees of polarity (hexane, chloroform and ethanol) were prepared in order to extract different classes of molecules algae and tested on tumor cell lines of rat glioma (C6), human glioblastoma multiforme (U87) and adenocarcinoma lung (A549). In order to evaluate the cytotoxicity of the extracts were evaluated the effects of the extracts on non-tumor cell lines of mouse astrocytes and human lung fibroblasts. Macroalgae, except *Palmaria decipiens* showed considerable antitumor activity, and the most promising extracts were nonpolar, demonstrating selective effect. Among the best results in the treatment of A549, the chloroform extracts of algae *Pyropia endiviifolia*, *Desmarestia anceps* and *Iridaea cordata* showed concentrations that inhibit 50% of cells (IC_{50}) 45.66 $\mu g.mL^{-1}$; 61.16 $\mu g.mL^{-1}$ and 67.54 $\mu g.mL^{-1}$, respectively; while the IC_{50} of hexane extract of chlorophyte *Prasiola crispa* was 93.02 $\mu g.mL^{-1}$. In relation to treatment with gliomas, which are more aggressive and malignant cells, the brown alga *D. anceps* showed inhibition of growth in all extracts analyzed, whereas effects were enhanced after 48 hours of exposure reaching 50% inhibition in 250$\mu g.mL^{-1}$ (hexane), 45% (chloroform) and 50% of inhibition in 500$\mu g.mL^{-1}$ at ethanolic extract. The crude chloroform extract of the red alga *I. cordata* inhibit 40% of growth in glioma 250$\mu g.mL^{-1}$, while and the hexane extract of the *Pyropia endiviifolia* inhibited in 10$\mu g.mL^{-1}$. Thus, the isolation of biologically active nonpolar molecules present in these extracts with potential antineoplastic effect becomes a next target for study of our research group.

Keywords: Algae. Antarctic continent. Antitumor activity. Endemism. Glioblastoma. Lung Cancer.

Lista de Figuras

Lista de Abreviaturas e Siglas

ADP – adenosina difosfato

APCs – células apresentadoras de antígenos

ATCC – Coleção de Cultura do Tipo Americana

ATP – adenosina trifosfato

BF_3 – trifluoreto de boro

bFGF – fator de crescimento de fibroblastos básico

Chl – clorofila

CTLA – antígeno associado a linfócito T citotóxico

DCs – células dendríticas

DMEM – meio de Eagle modificado por Dulbecco

DMSO – dimetilsulfóxido

EACF – Estação Antártica Comandante Ferraz

EFA – ácidos graxos essenciais

EGFR – receptor do fator de crescimento epidermal

EMT – transição epitelial-mesenquimal

EPA – ácido cis-5,8,11,14,17- eicosapentaenóico

FA – ácidos graxos

FAME – metil éster de ácidos graxos

FBS – soro fetal bovino

FDA – Food and Drug Administration

GBM – glioblastoma multiforme

GC-FID – cromatágrafo a gás com detector de ionização de chamas

GLSPs – polissacarídeo ácido

GTPase – guanina trifosfatase

HDL – lipoproteína de alta densidade

Hh – Hedgehog

HPLC – cromatografia líquida de alta eficiência

IC_{50} – concentração que inibe o crescimento de 50% das células

IGF – fator de crescimento semelhante à insulina

IL-2 – interleucina-2

IR – receptor de insulina

JNK – c-Jun N-terminal quinase

LC3 – cadeia de luz 3

LDL – lipoproteína de baixa densidade

Ma – milhões de anos

MHC – complexo de histocompatibilidade maior

MTIC – carboxamida-imidazol monometiltriazeno

MTT – brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio

NaCl – cloreto de sódio

NaOH – hidróxido de sódio

NK – natural killers

PI – propidium iodide

PI3K – fosfatidilinositol 3-quinase

PUFA – ácidos graxos poli-insaturados

ROS – espécies reativas de oxigênio

RTK – receptor de tirosina quinase

TAMs – macrófagos associados a tumor

TFA – ácidos graxos totais

TGF-β – fator de crescimento transformante beta

TILs – linfócitos infiltrantes de tumor

TNFR – receptor do fator de necrose tumoral

UFA – ácidos graxos insaturados

UHPLC – cromatógrafo líquido de alta ultra eficiência

VEGF – fator de crescimento endotelial vascular

WHO – Organização Mundial de Saúde

Sumário

1 Introdução

A Antártica e o sul do oceano foram reconhecidos como áreas de grande valor para a ciência em 1991 por meio do Protocolo de Proteção Ambiental para o Tratado Antártico, visto que a análise minuciosa das suas características em geral facilitará o compreendimento de processos globais, o estudo de organismos com características ecofisiológicas únicas devido ao longo período histórico evolutivo em isolamento e para oportunidades únicas de observações astronômicas, além de investigações da magnetosfera e ionosfera da Terra (BARGAGLI, 2008).

Uma característica impressionante do ecossistema Antártico é o evidente contraste entre o empobrecimento extremo do ecossistema terrestre e a riqueza de biomassa dos organismos marinhos (BARGAGLI, 2008). Segundo Wiencke & Clayton (2002), existe um elevado grau de endemismo na Antártica, cerca de um terço das 120 espécies de algas já registradas são endêmicas do continente Antártico.

Segundo Abida e colaboradores (2013), essa diversidade de espécies pode apresentar grande variedade de estruturas químicas com potencial aplicação na criação de novos fármacos. Nas últimas décadas, o crescente aumento de pesquisas com macroalgas resultou na descoberta de metabólitos com atividade biológica, destacando-se como uma notável fonte de diversidade química, responsável por 20% dos compostos relatados de origem marinha (BLUNT et al., 2014).

Os compostos das macroalgas são caracterizados pela sua origem biológica. As algas vermelhas (Rhodophyceae) produzem grandes quantidades de monoterpenos poli-halogenados, sesquiterpenos e acetogeninas. As algas marrons (Phaeophyceae) produzem primariamente diterpenos, apresentando também uma

riqueza de florotaninos - com potenciais atividades antioxidante, anti-inflamatória e antialergênica - quinonas preniladas ou hidroquinonas, além de diacilgliceróis, sargafuranos - possível base para os novos tratamentos de pele para prevenir ou melhorar a acne (KAMEI et al., 2009) - e brassinosteróides - recentemente utilizado em pesquisas de atividade citotóxica contra duas linhagens de células de câncer humanas (HAMDY et al., 2009). E as algas verdes (Chlorophyceae e Ulvophyceae) produzem sesqui- e diterpenos, sendo conhecidas por seus 1,4-dialdeídos, além dos depsipeptídeos e caulerpina - um inibidor da respiração mitocondrial, com potencial aplicação em fármacos anticâncer (MASCHEK & BAKER, 2008; BLUNT et al., 2011).

Além disso, sabe-se que as macroalgas são uma rica fonte de proteínas, representando o ideal material de base para a geração de peptídeos bioativos derivados de proteínas marinhas (HARNEDY & FITZGERALD, 2011). Esses peptídeos bioativos tem apresentado propriedades como inibidor acetilcolinesterase, antioxidante (CIAN et al., 2013), imunomodulatório (CIAN et al., 2012), antibacteriano (BALAKRISHNAN et al., 2011), antitrombótico (KOTB, 2013) e atividade anti-hipertensiva (FITZGERALD et al., 2012). Investigações recentes têm demonstrado que os polissacarídeos sulfatados de algas marinhas apresentam potenciais propriedades biológicas, dentre as quais se destacam atividades antitumoral, anticoagulante, antiviral, anti-hiperlipidêmica, antioxidante, anti-inflamatória e antinociceptiva sendo, portanto considerados moléculas farmacologicamente ativas (RWEHUMBIZA et al., 2013; TENG et al., 2013; BATISTA et al., 2014; LIANG et al., 2014; MOGHADAMTOUSI et al., 2014; RODRIGUEZ-JASSO et al., 2014).

Segundo Vishchuk e colaboradores (2011), as macroalgas marrons *Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, Druehl & G.W. Saunders e *Undaria pinnatifida* (Harvey) Suringar apresentam fucoidanas distintas capazes de inibir a proliferação e a formação de colônias das linhagens celulares de melanoma e câncer de mama de forma dose-dependente. Esses resultados demonstraram que o uso de polissacarídeos sulfatados dessas algas pode ser um potencial tratamento para o câncer. Muitas pesquisas têm registrado que as fucoidanas tem atividade antiproliferativa em células de câncer *in vitro*, assim como atividade inibitória no crescimento de tumores *in vivo* (SOUZA et al., 2007; YE et al., 2008; MOGHADAMTOUSI et al., 2014). As fucoidanas foram efetivas contra várias células

tumorais, incluindo células de câncer de cólon humano (DLD-1), de câncer de mama (T-47D), de melanoma (RPMI-7951), de leucemia humana (U937) e de carcinoma hepatocelular humano (SMMC-7721) (PARK et al., 2013; VISHCHUK et al., 2013; YANG et al., 2013).

A atividade antitumoral dos polissacarídeos sulfatados de macroalgas marrons é relacionada com vários parâmetros estruturais, incluindo o grau de sulfatação, a composição do monossacarídeo, assim como o tipo de ligação glicosídica (NISHINO et al., 1991; YANG et al., 2008). Segundo Ye e colaboradores (2008), existem muitos registros de que um dos fatores mais importantes para esses efeitos biológicos é o conteúdo de sulfato dos polissacarídeos.

Zhang e colaboradores (2008) demonstraram que as fucoxantinas exibem notáveis efeitos antiproliferativos em células EJ-1 de câncer de vesícula urinária de humanos (um tipo de câncer maligno, cujo exame e tratamento são muito caros) e reduzem a viabilidade dessas células pela indução da apoptose, a qual foi caracterizada por mudanças morfológicas na hélice do DNA, elevada percentagem de células haplodiplóides e ativação da atividade da caspase-3, com uma razão máxima de células apoptóticas de >93% com 20μM de fucoxantina. Dessa forma, as fucoxantinas tem despertado grande interesse devido às suas potentes bioatividades, destacando-se atividades antioxidante, anti-inflamatória, anticancer, anti-obesidade, antidiabética, antiangiogênica e anti-malária; e seu efeito protetivo no fígado, vasos sanguíneos do cérebro, ossos, pele e olhos (PENG et al., 2011).

Portanto, observa-se a crescente busca por potenciais substâncias biologicamente ativas para o tratamento de câncer, o qual atinge uma considerável parcela da população. Além disso, em detrimento dos limitados estudos na Península Antártica, busca-se caracterizar os materiais biológicos de lá provenientes, atribuindo-lhes importantes aplicações, a fim de que possam contribuir para futuros desenvolvimentos farmacológicos. Nessa perspectiva, considerando as condições ambientais extremas do continente Antártico, as quais afetam a produção de metabólitos secundários das algas, espera-se constatar atividade antitumoral a partir dos extratos das espécies de macroalgas analisadas.

2 Objetivos

Objetivos Gerais

- Avaliação dos componentes químicos das macroalgas da Antártica *Desmarestia anceps*, *Iridaea cordata*, *Palmaria decipiens*, *Prasiola crispa* e *Pyropia endiviifolia*;
- Comparação da atividade antitumoral dos extratos em diferentes culturas celulares.

Objetivos Específicos

- Realização de extrações sistemáticas a fim de analisar cada classe de moléculas presentes nos diferentes extratos;
- Identificação da fração lipídica via Cromatografia Gasosa da macroalga *Prasiola crispa*;
- Comparação de diferentes sistemas de extração a fim de otimizar os resultados;
- Análise dos extratos por Cromatografia Gasosa e Líquida acoplados a Espectrometria de Massas a fim de identificar os compostos químicos;
- Análise da atividade antitumoral em cultura de linhagens de glioma e câncer de pulmão (C6, U87 e A549).

3 Revisão de Literatura

O Continente Antártico

O continente Antártico atingiu sua atual posição há 45 milhões de anos (Ma) e tem sido isolado geograficamente dos outros continentes desde a separação da Península Antártica da América do Sul (há 30 Ma) (BARGAGLI et al., 2008). A abertura e o aprofundamento da Passagem do Drake permitiu o estabelecimento da Corrente Circumpolar Antártica e o Vórtice Ciclônico Circumpolar, os quais aumentaram o isolamento da Antártica e contribuíram para seu extraordinário resfriamento (SIEGERT et al., 2008; HOMMERSAND et al., 2011).

A Antártica é um continente coberto por gelo (98% do território, com espessura média de 2km e máxima de mais de 4km) cercado pelo Oceano Antártico sem qualquer conexão de terra com regiões temperadas desde o Mesozóico (BARGAGLI et al., 2008; CONVEY et al., 2008; STOREY et al., 2013). Ela pode ser considerada a região mais preservada no planeta e a mais vulnerável a mudanças globais, sendo inclusive definida como um Continente Científico (YONESHIGUE-VALENTIN et al., 2013).

O continente Antártico apresenta inúmeras perspectivas de estudos ambientais, dentre os quais geológico/paleontológico, geofísico, meteorológico, ecológico, botânico, zoológico e microbiológico, sendo uma verdadeira estação de pesquisa (ZACHER et al., 2009; RIBEIRO et al., 2011; RYAN et al., 2012). Em 1975, o Brasil aderiu ao Protocolo do Tratado da Antártica, iniciando suas pesquisas em 1982, com o surgimento do Programa Antártico Brasileiro. A base brasileira foi instalada em 1984, sendo denominada de Estação Antártica Comandante Ferraz

(EACF), localizada na parte interna da Baía do Almirantado, na Ilha Rei George (Fig. 1) (PROANTAR; STEFENON et al., 2013).

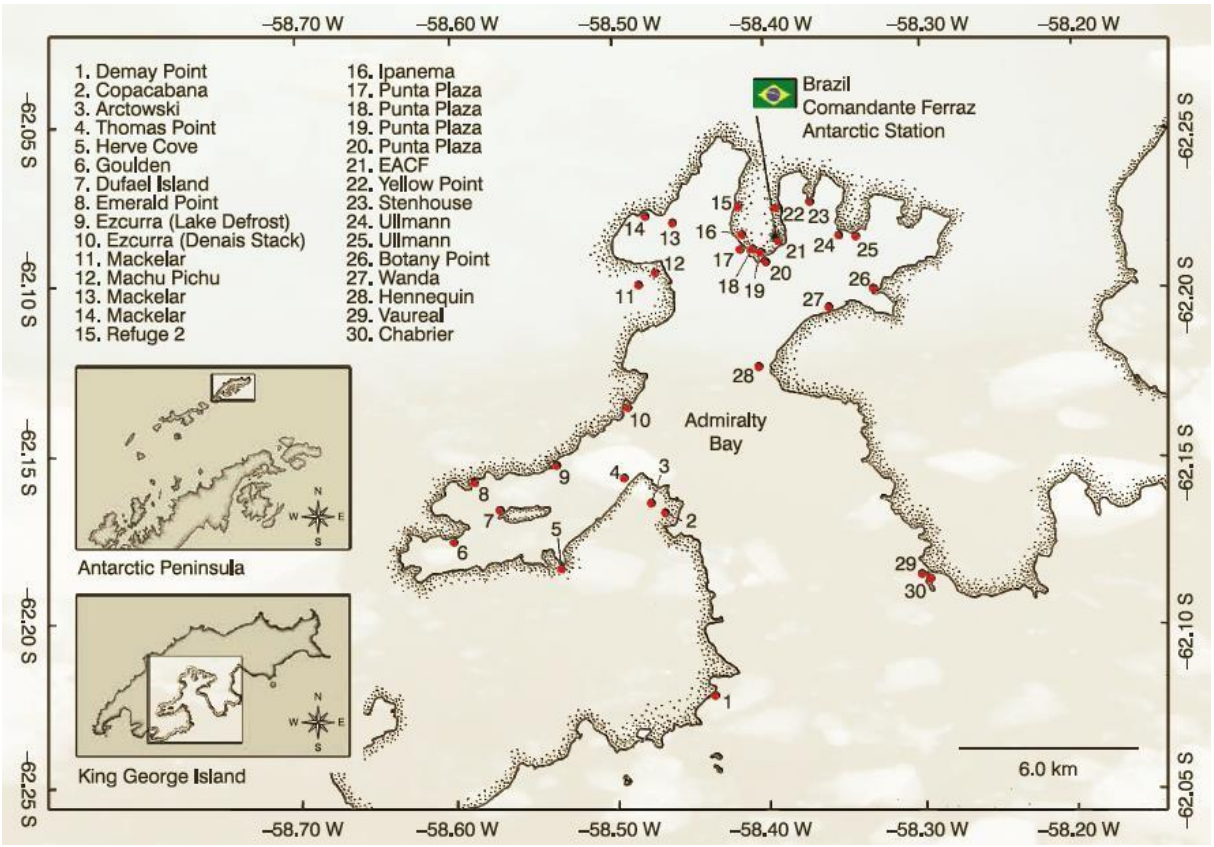

Figura 1. Mapa da Península Antártica e pontos de coleta na Baía do Almirantado, Ilha Rei George.
Fonte: YONESHIGUE-VALENTIN et al. (2013), pag.3.

Apesar do seu característico isolamento geográfico, essa região tem apresentado alterações ambientais em decorrência das mudanças climáticas globais e atividades antrópicas (CONVEY, 2011). A partir do século XX, iniciou-se a exploração da Antártica, acarretando a extinção de algumas espécies em detrimento do uso de transportes deliberadamente e da introdução de espécies invasivas. Dessa forma, gerando um considerável impacto na biodiversidade e funcionamento dos ecossistemas em muitas ilhas subantárticas (FRENOT et al., 2005).

De uma perspectiva terrestre e de água doce, o ambiente Antártico caracterizado pelo isolamento e com pouca energia resulta em uma pobreza de espécies (CHOWN, 2007). Característica que contrasta com a biodiversidade

encontrada no ambiente marinho (BRANDT, 2007). Em geral, os organismos marinhos são caracterizados por altos níveis de endemismo, gigantismo, longevidade, taxas lentas de crescimento, maturação tardia e ausência de estágios larvais pelágicos (FRENOT et al., 2005).

Macroalgas Marinhas e o Ambiente Antártico

Os costões rochosos ao longo do Oeste da Península Antártica são recobertas por extensivas assembleias de macroalgas de infralitoral, frequentemente fornecendo 70% ou mais de cobertura do fundo e com permanentes estoques de biomassa que rivalizam aqueles das florestas temperadas de kelps (AMSLER et al., 1995; KLÖSER et al., 1996; QUARTINO et al., 2008).

O ambiente natural das macroalgas polares é caracterizado por fortes condições de iluminação sazonal e temperaturas baixas constantes (ZACHER et al., 2011). Portanto, macroalgas podem ser expostas por longos períodos à escuridão e condições de luminosidade muito baixa (WIENCKE & AMSLER, 2012). Na Ilha Rei George, as temperaturas podem variar de 14°C nas piscinas de maré, enquanto as macroalgas expostas ao ar no supralitoral se submetem a temperaturas de até 30°C no verão e abaixo de -27°C no inverno (ZACHER et al., 2011).

O grande isolamento geográfico da região Antártica e a necessidade de reduzir as demandas de temperatura possuem relevantes efeitos na biodiversidade (WIENCKE & AMSLER, 2012). No continente Antártico, 35% das espécies são endêmicas. Dentre as espécies de algas 44% das Heterokontophyta (Phaeophyceae e Chrysophyceae), 36% das Rhodophyta e 18% das Chlorophyta são endêmicas e o número de espécies endêmicas tem crescido continuamente, sendo que há registro de 130 espécies (WIENCKE & CLAYTON, 2002; WULFF et al., 2011).

Algumas espécies endêmicas destacam-se por serem mais conhecidas cientificamente, como as feofíceas *Himantothallus grandifolius* (A. Gepp & E.S. Gepp) Zinova e *Cystosphaera jacquinotii* (Montagne) Skottsberg, a rodófita *Pyropia endiviifolia* A. Gepp & E.S. Gepp) Y.M. Chamberlain e a clorófita *Lambia antarctica* (Skottsberg) Delépine (WULFF et al., 2009). Alguns fatores determinam a distribuição das macroalgas na região, dentre os fatores abióticos destacam-se

radiação solar (FRANCELINO et al., 2007), salinidade, disponibilidade de nutrientes e tipos de substrato para fixação. Além disso, fatores bióticos como a herbivoria também exerce grande influência (WULFF et al., 2009).

Macroalgas crescendo em tais ambientes extremos necessitam se adaptar a essas condições. Um importante pré-requisito para o sucesso ecológico das macroalgas de águas polares é sua capacidade de utilizar, durante a maior parte do ano, as condições de baixa luminosidade predominantes, além de tolerar e utilizar de modo eficiente as condições de luminosidade elevada durante a primavera para a fotossíntese (WIENCKE & AMSLER, 2012).

Luminosidade suficiente é requerida para atingir o ponto de compensação para a fotossíntese, porém muita luminosidade pode induzir fotoinibição, e a radiação ultravioleta em águas rasas pode resultar em muitos efeitos prejudiciais nos níveis celular e molecular (BISCHOF et al., 2006). Dessa forma, a luminosidade pode determinar os limites superior e inferior de distribuição das macroalgas (HUOVINEN & GÓMEZ, 2013). Assim, vivendo em águas rasas e expostas à elevada radiação solar, algas de regiões polares apresentam diversas estratégias fisiológicas reguladoras a fim de evitar danos de moléculas sensíveis e processos, incluindo fotoinibição da fotossíntese ou dano resultantes do aumento da radiação ultravioleta (HANELT, 1998; ROLEDA et al., 2006; BECKER et al., 2011).

Em relação à adaptação ao frio, algas polares apresentam algumas estratégias metabólicas incluindo a) a manutenção da fluidez de membranas biológicas pela presença de ácidos graxos insaturados que previnem a rigidez da membrana lipídica; b) adaptações moleculares de enzimas a fim de manter taxas suficientes de reações catalisadas por enzimas de processos metabólicos chave; c) a evolução do choque térmico e proteínas anticongelante, e d) adaptações da cadeia de transporte de elétrons fotossintética para funcionar em temperaturas baixas (MORGAN-KISS et al., 2006). Essas características fisiológicas permitem que as algas da Antártica completem seus ciclos de vida *in situ* nas temperaturas próximo a 0°C.

Os mecanismos de resposta ao estresse por radiação podem variar conforme a profundidade de crescimento da macroalga na Antártica (HUOVINEN & GÓMEZ, 2013). A Rhodophyta *Palmaria decipiens*, endêmica da região Antártica,

como exemplo, mediante mudanças na luminosidade modifica a composição dos seus pigmentos (LÜDER et al., 2001) e dos lipídios de membrana, permitindo maior eficiência para aclimatação luminosa em temperaturas próximas a 0°C (BECKER et al., 2010).

Metabólitos Secundários

Os metabólitos secundários são produzidos por vias sintéticas derivadas do metabolismo primário e desempenham funções não essenciais para a sobrevivência do organismo, porém, contribuem para o sucesso da espécie no ambiente em que vivem ao mediarem interações ecológicas que trazem alguma vantagem seletiva aos organismos que os produzem (CRONIN, 2001; ARNOLD, 2003).

A maior parte dos metabólitos secundários relatados em macroalgas são isoprenóides, que consistem em terpenos, esteroides, carotenoides, quinonas preniladas e hidroquinonas (MASCHEK & BAKER, 2008). A segunda maior classe de compostos são os policetídeos, como os florotaninos, cujo mecanismo básico de alongamento da cadeia consiste na condensação de unidades de acetato fornecidas pela acetil coenzima A (MASCHEK & BAKER, 2008; BLUNT et al., 2011).

A maior produção de metabólitos secundários ocorre em representantes do filo Rhodophyta, sendo que as substâncias produzidas por elas podem pertencer a praticamente todas as classes químicas, desde hidrocarbonetos de baixo peso molecular, cetonas simples, fenóis e acetogeninas até sofisticados terpenos. Elas apresentam uma característica particular de incorporar três halogênios, cloro, iodo, e principalmente o bromo às moléculas orgânicas (FENICAL, 1975; CABRITA et al., 2010). Dentre os sesquiterpenos, o elatol apresenta particular interesse em detrimento das atividades biológicas já comprovadas, como tripanocida, leishmanicida, antibacteriana e antiherbivoria (PEREIRA et al., 2003; SANTOS et al., 2010; VEIGA-SANTOS et al., 2010; PARADAS et al., 2010; DESOTI et al., 2012).

Mais de 1140 metabólitos secundários já foram relatados em algas pardas, sendo os compostos característicos diterpenos, florotaninos e acetogeninas C_{11} pequenas, todas apresentando pouca halogenação (BLUNT et al., 2011). Os florotaninos são compostos que recebem peculiar destaque visto que podem

representar 10-20% do peso seco, sendo encontrados em quase todas as ordens das algas pardas (RAGAN & GLOMBITZA, 1986; AMSLER & FAIRHEAD, 2006), desempenhando atividade contra herbivoria e *biofouling* (ANK et al., 2013), como antioxidantes (CRUCES et al., 2012), e na proteção ultravioleta (MASCHEK & BAKER, 2008).

As algas verdes, por outro lado, apresentam apenas 300 compostos conhecidos, sendo eles similares àqueles produzidos pelas rodófitas, di- e sesquiterpenóides, com ausência das extensivas halogenações características dos compostos das algas vermelhas. A característica química das clorófitas é a presença do éster dienolato "1,4-diaceoxibutadieno" encontrado em muitos terpenos das algas verdes (BLUNT et al., 2011; MASCHEK & BAKER, 2008).

Devido às inúmeras situações de estresse às quais as macroalgas da Antártica estão submetidas como temperatura, luminosidade, radiação ultravioleta, salinidade, dessecação e predação, elas são capazes de produzir metabólitos secundários a fim de protegê-las, os quais podem apresentar potenciais atividades biológicas (MASCHEK & BAKER, 2008; KARSTEN et al., 2011; WIENCKE & AMSLER, 2012; IBAÑEZ & CIFUENTES, 2013).

As algas em situações de estresse por exposição à radiação ultravioleta são capazes de sintetizar substâncias que absorvem tal radiação (como os florotaninos nas algas pardas), permitindo lidar com tal situação extrema (HUOVINEN & GÓMEZ, 2013). Os florotaninos estão presentes em elevadas concentrações em uma variedade de Desmarestiales da Antártica, entretanto uma relação direta com a exposição à radiação ultravioleta e seu possível papel fotoprotetivo ainda não estão bem estabelecidos (FAIRHEAD et al., 2006).

Contudo, estudos recentes com kelps subantárticos enfatizaram a importância dos florotaninos como eficientes *scavengers* das Espécies Reativas de Oxigênio (CRUCES et al., 2012; 2013), os quais minimizaram os danos ao DNA e o decréscimo na fotossíntese em resposta ao estresse pela radiação durante maré baixa (GÓMEZ & HUOVINEN, 2010).

Importância Biológica das Macroalgas Marinhas

As macroalgas marinhas bentônicas são organismos fotossintéticos, ecologicamente importantes como produtores primários nas cadeias alimentares. Taxonomicamente, as algas estão agrupadas em três filos Rhodophyta (algas vermelhas), Heterokonthophyta (algas pardas) e Chlorophyta (algas verdes), de acordo com sua morfologia, pigmentação, função e ciclo de vida. Elas são consideradas engenheiras do ecossistema por desempenhar funções de extrema importância nos habitats costeiros, estendendo-se desde as florestas de kelps até os recifes de corais, determinando a estrutura física do habitat (SCHIEL & FOSTER, 2006; HARLEY et al., 2012; CHUNG et al., 2013).

As macroalgas atuam na manutenção da biodiversidade local (SCHIEL, 2006; SCHIEL & LILLEY, 2007), podendo servir como local de abrigo para muitas espécies de invertebrados, protegendo-os e como fonte de alimento, inclusive, quando em decomposição (RAYBAUD et al., 2013). Além disso, desempenham importante papel como indicadoras da qualidade da água, fornecendo informação para avaliar a condição ecológica dos costões rochosos, e indicadoras de mudanças climáticas (DÍEZ et al., 2012; BORJA et al., 2013).

Características das Macroalgas Vermelhas da Antártica (Rhodophyta)

Iridaea cordata (Turner) Bory de Saint-Vincent é uma espécie de clima temperado frio, cuja distribuição se estende do Leste da Antártica (Ross Sea) para Tierra del Fuego, nas ilhas subantárticas (WIENCKE & CLAYTON, 2002). *I. cordata* coloniza, frequentemente, piscinas de maré-baixa e fendas no eulitoral, sendo encontrada em abundância de 2-10m de profundidade, com densidades acima de 4000 indivíduos m^{-2} e níveis de biomassa úmida acima de 3,5kg.m^{-2} (CATTANEO-VIETTI et al., 2000). Morfologicamente, apresenta talo lamelar irregular, lâmina lisa e plana de forma foliácea em formato de coração (cordata) ou lanceolada (Fig. 2). Atinge comprimento de 15cm individualmente ou em torno de 30cm quando agrupada (ZIELINSKI, 1990).

Classificação	**Filo**	Rhodophyta
Taxonômica:	**Subfilo**	Eurhodophytina
	Classe	Florideophyceae
	Subclasse	Rhodymeniophycidae
	Ordem	Gigartinales
	Família	Gigartinaceae
	Gênero:	*Iridaea*

Figura 2. Rodófita *Iridaea cordata*.

Fonte: Núcleo de Pesquisa em Ficologia, Instituto de Botânica de São Paulo

Foto: Prof. Dra. Mutue Toyota Fujii

Palmaria decipiens (Reinsch) R.W. Ricker, endêmica da região Antártica e de algumas ilhas subantárticas: Iles Kerguelen, Ilha Macquarie e Ilha Campbell (WIENCKE & CLAYTON, 2002). Esta macroalga, uma das espécies dominantes na região em termos de biomassa, é encontrada em fendas no eulitoral, sendo abundante nas zonas entre marés, entre 10-20m. *P. decipiens* apresenta uma lâmina entre 8-15cm de largura, não ramificada, lanceolada, atingindo até 70cm no estágio adulto (Fig. 3). Temperaturas entre 0º e 10ºC se mostram ideais para um melhor crescimento e desenvolvimento desta espécie (BECKER et al., 2011).

Classificação	**Filo**	Rhodophyta
Taxonômica:	**Subfilo**	Eurhodophytina
	Classe	Florideophyceae
	Subclasse	Nemaliophycidae
	Ordem	Palmariales
	Família	Palmariaceae
	Gênero:	*Palmaria*

Figura 3. Rodófita *Palmaria decipiens*.

Fonte: Núcleo de Pesquisa em Ficologia, Instituto de Botânica de São Paulo

Foto: Prof. Dra. Mutue Toyota Fujii

Pyropia endiviifolia (A. Gepp & E. Gepp) H.G. Choi & M.S. Hwang rodófita endêmica da Antártica, ocorre em áreas protegidas, como fendas de rochas sombreadas, no sul da convergência da Antártica (WULFF et al., 2011). Apresenta talo lamelar séssil, com coloração verde oliva escura ficando acastanhado ao secar (Fig. 4). Lâmina brilhante com bordas finas, membranosas e onduladas, podendo ser inteira ou dividida profundamente em vários lobos, tendo de 2cm a 20cm de diâmetro e espessura entre 60µ e 70µ (WIENCKE & CLAYTON, 1998).

Classificação	**Filo**	Rhodophyta
Taxonômica:	**Subfilo**	Eurhodophytina
	Classe	Bangiophyceae
	Subclasse	Bangiophycidae
	Ordem	Bangiales
	Família	Bangiaceae
	Gênero:	*Pyropia*

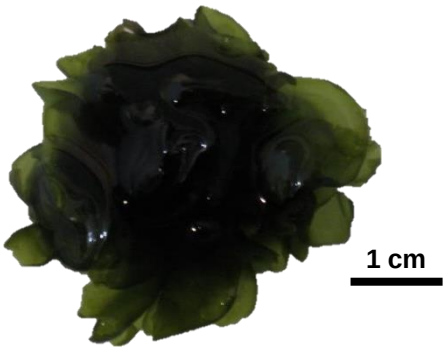

Figura 4. Rodófita *Pyropia endiviifolia*

Fonte: Núcleo de Pesquisa em Ficologia, Instituto de Botânica de São Paulo

Foto: Prof. Dra. Mutue Toyota Fujii

Características das Macroalgas Pardas da Antártica (Heterokontophyta)

Em geral, as assembleias de macroalgas de profundidade são dominadas por coberturas de grandes algas pardas, as quais são perenes, membros da ordem Desmarestiales, a qual substitui ecologicamente na Antártica as Laminariales (kelps) de ocorrência em águas temperadas, sendo considerado o grupo taxonômico predominante e importante fonte alimentar para as comunidades bentônicas da Antártica. *Desmarestia anceps*, *D. menziesii* e *Himantothallus grandifolius* são as principais espécies com as maiores biomassas ao longo da Península Antártica (CLAYTON, 1994; QUARTINO & BORASO DE ZAIXSO, 2008).

D. anceps Montagne é uma feófita endêmica da Antártica, predominante em águas de 10-20m, eventualmente sendo encontradas nas profundidades de 30m ou superiores (QUARTINO et al., 2008). Apresenta talo grande, em forma de pera, um

rizóide maciço passando para um caulóide achatado com ramos crescendo irregularmente em ambos os lados (Fig. 5). O esporófito possui mais que 4m de comprimentos, formando densas agregações no sublitoral. Encontrada em toda a Baía do Almirantado (ZIELINSKI, 1990; WIENCKE & CLAYTON, 2002). Possui uma vantagem competitiva em relação a outras algas de grandes profundidades por estar adaptada a baixa luminosidade (KLÖSER et al., 1994).

Classificação Taxonômica:	**Filo**	Heterokontophyta
	Classe	Phaeophyceae
	Ordem	Desmarestiales
	Família	Desmarestiaceae
	Gênero:	*Desmarestia*

Figura 5. Feófita *Desmarestia anceps*

Fonte: Núcleo de Pesquisa em Ficologia, Instituto de Botânica de São Paulo

Foto: Prof. Dra. Mutue Toyota Fujii

Características das Macroalgas Verdes da Antártica (Chlorophyta) Algas verdes trebouxiofíceas do gênero *Prasiola* estão entre as algas da Antártica melhor conhecida, encontrada em áreas terrestres e no supralitoral,

representando um dos produtores primários mais importantes (KOVÁČIK & PEREIRA, 2001; WIENCKE & CLAYTON, 2002; CONVEY, 2007).

A clorófita *Prasiola crispa* (Lightfoot) Kützing é reportada como uma espécie cosmopolita em áreas temperadas frias e polares, sendo encontrada na zona eulitoral (MONIZ et al., 2012; HUOVINEN & GÓMEZ, 2013). Essa alga nitrofílica cresce tipicamente em solos úmidos que são fertilizados pelo guano das aves, por exemplo, nas áreas adjacentes às colônias de pinguins (GRAHAM et al., 2009). Apresenta talo delicado, lamelar, irregularmente ondulado, com dezenas de milímetros de tamanho (Fig. 6) (ZIELINSKI, 1990).

P. crispa tolera repetidos ciclos de gelo/degelo na primavera e outono, congelamento durante o inverno e altos níveis de radiação ultravioleta durante o verão (JACKSON & SEPPELT, 1997; HOLZINGER et al., 2006; KOSUGI et al., 2010). Aminoácidos como prolina servem como crioprotetores e aminoácidos tipo micosporina absorvem a radiação ultravioleta (JACKSON & SEPPELT, 1997; KARSTEN et al., 2005).

Classificação Taxonômica:	**Filo**	Chlorophyta
	Classe	Trebouxiophyceae
	Ordem	Prasiolales
	Família	Prasiolaceae
	Gênero:	*Prasiola*

Figura 6. Clorófita *Prasiola crispa*

Fonte: Núcleo de Pesquisa em Ficologia, Instituto Botânico de São Paulo

Foto: Prof. Dra. Mutue Toyota Fujii

Importância Econômica das Macroalgas Marinhas

Macroalgas são usadas diretamente como alimento ("*nori" - Porphyra purpurea, "aonori" – Enteromorpha* sp*., "kombu" - Laminaria* sp*., "wakame" - Undaria pinnatifida*, uvas do mar – *Ulva* sp. dentre outras) ou indiretamente como agente de texturização, com propriedades de gelificação e espessamento (como carragenanas, ágar e alginatos) nas indústrias de biotecnologia, farmacêutica, cosméticos, têxtil e alimentos (PELLIZZARI & REIS, 2011).

A riqueza em nitrogênio, fósforo e potássio, além do alto conteúdo de elementos traços e metabólitos viabilizam a utilização das macroalgas como fertilizantes, alimento animal e biomassa para biocombustíveis (FLEURENCE et al., 2012; MICHALAK & CHOJNACKA, 2013; YANIK et al., 2013). A relevância ambiental, social e econômica do cultivo das macroalgas e seus potenciais usos, portanto, são bem conhecidos mundialmente. Além disso, a aquacultura das algas pode reduzir a pressão da pesca, reduzir a eutrofização e tornar-se uma ocupação alternativa e fonte de renda para os moradores do litoral (PELLIZZARI & REIS, 2011).

Importância Farmacológica das Macroalgas Marinhas

Nos últimos 50 anos o potencial de produtos naturais marinhos tem atraído muitos pesquisadores. Inspirados pela riqueza do ambiente marinho e imensidão dos oceanos, companhias farmacêuticas e instituições de pesquisa tem explorado o potencial farmacológico dos metabólitos secundários dos organismos marinhos (ABIDA et al., 2013).

Desde então, mais de 14000 produtos naturais diferentes de organismos marinhos tem sido descritos, e centenas de patentes descrevendo novos produtos naturais marinhos bioativos tem sido depositadas (RESEARCH CORP, 1982; HU et al., 2011; UNIV GEORGIA SYSTEM & UNIV VALDOSTA STATE, 2013; SMITH et al., 2014; ZHOU et al., 2014; KOLLÁR et al., 2014). Inúmeros produtos naturais marinhos estão atualmente em avaliação clínica e pré-clínica, especialmente nas áreas do câncer, dor e doenças inflamatórias (GLASER & MAYER, 2009; INDUMATHY & DASS, 2013; REZENDE et al., 2013).

Dentre os organismos marinhos, as macroalgas marinhas são uma das fontes mais biologicamente ativas da natureza, visto que possuem uma riqueza de compostos bioativos de grande importância farmacêutica e nutracêutica (KUMARI et al., 2013). Estudos recentes têm demonstrado potenciais atividades das biomoléculas de macroalgas como polissacarídeos, proteínas, lipídios e polifenóis (ZHANG et al., 2013; ARAVINDAN et al., 2013; VISHCHUK et al., 2013; KIM et al., 2013; WANG et al., 2013). Dentre as atividades que elas apresentam destacam-se antinociceptiva, anti-inflamatória, antioxidante, antiproliferativa, antimutagênica, antitumoral, antiviral, neuroprotetiva, antimicrobiana, antidiabética, anti-hipertensiva e anticoagulante (WIJESEKARA et al., 2011; WIJESINGHE & JEON, 2012; YUVARAJ et al., 2013; KWON et al., 2013; MURUGAN et al., 2013; PENG et al., 2013; JIN et al., 2013; DORE et al., 2013).

Câncer

O câncer é o principal problema de saúde que constantemente aflige a população humana. Evidências epidemiológicas demonstram que os atuais tratamentos do câncer como quimioterapia e cirurgia são pouco efetivos; assim enfatizando a necessidade de novos fármacos alternativos que apresentem menos efeitos colaterais que os atuais fármacos usados na quimioterapia (INDUMATHY & DASS, 2013).

As células cancerosas possuem determinadas características: proliferação contínua, capacidade de escapar da supressão de crescimento (escape da morte celular programada), invadir e realizar metástase, promover angiogênese, reprogramar o metabolismo energético e escapar da destruição imune (HANAHAN & WEINBERG, 2011).

Alguns fármacos derivados do ambiente marinho já foram aprovados e encontram-se no mercado. Dentre esses fármacos anticâncer encontram-se o Cytosar-U®, também conhecido como citarabina ou ara-C (1-barabinofuranosilcitosina), derivado da espongotimidina isolada da esponja *Cryptotethya crypta* (aprovada em 1969 pela *Food and Drug Administration* - FDA) utilizada no tratamento da leucemia mielóide aguda. A Yondelis®, também chamada

de trabectedina ou ecteinascidina 743, foi isolada de um tunicado do Caribe, *Ecteinascidia turbinata,* sendo empregado no sarcoma de tecido mole e câncer de ovário (aprovado em 2007 pela Agência Européia de Avaliação de Produtos Medicinais. A Halaven®, conhecida também como mesilato de eribulina ou halicondrina E7389, é um análogo da halicondrina B presente na esponja do mar *Halichondria okadai*, usada para tratar pacientes com câncer de mama metástico (aprovado em 2010 pela FDA) (MAYER et al., 2010; ABRAHAM et al., 2012; INDUMATHY & DASS, 2013).

Gliomas

Tumores cerebrais são os mais malignos dos tipos de câncer, apresentando, inclusive, grandes dificuldades para o tratamento (RÍOS-MARCO et al., 2013). Os gliomas são os mais comuns e o tipo mais malignos dentre os tipos de tumor cerebral primário em crianças e adultos (MACDONALD et al., 2011; SHAH et al., 2013). A invasão do glioma é um complexo fenômeno celular que envolve mudanças no sistema biomecânico intracelular e extracelular (NAKADA et al., 2007).

Os gliomas são tumores do tecido neuroepitelial e são classificados com base na sua morfologia: astrocitária, oligodendroglial, ependimal e tumores no plexo coroide (NAKADA et al., 2007). A classificação da Organização Mundial da Saúde utiliza uma escala de fraco (grau I) ao forte (grau IV), de acordo com os marcadores de aberrações histológicas do tumor: atipia nuclear, atividade mitótica, hiperplasia endotelial e necrose (KLEIHUES et al., 2002). Os astrocitomas de grau IV, também referidos como gliobastoma multiforme, manifestam três ou quatro das anormalidades morfológicas, sendo, portanto, o mais agressivo e letal dos tumores, com 75% dos casos de ocorrência em adultos (NAKADA et al., 2007; RÍOS-MARCO et al., 2013; TABOURET et al., 2013).

Embora a incidência do glioma seja menor que outros tipos de câncer, sua mortalidade é extremamente alta (LEE et al., 2013). Em 2005, um estudo de fase III demonstrou os benefícios de incluir temozolomida durante e após o tratamento radioterápico em glioblastoma multiforme (STUPP et al., 2005). Esse tratamento foi capaz de aumentar a média de sobrevivência de 12,1 para 14,6 meses, e as taxas

de sobrevivência de 2- e 5- anos de 8 para 26% e 3 para 10%, respectivamente. Em estudos de coorte, a sobrevivência associada ao glioblastoma multiforme varia de 9,1 para 15,8 meses, influenciada pela cirurgia, para pacientes passíveis a radioterapia e temozolomida (BAUCHET et al., 2010).

Apesar dos recentes progressos na terapia do tumor cerebral, incluindo tratamentos convencionais como ressecção, radioterapia e quimioterapia, além dos tratamentos biológicos como terapia gênica e terapias do sistema imunológico, o glioblastoma apresenta um prognóstico pouco satisfatório, uma das razões podendo estar associada à sua grande heterogeneidade (HDEIB & SLOAN, 2011; MARSH et al., 2013; MULLINS et al., 2013). A rápida proliferação, elevada agressividade e efeito de imunossupressão no organismo nos gliomas tem aumentado a preocupação de pesquisadores (WU et al., 2013).

Câncer de Pulmão

Atualmente, o câncer de pulmão é um dos mais malignos no mundo (SERENO, et al., 2013). Estimativas de 2013 indicaram 27000 novos casos do câncer de pulmão no Brasil, sendo que 63% dos diagnósticos foram para homens, sendo o terceiro tipo de tumor com maior incidência em homens e o quinto em mulheres (INCA, 2011).

Na maioria dos casos, o diagnóstico é realizado em estágios avançados, apresentando assim pobre prognóstico para pacientes (SIEGEL et al., 2012). Esse câncer tem apresentado dificuldades para ser controlado com abordagens cirúrgicas e a terapêutica convencional. Assim, a busca por agentes eficazes e seguros para prevenir, inibir ou reverter a carcinogênese no pulmão permanece prioridade das pesquisas no câncer de pulmão (LI et al., 2013). Em uma perspectiva médica, acredita-se que produtos naturais sejam uma abordagem alternativa para a utilização estratégica de materiais totalmente sintéticos, especialmente na criação de dispositivos médicos (ALVES et al., 2013).

Estudos anticâncer com Macroalgas

Algas marinhas têm evoluído suas próprias defesas químicas para sintetizar metabólitos secundários tóxicos e, portanto, sendo alvo de interesse para estudos anticâncer (FOLMER et al., 2010; MOHAMED et al., 2012). Atividades antitumorais têm sido observadas nas macroalgas. Recentemente, compostos de monoterpeno polihalogenados foram isolados das algas vermelhas *Plocamium suhrii*, *P. cornutum* e *P. corallorhiza* coletadas no litoral da África do Sul, os quais foram citotóxicos *in vitro* para as células de câncer de mama e de esôfago (ANTUNES et al., 2011; DE LA MARE et al., 2012).

Experimentos *in vitro* e *in vivo* sugeriram que o elatol, um composto (sesquiterpeno) isolado da alga *Laurencia microcladia*, tem propriedades antitumorais (CAMPOS et al., 2012). Nas macroalgas do gênero *Pyropia*, cujos principais componentes são porfirinas, polissacarídeos sulfatados demonstraram potencial atividade apoptótica contra células de câncer gástrico humano e não afetaram o crescimento das células normais (KWON & NAM, 2006). O efeito antitumoral também foi verificado na R-ficoeritrina da *Pyropia haitanensis* (PAN et al., 2013), assim como na ficocianina e um componente (PY-D2) em polissacarídeos da *Pyropia yezoensis* no tratamento de alguns tipos de câncer humano (ZHANG et al., 2011).

Fan e colaboradores (2012) isolaram da macroalga *Gracilaria lemaneiformis* (Bory de Saint-Vincent) Greville um polissacarídeo ácido (GLSPs) com conteúdo de carboidrato de 72,06% e de sulfato de 6,13%, o qual mostrou significativa inibição do crescimento tumoral, promoveu a proliferação das células do baço e fagocitose dos macrófagos, e aumentou o nível de IL-2 e células T $CD8^+$ no sangue que nutria o tumor em camundongos. Os resultados desse estudo sugerem que o GLSPs possuem notável atividade antitumoral e imunomodulatória.

Considerando os recentes estudos anticâncer com macroalgas, os nossos resultados foram organizados na forma de artigos científicos com a finalidade de podermos divulgar os nossos resultados promissores em relação à atividade antitumoral dos extratos obtidos a partir de macroalgas da Antártica.

4 Artigos Científicos

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Fatty acid and Chlorophyll *a* profile of Chlorophyta *Prasiola crispa* from Antarctica

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Abbreviations: **Chl**, chlorophyll; **EPA,** eicosapentaenoic acid; **FA**, fatty acid; **PUFA**, polyunsaturated fatty acid; **UFA,** unsaturated fatty acids; **GC-FID**, Gas Chromatography-Flame Ionization Detector

Abstract

The fatty acid and chlorophyll *a* profile of green macroalgae *Prasiola crispa* from Antarctica collected at two different and distinct sites (Arctowski and Demay Point located at the entrance to Admiralty Bay, King George Island , in the South Sheterland Islands) has been described in this paper. Fatty acids were extracted by Bligh Dyer method and analyzed by GC-FID (Gas Chromatography-Flame Ionization Detector) and chlorophyll *a* was obtained using 90% acetone and determined by spectrophotometry. Was possible to confirm the presence of high amounts of unsaturated fatty acids in nutraceutical chlorophyte *P. crispa*, which stand out for numerous health benefits. The major fatty acids were palmitic (C16:0) linoleic (C18:2n6c) and α-Linolenic (C18:3n3) in both sites. The sample from Arctowski showed levels of ω-3, ω-6 and ω-9 around of 24.9, 18.3 and 18.6%, respectively. Algae from Demay, the levels were 35.5, 25.1 and 4.6% (ω-3, 6 and 9, respectively). The determination of chlorophyll *a* showed 0.33±0.02 mg g^{-1} in Demay site and 0.46 ± 0.04 mg g^{-1} in Arctowski.

Practical applications: Fatty acids and omegas have nutraceutical importance, being used in the prevention of numerous diseases from cardiovascular as well as with fetal development. The amount of chlorophyll *a* in photosynthetic organisms is an important measurement that allow calculate photosynthetic and respiratory rates, the metabolically active biomass and the productivity of terrestrial and aquatic ecosystems. In the present work we investigate the levels of fatty acids of the Chlorophyta *Prasiola crispa*, once some of them have great potential for use in human food due to the beneficial effects, and the quantification of Chl *a* demonstrates the metabolic activity of the algae despite the low temperatures.

Keywords: Antarctic macroalgae / Chlorophyll a / Fatty acids / *Prasiola crispa*

Introduction

Benthic marine macroalgae, commonly known as seaweeds, are multicellular photosynthetic organisms ecologically important as primary producers. They play a key role in the food chain and are recognized as ecosystem engineers due to its central function in coastal habitats ranging from kelp forests to coral reefs, determining the physical structure of the habitat [1-3].

Seaweeds allow for the maintenance of local biodiversity [4-5], acting as nurseries and protective shelter for many invertebrate species [6]. Besides, they are useful indicators of

water quality, providing information to assess the ecological status of rocky shores, and indicators of climate change [7-8].

Macroalgae are source of bioactive compounds of immense pharmaceutical and nutraceutical importance [9]. They are rich sources of nutritionally beneficial components such as proteins, carbohydrates, polyunsaturated fatty acid (PUFA), antioxidants, minerals, dietary fibers and vitamins [10]. The nutritionally important C18 and C20 PUFA including *n*-3 PUFA, which are biosynthesized at chloroplasts in photosynthetic eukaryotes, with anti-inflammatory, anti-thrombotic and antiarrhythmic responses [11-14].

Kumari et al. [9] analyzed the FA compositions of 33 species of Chlorophyta belonging to the orders Ulvales, Ulotrichales, Bryopsidales, Siphonocladales and Cladophorales. According to the same author [9], the green algal samples showed higher contents of unsaturated fatty acids (UFA), ranged between 28% (*Ulva lactuca*) and 71% (*Caulerpa veravalensis*) of total FA (TF). The more abundant FA detected in Chlorophyta members were myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1, *n*-9), oleic (C18:1, *n*-9), linoleic (C18:2, *n*-6; LA), α-linolenic (18:3, *n*-3; ALA) and stearidonic acid (C18:4, *n*-3; STA) that collectively contributed to 58.7–88.9% of TFA pool. Interestingly, no trans FA were encountered in any green algae. It has been well reported that trans FA are responsible by increase the risk of cardiovascular diseases [10, 15].

According to Dawczynski et al. [16], marine macroalgae display a FA distribution with a higher levels of *n*-3 FA, where eicosapentaenoic acid (EPA) was as high as 50% of total FA content. Seaweeds can be used as sources of ω-3 (omega-3) fatty acids such as EPA [17]. However, the lipid and fatty acid contents vary according to the species, habitat conditions [18], season [19] and locations.

Antarctic environment is characterized by strong seasonal light conditions and constant low temperatures [20]. In conjunction with the strong geographic isolation of the Antarctic region, this has had great effects on biodiversity [21]. In the Antarctic, 44% of the Heterokontophyta (Phaeophyceae and Chrysophyceae), 36% of the Rhodophyta, and 18% of the Chlorophyta are endemic and the number of endemic species is continuously increasing [22]. In the Antarctic, species richness is high in the Antarctic Peninsula region and about 130 species have been well documented [21, 23].

Sanina et al. [24] observed an increase of unsaturation of FA in winter compared with summer and also in ω-3/ ω-6 ratio when evaluated five species of marine macrophytes. It is reported that ω-3 PUFA is related to the functioning of photosynthetic membranes in winter and may be connected with the defense role of these PUFAs against low-temperature

photoinhibition of photosynthesis [25], once that affect fluidization of thylakoid membranes and the number of double bonds in the content of ω-3 PUFA is important for electron-transport activity of photosystems [24]. The ω-3 PUFA are of particular importance as they cannot be synthesized by humans and are thus obtained only through dietary sources [9].

World Health Organization (WHO) suggests that dietary ω-6/ω-3 ratio should not exceed 10:1, although both PUFA are effective for reducing coronary diseases, diabetes and osteoarthritis [26-27]. Dietary ω-3 PUFA helps reduce heart disease risks, decrease low density lipoprotein (LDL) cholesterol but do not reduce high density lipoprotein (HDL) cholesterol. The incorporation of seaweeds in slimming food may help in weight management, since they have very low lipid, and high dietary fibers and proteins contents [28].

Considering the importance of fatty acids for the health and the possible increase of them at extreme temperatures, the goal of this research was to evaluate the fatty acids and chlorophyll *a* profile of Chlorophyta *Prasiola crispa* collected at two different sites at Antarctica.

2. Materials and Methods

Instrumentation and Apparatus

The following instruments and apparatus were used to sample preparation and analytical procedure. Analytical Knife mill brand QUIMIS Model A298A21 was used for cut samples. A magnetic stirrer, centrifuge Model 222T208, rotaevaporator brand BÜCHI (Switzerland) with vacuum pump Model V-700 and cooler distillation model B-741 were also used in sample preparation. A GC/FID with AOC-20i autosampler (Shimadzu, Japan) was used to analytical measurements. Refrigerated microcentrifuge CIENTEC Model 15000R (1500W) and Visible/UV spectrophotometer JENWAY Model 6705 were used for analysis of chlorophyll.

Reagents and solution

Chloroform, sodium chloride and anhydrous sodium sulfate (Proquimios); hexane and methanol (Panreac, grade HPLC); sodium hydroxide microbeads (Vetec) and BF_3(Boron trifluoride)-methanol (Merck); acetone (Synth) were commercially obtained. The fatty acid standard was Supelco 37

component FAME mix and was purchased from Sigma–Aldrich Inc., USA such as the internal standard nonadecanoate methyl ester.

Samples

Samples of Chlorophyta *Prasiola crispa* were used to evaluate the fatty acids profile and Chlorophyll *a* quantification. They were collected at sites Demay Point (62º13´7.54" × 58º26´29.02") and Arctowski (62º9´44.87" × 58º27´38.79") at Antarctica in 2012, January. The collected material was botanically identified by Dr. Mutue Toyota Fujii (IBt-SP). The algae were collected, frozen at – 70 °C and lyophilized at Comandante Ferraz Antarctic Station (EACF). The algal samples were ground in a knife mill to spray, stored in falcons packed with aluminum foil protected from light.

Analytical Procedure

Fatty acids determination

The lipid extraction followed the conventional method of Bligh & Dyer using 1g of algae [29]. This method is based on the extraction of FA using chloroform and methanol at room temperature. The samples were shaken for 30 minutes with the aid of magnetic stirrer bar, and the extractant composed of 30 mL of chloroform / methanol (1:2 v/v) and 10 mL of sodium sulphate 1.5% (w/v). After stirring were added 10 mL of chloroform and 10 mL of sodium sulphate 1.5% (w/v). The extracts were centrifuged at 3000 rpm (rotation per minute) for 30 minutes, after the organic phase was collected and dried.

Lipids extracted from biomasses were methylated and converted to their respective fatty acid methyl esters (FAME), according to the following methodology modified Moss, Lambert and Merwin (1974) [30]. In 100 mL flask containing the lipid was added 2 mL of NaOH solution (0.5 mol L^{-1}) in methanol 2% (w/v) under stirring and heating to 100 °C, being for a period of 5 minutes at reflux. BF_3 was added 3 mL (Lewis acid) followed by stirring for 2 minutes to acid catalysis to occur, and after added 3 mL of NaCl solution at 20% (w/v). The sample was allowed to stand to room temperature, and was then transferred to a separatory funnel with 20 mL of hexane. The organic phase was separated in hexane (15 mL) and dried

with 2 g of anhydrous sodium sulfate. Evaporation of solvent was carried out with N_2 (g) and weighed sample for subsequent analysis. Samples were analyzed in triplicate.

The quantitative GC analyses have been performed according to the following conditions described: a gas chromatograph GC/FID with AOC-20i autosampler (Shimadzu 2010, Japan) equipped with a fused-silica capillary column (Rtx-WAX, with dimensions of 30 m x 0.25 mm I.D. x 0.25 µm film thickness). Injections were performed with a 1:25 split ratio and hydrogen was used as carrier gas under constant flow mode at 1.2 mL/min. Injector was heated at 250 °C and a flame-ionization detector operated at 250 °C. Oven initial temperature programming used was 100 °C at 7 ºC/min to 200 °C and then increased at 5 °C min^{-1} to 202.6 ºC, isothermal for 2 min at this temperature, and then increased to 5 °C min^{-1} at 222.9 ºC and held isothermal for 2 min and then increased to 230 °C at 5 °C min^{-1} and held isothermal for 10 min at 230 °C [31]. The internal standard solution containing nonadecanoate methyl ester in n-hexane was prepared at a concentration of 2 mg mL^{-1}.

2.5 Chlorophyll *a*

The Chlorophyll *a* (Chl) was obtained directly from the algal biomass previously lyophilized and macerated in N_2 (l). The extraction and spectrophotometric measurements were adapted from Martins et al. [32]. The mixture of the 50 mg of the sample and 1.6 mL of 90% acetone distilled was centrifuged at 10,000 rpm during 15 minutes under cooling (3 °C). The supernatant containing the chlorophyll was transferred to test tubes, sealed and kept protected from light. In following, the chlorophyll concentration was determined by spectrophotometry and quantitated by measuring the absorbance at 630, 664 and 647 nm according to the equation used by Jeffrey & Humphrey [33].

$$[\text{Chl a}]\ \mu g\ L^{-1} = \frac{(11.85\times A - 1.54\times B - 0{,}08\times C)\ .\ v}{V}$$

*where A = absorbance in 664 nm; B = absorbance in 647 nm; C = absorbance in 630 nm; v = acetone volume; V = total volume of extraction (L)

All measurements were performed at room temperature (22±2 °C) in triplicates.

3. Results

Fatty acids

The FA composition of *Prasiola crispa* from Antarctic are summarized in Table 1. The major fatty acids were palmitic (C16:0), linoleic (C18:2n6c) and α-Linolenic (C18:3n3)in both sites, present in concentrations averaging of 9, 15.1 and 26.3% respectively. Differences were observed with respect to palmitoleic (C16:1) and oleic (C18:1n9c) acids, which showed higher concentrations in Arctowski Point, while heptadecenoic acid (C17:1) presents in Demay with 13.4% was not detected in Arctowski. The level of the C16:1 were 8.1 and 2.0% from Arctowski and Demay, respectively. The level of the C18:1n9c were 18.6 and 4.6% from Arctowski and Demay, respectively.

Additionally, it is possible to observe in Table 1, that among theses fatty acids, *ω*-3, *ω*-6 and *ω*-9 are the majority compounds in both collection sites. They represent 61.8 and 65.2% of the total fatty acids from Arctowski and Demay, respectively. The macroalgae *Prasiola crispa* from Arctowski showed *ω*-3, *ω*-6 and *ω*-9 levels around of 24.9, 18.3 and 18.6, respectively. Already from Demay, the levels were 35.5, 25.1 and 4.6% (*ω* 3, 6 and 9, respectively). The relative difference was 10% between the two points, at which Demay Point there was a predominance of omegas (*n*-3 and *n*-6) and at Arctowski Point the amount of omega -9 found in *P. crispa* was higher (18.6) in which Demay (4.6). Besides, the *ω*-6/*ω*-3 ratio is around 0.7.

Chlorophyll *a*

Estimates of chlorophyll *a* from green macroalgae *Prasiola crispa* showed 0.33 ± 0.02 mg g^{-1} of Chl *a* in Demay Point and 0.46 ± 0.04 mg g^{-1} in Arctowski.

4. Discussion

Fatty acids

Different species of macroalgae have been studied as a source of fatty acids [11, 16-19, 34]. However, the use of *P. crispa* to this purpose is still barely explored. The results summarized in Tables 1 shown that the major fatty acids determined in our experiments were C16:0, C16:1, C17:1, C18:1n9c, C18:2n6c and C18:3n3. Graeve and coworkers [35] studied

the fatty acid composition of Arctic and Antarctic macroalgae among than, *P. crispa* using dichloromethane/methanol (2:1, v/v). They showed that the fatty acids predominant in *P. crispa* from Arctic were C16:0; C18:3(n-3) and C18:1(n-7), (28.6, 26.1 and 20.9%, respectively). The results to C16:0 shown in Graeve and coworkers [35] studies from *P. crispa* are distinct to our studies (9% on average). However, the levels of C18:1n9c and C18:2n6c were lower (1.7% and 6.3% on average, respectively) than in our experiment (11.6% and 15.1%, respectively). The absence of C17: 1 observed in Arctowski Point was also observed by Graeve and coworkers [35].

Additionally, it is possible to observe, that among theses fatty acids, omegas 3, 6 and 9 are the majority compounds in both collection sites. Previously, Graeve and coworkers [35] reported that this macroalgae had levels of 39.4 and 8.7% of the ω-3 and ω-6, respectively; and they did not determined ω-9 in it.

Fatty acids unsaturated and omegas have nutraceutical importance, being used in the prevention of numerous diseases from cardiovascular as well as with fetal development. The amount of chlorophyll *a* in photosynthetic organisms is an important measurement that allow calculate photosynthetic and respiratory rates, the metabolically active biomass and the productivity of terrestrial and aquatic ecosystems. In the present work we investigate the levels of fatty acids of the Chlorophyta *Prasiola crispa*, once some of them have great potential for use in human food due to the beneficial effects, and the quantification of Chl *a* demonstrates the metabolic activity of the algae despite the low temperatures.

The studies about obtainment of omegas are very important once these compounds are known for its beneficial properties. From a nutritional point of view, ω-6 and ω-3 are commonly considered as ‘‘essential’’ EFA, since they are not synthesized in the human body and are mostly obtained from the diet [9, 26].

Several authors tended to explain the EFA/PUFA effects in terms of a balance between total ω-6 and ω-3, rather than the absolute amount of each single molecule [9]. The importance of the ω-6/ω-3 ratio has been evoked not only in the pathogenesis of cardiovascular diseases, but also in cancer, inflammatory and autoimmune diseases [12, 17, 26, 27]. In the most simplistic interpretation, a very high ω-6/ω-3 ratio is considered detrimental for human health, while a value as much as possibly close to 1 is considered protective against degenerative pathologies [9].

Once the ω-6/ω-3 ratio, which the WHO currently recommends should be no higher than 10 in the diet [36] we can infer that the biomass obtained from *P. crispa* showed in this studied can be used for reduction of ω-6/ω-3 ratio (0.7%) and consequently applied in the alimentary diet.

Chlorophyll *a*

Chlorophyll *a* is the primary photosynthetic pigment in nearly all known oxygenic photosynthetic organisms, and is a fundamental measurement in many branches of plant biology and ecology [37]. Acetone was the solvent chosen since it is well referenced in the literature for gives very sharp Chl absorption peaks and so used for Chl assays [33, 37-40].

Chl *a* (or sometimes total Chl) are usually used as the bases for the calculation of photosynthetic and respiratory rates, the metabolically active biomass, and the productivity of terrestrial and aquatic ecosystems [41]. In the Antarctic green algae *Prasiola* photosynthetic activity was observed down to -15°C [42], however the physiological basis of this extraordinary capacity is not known yet [22]. The quantities of Chl *a* extracted from the macroalgae at distinct sites showed a small variation of values at Arctowski (0.46 ± 0.04 mg g^{-1}) than at Demay Point (0.33 ± 0.02 mg g^{-1}), suggesting higher metabolic rates in Arctowski.

Considering their high metabolic activity at extreme low temperatures, PUFA play an important role as defense of photoinhibition of photosynthesis, acting at electron-transport activity of photosystems and ensure fluidization of thylakoid membranes as seen previous [24-25].

5. Conclusions:

The Chlorophyta *Prasiola crispa* collected at two locations at Antarctica showed high proportions of ω-3 and ω-6, which has demonstrated high nutraceutical importance. Besides, the analysis of chlorophyll demonstrates its high metabolic activity despite the freezing conditions of the region.

6. Acknowledgements

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7. Conflict of interest

There are no conflicts of interest among the authors.

8. References

[1] Schiel, D. R., Foster, M. S., The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. *Annu. Rev. Ecol. Evol. S.* 2006, *37*, 343–372.

[2] Harley, C. D. G., Anderson, K. M., Demes, K. W., Jorve, J. P., Kordas, R. L., Coyle, T. A., Effects of climate change on global seaweed communities. *J. Phycol.* 2012, *48*, 1064-1078.

[3] Chung, I. K., Oak, J. H., Lee, J. A., Shin, J. A., Kim, J. G., Park, K. -S., Installing kelp forests/seaweed beds for mitigation and adaptation against global warming: Korean Project Overview. *ICES J. Mar. Sci.* 2013, *70*, 1038-1044.

[4] Schiel, D. R., Rivets or bolts? When single species count in the function of temperate rocky reef communities. *J. Exp. Mar. Biol. Ecol.* 2006, *338*, 233-252.

[5] Schiel, D. R., Lilley, S., Gradients of disturbance to an algal canopy and the modification of an intertidal community. *Mar. Ecol-Prog. Ser.* 2007, *339*, 1-11.

[6] Raybaud, V., Beaugrand, G., Goberville, E., Delebecq, G., Destombe, C., Valero, M., Davoult, D., Morin, P., Gevaert, F., Decline in Kelp in West Europe and Climate. *Plos One.* 2013, *8*, e66044.

[7] Díez, I., Muguerza, N., Santolaria, A., Ganzedo, U., Gorostiaga, J. M., Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. *Estuar. Coast. Shelf S.* 2012, *99*, 108–120.

[8] Borja, Á., Fontán, A., Muxika, I., Interactions between climatic variables and human pressures upon a macroalgae population: Implications for management. *Ocean Coast. Manage.* 2013, *76*, 85–95.

[9] Kumari, P., Bijo, A. J., Mantri, V. A., Reddy, C. R. K., Jha, B., Fatty acid profiling of tropical marine macroalgae: An analysis from chemotaxonomic and nutritional perspectives. *Phytochemistry*. 2013, *86*, 44–56.

[10] Mohamed, S., Hashim, S. N., Rahman, H. A., Seaweeds: a sustainable functionalfood for complementary and alternative therapy. *Trends Food Sci. Tech.* 2012, *23*, 83–96.

[11] Khan, M. N. A., Cho, J. Y., Lee, M. C., Kang, J. Y., Park, N. G., Fujii, H., Hong, Y. K., Isolation of two anti-inflammatory and one pro-inflammatory polyunsaturated fatty acids from the brown seaweed *Undaria pinnatifida*. *J. Agr. Food Chem.* 2007, *55*, 6984-6988.

[12] Richard, D., Bausero, P., Schneider, C., Visioli, F., Polyunsaturated fatty acids and cardiovascular disease. *Cell. Mol. Life Sci.* 2009, *66*, 3277–3288.

[13] Kumari, P., Kumar, M., Gupta, V., Reddy, C. R. K., Jha, B., Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chem.* 2010, *120*, 749–757.

[14] Martinez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Vecino, J. G., Ruohonen, K., Bell, J. G., Tocher, D. R., Effect of functional feeds on fatty acid and eicosanoid metabolism in liver andhead kidney of Atlantic salmon (*Salmo salar* L.) with experimentally induced Heart and Skeletal Muscle Inflammation. *Fish Shellfish Immun.* 2013, *34*, 1533-1545.

[15] Ferreri, C., Chatgilialoglu, C., Geometrical trans lipid isomers: a new target for lipidomics. *ChemBiochem*. 2005, *6*, 1722–1734.

[16] Dawczynski, C., Schubert, R., Jahreis, G., Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.* 2007, *103*, 891-899.

[17] Polat, S., Ozogul, Y., Seasonal proximate and fatty acid variations of some seaweeds from the northeastern Mediterranean coast. *Oceanologia.* 2013, *55*, 375–391.

[18] Khotimchenko, S. V., Lipids from the marine alga *Gracilaria verrucosa*. *Chem. Nat. Compd.* 2005, *41*, 285–288.

[19] Kendel, M., Couzinet-Mossion, A., Viau, M., Fleurence, J., Barnathan, G., Wielgosz-Collin, G., Seasonal composition of lipids, fatty acids, and sterolsin the edible red alga *Grateloupia turuturu*. *J. Appl. Phycol.* 2013, *25*, 425-432.

[20] K. Zacher, R. Rautenberger, D. Hanelt, A. Wulff, C. Wiencke: The abiotic environment of polar benthic algae. In: Biology of polar benthic algae. Ed. C. Wiencke, De Gruyter, Berlin (Germany) 2011, pp.9–22.

[21] A. Wulff, K. Iken, M. L. Quartino, A. Al-Handal, C. Wiencke, M. N. Clayton: Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. In: Biology of polar benthic algae. Eds. C. Wiencke, De Gruyter, Berlin (Germany) 2011, pp. 23–52.

[22] C. Wiencke, Amsler, C. D.: Seaweeds and their communities in polar regions. In: Seaweed biology: novel insights into ecophysiology, ecology and utilization Eds. C. Wiencke, K. Bischof, Springer-Verlag, Berlin (Germany) 2012, pp.265-291.

[23] C. Wiencke, M. N. Clayton: Antarctic Seaweeds. ARG Gantner Verlag, KG (Ruggell) 2002.

[24] Sanina, N. M., Goncharova, S. N., Kostetsky, E. Y., Seasonal changes of fatty acid composition and thermo tropic behavior of polar lipids from marine macrophytes. *Phytochemistry.* 2008, *69*, 1517–1527.

[25] R. E. Blankenship: Molecular Mechanisms of Photosynthesis. Blackwell Science Ltd., Oxford (England) 2002.

[26] Russo, G. L. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem. Pharmacol.* 2009, *77*, 937-946.

[27] Simopoulos, A. P., Omega-6/omega-3 essential fatty acids: biological effects. *World Rev. Nutr. Diet.* 2009, *99*, 1-16.

[28] Matanjun, P., Mohamed, S., Mustapha, N. M., Muhammad, K., Nutrient content of tropical edible seaweeds, *Euchema cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *J. Appl. Phycol.* 2009, *21*, 75–80.

[29] Bligh, E. G., Dyer, W. J., A rapid method for total lipid extraction and purification. *Can. J. Biochem. Phys.* 1959, *37*, 911-917.

[30] Moss, C. W., Lambert, M. A., Merwin, W. H., Comparation of rapid methods for analysis of bacterial fatty acids. *J. Appl. Microbiol.* 1974, *28*, 80-85.

[31] Tang, B., Row, K. H., Development of gas chromatography analysis of fatty acids in marine organisms. *J. Chromatogr. Sci.* 2013, *51*, 599-607.

[32] Martins, A. P., Necchi Junior, O., Colepicolo, P., Yokoya, N. S., Effects of nitrate and phosphate availabilities on growth, photosynthesis and pigment and protein contents in colour strains of *Hypnea musciformis* (Wulfen in Jacqu.) J.V. Lamour. (Gigartinales, Rhodophyta). *Braz. J. Pharmacog.* 2011, *21*, 340-348.

[33] Jeffrey, S. W., Humphrey, G. F., New spectrophotometric equations for determining chlorophyll *a*, *b*, *c1* and *c2* in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pfl.* 1975, *167*, 191-194.

[34] Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., Paseiro-Losada, P., Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.* 2004, *85*, 439–444.

[35] Graeve, M., Kattner, G., Wiencke, C., Karsten, U., Fatty acid composition of Arctic and Antarctic macroalgae: indicator of phylogenetic and trophic relationships. *Mar. Ecol-Prog. Ser.* 2002, *231*, 67–74.

[36] Folch, J., Lees, M., Sloane-Stanley, G. H., A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 1957, *226*, 497–509.

[37] Ritchie, R. J., Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynth. Res.* 2006, *89*, 27–41.

[38] Arnon, D. I., Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 1949, *24*,1-15.

[39] R. J. Porra: Spectrometric Assays for Plant, Algal and Bacterial Chlorophylls. In: Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and

Applications. Eds. B. Grimm, R. J. Porra, W. Rüdiger, H. Scheer, Springer (Netherlands) 2006, pp.95–107.

[40] Su, S., Zhou, Y., Qin, J. G., Yao, W., Ma, Z., Optimization of the Method for Chlorophyll Extraction in Aquatic Plants. *J. Freshwater Ecol.* 2010, *25*, 531-538.

[41] Z. Šesták: Determinations of chlorophylls *a* and *b*. In: Plant Photosynthetic Production: Manual of Methods. Eds. Z. Šesták, J. Čatský, P. G. Jarvis, Dr W. Junk N.V. Publ., The Hague (Netherland) 1971, pp.672-701.

[42] Becker, E. W., Physiological studies on Antarctic *Prasiola crispa* and *Nostoc* commune at low temperatures. *Polar Biol.* 1982, *1*, 99–104.

Table 1. Fatty acid composition (%) of Antarctic Chlorophyta *Prasiola crispa* from different collection sites.

Fatty acids	*Prasiola crispa** (%)	
	Arctowski	Demay
C 14:0	2.6	3.6
C 16:0	9.3	8.8
C 16:1	8.1	2.0
C17:1	-	13.4
C 18:0	1.5	1.5
C 18:1n9c	18.6	4.6
C 18:2n6c	13.0	17.3
C 18:3n6	-	1.2
C 18:3n3	22.7	30.0
C 20:0	-	1.1
C 20:2	-	1.5
C 20:3n6	0.8	1.2
C 20:4n6	4.5	6.6
C 20:3n3	1.9	2.7
C 20:5n3	0.3	2.8
C 22:0	-	1.3
C 23:0	1.5	-
C24:0	5.2	-
ω-3	24.9	35.5
ω-6	18.3	25.1
ω-9	18.6	4.6
ω-6/ ω-3	0.7	0.7

* n=3, Standard desviation slower than 0.23

4.2 Manuscritos a serem submetidos no periódico “Polar Biology”

Antitumor activity of extracts from Antarctic macroalgae *Iridaea cordata* and *Desmarestia anceps*

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Abstract

The marine environment is home to a wide diversity of algal species, which can provide a variety of chemical compounds which may have pharmacological properties. This research aimed evaluates the antitumor activity against the malignant tumor Glioblastoma Multiforme of extracts from Antarctic seaweeds *Iridaea cordata* and *Desmarestia anceps* collected at Peninsula Antarctica. Hexane, chloroform and ethanol extracts were evaluated in rat glioblastoma, human glioblastoma and astrocytes cell lineage in order to analyze the cytotoxicity. All extracts showed significant antiproliferative effect in glioma cells, without showing cytotoxicity for healthy cell (astrocytes), which were evaluated by the MTT and propidium iodide assay. Despite the observed effects, the human glioblastoma showed an inhibition of less than that evidenced in rat glioblastoma growth. Based on these promising data, it is expected that future studies achieve the isolation and therefore the synthesis of biologically active molecules.

Keywords: Algae. Antiproliferative effect. Endemic brown algae. Glioblastoma. Rhodophyta.

1. Introduction

The oceans harbor 90 % of the world’s living biomass, which makes up about half of the total global biodiversity (Kim and Wijesekara 2010). Among which algae have been estimated to include anything from 30,000 to more than 1 million species. A conservative approach results in an estimate of 72,500 algal species (Guiry 2012). These species survive and live within complex communities and in close association with other

organisms. This diversity of living systems and habitats defines the basis of the wide variety of chemical classes typical of marine derived molecules (Alves et al. 2013).

In polar regions, the environment is characterized by strong seasonal light conditions, constant low temperatures, besides another stresses situations that seaweeds are exposed such as desiccation, salinity, radiation and predation (Karsten et al. 2011; Zacher et al. 2011; Wiencke and Amsler 2012). Seaweeds growing in such extreme environment have to be adapted to these conditions and, consequently, produce a great variety of secondary metabolites to protect them, which may have potent biological activities (Maschek and Baker 2008; Ibañez and Cifuentes 2013). Recently has been described many biological activities of seaweeds as antinociceptive, anti-inflammatory, antioxidant, antifungal, antibacterial, renin inhibitory, antimalarial, gastroprotective, anti-ulcerogenic and antitumor activities (Alves et al. 2012; Cavalcante-Silva et al. 2012; Fitzgerald et al. 2012; Genovese et al. 2012; Guedes et al. 2012; Stout et al. 2010; Yu et al. 2012; Shu et al. 2013).

In special, the cold-temperate Rhodophyta species *Iridaea cordata*, whose distribution extends from the Ross Sea in East Antarctica to Tierra del Fuego and on subantarctic islands (Wiencke and Clayton 2002), frequently colonize low intertidal pools and crevices in the upper sublittoral (Wiencke and Amsler 2012). *I. cordata* is more abundant in the 2–10 m depth range with densities up to 4,000 individuals m^{-2} and wet biomass levels up to 3.5 kg m^{-2} (Cattaneo-Vietti et al. 2000). *Desmarestia anceps*, an endemic Antarctic Phaeophyta, is a deeper species, usually dominate in waters to 10–20 m, sometimes can be found at 30 m or more. Besides, it is one of the main species with the highest macroalgal biomass along the Antarctic Peninsula (Quartino et al. 2008). Ecological studies about the species have been conducted (Wiencke et al. 1996; Iken et al. 2009; Aumack et al. 2011; Amsler et al. 2012). However, due to be endemic to a poorly studied region, little is known about pharmacological properties of this macroalgae.

Human glioblastoma multiforme (GBM) is the most common, aggressive, and deadly form of brain cancer (Pedron et al. 2013). Despite increasing knowledge of the genetic and molecular changes associated with GBM pathogenesis and the development of multimodal therapies including surgery, chemotherapy and radiotherapy, the tumor invariably recurs (Stupp et al. 2009). The poor prognosis for GBM has led investigators to seek new, innovative treatments with minimal side effects (Li et al. 2013). Considering the wide spectrum of biological activities that algae present and the possibility of formation of new molecules in polar environments, the goal of our research is evaluate the antitumor activity of seaweeds *Iridaea cordata* and *Desmarestia anceps* collected at Antarctica against GBM.

2. Materials and Methods

Chemicals

Dulbecco's modified eagle's medium–high glucose (DMEM), trypsin–EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), propidium iodide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Heat-inactivated fetal bovine serum (FBS) was purchased from Cultilab Lab Inc. (Campinas, SP, BR). Petroleum ether, hexane, chloroform and ethanol were obtained from Synth. All other chemicals were of analytical grade and HPLC grade solvents.

Algal Sample

The Rhodophyta (Gigartinales) *Iridaea cordata* (Turner) Bory de Saint-Vincent and Phaeophyta *Desmarestia anceps* Montagne weres collected in the Demay site in Admiralty Bay, King George Island (62º13´ S × 58º26´ W) at Antarctica in January, 27 in 2012. The algae fixed to the substrate was collected during periods of low tide; part of the material was preserved in 4 % formaldehyde solution in seawater for identification and the preparation of herbarium specimen, and part was washed with seawater to remove epiphytes, frozen at -20

°C, and lyophilized for biochemical analyzes. Species identification was performed according to specimen morphology and the classification system proposed by Wynne (2005), and herbarium specimen was deposited in the Herbarium of Instituto de Botânica, São Paulo.

Preparation of extracts

The extraction was performed by depletion twice, using three extractions with solvents: hexane, chloroform and ethanol, using two systems: Ultrasound and Soxhlet, according to Agência Nacional de Vigilância Sanitária - National Health Surveillance Agency (2010). In the system Soxhlet, prewashed with petroleum ether, 1 g of macroalgae were used for each 150 mL solvent extraction, held for 4 hours and the cartridge with the biomass of macroalgae remained submerged in the solvent overnight (method adapted from Crespo and Yusty 2006).

In Ultrasound probe (SONICS Vibra-Cell, 500Watt), with amplitude of 40 %, the seaweed was submitted to extraction for 30 minutes with the solvents mentioned previously (40 mL), using the same technique depletion in three cycles with renewal of solvent and evaporation of the extract, and ice bath, avoiding large swings in temperature (method Stein et al. 2011 - modified). The extracts were evaporated (Büchi Rotaevaporador RII) at the end to remove the solvents.

Liquid chromatography analysis of extracts

To analyze extracts was used Liquid Chromatography Ultra High Efficiency (UHPLC) coupled to a Mass Spectrometer for High Resolution (type quadrupole-time of flight), Shimadzu Nexera, Bruker Impact. The analysis used was the positive ionisation mode, with a total time of 42 min, flow 0.2 $mL.min^{-1}$ and the column Shimadzu Shim-Pack XR-ODS III 150 x 2.0 mm. The Impact mass spectrometer was calibrated of m/z 90-1200 using 10 mM sodium formate. To assist in proposing molecular formulas was used an automatic method of fragmentation of ions along the chromatographic run [AutoMS(n)].

Cell Culture

The rat (C6) and human (U87) malignant glioblastoma multiforme cell lines, a grade IV glioma, were obtained from ATCC (CCL-107™ and HTB-14™ number respectively). The rat astrocytes were prepared in our laboratory. Cells were cultured in DMEM supplemented with 5% heat-inactivated FBS. Cells were maintained in a humidified atmosphere at 37 °C, in 5% CO_2, and 95% air. The cytotoxicity study was performed when the cells reached 70-80% confluence. The cells (5×10^3) were seeded in 96-well plates with 100 µl media per well, and after exposed to different extracts at concentrations of 10-1000 $\mu g.mL^{-1}$ for 24 and 48 hours.

MTT Assay

Cell viability was assessed by the 3-(4,5-dimethyl)-2,5 diphenyl tetrazolium bromide (MTT) assay. This method is based on the ability of viable cells to reduce MTT and form a blue formazan product. MTT solution (sterile stock solution of 5 $mg.mL^{-1}$) was added to the incubation medium in the wells at a final concentration of 0.5 mg/ml. The cells were left for 2 hours at 37 °C in a humidified 5% CO_2 atmosphere. The medium was then removed, after drying, was added DMSO. The optical density of each well was measured at 405 nm. Results were expressed as the percentage of cell inhibition against the control (DMEM + DMSO).

Propidium iodide Assay (PI)

C6 cells were subcultured into 24-well tissue culture plates containing 1×10^4 cells per well and reaching subconfluence after 24 h of culture. Cells were treated with chloroform extract (1, 10, 50, 100, 250 and 1000 $\mu g.mL^{-1}$) for 48 h. Following the treatments, cells were incubated with propidium iodide (PI) (7.5 mM) for 1 h. PI fluorescence was excited at 515–560 nm using an inverted microscope (Olympus IX71, Tokyo, Japan) fitted with a standard rhodamine filter. Images were captured using a digital camera connected to the microscope. This indicator of cell viability monitor plasma integrity through nuclear staining by membrane-impermeant dyes (Nieminen et al. 1992).

Statistical analysis

All experiments were performed in quadruplicate. Results are expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance ANOVA, with Tukey's post hoc multiple comparisons test to assess statistical differences in case of normal distribution. Differences were considered to be statistically significant if $P < 0.05$.

3. Results

Antitumor activity

Initially, we conducted a screening of extracts with the strain of rat glioblastoma (C6), which was submitted to three different extracts (hexane, chloroform and ethanol) of Soxhlet and Ultrasound from *I. cordata* and *D. anceps* (Table 1).

Table 1. Inhibition of growth of rat gliomas with hexane, chloroform and ethanol extracts at Soxhlet (%).

Algae	Extract	Exposure Time (h)	10 $\mu g.mL^{-1}$	100 $\mu g.mL^{-1}$	250 $\mu g.mL^{-1}$	500 $\mu g.mL^{-1}$	1000 $\mu g.mL^{-1}$
D. anceps	**Hexane**	24	0.90±1	22.04±1	22.93±0	25.28±1	24.99±1
		48	7.49±4	43.40±3	51.19±2	50.72±2	49.72±1
	Chloroform	24	4.93±4	25.99±1	26.99±1	28.22±1	28.83±1
		48	12.25±4	41.31±3	44.41±4	45.07±4	45.02±2
	Ethanol	24	0.00±0	5.96±1	18.08±1	20.77±1	24.42±1
		48	0.00±0	7.51±4	30.85±3	49.11±2	45.69±1
I.cordata	**Hexane**	24	0.00±0	0.00±0	0.00±0	0.00±0	8.89±1
		48	2.45±3	11.38±3	24.08±6	28.13±3	26.35±3
	Chloroform	24	4.67±0	17.72±3	29.97±1	30.12±1	41.52±1
		48	8.04±2	32.87±1	38.46±1	38.81±1	44.75±1
	Ethanol	24	0.00±0	0.00±0	0.00±3	2.24±2	5.50±1
		48	0.00±0	2.90±2	9.15±4	19.76±4	26.48±3

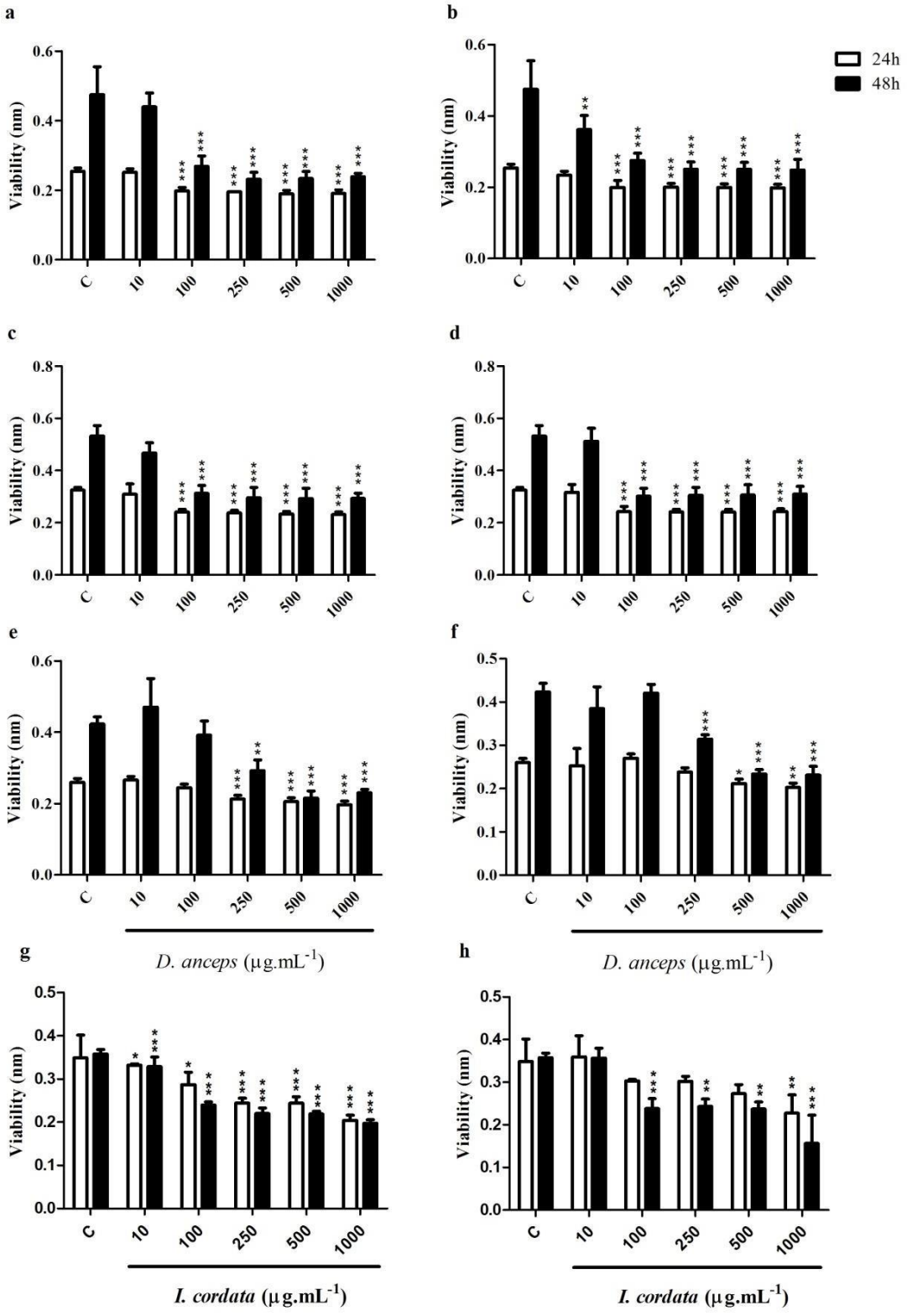

Figure 1. Viability of rat glioblastoma in hexane (a and b), chloroform (c, d, g and h) and ethanol (e and f) extracts from soxhet (left column) and ultrasound (right column).

*P<0.05; **P<0.005; ***P<0.001 compared to control in time corresponding analysis

The results obtained showed very similar responses from extracts obtained by soxhlet and by ultrasound. All extracts of *D. anceps* showed antiproliferative activity varying degrees. The hexane extract inhibited approximately 25 % growth of C6 in 100 µg.mL^{-1} after 24 hours of exposition and 50 % in 250 µg.mL^{-1} after 48 hours. The chloroform extract showed similar results. While ethanol extract inhibited 25% of growth only in 1000 µg.mL^{-1} after 24 hours and 50 % in 500 µg.mL^{-1} after 48 hours (Fig. 1). The best extract of *I. cordata* was chloroform in Soxhlet after 48h of exposure, which inhibited 32.9 % of cell growth in 100 µg.mL^{-1} and 45 % in 1000 µg.mL^{-1} (Fig. 1). After, an initial screening of different extracts, were chosen those obtained by soxhlet, once that they had best result in ethanol at 24 hours in *D. anceps*, and treated U87 with extracts that had best inhibition (Table 2).

Table 2. Inhibition of growth of human glioma with best extracts at soxhlet after 24 hours (%).

Algae	Extract	10 µg.mL^{-1}	100 µg.mL^{-1}	250 µg.mL^{-1}	500 µg.mL^{-1}	1000 µg.mL^{-1}
D. anceps	**Hexane**	13.83±4	17.49±4	20.40±5	22.34±4	21.89±4
	Chloroform	7.50±3	10.38±4	11.28±4	8.46±4	66.03±1
	Ethanol	12.97±4	12.97±4	15.66±4	22.51±4	22.51±4
I. cordata	**Chloroform**	9.74±4	9.04±5	16.47±4	18.91±4	18.46±4

The U87 cells showed distinct effects, the hexane extract of *D. anceps* inhibited 20 % growth in 250 µg.mL^{-1} after 24 hours, chloroform extract inhibited 11 % and ethanol extract inhibited 16 %; while chloroform extract from *I. cordata* inhibited 16 %.

Cytotoxic activity

After finding statistically significant antitumor activity of extracts obtained by Soxhlet, in order to demonstrate the efficiency of them for use in treatment of gliomas, their cytotoxicity was evaluated on healthy cells (rat astrocytes) by MTT assay (Fig. 2) and propidium iodide assay (Online Resource 1 and 2). When comparing both tests, it appears that from the 100 µg.mL^{-1} there is considerable activity of the extract.

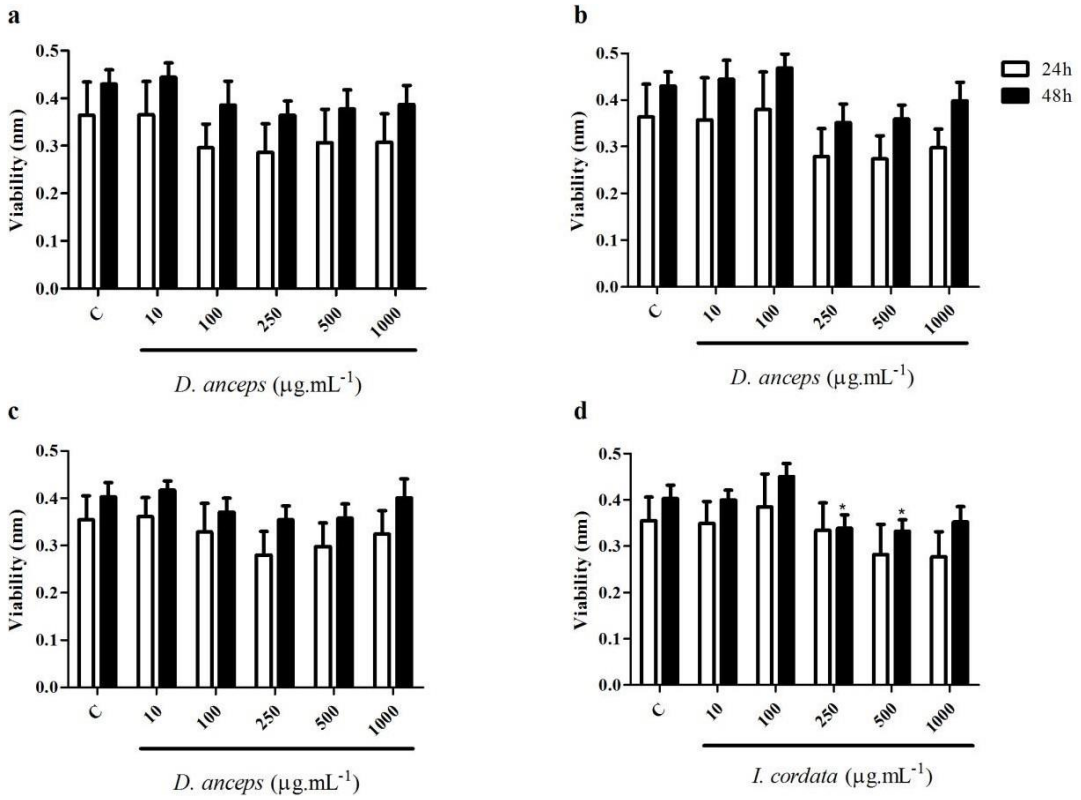

Figure 2. Cytotoxic activity of extracts in rat astrocytes: (a) hexane, (b) ethanol, (c and d) chloroform.

Chromatographic analysis

The chemical characterization of the extracts from the Soxhlet, which were selected to continue the tests, was performed by liquid chromatography (UHPLC) (Figs. 4, 5,6 and 7).

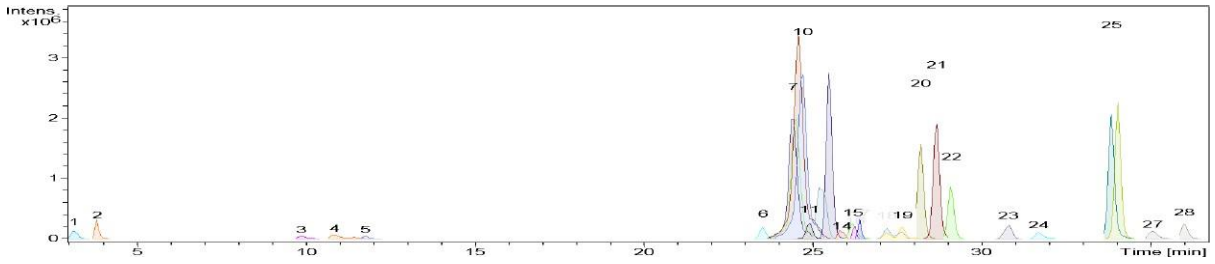

Figure 4. Chromatogram of the hexane extract.

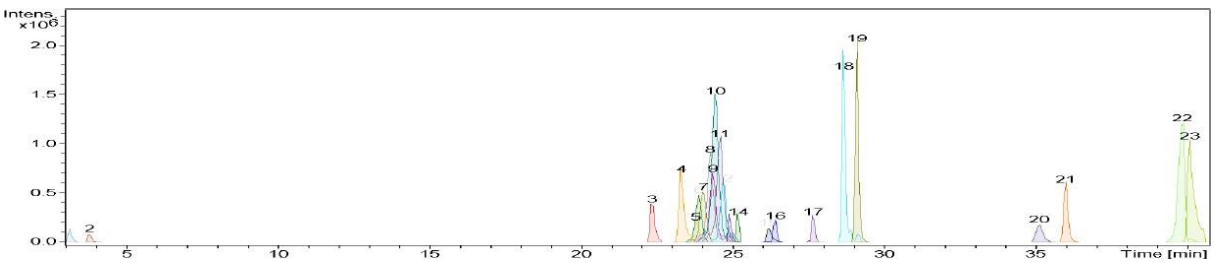

Figure 5. Chromatogram of the chloroform extract.

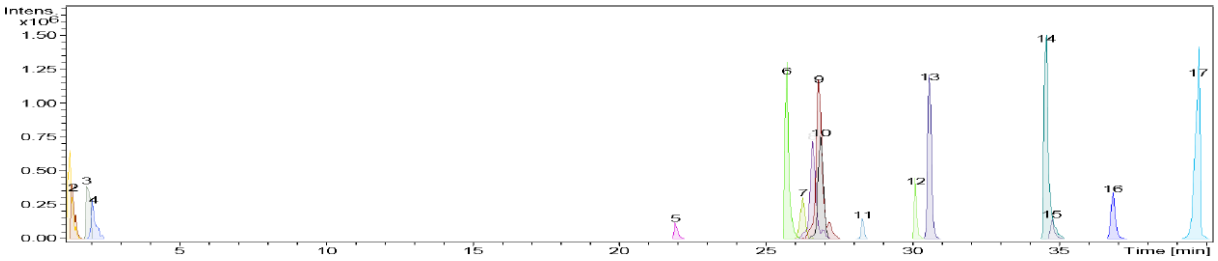

Figure 6. Chromatogram of the ethanol extract.

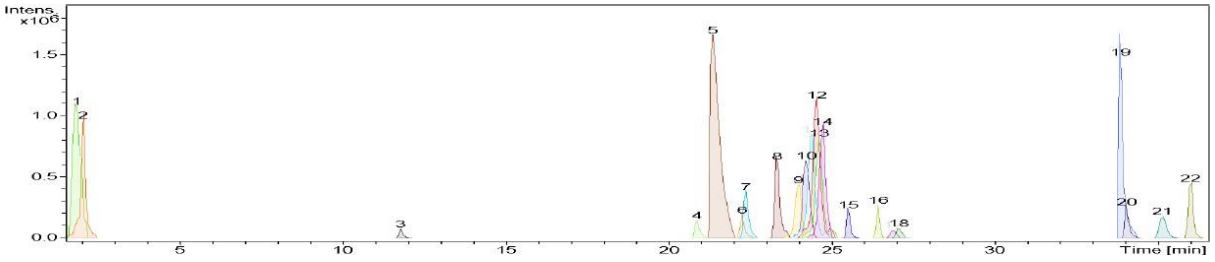

Figure 7. Chromatogram of the chloroform extract.

4. Discussion

Despite advances in therapeutic, patients with malignant gliomas still have a poor prognosis (Mohrenz et al. 2013). The intrinsic resistance to apoptosis plays a fundamental role for the therapy resistance of malignant gliomas (Ziegler et al. 2008). The discovery of the DNA-methylating agent temozolomide was a recent advance in chemotherapy for malignant gliomas, however the drug resistance is a recurring problem (Hayashi et al., 2013). Bioprospecting the oceans, in particular drawing on the diversity of molecules, enzymes and genes found in largely unexplored groups of marine organisms could provide new insights (Abida et al. 2013). Algae exhibit a variety of bioactive molecules, those exposed to the harsh conditions of the Antarctic environment are able to produce numerous secondary metabolites to adapt to extreme environmental changes (Wiencke and Amsler 2012). Thus, at this research we seek to obtain an extract of macroalgae from Antarctica *Desmarestia anceps* and *Iridaea cordata* that were able to inhibit the growth of gliomas.

Among results of different extracts of *D. anceps*, the human malignant glioblastoma multiforme showed less inhibition (13 % in hexane) than rat glioblastoma (51 % in hexane), once that U87 is a more complex lineage. The discrepancy observed effect concentration of 1000 µg.mL^{-1} at 24h (66 %) may result from high concentrations of the extract have caused changes in the absorbance reading of the plate, whereas inhibition after 48 h and 19 %. Among the extracts of *I. cordata* tested, chloroform extract had the best effect on the inhibition of cell growth, once hexane and ethanol extracts showed an effect only after 48 hours of exposure and at higher concentrations than chloroform. Chloroform extract showed 33 % of inhibition in 100 µg.mL^{-1} after 24 hours and initial answer in 10 µg.mL^{-1} after 48 hours at Soxhlet,

In general, both extracts of *D. anceps* as *I. cordata*, showed better inhibition after 48 hours of exposure in the rat gliomas, while in human gliomas better response to treatment was observed after 24 hours. Besides, the extracts obtained by two systems (soxhlet and ultrasound) showed similar effects (P<0.05). Despite high extraction yields of good quality from ultrasonic-assisted extraction, besides it finish in shorter periods of time and uses lower amounts of solvent than traditional processes, as soxhlet (Barrera Vázquez et al. 2014), from the results we chose to use the classic method of Soxhlet in tests. However, the extract from ultrasound (data not shown) wasn't choose to analyze the cytotoxicity, due to the fact that the chromatography identified long chain

alkanes at this extracts, which are described in the literature showing non-selective toxic effect (Cooley et al. 2001).

Thus, as we seek an antitumor activity with selective effect, we chose to use the extracts of the soxhlet. Since presenting the best inhibition effects tumor cells, the chloroform of *I. cordata* and all extracts of *D. anceps* from Soxhlet were tested in astrocytes, as control cells. There was a small inhibition at 250 and 500 $\mu g.mL^{-1}$ after 48 hours of exposure in chloroform extract from *I. cordata*, but this effect is observed at concentrations above those already present effect in gliomas. While extracts of *D. anceps* showed no significant inhibition of the cells compared to tumor cell lines.

In the assay with propidium iodide concentrations below those used in cell viability test were used to accompany the onset of the apoptotic effect. Thus, it was possible to observe the onset of effect of the hexane extract of *D. anceps* at 50 $\mu g.mL^{-1}$ in 24 h as well as gradual change in morphology of the cells increased as the concentration. The chloroform extract also initiated the apoptotic effect in 50 $\mu g.mL^{-1}$, but from 48h with clear morphological change. In contrast, shows a more potent effect as compared to 100 $\mu g.mL^{-1}$ of hexane extract. While the ethanolic extract already presents in the first concentrations gap formation between cells, reducing cell proliferation from 10 $\mu g.mL^{-1}$, being stained cells with iodide only from 100 $\mu g.mL^{-1}$. At chloroform extract of *I.* cordata, as low as 100 $\mu g.mL^{-1}$ showed a clear cell death using propidium iodide assay with a great change in cell morphology. Thus, we decided to evaluate the effect at lower concentrations. Despite lower concentrations do not show cells stained by propidium iodide was possible to follow the confluence reduction of the cells, formation of gaps and concentration-dependent effect.

The non-polar extracts (hexane and chloroform) showed the highest inhibition of growth of tumor cells, although ethanol extract also demonstrated potential antiproliferative activity. The extraction of secondary metabolites belong to various chemical classes is based on their polarity. Usually, the polar metabolites are extracted using water, methanol and ethanol, while non-polar metabolites are extracted using chloroform, dichloromethane and hexane solvents (Jones and Kinghorn 2005; Rispail et al. 2005). In nonpolar extract the most common classes of metabolites obtained are phenolics, quinones, terpenoids, sterols, esters of terpenes, fatty acids and hydrocarbons (Bony et al. 2014). Phenols and flavonoids have been reported to exert their antiproliferative effects by regulating apoptosis via loss of mitochondrial membrane permeability and subsequent release of cytochrome c, decreasing Bcl-2 levels and increasing Bax levels, caspase-8 and caspase-10 levels, and Fas death receptor signaling (Murugan and Yver 2013).

Lipophilic molecules are interesting in the therapeutic area, which are able to enter the central nervous system passively by diffusion, once that blood brain barrier exhibits low permeability to hydrophilic molecules (Dyrna et al. 2013). According El Baz et al. (2013), sulfolipid fractions showed potential antitumor activity. The Rhodophyta *Laurencia papillosa* (Gigartinales) presented high inhibition percentages (94.19%) toward the human breast carcinoma (MCF7), 79.89% of inhibition against human hepato carcinoma (Hep G2).

Brown seaweeds have high concentrations of polyphenols, such as phlorotannins that consist of polymers of phloroglucinol, which are formed in the acetate-malonate pathway in marine algae (Targett and Arnold 1998; Wijesekara and Kim 2010). They are highly hydrophilic components, so extracted in polar solvents. According Iken et al. (2009), phlorotannins from *D. anceps* showed antimicrobial effects against four Antarctic bacterial strains. These compounds isolated from other seaweeds have beneficial biological activities including anti-HIV (Artan et al. 2008), antiproliferative (Kong et al. 2009), anti-inflammatory (Kim et al. 2013), radioprotective (Zhang et al. 2008), anti-Alzheimer disease (Kannan et al. 2013), antimicrobial (Eoma et al. 2012), anticancer (Park and Pezzuto 2013), antioxidant (Kellogg and Lila 2013) and anti-diabetic (Lee and Jeon 2013) activities.

Many authors have correlated the antioxidant activity of macroalgae to phenolic compounds and sulfated polysaccharides, although both have the ability to neutralize free radicals (Imbs et al. 2013). An important relationship is that oxidative stress resulting from excessive reactive oxygen/nitrogen/electrophilic species (ROS/RNS/RES) can lead to diseases such as cancer (Park and Pezzuto 2013). In this research line, Subbiah and Sundaresan (2012) reported antitumor and antioxidant activities of the methanol extract of *Spyridia fusiformis*.

Some evidence has suggested that phenolic compounds inhibit telomerase activity in tumor cells (Naasani et al. 1998; Chakraborty et al. 2006).

Heterokontophyta are also a good source of fucoxanthin, as observed at ethanol extracts of *Fucus evanescens* C Agardh that presents too high unsaturated monogalactosyldiacylglycerol. They both showed *in vitro* antitumor activity (Imbs et al. 2013). The sulfated polysaccharide of fucoidan and carotenoid of fucoxanthin have been demonstrated important active metabolites of brown algae as potential chemotherapeutic or chemopreventive agents (Moghadamtousi et al. 2014).

According Yuan et al. (2011) *κ*-carrageenan oligosaccharides from *Kappaphycus striatum* (Gigartinales) have antitumor and immunomodulation effects on S180-bearing mice. Authors suggested that chemical modification, as sulfation, of carrageenan oligosaccharides can enhance their antitumor effect (54 % of inhibition) and stimulate their antitumor immunity, including on macrophage phagocytosis, and cellular immunity, mainly on spleen lymphocyte proliferation in mices.

Some natural sulphated polysaccharides are able to affecting the early stages of carcinogenesis and neoplastic progression in the period prior to the appearance of an invasive malignant tumor. Thus, this substance can be used as a drug carrier for therapy of malignant tumors and as a prophylactic with low toxicity, decreasing the side effects of cytostatic drugs (Khotimchenko 2010).

Fan et al. (2012) isolated an acidic polysaccharide (GLSPs) from *Gracilaria lemaneiformis* (Rhodophyta), chiefly composed of galactose, and contained a small amount of rhamnose, arabinose, xylose and mannose. This polysaccharide significantly inhibited the growth of tumor, promoted splenocytes proliferation and macrophage phagocytosis, and increased the level of IL^{-2} and CD^{8+} T cells in blood of tumor-bearing mice.

Despite these authors demonstrate antitumoral activity on polysaccharide (polar extracts), our results showed that the same effect is not observed in glioma cells, which were more susceptible to non-polar extracts, especially chloroform. Theses extracts are particularly rich in apolar phytochemicals of interest such as terpenoids, neophytadiene, phytol, (-)-loliolide, phenolic compounds, alkaloids, fatty acids, halogenated compounds, lignoids, steroids, esters, amides, ketones, carboxylic acids, aldehydes and alcohols (Rocha et al. 2011) which may be possibly responsible for pharmacological activities among them anti-inflammatory, antioxidant, anti-cholinesterase, antiprotozoal and antifungal (De Felício et al. 2010; Jung et al. 2013; Syad et al. 2013).

5. Conclusion

Although the metabolites that showed activity have not been identified by chromatography in this study, the data suggest the occurrence of several secondary compounds with low polarity, which are disperse more easily in cell membranes than the more polar. All the extracts of brown seaweed endemic of Antarctica, *Desmarestia anceps*, showed potential antiglioma activity (25 % of inhibiotin in 100 $\mu g.mL^{-1}$ after 24 hours and 50 % in 250 $\mu g.mL^{-1}$ after 48 hours in hexane and chloroform extracts), and chloroform extract of *Iridaea cordata* showed the most significant antiproliferative activity, seen to be a type of highly malignant and aggressive cancer. Therefore, they are promising enough to warrant the isolation and elucidation of the active constituents in this marine algae, aiming future syntheses of molecules of interest. From these data it is expected that the class of compounds having activity may be isolated in order to contribute to the clinical research.

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References

Chakraborty S, Ghosh U, Bhattacharyya NP, Bhattacharya K, Roy M (2006) Inhibition of telomerase activity and induction of apoptosis by curcumin in K-562 cells. Mutat Res 596:81-90. doi: 10.1016/j.mrfmmm.2005.12.007

Naasani I, Seimiya H, Tsuruo T (1998) Telomerase inhibition, telomere shortening, and senescence of cancer cells by tea catechins. Biochem. Biophys Res Commun 249: 391-396. doi: 10.1006/bbrc.1998.9075

Imbs TI, Ermakova SP, Fedoreyev SA, Anastyuk SD, Zvyagintseva TN (2013) Isolation of Fucoxanthin and Highly Unsaturated Monogalactosyldiacylglycerol from Brown Alga *Fucus evanescens* C Agardh and *In Vitro* Investigation of Their Antitumor Activity. Mar Biotechnol 15:606-612. doi: 10.1007/s10126-013-9507-2

Kannan RRR, Aderogba MA, Ndhlala AR, Stirk WA, Van Staden J (2013) Acetylcholinesterase inhibitory activity of phlorotannins isolated from the brown alga, *Ecklonia maxima* (Osbeck) Papenfuss. Food Res Int 54:1250-1254. doi: 10.1016/j.foodres.2012.11.017

Park E-J, Pezzuto JM (2013) Antioxidant Marine Products in Cancer Chemoprevention. Antioxid Redox Signaling 19:115-138. doi: 10.1089/ars.2013.5235

Zhang R, Kang KA, Piao MJ, Ko, DO, Wang ZH, Lee IK, Kim, BJ; Jeong IY, Shin T, Park JW, Lee NH, Hyun JW (2008) Eckol protects V79-4 lung fibroblast cells against ray radiation-induced apoptosis via the scavenging of reactive oxygen species and inhibiting of the c-Jun NH2-terminal kinase pathway. Eur J Pharmacol 591:114-123. doi: 10.1016/j.ejphar.2008.06.086

Kellogg J, Lila MA (2013) Chemical and in Vitro Assessment of Alaskan Coastal Vegetation Antioxidant Capacity. J Agric Food Chem 61:11025-11032. doi: 10.1021/jf403697z

Lee S-H, Jeon Y-J (2013) Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. Fitoterapia 86:129-136. doi: 10.1016/j.fitote.2013.02.013

Eoma S-H, Kim Y-M, Kim S-K (2012) Antimicrobial effect of phlorotannins from marine brown algae. Food Chem Toxicol 50:3251-3255. doi: 10.1016/j.fct.2012.06.028

Kim M-J, Kim K-B-W-R, Jeong D-H, Ahn D-H (2013) Anti-inflammatory Activity of Ethanolic Extract of *Sargassum sagamianum* in RAW 264.7 Cells. Food Sci Biotechnol 22:1113-1120. doi: 10.1007/s10068-013-0191-9

Artan M, Li Y, Karadeniz F, Lee SH, Kim MM, Kim SK (2008) Anti-HIV-1 activity of phloroglucinol derivative, 6,60-bieckol, from *Ecklonia cava*. Bioorg Med Chem 16:7921-7926. doi: 10.1016/j.bmc.2008.07.078

Iken K, Amsler CD, Amsler MO, Mcclintock JB, Baker BJ (2009) Field studies on deterrent properties of phlorotannins in Antarctic brown algae. Bot Mar 52:547-557. doi: 10.1515/BOT.2009.071

Kong CS, Kim JA, Yoon NY, Kim SK (2009) Induction of apoptosis by phloroglucinmol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. Food Chem Toxicol 47:1653-1658. doi: 10.1016/j.fct.2009.04.013

Targett NM, Arnold TM (1998) Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. J Phycol 34:195-205. doi: 10.1046/j.1529-8817.1998.340195.x

Wijesekara I, Kim, S-K (2010) Angiotensin-I-Converting Enzyme (ACE) Inhibitors from Marine Resources: Prospects in the Pharmaceutical Industry. Mar Drugs 8:1080-1093. doi:10.3390/md8041080

Moghadamtousi SZ, Karimian H, Khanabdali R, Razavi M, Firoozinia M, Zandi K, Kadir HA (2014)Anticancer and Antitumor Potential of Fucoidan and Fucoxanthin, Two Main Metabolites Isolated from Brown Algae. The Scientific World Journal 2014:1-10. doi: 10.1155/2014/768323

Abida H, Ruchaud S, Rios L, Humeau A, Probert I, De Vargas C, Bach S, Bowler C (2013) Bioprospecting Marine Plankton. Mar. Drugs 11:4594-4611. doi:10.3390/md11114594

Agência Nacional de Vigilância Sanitária (2010) Farmacopéia Brasileira. Anvisa, Brasília.
Alves A, Sousa RA, Reis RL (2013) A practical perspective on ulvan extracted from green algae. J Appl Phycol 25:407-424. doi: 10.1007/s10811-012-9875-4

Alves MGCF, Dore CMP, Castro AJG, Nascimento MS, Cruz AKM, Soriano EM, Benevides NMB, Leite EL (2012) Antioxidant, cytotoxic and hemolytic effects of sulfated galactans from edible red alga *Hypnea musciformis*. J Appl Phycol 24:1217-1227. doi: 10.1007/s10811-011-9763-3

Amsler CD, Mcclintock JB, Baker BJ (2012) Palatability of living and dead detached Antarctic macroalgae to consumers. Antarctic Sci 24:589–590. doi:10.1017/S0954102012000624

Argandoña V, Del Pozo T, San-Martín A, Rovirosa J (2000) Insecticidal activity of *Plocamium cartilagineum* monoterpenes. Bol Soc Chil Quim 45:339-344. doi: 10.4067/S0366-16442000000300006

Aumack CF, Amsler CD, Mcclintock JB (2011) Impacts of mesograzers on epiphyte and endophyte growth associated with chemically defended macroalge from the Western Antarctic Peninsula: a mesocosm experiment. J Phycol 47:36–41. doi: 10.1111/j.1529-8817.2010.00927.x

Cattaneo-Vietti R, Chiantore M, Gambi MC, Albertelli G, Cormaci M, Di Geronimo I (2000) Spatial and vertical distribution of benthic littoral communities in Terra Nova Bay. In: Faranda FM, Guglielmo L, Ianora A (eds) Ross sea ecology: Italiantartide expeditions (1985–1995). Springer-Verlag, Berlin, pp 503-514

Cavalcante-Silva LHA, Matta CBB, Araújo MV, Barbosa-Filho JM, Lira DP, Santos BVO, Miranda GEC, Alexandre-Moreira MS (2012) Antinociceptive and Anti-Inflammatory Activities of Crude Methanolic Extract of Red Alga *Bryothamnion triquetrum*. Mar Drugs10:1977-1992. doi: 10.3390/md10091977

Cooley HM, Fisk AT, Wiens SC, Tomy GT, Evans RE, Muir DCG (2001) Examination of the behavior and liver and thyroid histology of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to high dietary concentrations of C_{10}-, C_{11}-, C_{12}- and C14-polychlorinated n-alkanes. Aquatic Toxicology 54:81-99. doi: 10.1016/S0166-445X(00)00172-7.

Crespo MOP, Yusty MAL (2006) Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of aliphatic hydrocarbons in seaweed samples. Ecotoxicology and Environment Safety 64:400-405. doi: 10.1016/j.ecoenv.2005.04.010

De Felício R, Albuquerque S, Young MCM, Yokoya NS, Debonsi HM (2010) Trypanocidal, leishmanicidal and antifungal potential from marine red alga *Bostrychia tenella* J. Agardh (Rhodomelaceae, Ceramiales). J Pharm Biom Anal 52:763-769. doi: 10.1016/j.jpba.2010.02.018

Dyrna F, Hanske S, Krueger M, Bechmann I (2013) The Blood-Brain Barrier. J Neuroimmune Pharmacol 8:763-773. doi: 10.1007/s11481-013-9473-5

El Baz FK, El Baroty GS, Abd El Baky HH, Abd El-Salam OI, Ibrahim EA (2013) Structural characterization and Biological Activity of Sulfolipids from selected Marine Algae. Grasas aceites 64:561-571. doi: 10.3989/gya.050213

Fan Y, Wang W, Song W, Chen H, Teng A, Liu A (2012) Partial characterization and anti-tumor activity of an acidic polysaccharide from *Gracilaria lemaneiformis*. Carbohyd Polym 88:1313-1318. doi: 10.1016/j.carbpol.2012.02.014

Fitzgerald C, Mora-Soler L, Gallagher E, O'connor P, Prieto J, Soler-Vila A, Hayes M (2012) Isolation and Characterization of Bioactive Pro-Peptides with *in Vitro* Renin Inhibitory Activities from the Macroalga *Palmaria palmata*. J Agr Food Chem 60:7421-7427. doi: 10.1021/jf301361c

Genovese G, Faggio C, Gugliandolo C, Torre A, Spanò A, Morabito M, Maugeri TL (2012) *In vitro* evaluation of antibacterial activity of *Asparagopsis taxiformis* from the Straits of Messina against pathogens relevant in aquaculture. Mar Environ Res 73:1-6. doi: 10.1016/j.marenvres.2011.10.002

Guedes EAC, Araújo MAS, Souza AKP, Souza LIO, Barros LD, Maranhão FCA, Sant'ana AEG (2012) Antifungal Activities of Different Extracts of Marine Macroalgae Against Dermatophytes and *Candida* Species. Mycopathologia 174:223-232. doi: 10.1007/s11046-012-9541-z

Guiry MD (2012) How many species of algae are there? J Phycol 48:1057-1063. doi: 10.1111/j.1529-8817.2012.01222.x

Hayashi T, Adachi K, Ohba S, Hirose Y (2013) The Cdk inhibitor flavopiridol enhances temozolomide-induced cytotoxicity in human glioma cells. J Neurooncol 115:169-178. doi: 10.1007/s11060-013-1220-5

Ibañez E, Cifuentes A (2013) Benefits of using algae as natural sources of functional ingredients. J Sci Food Agr 93:703-709. doi: 10.1002/jsfa.6023

Iken K, Amsler CD, Amsler MO, Mcclintock JB, Baker BJ (2009) Field studies on deterrent properties of phlorotannins in Antarctic brown algae. Bot Mar 52:547-557. doi: 10.1515/BOT.2009.071

Jung HA, Jin SE, Ahn BR, Lee CM, Choi JS (2013) Anti-inflammatory activity of edible brown alga *Eisenia bicyclis* and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264.7 macrophages. Food Chem Toxicol 59:199-206. doi: 10.1016/j.fct.2013.05.061

Karsten U, Wulff A, Roleda MY, Müller R, Steinhoff FS, Fredersdorf J, Wiencke C (2011) Physiological responses of polar benthic algae to ultraviolet radiation. In: Wiencke C (ed). Biology of polar benthic algae. De Gruyter, Berlin, pp 271-298

Khotimchenko YS (2010) The Antitumor Properties of Nonstarch Polysaccharides: Carrageenans, Alginates, and Pectins. Russ J Mar Biol 36:401-412. doi: 10.1134/S1063074010060015

Kim S, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: A review. J Funct Foods 2:1-9. doi: 10.1016/j.jff.2010.01.003

Li YM, Vallera DA, Hall WA (2013) Diphtheria toxin-based targeted toxin therapy for brain tumors. J Neurooncol 114:155-164. doi: 10.1007/s11060-013-1157-8

Maschek JA, Baker BJ (2008) The chemistry of algal secondary metabolism. In: Amsler CD (ed) Algal Chemical Ecology, 1st edn. Springer-Verlag, Berlin, pp 1-24

Mohrenz IV, Antonietti P, Pusch S, Capper D, Balss J, Voigt S, Weissert S, Mukrowsky A, Frank J, Senft C, Seifert V, Von Deimling A, Kögel D (2013) Isocitrate dehydrogenase 1 mutant R132H sensitizes glioma cells to BCNU-induced oxidative stress and cell death. Apoptosis 18:1416-1425. doi: 10.1007/s10495-013-0877-8

Nieminen AL, Gores GJ, Bond JM, Imberti R, Herman B, Lemasters JJ (1992) A novel cytotoxicity screening assay using a multiwell fluorescence scanner. Toxicol Appl Pharm 115:147-155. doi: 10.1016/0041-008X(92)90317-L

Pedron S, Becka E, Harley BAC (2013) Regulation of glioma cell phenotype in 3D matrices by hyaluronic acid. Biomaterials 34:7408-7417. doi: 10.1016/j.biomaterials.2013.06.024

Quartino ML, Boraso De Zaixso AL (2008) Summer macroalgal biomass in Potter Cove, South Shetland Islands. Antarctica: its production and flux to the ecosystem. Polar Biol 31:281-294. doi: 10.1007/s00300-007-0356-1

Rocha, OP, Felício R, Rodrigues AHB, Ambrósio DL, Cicarelli RMB, Albuquerque S, Young MCM, Yokoya NS, Debonsi HM (2011) Chemical Profile and Biological Potential of Non-Polar Fractions from *Centroceras clavulatum* (C. Agardh) Montagne (Ceramiales, Rhodophyta). Molecules 16:7105-7114. doi:10.3390/molecules16087105

Shu M-H, Appleton D, Zandi K, Abubakar S (2013) Anti-inflammatory, gastroprotective and antiulcerogenic effects of red algae *Gracilaria changii* (Gracilariales, Rhodophyta) extract. BMC Complement Altern Med 13:1-13. doi: 10.1186/1472-6882-13-61

Stein EM, Andreguetti DX, Rocha CS, Fujii MT, Baptista MS, Colepicolo P, Indig GL (2011) Search for cytotoxic agents in multiple Laurencia complex seaweed species (Ceramiales, Rhodophyta) harvested from the Atlantic Ocean with emphasis on the Brazilian State of Espirito Santo. Braz J Pharmacog 21:239-243. doi: 10.1590/S0102-695X2011005000069

Stout EP, Prudhomme J, Le Roch K, Fairchild CR, Franzblau SG, Aalbersberg W, Hay ME, Kubanek J (2010) Unusual antimalarial meroditerpenes from tropical red macroalgae. Bioorg Med Chem Lett 20:5662-5665. doi: 10.1016/j.bmcl.2010.08.031

Stupp R, Hegi ME, Mason WP, Van Den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomized phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459-466. doi: 10.1016/S1470-2045(09)70025-7

Syad AN, Shunmugiah KP, Kasi PD (2013) Antioxidant and anti-cholinesterase activity of *Sargassum wightii*. Pharm Biol 51:1401-1410. doi:10.3109/13880209.2013.793721

Tang B, Row KH (2013) Development of gas chromatography analysis of fatty acids in marine organisms. J Chromatogr Sci 51:599-607. doi: 10.1093/chromsci/bmt005

Wiencke C, Amsler CD (2012) Seaweeds and their communities in polar regions. In: Wiencke C, Bischof K (eds) Seaweed biology: novel insights into ecophysiology, ecology and utilization. Springer-Verlag, Berlin, pp 265-291

Wiencke C, Clayton M, Langreder C (1996) Life History and Seasonal Morphogenesis of the Endemic Antarctic Brown Alga *Desmarestia anceps* Montagne. Bot Mar 39:435-444. doi: 10.1515/botm.1996.39.1-6.435

Wiencke C, Clayton MN (2002) Antarctic Seaweeds. ARG Gantner Verlag, KG Ruggell

Wynne MJ (2005) A checklist of benthic marine algae of the tropical and subtropical western Atlantic: second revision. Beihefte zur Nova Hedwigia, Berlin

Yu Q, Yan J, Wang S, Ji L, Ding K, Vella C, Wang Z, Hu Z (2012) Antiangiogenic effects of GFP08, an agaran-type polysaccharide isolated from *Grateloupia filicina*. Glycobiology 22:1343-1352. doi: 10.1093/glycob/cws096

Yuan H, Song J, Li X, Li N, Liu S (2011) Enhanced immunostimulatory and antitumor activity of different derivatives of κ-carrageenan oligosaccharides from *Kappaphycus striatum*. J Appl Phycol 23:59-65. doi: 10.1007/s10811-010-9536-4

Zacher K, Rautenberger R, Hanelt D, Wulff A, Wiencke C (2011) The abiotic environment of polar benthic algae. In: Wiencke C (ed). Biology of polar benthic algae. De Gruyter, Berlin, pp 9-22

Ziegler DS, Kung AL, Kieran MW (2008) Anti-Apoptosis Mechanisms in Malignant Gliomas. J Clin Oncol 26:493-500. doi: 10.1200/JCO.2007.13.9717

Jones WP, Kinghorn AD (2005) Extraction of plant secondary metabolites. In: Sarker SD, Latif Z, Gray AI (eds). Natural Products Isolation. Humana Press, Totowa, pp 323-351

Bony NF, Libong D, Solgadi A, Bleton J, Champy P, Malan AK, Chaminade P (2014) Establishing high temperature gas chromatographic profiles of non-polar metabolites for quality assessment of African traditional herbal medicinal products. J Pharm Biomed 88:542-551. doi: 10.1016/j.jpba.2013.10.013

Murugan K, Iyer VV (2013) Differential growth inhibition of cancer cell lines and antioxidant activity of extracts of red, brown, and green marine algae. In Vitro Cell Dev Biol Anim 49:324-334. doi: 10.1007/s11626-013-9603-7

Rispail N, Nash R, Webb J (2005) Secondary metabolic profiling. In: Márquez AJ, Stougaard J, Udvardi M, Parniske M, Spaink H, Saalbach G, Webb J, Chiurazzi M (eds). Lotus Japonicus Handbook. Springer, Netherlands, pp 341-348

POLAR BIOLOGY

Antitumor activity of extracts from Antarctic macroalgae *Iridaea cordata* and *Desmarestia anceps*

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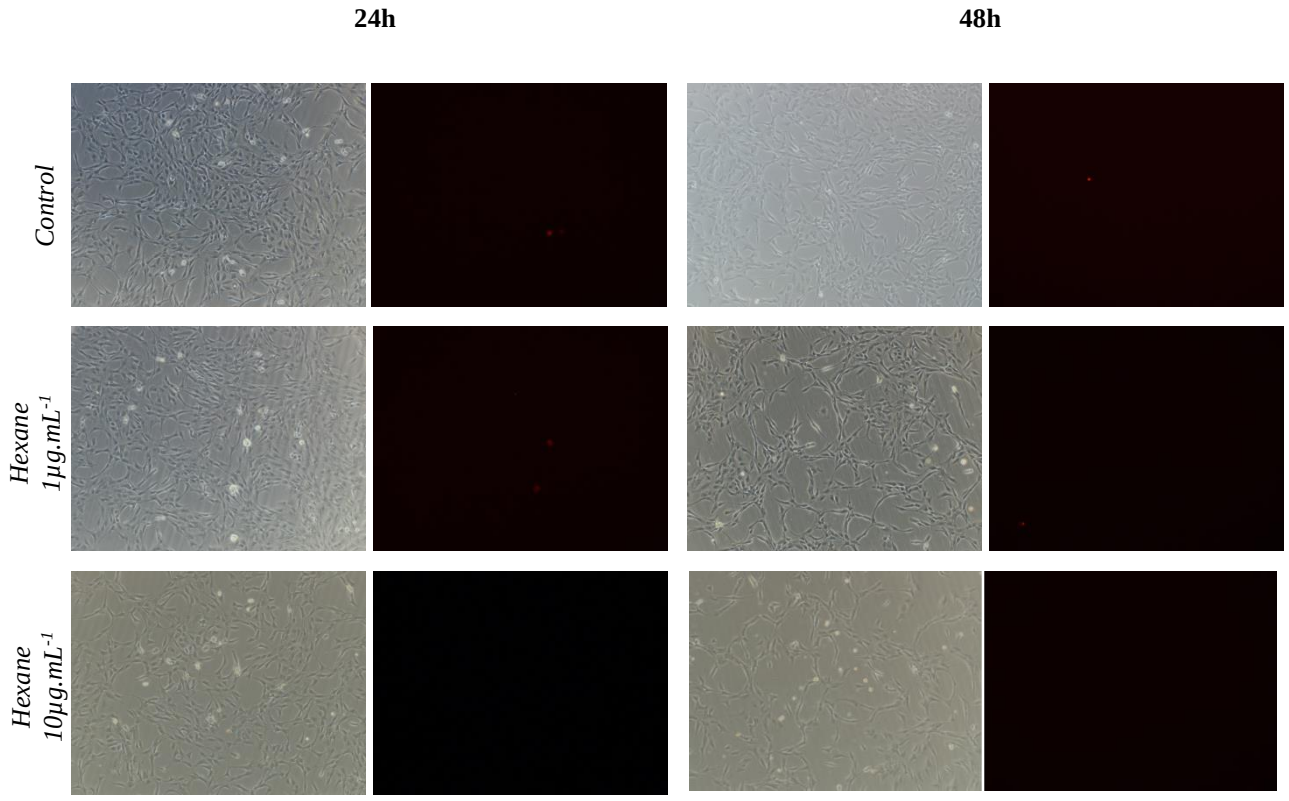

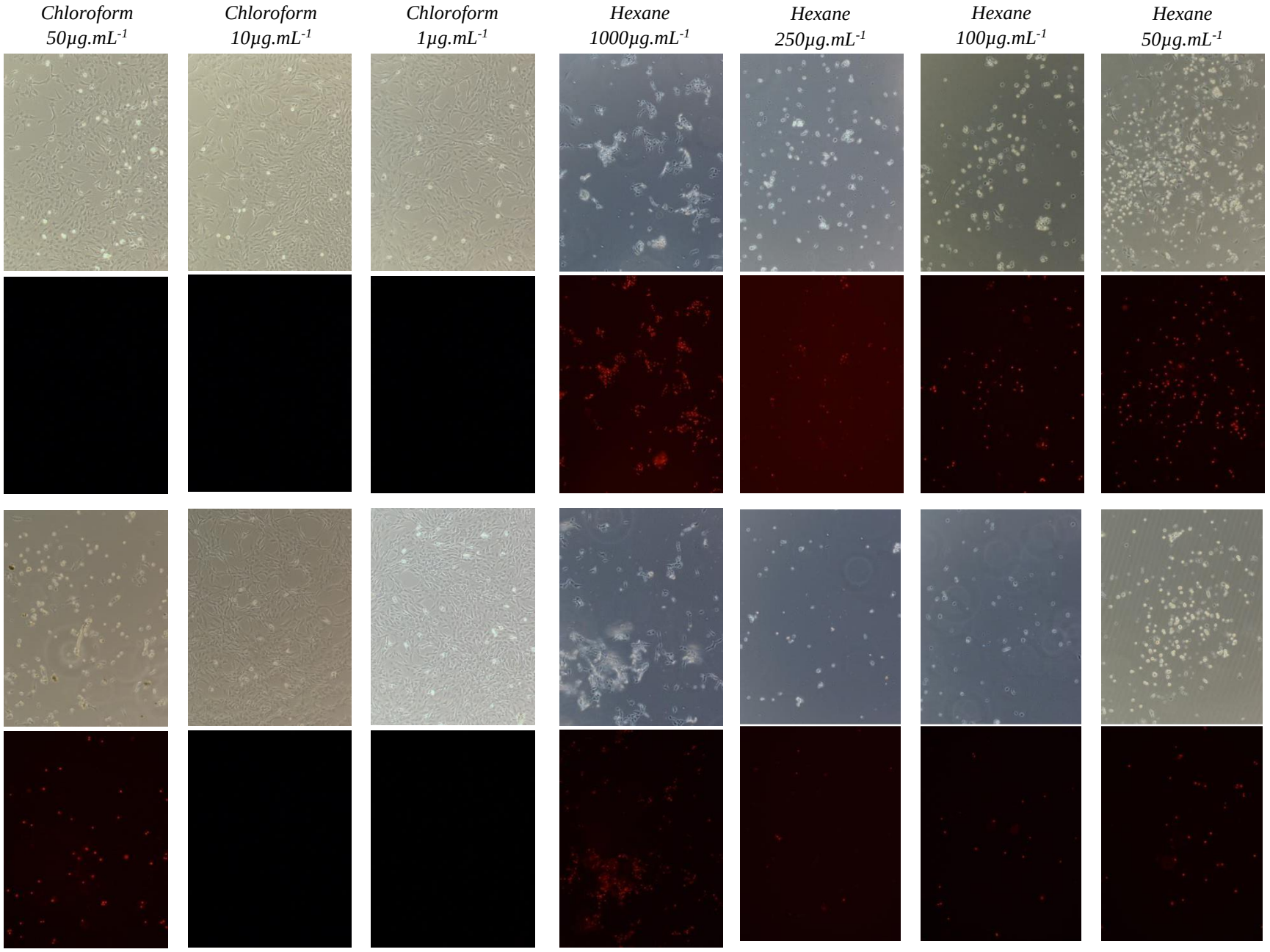
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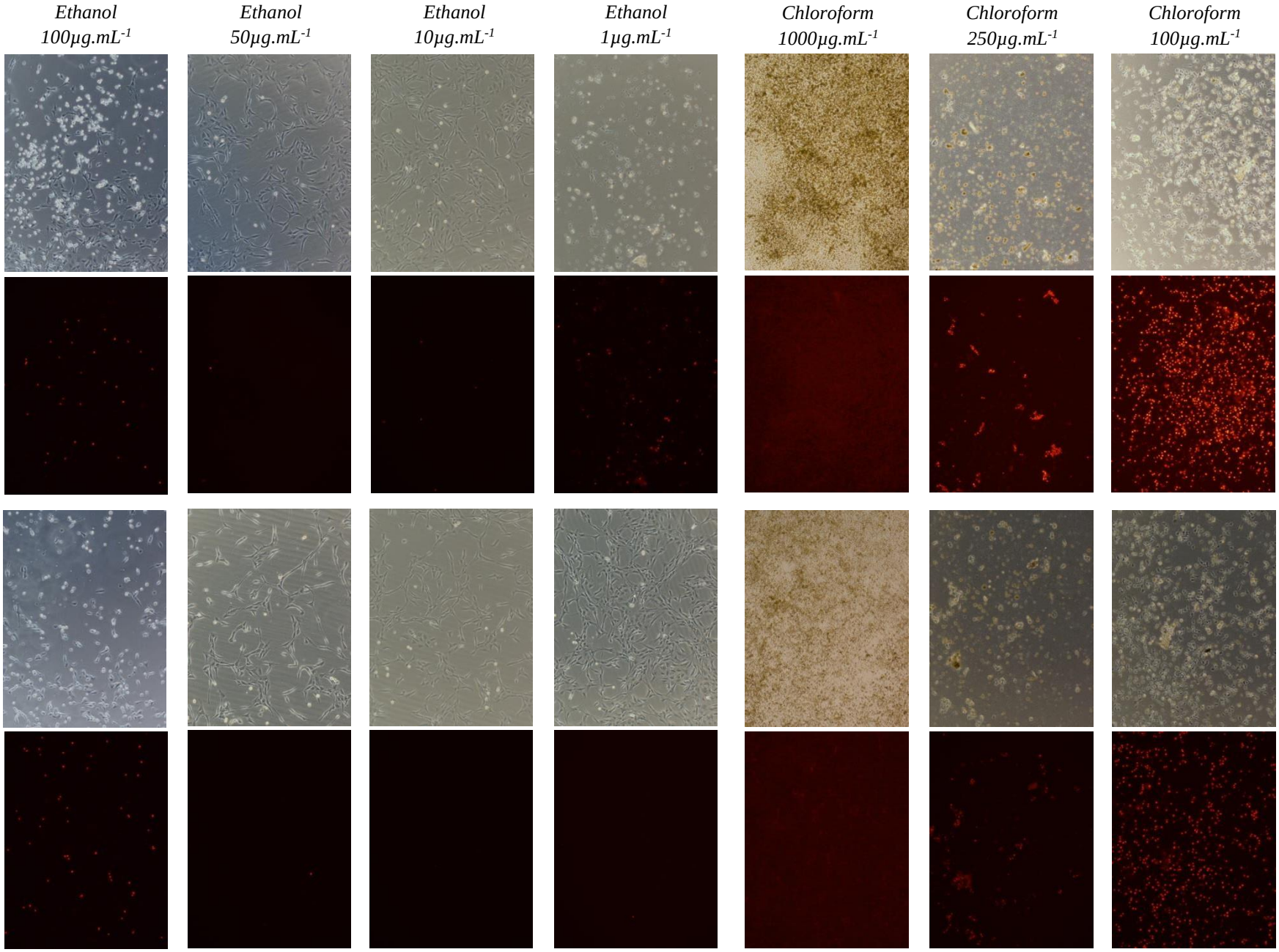
Hexane 50µg.mL-1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Ethanol *50µg.mL^{-1}*
Ethanol *10µg.mL^{-1}*
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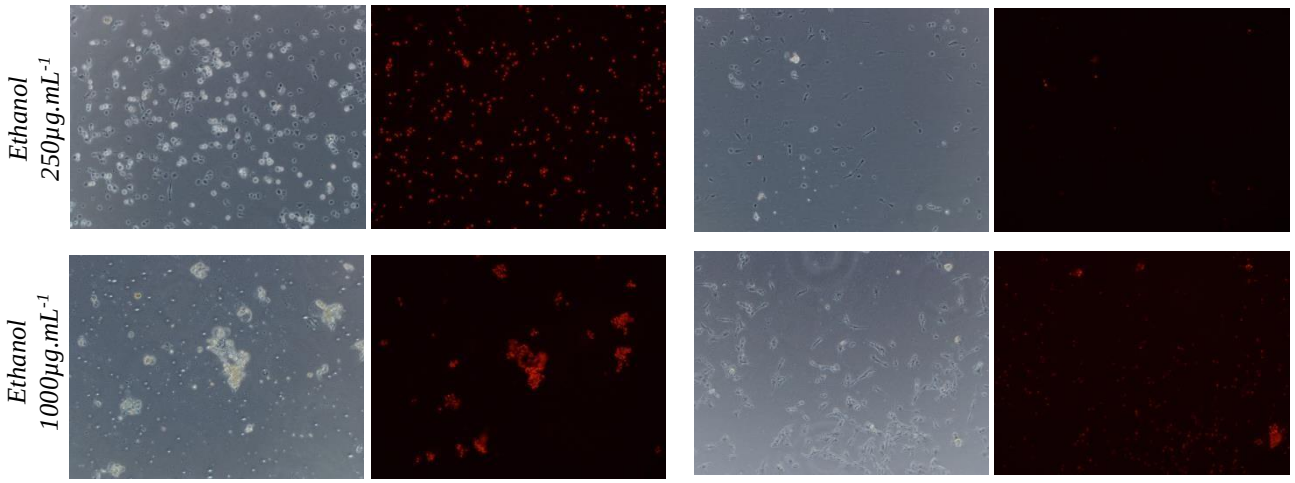
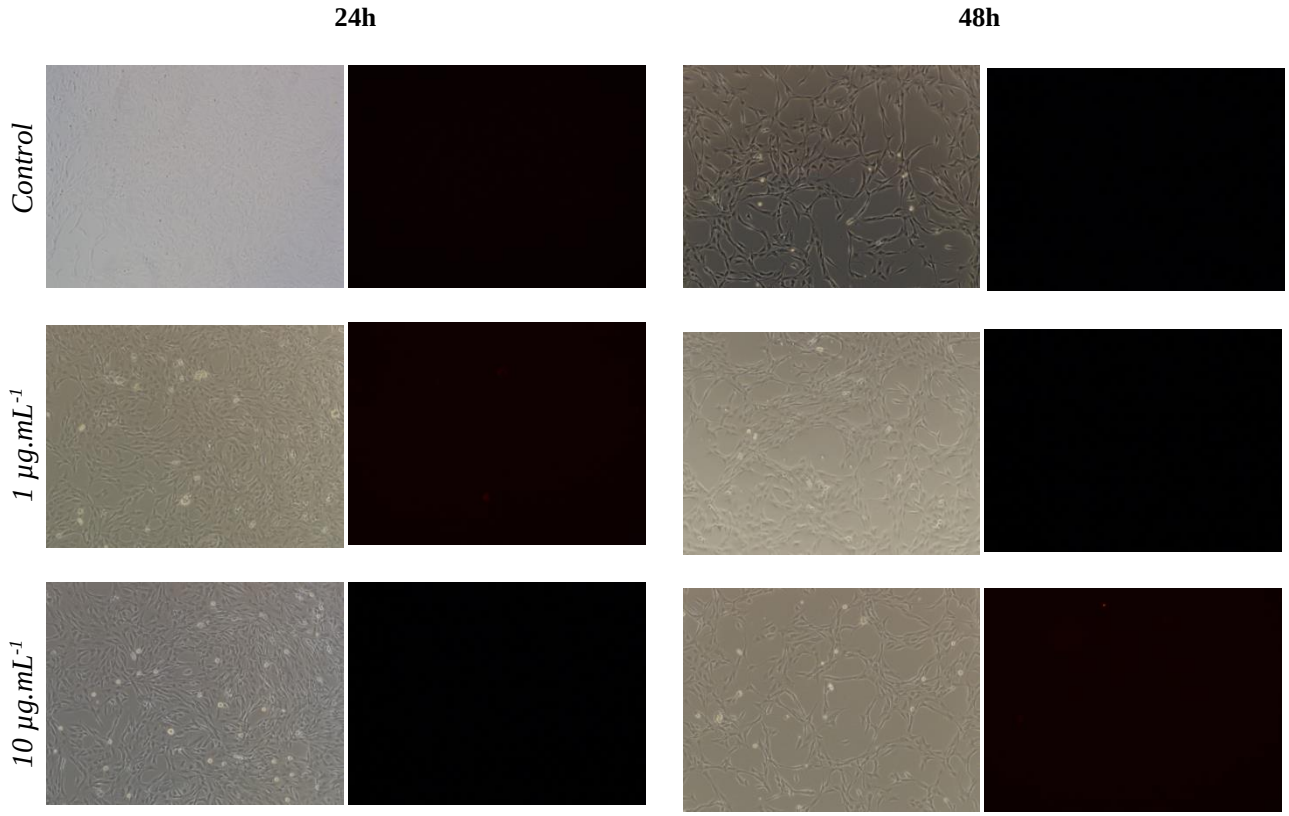
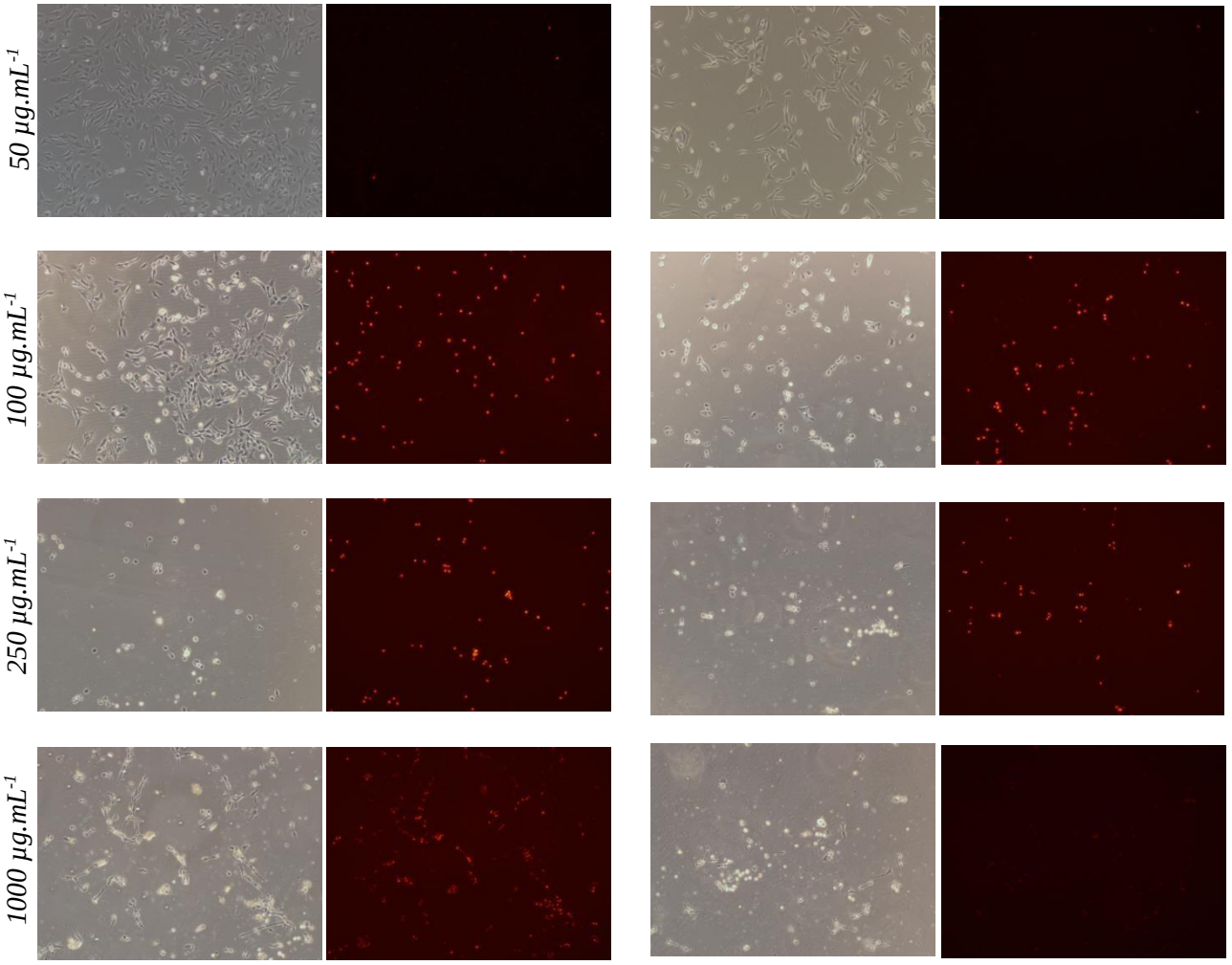
Chloroform *250µg.mL^{-1}*
Chloroform *100µg.mL^{-1}*



Online Resource 1. Pictures of propidium iodide assay in rat astrocytes (C6) in different extracts of *Desmarestia anceps*.





Online Resource 2. Representative pictures of C6 cell culture stained with propidium iodide (PI) treated for 24 and 48 hours with chloroform extract from *I. cordata*.

Antitumor activity of seaweeds from Antarctica: *Desmarestia anceps, Iridaea cordata, Palmaria decipiens, Prasiola crispa* and *Pyropia endiviifolia*

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Abstract

Lung carcinoma is one of the most frequent type of cancer in Brazil, due to the difficulty in controlling it, there is a continuous search for new drugs. In this perspective, considering the diversity of algae is still little explored this biomass emerges as a possible source of new biologically active molecules. The Antarctic continent by presenting a peculiar microenvironment and extreme conditions of survival for macroalgae, may facilitate the production of new metabolites. Thus, this study intends to evaluate the structural variety of five seaweeds from Antarctica and their biological potential as prototypes for the pharmaceutical application. Extracts of different polarities were prepared (hexane, chloroform and ethanol) to make a selection of molecules that may provide antitumor activity against human lung adenocarcinoma epithelial cell. The best cytotoxicity activities were observed in the chloroform extracts of algae *D. anceps* (61.16 µg.mL^{-1}), *I. cordata* (67.54 µg.mL^{-1}) and *Pyropia endiviifolia* (45.66 µg.mL^{-1}), and in the hexane extract of *Prasiola crispa* (93.02 µg.mL^{-1}). Thus, the isolation of these molecules with potential anticancer activity is the next target of our study group in order to contribute to a future drug use.

Keywords: Antarctic macroalgae. Anticancer activity. Bioactive molecules. Lung carcinoma.

1. Introduction

Cancer is a major world public health problem. In Brazil, the estimates for the year 2012 indicate the occurrence of approximately 518,510 new cases of cancer, among them 27,320 of lung carcinoma, being a highly lethal disease once that it has mortality/incidence ratio about 86 % (Inca 2011). This cancer has proven difficult to control with conventional therapeutic and surgical approaches, thus the searching for efficacious and safe agents to prevent, inhibit, or reverse lung carcinogenesis remains the priority of lung cancer research (Li et al. 2013). There is a continuing need to identify new drugs with innovative mechanisms of action (Demedts et al. 2010).

In a medical perspective, nature-based products are believed to be a strategic alternative approach to the use of fully synthetic materials, particularly in the design of medical devices. In the past decades, marine organisms have become the focus of considerable attention as potential sources of valuable materials. In particular, the sustainable exploitation and valorization of natural marine compounds are a highly attractive and strategic platform for the development of novel biomaterials, with both economic and environmental benefits (Alves et al. 2013)

Biodiversity within red (Rhodophyta), green (Chlorophyta) and brown (Phaeophyta) macroalgae offers the possibility of finding a wide variety of natural compounds with interesting properties (Barsanti and Gualtieri 2006; Almeida et al. 2011). In fact, more than 15,000 primary and secondary metabolites from different metabolic pathways have been reported for macroalgae and different applications were assigned to them (Grosso 2011). From human health point of view, both types of metabolites are of extreme importance since some of them can display remarkable positive effects on organism (Andrade et al. 2013). The metabolites found in macroalgae are described as having anti-inflammatory, antimutagenic, antitumor, antidiabetic, antihypertensive, anticoagulant, antibacterial, antiviral, apoptotic activities and antioxidant potential (Foley et al. 2011; Wijesekara et al. 2011; Elizondo-Gonzalez et al. 2012; Lopes et al. 2012; Wijesinghe and Jeon 2012; Auezova et al. 2013; Cavallo et al. 2013; Dore et al. 2013; Sousa et al. 2013). Furthermore, they are hepatoprotectors, and can also inhibit the lipoxygenase, aldose reductase and cholinesterases (El Gamal 2010; Pangestuti and Kim 2011).

In special, in polar regions, adaptation to the strong seasonality of the light regime is one of the most important prerequisites for the ecological success of seaweeds (Wiencke et al. 2011). The strong adaptation of Antarctic seaweeds to low temperatures is also reflected in their photosynthetic performance (Wiencke and Amsler 2012). In the Antarctic, species richness and endemism (33 %) is high in the Antarctic Peninsula region and about 130 species have been documented (Wiencke and Clayton 2002; Wulff et al. 2011). In this context, subjected to severe environmental stresses such as desiccation, salinity, radiation, extreme temperatures and predation, the macroalgae produce secondary metabolites to protect them, which may have potential biological activities (Maschek and Baker 2008; Karsten et al. 2011; Wiencke and Amsler 2012).

The importance of these organisms and their constituents to humanity has justified intense research work; however, the full potential of algae molecules is yet to be unveiled (Alves et al. 2013). Thus, the aim of this study is evaluate the chemical composition and antitumor activity against human lung adenocarcinoma epithelial cell line from five seaweeds collected from Antarctica.

2. Materials and Methods

Reagents

Dulbecco's modified eagle's medium–high glucose (DMEM), trypsin–EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Heat-inactivated fetal bovine serum (FBS) was purchased from Cultilab Lab Inc. (Campinas, SP, BR). Petroleum ether, hexane, chloroform and ethanol were obtained from Synth. All other chemicals were of ultrapure grade and obtained from Sigma–Aldrich (St. Louis, MO, USA).

Seaweeds

Five seaweeds were used, which were collected in the Demay site in Admiralty Bay, King George Island (62º23 S, 58º27 W) at Antarctica in January 2012. Macroalgae fixed to the substrate were collected during periods of low tide; part of the material was preserved in 4 % formaldehyde solution in seawater for identification and the preparation of herbarium specimens, and part was washed with seawater to remove epiphytes, frozen at -20 °C, and lyophilized for biochemical analyzes. Species identification was performed according to specimen morphology and the classification system proposed by Wynne (2005), and herbarium specimens were deposited in the Herbarium of Instituto de Botânica, São Paulo.

Among the macroalgae: three species of red algae (Rhodophyta) are *Iridaea cordata* (Turner) Bory de Saint-Vincent, *Palmaria decipiens* (Reinsch) R.W. Ricker, and *Pyropia endiviifolia* (A. Gepp & E. Gepp) H.G. Choi & M.S. Hwang; one species of brown algae (Heterokontophyta) *Desmarestia anceps* Montagne; one species of green algae (Chlorophyta) *Prasiola crispa* (Lightfoot) Kützing.

Preparation of extracts

The extraction was performed by depletion, three sequential extractions with solvents: hexane, chloroform and ethanol, using two systems: Ultrasound bath and Soxhlet were realized twice.

In the system Soxhlet, prewashed with petroleum ether, 5 g of seaweed were used for each 300 mL solvent extraction and held for 4 hours (method adapted from Crespo & Yusty 2006). In Ultrasound Bath (Model USC 1400A), with a frequency of 40KHz, the seaweed was submitted to extraction for 1 hour with the solvents mentioned previously (40mL), using the same technique depletion in three cycles with renewal of solvent and

evaporation of the extract, and ice bath, avoiding large swings in temperature (method Stein et al., 2011 - modified). Extracts were evaporated at the end to remove the solvents.

Chemical analysis of extracts

The dry extracts were dissolved in a solution of 50 % (v/v) chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA), 50 % (v/v) deionized water and 0.1% formic acid. The solutions were infused directly individually or with HPLC (Shymadzu) assistance into the ESI source by means of a syringe pump (Harvard Apparatus) at a flow rate of 10 µL min^{-1}. ESI(+)-MS and tandem ESI(+)-MS/MS were acquired using a hybrid high-resolution and high accuracy (5 $\mu L.L^{-1}$) microTof (Q-TOF) mass spectrometer (Bruker Scientific) under the following conditions: capillary and cone voltages were set to + 3500 V and +40 V, respectively, with a de-solvation temperature of 100 ◦C. For ESI(+)-MS/MS, the energy for the collision induced dissociations (CID) was optimized for each component. Diagnostic ions in different fractions were identified by the comparison of their ESI(+)-MS/MS dissociation patterns with compounds identified in previous studies (Table). For data acquisition and processing, Hystar software (Bruker Scientific) was used. The data were collected in the m/z range of 70–700 at the speed of two scans per second, providing the resolution of 50,000 (FWHM) at m/z 200. No important ions were observed below m/z 180 or above m/z 650, therefore ESI(+)-MS data is shown in the m/z 180−650 range.

Cell Culture

A549 (human lung adenocarcinoma epithelial cell) cancer cell line and MRC-5 (human lung fibroblast cell) non-tumor cell line purchased from American Type Culture Collection (ATCC), Manassas, VA, USA. Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS. Cells were maintained in a humidified atmosphere at 37 °C, in 5 % CO_2, and 95 % air. The cytotoxicity study was performed when the cells reached 70-80 % confluence.

Cytotoxic assay

Cell viability was measured using the MTT assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenases (Mosmann 1983). Briefly, cells were seeded into the 96-well plates at a density of 7.0×10^4 – $cells.mL^{-1}$, in a volume of 100 µL of supplemented culture media. After 24 h, cells were treated with different concentrations (10-250 $\mu g.mL^{-1}$) of seaweeds extracts and incubated at 37 °C in 5 % CO_2 for 24 h. Negative controls were treated with ethanol/DMSO (2 %) solution. Then, after 24 h, the medium was removed and 1 $mg.mL^{-1}$ MTT dye in serum-free medium was added to the wells. Plates were incubated at 37 °C for 2 h in humidified 5 % CO_2 atmosphere. Subsequently, the MTT solution was removed and the obtained formazan violet product was dissolved in 100 µL DMSO. Absorbance was measured using a microplate reader (Spectra Max 190, Molecular Devices, USA) at 570 nm. All readings were compared with the control, which represented 100% viability. The percentage growth inhibition was calculated using the equation developed by Monks et al. (1991): cell viability (%) = (absorbance of experimental wells/absorbance of control wells) × 100. The IC50 (concentration µg/mL that inhibits cell growth by 50 %) ratio of cancerous (A549) and non-tumor (MRC-5) cell was also calculated. Each experiment was performed in triplicate. The data were expressed as means of at least three independent experiments.

Statistical analysis

The results were expressed as the means ± SD. Statistical analysis was performed using SPSS version 15.0 and GaphPad Prism version 5.0. Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk), Student's t-test for independent samples, and one-way analysis of variance ANOVA, with Tukey's post hoc multiple comparisons test were performed. The significance of difference was considered to include values of $P < 0.05$.

3. Results

Algal screening and Antitumor activity

During the screening process of different extracts, five species of algae were tested for their antitumor activities using the A549 and MRC-5 cell lines. The concentration that inhibited 50% of cell growth (IC_{50}) is shown in Table 1 and 2.

Table 1. IC_{50} extracts of macroalgae at Soxhlet toward tumor and normal cells ($\mu g.mL^{-1}$).

Algae	Hexane		Chloroform		Ethanol	
	A549	**MRC-5**	**A549**	**MRC-5**	**A549**	**MRC-5**
Desmarestia anceps	70.02 ±0.96	90.96 ±1.71	61.16 ±0.65	123.30 ±1.78	424.90 ±2.26	651.00 ±0.64
Iridaea cordata	156.90 ±0.74	298.20 ±0.52	67.54 ±0.64	95.41 ±1.53	323.10 ±2.06	258.20 ±1.67
Palmaria decipiens	61.32 ±0.55	21.16 ±0.55	51.42 ±0.84	47.50 ±0.83	665.30 ±1.41	477.10 ±1.31
Pyropia endiviifolia	198.20 ±0.83	212.40 ±0.73	276.50 ±0.24	430.90 ±0.11	524.30 ±1.70	591.90 ±1.15
Prasiola crispa	94.38 ±0.78	197.20 ±0.85	185.10 ±0.61	162.30 ±0.82	442.60 ±2.41	250.70 ±1.12

Table 2. IC_{50} extracts of macroalgae at Ultrasound bath toward tumor and normal cells ($\mu g.mL^{-1}$).

Algae	Hexane		Chloroform		Ethanol	
	A549	**MRC-5**	**A549**	**MRC-5**	**A549**	**MRC-5**
Desmarestia anceps	382.4 ±0.71	130.80 ±1.16	95.96 ±1.05	69.94 ±1.73	339.60 ±2.57	322.60 ±1.53
*Iridaea cordata**	-	-	56.52 ±0.93	55.12 ±0.92	425.80 ±1.58	350.70 ±2.20
Palmaria decipiens	144.2 ±2.20	106.60 ±1.28	36.47 ±0.69	29.96 ±0.69	477.60 ±1.24	324.70 ±0.81
Pyropia endiviifolia	123.7 ±1.20	91.35 ±0.78	45.66 ±0.65	87.47 ±1.24	146.70 ±1.05	136.30 ±0.85
Prasiola crispa	93.02 ±1.49	125.20 ±1.62	81.09 ±1.09	69.76 ±0.78	139.40 ±1.11	84.40 ±0.64

*no significant inhibition

The ethanolic extract did not inhibit the growth of cells at higher concentrations than the nonpolar extracts, being also more cytotoxic to tumor cells did not. Among the nonpolar extracts of brown seaweed *D. anceps*, the chloroform extract showed greater antitumor activity and selective effect with IC_{50} of 61.16 $\mu g.mL^{-1}$ (A549) and 123.30 $\mu g.mL^{-1}$ (MRC-5) than the hexane extract: A549 (70.02 $\mu g.mL^{-1}$) and MRC-5 (90.96 $\mu g.mL^{-1}$). Followed by chloroform extract of *I. cordata*, wherein A549 showed IC_{50} of 67.54 $\mu g.mL^{-1}$ and MRC-5 of 95.41 $\mu g.mL^{-1}$ (Figure 1), while the hexane extract despite it has selective effect the concentrations required for inhibition are higher, 156.90 $\mu g.mL^{-1}$ (A549) and 298.20 $\mu g.mL^{-1}$ (MRC-5).

A similar situation is observed for the Rhodophyta *Pyropia endiviifolia*, with concentrations that inhibited the growth of lung adenocarcinoma were high: 198.20 $\mu g.mL^{-1}$ (hexane extract) and 276.50 $\mu g.mL^{-1}$ (chloroform extract). The chlorophyte *Prasiola crispa* showed cytotoxicity, with selective effect only in the hexane extract with IC_{50} of A549 was 94.38 $\mu g.mL^{-1}$ while for MRC-5 was 197.20 $\mu g.mL^{-1}$. The macroalgae *Palmaria decipiens* showed greater cytotoxic effect on non-tumor cell line in all tested extracts.

The extracts obtained by the ultrasound in bath system, contrasting way in relation to the aforementioned soxhlet presented almost in its entirety more cytotoxic effect on lung fibroblasts. The exceptions were the chloroform extract of *Pyropia endiviifolia* where the IC_{50} was 45.66 $\mu g.mL^{-1}$ A549 and MRC-5 was 87.47 $\mu g.mL^{-1}$, nearly twice the concentration as observed in chloroform extract of *D. anceps* obtained by soxhlet; and the hexane extract of *Prasiola crispa* A549 (93.02 $\mu g.mL^{-1}$) e MRC-5 (125.20 $\mu g.mL^{-1}$).

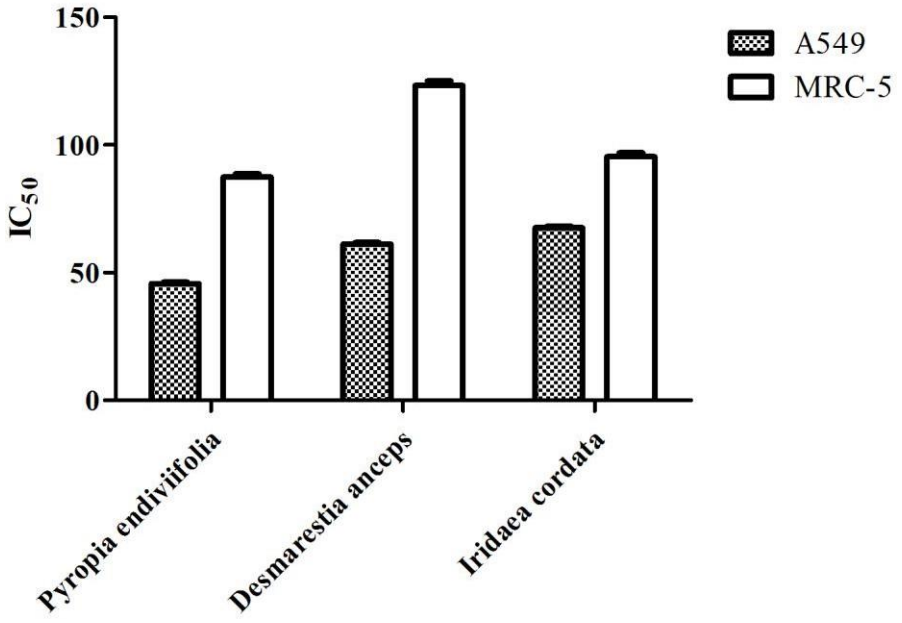

Figure 1. IC_{50} values of the extracts chloroform (*Pyropia endiviifolia*, *D. anceps* and *I. cordata*) that had better activity and selectivity in cell lines A549 and MRC-5.

Effects of extraction

The best cytotoxicity activities were observed in the chloroform extract of *Pyropia endiviifolia* (ultrasound), *D. anceps* and *I. cordata* (soxhlet). These extracts showed high inhibition at low concentrations and selectivity effect as observed in Figure 2. Hexane extract in Soxhlet of *Prasiola crispa* also showed inhibition, but reaching IC_{50} at higher concentration (94.38µg.mL^{-1}).

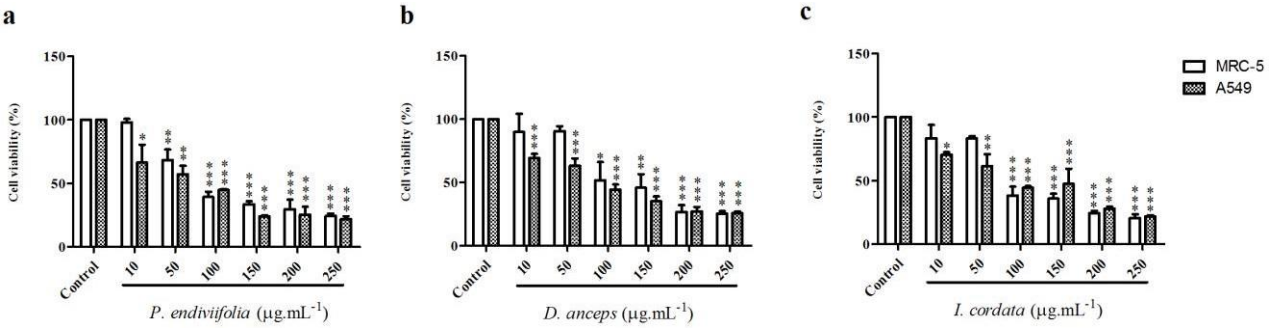

Figure 2. Cell viability of the A549 and MRC-5 cell lines in chloroform extracts that had best activity. *P<0.05; **P<0.005; ***P<0.001 compared to control in time corresponding analysis

Chromatographic analysis

The extracts were analyzed by liquid chromatography coupled to mass spectrometer to enable the identification of biologically active molecules present in extracts that had better activities. The chromatograms of chloroform extracts of algae *D. anceps*, *I. cordata* and *Pyropia endiviifolia* can be analyzed in figures 3, 4 and 5, respectively.

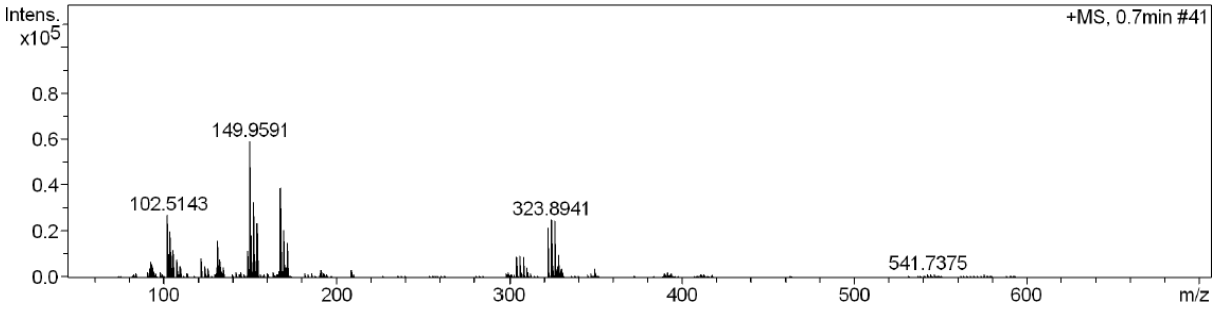

Figure 3. Mass spectrum of chloroform extract of *D. anceps* via soxhlet.

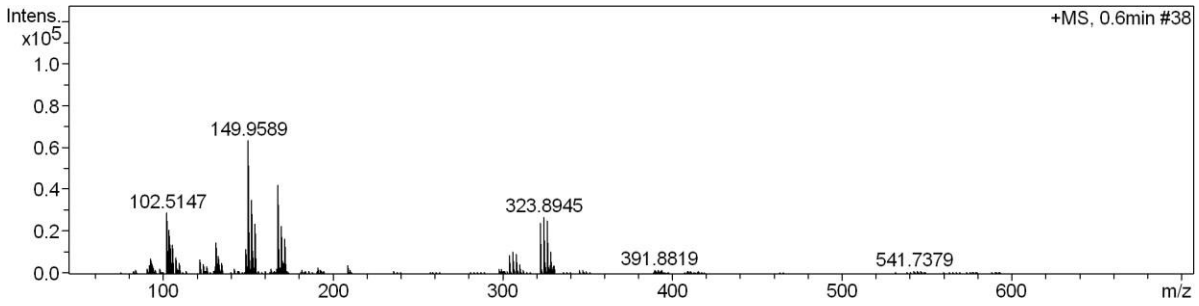

Figure 4. Mass spectrum of chloroform extract of *I. cordata* via soxhlet.

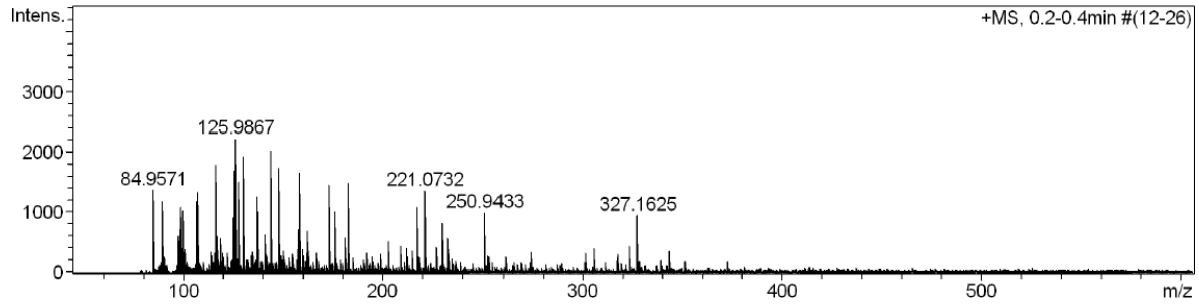

Figure 5. Mass spectrum of chloroform extract of *Pyropia endiviifolia* via ultrasound.

4. Discussion

Considering the need for alternative drugs for the treatment of lung cancer, we evaluated the cytotoxic effect of different extracts of five macroalgae collected in the Antarctic environment could have on cell lineage of lung adenocarcinoma. Our study showed that the nonpolar extracts were most promising, showing growth inhibition of lung adenocarcinoma cells with dose-dependent effect. The chloroform extracts of algae *D. anceps* (61.16 µg.mL^{-1}), *I. cordata* (67.54 µg.mL^{-1}) and *Pyropia endiviifolia* (45.66 µg.mL^{-1}), and the hexane extract of *Prasiola crispa*, at higher concentration, (93.02 µg.mL^{-1}) were able to affect cell viability at low concentrations, making it a significant result since it presents selective effect by tumor cell lines.

Many studies have reported biological activities from nonpolar extracts (Stein et al. 2011; De Felício et al. 2010; Jung et al. 2013; Syad et al. 2013). According to Murugan and Iyer (2013) at chloroform extract is common to obtain compounds as phenols and flavonoids, which showed higher activity in many assays, including in tests with A549, that evaluated the activity of different extracts of algae in 100 µg.mL^{-1}. These data corroborate our research, which found even IC50 values less than 100 µg.mL^{-1}. In general, the bioactive compounds present in these extracts, which vary widely in their chemical polarity, must be purified for better results.

In general, activity variation was observed between different extracts, by changing the polarity of the solvents, as well as between the extraction systems. Soxhlet is a conventional extraction system in which the

sample is repeatedly brought into contact with solvent and works relatively high temperature by effect of the heat applied to the distillation flask. Moreover, after extraction is not need filtration and sample throughput can be increased by performing several simultaneous extractions in parallel, which is facilitated by the low cost of the basic equipment. It should be considered that large amounts of biomass can be used in soxhlet compared with other extraction systems (Luque de Castro and Priego-Capote, 2010).

However, the long extraction time and volume of solvent required by soxhlet represent, plus spending on technical, environmental problems (Virot et al. 2008). Thus, new systems of extraction, such as ultrasound, represent a more advantageous means in front of such limiting features. The main advantage of ultrasound water-bath system is the possibility of temperature control, compensating the increase on temperature as consequence of ultrasounds irradiation, which can lead to analyte alteration (Peña-Farfal et al. 2005). From the results obtained in soxhlet and ultrasound, future studies will be used to evaluate different methods for extracting, as there was a change of antitumor activity as the system has changed.

Our studies are in association with current research of biomolecules with anticancer activity, once that researchers are looking for new anticancer drugs that must kill or disable tumor cells in the presence of normal cells without displaying toxicity (Mary et al. 2012; Patra and Muthuraman 2013; Shao et al. 2013; Xue et al. 2013; Kollár et al. 2014; Menshova et al. 2014). This discovery, especially from marine origin, is potential challenge for therapeutic care.

5. Conclusion

Nonpolar extracts of seaweeds from Antarctica showed potential antitumor activity against human lung adenocarcinoma. Despite molecules present in the extracts analyzed have not yet been identified, these unfractionated nonpolar extracts showed in this work might be an adequate candidate to purification since it shows an effective inhibition of tumor growth and selectivity, desired characteristics in pharmacological studies.

6. Acknowledgments

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References

Almeida CLF, Falcão HS, Lima GRM, Montenegro CA, Lira NS, Athayde-Filho PF, Rodrigues LC, Souza MFV, Barbosa-Filho JM, Batista LM (2011) Bioactivities from Marine Algae of the Genus *Gracilaria*. Int J Mol Sci 12:4550-4573. doi: 10.3390/ijms12074550

Alves A, Sousa RA, Reis RL (2013) A practical perspective on ulvan extracted from green algae. J Appl Phycol 25:407-424. doi: 10.1007/s10811-012-9875-4

Andrade PB, Barbosa M, Matos RP, Lopes G, Vinholes J, Mouga T, Valentão P (2013) Valuable compounds in macroalgae extracts. Food Chem 138:1819-1828. doi: 10.1016/j.foodchem.2012.11.081

Auezova L, Najjar F, Selivanova O, Moussa EH, Assaf MD (2013) Antioxidant activity of brown alga *Saccharina bongardiana* from Kamchatka (Pacific coast of Russia): A methodological approach. J Appl Phycol 25:1189-1196. doi: 10.1007/s10811-012-9932-z

Barsanti L, Gualtieri P (2006) Algae: anatomy, biochemistry and biotechnology. Taylor & Francis Group, New York

Cavallo RA, Acquaviva MI, Stabili L, Cecere E, Petrocelli A, Narracci M (2013) Antibacterial activity of marine macroalgae against fish pathogenic *Vibrio* species. Cent Eur J Biol 8:646-653. doi: 10.2478/s11535-013-0181-6

Crespo MOP, Yusty MAL (2006) Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of aliphatic hydrocarbons in seaweed samples. Ecotox Environ Safe 64:400-405. doi: 10.1016/j.ecoenv.2005.04.010

De Felício R, Albuquerque S, Young MCM, Yokoya NS, Debonsi HM (2010) Trypanocidal, leishmanicidal and antifungal potential from marine red alga *Bostrychia tenella* J. Agardh (Rhodomelaceae, Ceramiales). J Pharm Biom Anal 52:763-769. doi: 10.1016/j.jpba.2010.02.018

Demedts IK, Vermaelen KY, Van Meerbeeck JP (2010) Treatment of extensive stage small-cell lung carcinoma: current status and future prospects. Eur Respir J 35:202-215. doi: 10.1183/09031936.00105009

Dore CMPG, Alves MGCF, Will LSEP, Costa TG, Sabry DA, Rêgo LARS, Accardo CM, Rocha HAO, Filgueira LGA, Leite EL (2013) A sulfated polysaccharide, fucans, isolated from brown algae *Sargassum vulgare* with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. Carbohydr Polym 91:467-475. doi: 10.1016/j.carbpol.2012.07.075

El Gamal AA (2010) Biological importance of marine algae. Saudi Pharm J 18:1-25. doi: 10.1016/j.jsps.2009.12.001

Elizondo-Gonzalez R, Cruz-Suarez LE, Ricque-Marie D, Mendoza-Gamboa E, Rodriguez-Padilla C, Trejo-Avila LM (2012) *In vitro* characterization of the antiviral activity of fucoidan from *Cladosiphon okamuranus* against Newcastle Disease Virus. Virol J 9:1-9. doi: 10.1186/1743-422X-9-307

Foley SA, Szegezdi E, Mulloy B, Samali A, Tuohy MG (2011) An unfractionated Fucoidan from *Ascophyllum nodosum*: extraction, characterization, and apoptotic effects in vitro. J Nat Prod 74:1851-1861. doi: 10.1021/np200124m

Grosso C, Vinholes J, Valentão P, Andrade PB (2011) Halogenated compounds from seaweed, a biological overview. In: Pomin VH (ed) Seaweed: Ecology, nutrient composition and medicinal uses. Nova Science Publishers, New York, pp 163-184

Instituto Nacional De Câncer (Brasil) (2011) Estimativa 2012: Incidência do Câncer no Brasil. INCA, Rio de Janeiro

Jung HA, Jin SE, Ahn BR, Lee CM, Choi JS (2013) Anti-inflammatory activity of edible brown alga *Eisenia bicyclis* and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264.7 macrophages. Food Chem Toxicol 59:199-206. doi: 10.1016/j.fct.2013.05.061

Karsten U, Wulff A, Roleda MY, Müller R, Steinhoff FS, Fredersdorf J, Wiencke C (2011) Physiological responses of polar benthic algae to ultraviolet radiation. In: Wiencke C (ed) Biology of polar benthic algae. De Gruyter, Berlin pp 271-298

Kollár P, Rajchard J, Balounová Z, Pazourek J (2014) Marine natural products: Bryostatins in preclinical and clinical studies. Pharm Biol 52:237-242. doi: 10.3109/13880209.2013.804100

Li Y, Gong Y, Li L, Abdolmaleky HM, Zhou JR (2013) Bioactive Tanshinone I inhibits the growth of lung cancer in part via down regulation of Aurora A function. Mol Carcinogen 52:535-543. doi: 10.1002/mc.21888

Lopes G, Sousa C, Silva LR, Pinto E, Andrade PB, Bernardo J, Mouga T, Valentão P (2012) Can phlorotannins purified extracts constitute a novel pharmacological alternative for microbial infections with associated inflammatory conditions? PLoS ONE 7:e31145. doi: 10.1371/journal.pone.0031145

Luque de Castro MD, Priego-Capote F (2010) Soxhlet extraction: Past and present panacea. *J. Chromatogr. A*, 1217:2383-2389. doi: 10.1016/j.chroma.2009.11.027

Mary JS, Vinotha P, Pradeep AM (2012) Screening for *in vitro* Cytotoxic Activity of Seaweed, *Sargassum* sp. Against Hep-2 and MCF-7 Cancer Cell Lines. Asian Pac J Cancer Prev 13:6073-6076. doi: 10.7314/APJCP.2012.13.12.6073

Maschek JA, Baker BJ (2008) The chemistry of algal secondary metabolism. In: Amsler CD (ed) Algal Chemical Ecology, 1st edn., Springer-Verlag, Berlin, pp 1-24

Menshova RV, Ermakova SP, Anastyuk SD, Isakova VV, Dubrovskaya YV, Kusaykin MI, Umb B-H, Zvyagintseva TN (2014) Structure, enzymatic transformation and anticancer activity of branched high molecular weight laminaran from brown alga *Eisenia bicyclis*. Carbohydr Polym 99:101-109. doi: 10.1016/j.carbpol.2013.08.037

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M (1991) Feasibility of a high-flux anticancer drug screen

using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst 83:757-766. doi: 10.1093/jnci/83.11.757

Mosmann, T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55-63. doi: 10.1016/0022-1759(83)90303-4

Murugan K, Iyer VV (2013) Differential growth inhibition of cancer cell lines and antioxidant activity of extracts of red, brown, and green marine algae. In Vitro Cell Dev Biol Anim 49:324-334. doi: 10.1007/s11626-013-9603-7

Pangestuti R, Kim S-K (2011) Neuroprotective effects of marine algae. Mar Drugs 9:803-818. doi: 10.3390/md9050803

Patra S, Muthuraman MS (2013) *Gracilaria edulis* extract induces apoptosis and inhibits tumor in Ehrlich Ascites tumor cells in vivo. BMC Complement Altern Med 13:1-12. doi: 10.1186/1472-6882-13-331

Peña-Farfal C, Moreda-Piñeiro A, Bermejo-Barrera A, Bermejo-Barrera P, Pinochet-Cancino H, Gregori-Henríquez I (2005) Speeding up enzymatic hydrolysis procedures for the multi-element determination in edible seaweed. Anal Chim Acta 548:183-191. doi: 10.1016/j.aca.2005.06.004

Shao P, Chen X, Sun P (2013) In vitro antioxidant and antitumor activities of different sulfated polysaccharides isolated from three algae. Int J Biol Macromol 62:155-161. doi: 10.1016/j.ijbiomac.2013.08.023

Sousa AAS, Benevides NMB, Pires AF, Fiúza FP, Queiroz MGR, Morais TMF, Pereira MG, Assreuy MAS (2013) A report of a galactan from marine alga *Gelidium crinale* with *in vivo* anti-inflammatory and antinociceptive effects. Fundamen Clin Pharm 27:173-180. doi: 10.1111/j.1472-8206.2011.01001.x

Stein EM, Andreguetti DX, Rocha CS, Fujii MT, Baptista MS, Colepicolo P, Indig GL (2011) Search for cytotoxic agents in multiple *Laurencia* complex seaweed species (Ceramiales, Rhodophyta) harvested from the Atlantic Ocean with emphasis on the Brazilian State of Espirito Santo. Braz J Pharmacog 21:239-243. doi: 10.1590/S0102-695X2011005000069

Syad AN, Shunmugiah KP, Kasi PD (2013) Antioxidant and anti-cholinesterase activity of *Sargassum wightii*. Pharm Biol 51:1401-1410. doi:10.3109/13880209.2013.793721

Virot M, Tomao V, Ginies C, Visinoni F, Chemat F (2008) Green procedure with a green solvent for fats and oils' determination Microwave-integrated Soxhlet using limonene followed by microwave Clevenger distillation. J Chromatogr A, 1196-1197:147-152. doi: 10.1016/j.chroma.2008.04.035

Wiencke C, Amsler CD (2012) Seaweeds and their communities in polar regions. In: Wiencke C, Bischof K (eds) Seaweed biology: novel insights into ecophysiology, ecology and utilization. Springer-Verlag, Berlin, Germany, pp 265-291

Wiencke C, Clayton MN (2002) Antarctic Seaweeds. ARG Gantner Verlag, KG Ruggel

Wiencke C, Gómez I, Dunton K (2011) Phenology and seasonal physiological performance of polar seaweeds. In: Wiencke C (ed) Biology of Polar Benthic Algae. De Gruyter, Berlin, pp 181-194

Wijesekara I, Qian ZJ, Ryu B, Ngo DH, Kim SK (2011) Purification and identification of antihypertensive peptides from seaweed pipefish (*Syngnathus schlegeli*) muscle protein hydrolysate. Food Res Int 44:703-707. doi: 10.1016/j.foodres.2010.12.022

Wijesinghe WAJP, Jeon YJ (2012) Exploiting biological activities of brown seaweed *Ecklonia cava* for potential industrial applications: a review. Int J Food Sci Nutr 63:225-235. doi: 10.3109/09637486.2011.619965

Wulff A, Iken K, Quartino ML, Al-Handal A, Wiencke C, Clayton MN (2011) Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. In: Wiencke C (ed) Biology of polar benthic algae. De Gruyter, Berlin, pp 23-52

Wynne MJ (2005) A checklist of benthic marine algae of the tropical and subtropical western Atlantic: second revision. Beihefte zur Nova Hedwigia, Berlin

Xue M, Ge Y, Zhang J, Liu Y, Wang Q, Hou L, Zheng Z (2013) Fucoidan Inhibited 4T1 Mouse Breast Cancer Cell Growth *In Vivo* and *In Vitro* Via Downregulation of Wnt/*β*-Catenin Signaling. Nutr Cancer 65:460-468. doi:10.1080/01635581.2013.757628

4.3 Manuscrito a ser submetido no periódico “Polar Research”

Article

Antiglioma activity of red macroalgae from Antarctica *Pyropia endiviifolia*

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Abstract: Glioblastoma, a grade IV glioma, is a type of primary malignant brain tumor which shows a higher incidence in adults. Because of its poor prognosis, there is an incentive for new drug discovery. Accordingly, knowing the anticancer potential of some compounds isolated from seaweeds, our goal is evaluate the antiglioma activity from endemic Rhodophyta from Antarctica *Pyropia endiviifolia.* Four extracts were prepared with solvents hexane, chloroform, ethanol and distilled water, using two systems: Ultrasound and Soxhlet. The rat (C6) malignant glioblastoma multiforme and astrocytes cell lines were exposed to different extracts at concentrations of 10-1000µg.mL^{-1} for 24 and

48 hours. The cytotoxicity was measured by MTT assay. The best results were observed in the hexane and ethanol extracts out 10 μg.mL^{-1}. It is expected that these initial findings are likely to stimulate the isolation of compounds with activities of this macroalgae and intensify new drug discovery efforts.

Keywords: Biological activity; glioblastoma; Rhodophyta; seaweeds; tumor

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1. Introduction

Brain tumors are the most malignant of cancers, posing major health problems and presenting especially difficult challenges to therapy (Río-Marco et al. 2013). Glioma is the most common and highly malignant type of primary brain tumor in children and adults (MacDonald et al. 2011; Shah et al. 2013). Even though glioma incidence is far lower than other cancers, its mortality is extremely high (Lee et al. 2013).

Although recent progress in brain tumor therapy including conventional treatments such as resection, radiotherapy, and chemotherapy and biological treatments such as gene therapy and immune therapy, the prognosis of malignant glioma patients still does not provide satisfactory levels (Hdeib & Sloan 2011; Marsh et al. 2013). Rapid proliferation, high aggressiveness, and immunosuppression effect to organism in glioma have become an increasing concern for researchers in recent years (Wu et al. 2013).

Once that the use of a lot of anticancer drugs is associated with many undesirable side effects, there is an urgent need for the discovery of new, better, and specific anticancer compounds (Murugan & Iyer 2013). Several anticancer drugs are derived from a variety of natural sources like plants, animals and microorganisms (Nobili et al. 2009). In special, marine algae have evolved their own chemical defense by synthesizing toxic secondary

metabolites and, therefore, the target of interest for anticancer studies (Folmer et al. 2010; Mohamed et al, 2012).

Antitumor activity has been observed in red algae (Rhodophyta). Recently, a number of polyhalogenated monoterpene compounds were isolated from the red algae *Plocamium suhrii*, *P. cornutum* and *P. corallorhiza* collected from the South African coastline, which were cytotoxic to oesophageal and breast cancer cells *in vitro* (Antunes et al. 2011; De La Mare et al. 2012). Experiments *in vitro* and *in vivo* suggested that elatol, a compound (sesquiterpene) isolated from algae *Laurencia microcladia*, has antitumor properties (Campos et al. 2012).

Particularly, in red seaweeds *Porphyra*, redefined as the genus *Pyropia* (Sutherland et al. 2011), their main components, porphyrans, sulfated polysaccharides showed potential apoptotic activities against human gastric cancer cells and didn't affect the growth of normal cells (Kwon & Nam 2006). The antitumor effect was verified too of R-Phycoerythrin from *Pyropia haitanensis* (Pan et al. 2013), as in phycocyanin and one component (PY-D2) in polysaccharide from *Pyropia yezoensis* in the treatment of some kinds of human cancers (Zhang et al. 2011).

In the polar regions, both light conditions and temperatures are exceptionally low for a large part of the year, with a strong seasonality (Zacher et al. 2011). At these extreme conditions at Antarctica, subjected to severe environmental stresses such as desiccation, salinity, radiation, extreme temperatures and predation, seaweeds produce secondary metabolites to protect them, which may have potential biological activities (Maschek & Baker 2008; Karsten et al. 2011; Wiencke & Amsler 2012).

The Antarctic marine flora is characterized by a relatively high proportion of endemism (Wiencke & Clayton 2002). Thus, our main goal is asses the antiglioma activity from endemic Rhodophyta from Antarctica *Pyropia endiviifolia*, that is found at protected areas, as shaded

rock crevices. Despite that there are already studies of antitumor activity from algae, its biological activity has not been extensively examined in glioma.

2. Material and Methods

Reagents

Dulbecco's modified eagle's medium–high glucose (DMEM), trypsin–EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Heat-inactivated fetal bovine serum (FBS) was purchased from Cultilab Lab Inc. (Campinas, SP, BR). Petroleum ether, hexane, chloroform and ethanol were obtained from Synth. All other chemicals were of analytical grade and HPLC grade solvents.

Algal Sample

The red macroalgae *Pyropia endiviifolia* (A. Gepp & E. Gepp) H.G. Choi & M.S. Hwang was collected in the Demay site in Admiralty Bay, King George Island (62º13´13.93" S, 58º26´17.85 W) at Antarctica in 27 January 2012. The algae fixed to the substrate was collected during periods of low tide; part of the material was preserved in 4% formaldehyde solution in seawater for identification and the preparation of herbarium specimen, and part was washed with seawater to remove epiphytes, frozen at -20°C, and lyophilized for biochemical analyzes. Species identification was performed according to specimen morphology and the classification system proposed by Wynne (2005), and herbarium specimen was deposited in the Herbarium of Instituto de Botânica, São Paulo.

Algal extracts preparation

The extraction was performed by depletion, performing four extractions with solvents: hexane, chloroform, ethanol and distilled water, using two systems: Ultrasound and Soxhlet. The aqueous extraction as the exception rather than the Soxhlet extractor is performed by reflux in an oil bath at 100 °C, at the end are frozen at -70 in ultra-freezer and lyophilized (Model Liotop L101 with vacuum pump IP 21 – Potency 15.4W).

In the system Soxhlet, prewashed with petroleum ether, 1 g of seaweed were used for each 100 mL solvent extraction, held for 4 hours and the cartridge with the biomass of macroalgae remained submerged in the solvent overnight, method adapted from Crespo & Yusty (2006).

Ultrasound probe extraction was performed with a Model VCX-500 ultrasonic processor with a probe horn of 20 kHz frequency and 500 W power (Sonics & Materials Inc., Newton, USA). A 13 mm-diameter horn tip was used in the extractions with the power fixed at 25 % amplitude and the total irradiation period at 30 min with the solvents mentioned previously (40mL), using the same technique depletion in three cycles with renewal of solvent and evaporation of the extract. The sample bottle was immersed in ice, avoiding large swings in temperature, and the maximum temperature was below 20 ◦C throughout the extraction period, method Stein et al. (2011) modified. The extracts were evaporated (Büchi Rotaevaporador RII) at the end to remove the solvents.

The lipid extraction followed the conventional method of Bligh & Dyer (1959), using 1g of algae. This method is based on the extraction of fatty acids (FA) using chloroform and methanol at room temperature. The samples were shaken for 30 minutes with the aid of magnetic stirrer bar mark model QUIMIS Q-225M, and the extractant composed of 30 ml of chloroform / methanol (1:2 v/v) and 10 ml of sodium sulphate (1.5% w/v). After stirring were

added 10 ml of chloroform and 10 ml of sodium sulphate 1.5% (w/v). The extracts were centrifuged at 3000 rpm for 30 min, after the organic phase was collected and dried.

Lipids extracted from biomasses were methylated and converted to their respective fatty acid methyl esters (FAME), according to the following methodology modified Moss et al. (1974). In 100 mL flask containing the lipid was added 2 ml of NaOH solution (0.5 M) in methanol 2 % (w/v) under stirring and heating to 100 °C, being for a period of 5 minutes at reflux. BF_3 was added 3 mL (Lewis acid) followed by stirring for 2 minutes to acid catalysis to occur, and after added 3 ml of NaCl solution at 20% (w/v). The sample was allowed to stand to room temperature, and was then transferred to a separatory funnel with 20 mL of hexane. The organic phase was separated and dried with 2 g of anhydrous sodium sulfate. Evaporation of solvent was carried out with N_2 (g) and weighed sample for subsequent analysis.

Chromatographic analysis of extracts

To analyze extracts was used Liquid Chromatography Ultra High Efficiency (UHPLC) coupled to a Mass Spectrometer for High Resolution (type quadrupole-time of flight), Shimadzu Nexera, Bruker Impact. The analysis used was the positive ionisation mode, with a total time of 42 min, flow 0.2 mL. min^{-1} and the column Shimadzu Shim-Pack XR-ODS III 150 x 2.0 mm. The Impact mass spectrometer was calibrated of m/z 90-1200 using 10 mM sodium formate. To assist in proposing molecular formulas was used an automatic method of fragmentation of ions along the chromatographic run [AutoMS(n)].

Lipids extracts (hexane extract) was re-suspended in HPLC grade solvent for analysis. Fatty acids methyl esters (FAMEs) were separated, identified and quantified by Gas Chromatography with Flame Ionization Detector (GC/FID), qualitatively and quantitatively analyzed by normatized area. The quantitative GC analyses have been performed according to

the following conditions described: a gas chromatograph GC/FID with an AOC-20i auto sampler (Shimadzu, Japan) equipped with a fused-silica capillary column (Rtx-WAX, with dimensions of 30 m x 0.25 mm I.D. x 0.25 µm film thickness). Injections were performed with a 1:25 split ratio and hydrogen was used as carrier gas under constant flow mode at 1.2 mL/min. Injector was heated at 250 °C and a flame-ionization detector operated at 250 °C. Oven initial temperature programming used was 100 °C at 7 ºC/min to 200 °C and then increased at 5 °C/min to 202.6 ºC, isothermal for 2 min at this temperature, and then increased to 5 °C/min at 222.9 ºC and held isothermal for 2 min and then increased to 230 °C at 5 °C/min and held isothermal for 10 min at 230 °C (Tang & Row 2013). The internal standard solution containing nonadecanoate methyl ester (C19:0 ≥ 99.0 %, Aldrich-Sigma) was prepared at a concentration of 2 $mg.mL^{-1}$, where was dissolved 20 mg methyl nonadecanoate in 10 mL of n-hexane in the volumetric flask.

Cell Culture

The rat (C6) malignant glioblastoma multiforme cell line, a grade IV glioma, was obtained from ATCC (CCL-107™). The rat astrocytes were prepared in our laboratory (primary culture). Cells were cultured in DMEM supplemented with 5 % heat-inactivated FBS. Cells were maintained in a humidified atmosphere at 37 °C, in 5 % CO_2, and 95% air. The cytotoxicity study was performed when the cells reached 70-80 % confluence. The cells ($5x10^3$) were seeded in 96-well plates with 100 µl media per well, and after exposed to different extracts at concentrations of 10-1000 $µg.mL^{-1}$ for 24 and 48 hours.

MTT Assay

Cell viability was assessed by the 3-(4,5-dimethyl)-2,5 diphenyl tetrazolium bromide (MTT) assay in quadruplicate. This method is based on the ability of viable cells to reduce MTT and form a blue formazan product. MTT solution (sterile stock solution of 5 $mg.mL^{-1}$)

was added to the incubation medium in the wells at a final concentration of 0.5 mg.mL^{-1}. The cells were left for 2 hours at 37 °C in a humidified 5% CO_2 atmosphere. The medium was then removed, after drying, was added DMSO. The optical density of each well was measured at 405 nm. Results were expressed as the percentage of cell inhibition against the control.

Statistical analysis

All experiments were performed in quadruplicate. Results are expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance ANOVA, with Tukey's post hoc multiple comparisons test to assess statistical differences in case of normal distribution. Differences were considered to be statistically significant if $P < 0.05$ or $P < 0.001$.

3. Results

Antiglioma Activity

In order to assess the antiglioma activity of the extracts of seaweed *Pyropia endiviifolia*, the rat glioblastomas (C6) were exposed to different extracts for a treatment period of 24 h and 48 h. The percentage of growth inhibition was evaluated in MTT assay, as depicted in Tables 1 and 2.

Table 1. Inhibition of growth of gliomas with hexane extract (%).

System	Exposure Time (h)	10 µg.mL^{-1}	100 µg.mL^{-1}	250 µg.mL^{-1}	500 µg.mL^{-1}	1000 µg.mL^{-1}
Soxhlet[a]	24	7.92±4	11.95±8	21.47±8	38.40±3	53.49±3
	48	4.73±6	6.58±3	18.66±4	28.88±3	42.70±2
Ultrasound[b]	24	36.64±5	40.60±3	40.56±2	49.02±7	59.72±1
	48	11.63±6	14.37±3	21.06±4	29.05±4	34.16±4

[a] 4 hours of extraction (1 day); [b] 30 minutes of extraction (3 days)

Table 2. Inhibition of growth of gliomas with ethanol extract (%).

System	Exposure Time (h)	10 µg.mL^{-1}	100 µg.mL^{-1}	250 µg.mL^{-1}	500 µg.mL^{-1}	1000 µg.mL^{-1}
Soxhlet[a]	24	28.53±9	32.27±7	41.30±2	49.05±5	48.54±4
	48	9.21±5	12.45±2	28.09±4	37.49±3	52.07±3
Ultrasound[b]	24	38.82±3	37.99±2	40.64±2	34.35±4	50.54±5
	48	16.99±4	17.70±4	20.08±3	29.77±4	44.50±2

[a] 4 hours of extraction (1 day); [b] 30 minutes of extraction (3 days)

After the cytotoxicity assay, was noted that the aqueous extract didn't present good results since that at concentration 1000 µg.mL^{-1} it didn't inhibit the growth of tumor cells (data no showed), also it induces cell growth. The result for reflux was -10.48% (24 h) and for ultrasound was -4.66% (24 h). The chloroform extract also did not show satisfactory results, since whether to 50% inhibition of cell at lower concentrations of extract. At the highest concentration (1000 µg.mL^{-1}) the chloroform extract in Soxhlet inhibited 27.23% and 35.23%, 24 and 48 hours respectively; while in Ultrasound inhibited 17.61% and 27.33%, 24 and 48 hours respectively.

The hexane extract demonstrated significant results in lower concentrations at Ultrasound system in 24 hours (36.64% of inhibition at 10 µg.mL^{-1}, $P<0.001$), when compared with the extraction in Soxhlet (7.92% at 10 µg.mL^{-1}). Similar condition is observed in ethanol extract (38.82% of inhibition at 10 µg.mL^{-1} in Ultrasound, $P<0.001$), while at Soxhlet (28.53% at 10 µg.mL^{-1}). Marked contrast is observed in the inhibition of cells exposed to the extracts for 48 hours, in the hexane extract the inhibition reduced from 36.64% to 11.63%, while in the ethanol extract decreased from 38.82% to 16.99%. Thus, the best activity was observed in hexane and ethanol extracts of seaweed *P. endiviifolia* and the best extraction system, considering the cytotoxicity of the extract, was the ultrasound.

Cytotoxicity Activity

In order to assess whether the activity of the best extracts in the treatment of gliomas also shows cytotoxicity to healthy cells, we evaluated the response in rat astrocytes by exposure to hexane and ethanol extracts for 24 and 48 hours in quadruplicate (Table 3). The relevant inhibition started in 500 µg.mL^{-1} for both extracts, being that hexane showed 20% and 15% of reduction in growth at 24 and 48 h respectively, while ethanol presented 15% and 11% of inhibition. Thus it was confirmed that the growth inhibition at concentrations well above those that took effect in glioblastomas, and the best antitumor activity was after 24 hours (Figures 1 and 2).

Table 3. Inhibition of growth of rat astrocytes with hexane and ethanol extract in ultrasound (%).

Extract	Exposure Time (h)	10 µg.mL^{-1}	100 µg.mL^{-1}	250 µg.mL^{-1}	500 µg.mL^{-1}	1000 µg.mL^{-1}
Hexane	24	0.00±0	0.00±0	1.41±5	20.23±4	15.47±4
	48	0.00±0	0.00±0	0.00±0	14.78±3	9.11±3
Ethanol	24	0.00±0	0.00±0	2.00±6	14.74±6	17.96±5
	48	0.20±3	0.00±0	0.00±0	11.23±3	9.92±4

Figure 1. Comparison of hexane extracts (ultrasound) in inhibition of growth between rat glioblastoma and astrocytes.

Figure 2. Comparison of ethanol extracts (ultrasound) in inhibition of growth between rat glioblastoma and astrocytes.

3.3 Chromatographic analysis

After the observation of antitumor activity, we performed chemical characterization by liquid chromatography. Chromatograms of hexane and ethanol extracts are reported in Figures 4 and 5. Considering the remarkable activity of hexane extract, which is a nonpolar solvent, it was performed chromatographic profile of the major fatty acids of *P. endiviifolia*, as shown in Figure 6. The concentration of each fatty acid can be displayed in Table 4. Fatty

acids with highest proportion were palmitic acid - C16:0 (23.70%) and cis-5,8,11,14,17-Eicosapentaenoic (EPA) - C20:5n3 (60.00%).

Figure 3. Chromatogram of hexane extract from Ultrasound probe.

Figure 4. Chromatogram of ethanol extract from Ultrasound probe.

Figure 5. Chromatographic profile of the major fatty acids found in *P. endiviifolia*. Peaks **1.** C12:0; **2.** C14:0; **3.** C15:0; **4.** C16:0; **5.** C16:1; **6.** C17:0; **7.** C18:0; **8.** C18:1n9c; **9.** C18:2n6c; **10.** C18:3n6; **11.** C18:3n3; **12.** C20:0; **13.** C20:1n9; **14.** C20:2; **15.** C20:3n6; **16.** C20:4n6; **17.** C20:3n3; **18.** C20:5n3; **19.** C22:1n9

Table 3. The fatty acid composition (%) in Rodophyta *Pyropia endiviifolia*.

Fatty acids	Concentration
C12:0 (Lauric)	0.10
C14:0 (Myristic)	0.24
C15:0 (Pentadecanoic)	0.09
C16:0 (Palmitic**)**	23.70
C16:1 (Palmitoleic)	0.69
C17:0 (Margaric)	0.07
C18:0 (Stearic)	1.46
C18:1n9c (Oleic)	1.96
C18:2n6c (Linoleic)	1.61
C18:3n6 (Gamma-linolenic)	0.52
C18:3n3 (Alpha-Linolenic)	0.15
C20:0 (Arachidic)	0.10
C20:1n9 (cis-11-Eicosenoic)	2.77
C20:2 (cis-11,14-Eicosadienoic)	0.56
C20:3n6 (cis-8,11,14-Eicosatrienoic)	2.25
C20:4n6 (Arachidonic)	3.44
C20:3n3 (cis-11,14,17-Eicosatrienoic)	0.10
C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic)	60.0
C22:1n9 (Erucic)	0.18

4. Discussion

The identification of natural compounds and development of their derivatives have contributed to progress in antitumor drug research and many of these compounds are now being used in clinical practice (Nobili et al. 2009). Glioma due to its infiltrative and heterogeneous nature arising from its malignancy is difficult to treat, being remarkable its resistant to all current modalities of cancer therapy focuses on maximal surgical resection followed by radiation therapy and chemotherapy (Ashby & Ryken 2006; Chamberlain 2006). In 2005, a phase III study performed by European Organization for Research and Treatment of Cancer (EORTC) showed that concurrent chemo-radiotherapy with temozolomide, followed by adjuvant temozolomide increased survival from 12.1 months to 14.6 months, with a two-year survival rate of 26.5% and thus, defined the new standard first-line treatment (Stupp et al. 2005; Tabouret et al. 2013).

Temozolomide is the only chemotherapeutic agent used in the clinical treatment of gliomas. Thus, our research aimed to find new potential drugs or compounds against gliomas, exploring the possible antiglioma activity of macroalgae *Pyropia endiviifolia*, since other species of the genus showed antitumor activity (Kwon & Nam 2006; Zhang et al. 2011; Pan et al. 2013). In order to evaluate the antiglioma activity and cytotoxicity of extracts of seaweeds were used rat malignant glioblastoma multiforme cell line and rat astrocytes. The hexane and ethanol extracts have shown to be promising for treatment as cell inhibition startup of 10 $\mu g.mL^{-1}$. Both extracts demonstrated selective effect when comparing the results of astrocytes, whose effect is not significant.

Conventional Soxhlet extraction remains at a relatively high temperature, long time is required for extraction and large amount of extractant is waste. Thus, samples are usually extracted at the solvent boiling point over long periods, which can result in thermal decomposition of thermolabile target species (Luque de Castro & Priego-Capote 2010). While

ultrasound has been showed to be more economical and eco-friendly, the process can be completed in a few minutes instead of a few hours, as observed at Soxhlet, with high reproducibility. It also reduces the amount of solvent (sometimes no solvent is used) as well as the energy required compared to conventional methods, by working at lower temperatures (Chemat et al. 2011).

In our research, we compared the two systems and observed that extracts obtained from ultrasound probe had better activity than Soxhlet's extracts, showing inhibition effects in lower concentrations. The hexane extract that inhibited the cell growth in 10 $\mu g.mL^{-1}$ (ultrasound), had a significant effect in 250 $\mu g.mL^{-1}$ at Soxhlet, the same behavior was observed for the ethanol extract. The effect of ultrasound on extraction yields is attributed to the micro streaming and heightened mass transfer produced by cavitation and bubbles collapse, resulting in cells destruction (Adam et al. 2012). It's reported that ultrasound enhanced the extraction rate by disrupting plant cell walls leading to increased diffusion of cell contents into extraction solution (Jerman et al. 2010). Although the outcome of the aqueous extract did not exhibit antitumor activity, desired effect in the present study, induction of cell growth observed in treatments can be used in cases of degenerative diseases or in the treatment of burns, leading to a higher rate of cell growth and consequent tissue reconstruction, at this way another studies of bioassay can be explored.

Another point to discuss the differences of responses ranging system and, consequently, the types of compounds extracted is the temperature of extraction, remembering that Soxhlet performs extraction at high temperatures (boiling temperature of the solvent used) and Ultrasound probe had the temperature controlled in ice bath. The importance of temperature extraction relies on the usefulness of the active compounds based on their mechanism of action. As stated by others, the mechanism may vary depending on the temperature at which they were extracted due to differences in molecular weight (Guerra-Rivas et al. 2011).

According an experiment realized by the same authors (Guerra-Rivas et al. 2011), in hot water extracts from the seaweeds are to be expected high molecular weight components, while for the cold extracts, compounds with a lower molecular weight.

In the analysis of gas chromatography of fatty acids composition the main compounds with high proportion were palmitic acid (C16:0) and cis-5,8,11,14,17-Eicosapentaenoic – EPA (C20:5n3). In this context, is known in literature anti-angiogenic, antioxidant, anti-inflammatory, anti-proliferative properties of omega-3 polyunsaturated fatty acids (ω-3), so they can exert antitumor activities, as found in colorectal cancer stem cells (SW620), B-leukemic cell lines (EHEB, JVM-2 and MEC-2), pancreatic cancer, in a rat model of bladder cancer, hepatocellular carcinoma cells, prostate cancer cells, breast cancer cells, human neuroblastoma cell lines, human renal cell carcinoma cell (Gleissman et al. 2011; Ewaschuk et al. 2012; Yang et al. 2013; Fahrmann & Hardman 2013; Arshad et al. 2013; Parada et al. 2013; Sun et al. 2013; Hu et al. 2013; Yang et al. 2013).

Concerning ethanol extract, our studies of composition are still in current. In particular, on extraction of natural products the use of hexane is classic to extract nonpolar substances, especially lipids. According to a study conducted by Tsai and Pan (2012) with 21 species of algae showed that the red algae *Porphyra crispata* had the highest lipid content (28.34 ± 2.98 mg/g dry basis), as well as the highest n-3/n-6 ratio (4.11). These data can be correlated with its health benefits, since that epidemiological and at the molecular level studies evidence the importance of diet with an optimum ratio of n-3 to n-6 essential fatty acids close to 4:1 (Candela et al. 2011).

These data corroborate the relationship established in this work, as they confirm high quantity of omegas present in the genus of algae studied, the potential antitumor activity of omegas 3 observed in the literature and the action of the hexane extract in inhibiting the growth of gliomas in low concentrations, associated with high levels of EPA. Regarding the

chemical of fatty acids, we proposed the protocol to evaluation of chemical substances, as fatty acids and derivatives. Therefore, our study demonstrated the potential antiglioma activity of hexane and ethanol extracts of the endemic Antarctic algae *P. endiviifolia*.

5. Conclusions

The results of this study indicate the potential antiglioma activity of hexane and ethanol extracts of the red macroalgae from Antarctica *Pyropia endiviifolia*. These initial findings are likely to stimulate the isolation of compounds with activities of this macroalgae and intensify new drug discovery efforts.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Adam F., Abert-Vian M., Peltier G. & Chemat F. 2012. ‘‘Solvent-free’’ ultrasound-assisted extraction of lipids from fresh microalgae cells: A green, clean and scalable process. *Bioresource Technology 114*, 457–465.

Antunes E.M., Afolayan A.F., Chiwakata M.T., Fakee J., Knott M.G., Whibley C.E., Hendricks D.T., Bolton J.J. & Beukes D.R. 2011. Identification and in vitro anti-esophageal

cancer activity of a series of halogenated monoterpenes isolated from the South African seaweeds *Plocamium suhrii* and *Plocamium cornutum*. *Phytochemistry 72*, 769-772.

Arshad A., Chung W.Y., Steward W., Metcalfe M.S. & Dennison A.R. 2013. Reduction in circulating pro-angiogenic and pro-inflammatory factors is related to improved outcomes in patients with advanced pancreatic cancer treated with gemcitabine and intravenous omega-3 fish oil. *HPB 15*, 428–432.

Ashby L.S. & Ryken T.C. 2006. Management of malignant glioma: steady progress with multimodal approaches. *Neurosurgical Focus 20*, (E3)1-13.

Bligh E.G. & Dyer W.J. 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology 37*, 911-917.

Campos A., Souza C.B., Lhullier C., Falkenberg M., Schenkel E.P., Ribeiro-Do-Valle R.M. & Siqueira J.M. 2012. Anti-tumour effects of elatol, a marine derivative compound obtained from red algae *Laurencia microcladia*. *Journal of Pharmacy and Pharmacology 64*, 1146-1154.

Candela C.G., Lopez L.M.B. & Kohen, V.L. 2011. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health: Nutritional recommendations. *Nutrición Hospitalaria 26*, 323-329.

Chamberlain M.C. 2006. Treatment options for glioblastoma. *Neurosurgical Focus 20*, (E19)1-9.

Chemat F., Zill-.E H. & Khan M.K. 2011. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics Sonochemistry 18*, 813–835.

Crespo M.O.P. & Yusty M.A.L. 2006. Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of aliphatic hydrocarbons in seaweed samples. *Ecotoxicology and Environmental Safety 64*, 400-405.

De La Mare J., Lawson J.C., Chiwakata M.T., Beukes D.R., Edkins A.L. & Blatch G.L. 2012. Quinones and Halogenated Monoterpenes of Algal Origin show Anti-proliferative Effects against Breast Cancer Cells *in vitro*. *Investigational New Drug 30*, 2187–2200.

Ewaschuk J.B., Newell M. & Field C.J. 2012. Docosahexanoic Acid Improves Chemotherapy Efficacy by Inducing CD95 Translocation to Lipid Rafts in ER2 Breast Cancer Cells. *Lipids 47*, 1019–1030.

Fahrmann J.F. & Hardman W.E. 2013. Omega 3 fatty acids increase the chemo-sensitivity of B-CLL-derived cell lines EHEB and MEC-2 and of B-PLL-derived cell line JVM-2 to anti-cancer drugs doxorubicin, vincristine and fludarabine. *Lipids in Health and Disease 12*, 1-13.

Folmer F., Japars M., Dictato M. & Diederich, M. 2010. Photosynthetic marine organisms as a source of anticancer compounds. *Phytochemistry Reviews 9*, 557–579.

Gleissman H., Segerström L., Hamberg M., Ponthan F., Lindskog M., Johnsen J.I. & Kogner P. 2011. Omega-3 fatty acid supplementation delays the progression of neuroblastoma *in vivo*. *International Journal of Cancer 128*, 1703–1711.

Guerra-Rivas G., Gómez-Gutiérrez C.M., Alarcón-Arteaga G., Soria-Mercado I.E. & Ayala-Sánchez N.E. 2011. Screening for anticoagulant activity in marine algae from the Northwest Mexican Pacific coast. *Journal of Applied Phycology 23*, 495–503.

Hdeib A. & Sloan A.E. 2011. Convection-enhanced delivery of (131)IchTNT- 1/B mAB for treatment of high-grade adult gliomas. *Expert Opinion on Biological Therapy 11*, 799–806.

Hu Y., Sun H., O'Flaherty J.T. & Edwards I.J. 2013. 15-Lipoxygenase-1-mediated metabolism of docosahexaenoic acid is required for syndecan-1 signaling and apoptosis in prostate cancer cells. *Carcinogenesis 34*, 176–182.

Jerman T., Trebše P. & Mozetič Vodopivec B. 2010. Ultrasound-assisted solid liquid extraction (USLE) of olive fruit (*Olea europaea*) phenolic compounds. *Food Chemistry 123*, 175–182.

Karsten U., Wulff A., Roleda M.Y., Müller R., Steinhoff F.S., Fredersdorf J. & Wiencke C. 2011: Physiological responses of polar benthic algae to ultraviolet radiation. In C. Wiencke (ed.): *Biology of polar benthic algae.* Pp. 271–298. Berlin: De Gruyter.

Kwon M.-J. & Nam T.-J. 2006. Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines. *Life Sciences 79*, 1956–1962.

Lee S.-H., Kim J.K., Kim D.W., Hwang H.S., EumW.S., Park, J., Han K.H., Oh J.S. & Choi S.Y. 2013. Antitumor activity of methyl gallate by inhibition of focal adhesion formation and Akt phosphorylation in glioma cells. *Biochimica et Biophysica Acta 1830*, 4017–4029.

Luque De Castro M.D. & Priego-Capote F. 2010. Soxhlet extraction: Past and present panacea. *Journal of Chromatography A 1217*, 2383–2389.

MacDonald T.J., Aguilera D. & Kramm C.M. 2011. Treatment of a high-grade glioma in children and adolescents. *Journal of Neuro-Oncology 13*, 1049–1058.

Marsh J.C., Goldfarb J., Shafman T.D. & Diaz A.Z. 2013. Current Status of Immunotherapy and Gene Therapy for High-Grade Gliomas. *Cancer Control 20*, 43-48.

Maschek J.A. & Baker B.J. 2008: The chemistry of algal secondary metabolism. In C.D. Amsler (ed.): *Algal Chemical Ecology*. Pp.1–24. Berlin: Springer-Verlag.

Mohamed S., Hasim S.N. & Rahman H.A. 2012. Seaweeds: a sustainable functional food for complementary and alternative therapy. *Trends in Food Science & Technology 23*, 83-96.

Moss C.W., Lambert M.A. & Merwin W.H. 1974. Comparation of rapid methods for analysis of bacterial fatty acids. *Journal of Applied Microbiology 28*, 80-85.

Murugan K. & Iyer V.V. 2013. Differential growth inhibition of cancer cell lines and antioxidant activity of extracts of red, brown, and green marine algae. *In Vitro Cellular & Developmental Biology-Animal 49*, 324-334.

Nobili S., Lippi D., Witort E., Donnini M., Bausi L., Mini E. & Capaccioli S. 2009. Natural compounds for cancer treatment and prevention. *Pharmacological Research 59*, 365–378.

Pan Q.W., Chen M.Z., Li J., Wu Y., Zhen C. & Liang B. 2013. Antitumor function and mechanism of phycoerythrin from *Porphyra haitanensis*. *Biological Research 46*, 87-95.

Parada B., Reis F., Cerejo R., Garrido P., Sereno J., Xavier-Cunha M., Neto P., Mota A., Figueiredo A. & Teixeira F. 2013. Omega-3 Fatty Acids Inhibit Tumor Growth in a Rat Model of Bladder Cancer. *BioMed Research International 2013*, 1-11.

Ríos-Marco P., Martín-Fernández M., Soria-Bretones I., Ríos A., Carrasco M.P. & Marco C. 2013. Alkylphospholipids deregulate cholesterol metabolism and induce cell-cycle arrest and autophagy in U-87 MG glioblastoma cells. *Biochimica et Biophysica Acta 1831*, 1322–1334.

Shah A.H., Snelling B., Bregy A., Patel P.R., Tememe D., Bhatia R., Sklar E. & Komotar, R.J. 2013. Discriminating radiation necrosis from tumor progression in gliomas: a systematic review what is the best imaging modality? *Journal of Neuro-Oncology 112*, 141-152.

Stein E.M., Andreguetti D.X., Rocha C.S., Fujii M.T., Baptista M.S., Colepicolo, P. & Indig, G.L. 2011. Search for cytotoxic agents in multiple *Laurencia* complex seaweed species

(Ceramiales, Rhodophyta) harvested from the Atlantic Ocean with emphasis on the Brazilian State of Espirito Santo. *Brazilian Journal of Pharmacognosy 21*, 239-243.

Stupp R., Mason W.P., Van Den Bent M.J., Weller M., Fisher B., Taphoorn M.J., Belanger K., Brandes A.A., Marosi C., Bogdahn U., Curschmann J., Janzer R.C., Ludwin S.K., Gorlia T., Allgeier A., Lacombe D., Cairncross J.G., Eisenhauer E. & Mirimanoff R.O. 2005. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. The *New England Journal of Medicine 352*, 987–996.

Sun S.-N., Jia W.-D., Chen H., Ma J.-L., Ge Y.-S., Yu J.-H. & Li J.-S. 2013. Docosahexaenoic acid (DHA) induces apoptosis in human hepatocellular carcinoma cells. *International Journal of Clinical and Experimental Pathology 6*, 281–289.

Sutherland J.E., Lindstrom S.C., Nelson W.A., Brodie J., Lynch M.D.J., Hwang M.S., Choi H.-G., Miyata M., Kikuchi N., Oliveira M.C., Farr T., Neefus C., Mols-Mortensen A., Milstein D. & Müller K.M. 2011. A new look at an ancient order: generic revision of the Bangiales (Rhodophyta). *Journal of Phycology 47*, 1131–1151.

Tabouret E., Barrie M., Thiebaut A., Matta M., Boucard C., Autran D., Loundou A. & Chinot O. 2013. Limited impact of prognostic factors in patients with recurrent glioblastoma multiforme treated with a bevacizumab-based regimen. *Journal of Neuro-Oncology 114*, 191–198.

Tang B. & Row K.H. 2013. Development of gas chromatography analysis of fatty acids in marine organisms. *Journal of Chromatographic Science 51*, 599-607.

Tsai C.-J. & Pan B.S. 2012. Identification of Sulfoglycolipid Bioactivities and Characteristic Fatty Acids of Marine Macroalgae. *Journal of Agricultural and Food Chemistry 60*, 8404−8410.

Wiencke C. & Amsler C.D. 2012: Seaweeds and their communities in polar regions. In C. Wiencke & K. Bischof (eds.): *Seaweed biology: novel insights into ecophysiology, ecology and utilization*. Pp. 265–291. Berlin: Springer-Verlag.

Wiencke C. & Clayton M.N. 2002. *Antarctic Seaweeds*. Ruggell: ARG Gantner Verlag KG.

Wu X., Hu A., Zhang M. & Chen, Z. 2013. Effects of Rab27a on proliferation, invasion, and anti-apoptosis in human glioma cell. *Tumor Biology 34*, 2195–2203.

Wynne M.J. 2005. *A checklist of benthic marine algae of the tropical and subtropical western Atlantic: second revision.* Berlin: Beihefte zur Nova Hedwigia.

Yang L., Yuan J., Liu L., Shi C., Wang L., Tian F., Liu F., Wang H., Shao C., Zhang Q., Chen Z., Qin W. & Wen W. 2013. α-linolenic acid inhibits human renal cell carcinoma cell proliferation through PPAR-γ activation and COX-2 inhibition. *Oncology Leters 6*, 197-202.

Yang T., Fang S., Zhang H-X., Xu L-X., Zhang Z-Q., Yuan K-T., Xue C-L., Yu H-L., Zhang S., Li Y-F., Shi H-P. & Zhang Y. 2013. N-3 PUFAs have antiproliferative and apoptotic effects on human colorectal cancer stem-like cells *in vitro*. *The Journal of Nutritional Biochemistry 24*, 744–753.

Zacher K., Rautenberger R., Hanelt D., Wulff A. & Wiencke C. 2011: The abiotic environment of polar benthic algae. In C. Wiencke (ed.): *Biology of polar benthic algae*. Pp.9–22. Berlin: De Gruyter.

Zhang L.X., Cai C.E., Guo T.T., Gu J.W., Xu H.L., Zhou Y., Wang Y., Liu C.C. & He P.M. 2011. Anti-cancer effects of polysaccharide and phycocyanin from *Porphyra yezoensis*. *Jurnal of Marine Science and Technology 19*, 377-382.

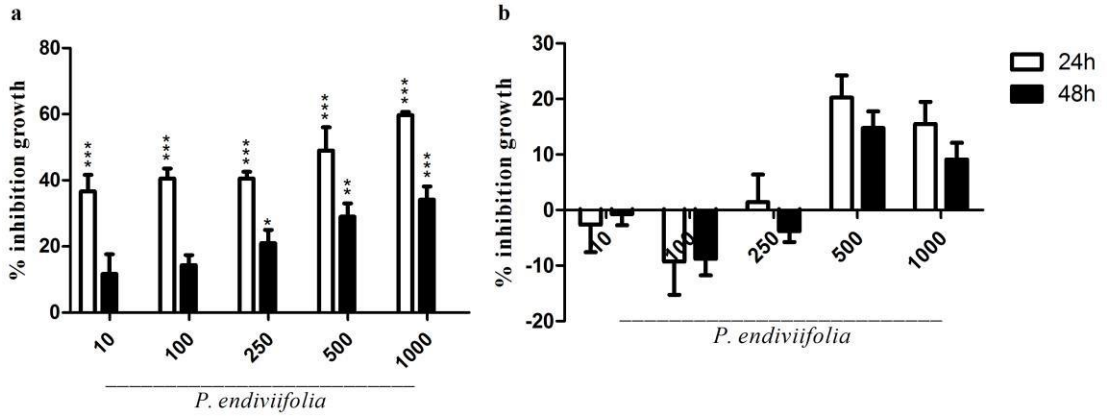

Figure 1. Comparison of hexane extracts (ultrasound) in inhibition of growth between rat glioblastoma (a) and astrocytes (b). *P<0.05; **P<0.005; ***P<0.001 compared to control in time corresponding analysis

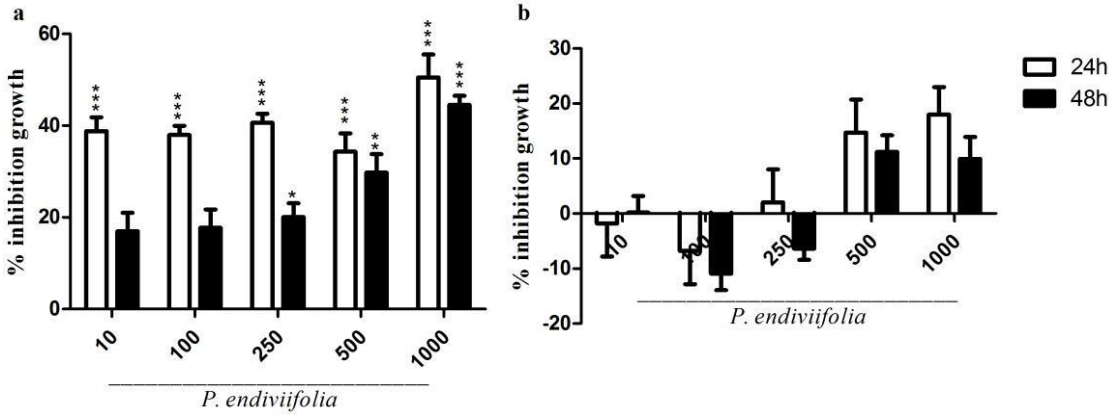

Figure 2. Comparison of ethanol extracts (ultrasound) in inhibition of growth between rat glioblastoma (a) and astrocytes (b).

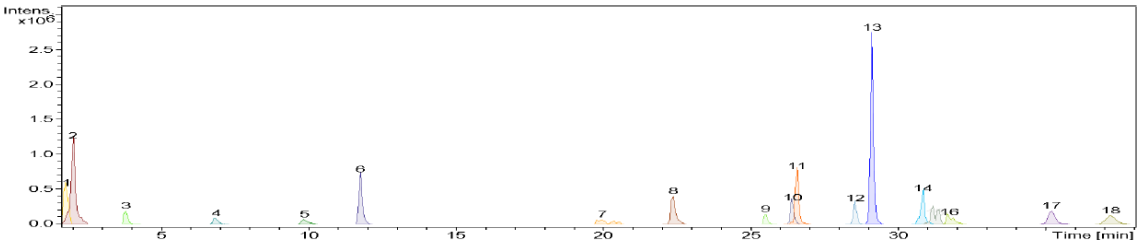

Figure 3. Chromatogram of hexane extract from Ultrasound probe.

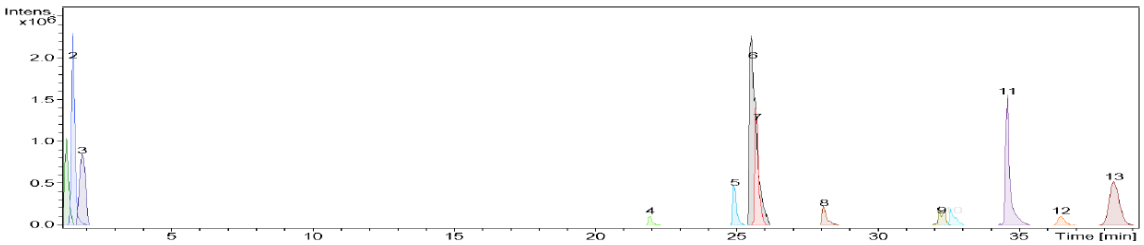

Figure 4. Chromatogram of ethanol extract from Ultrasound probe.

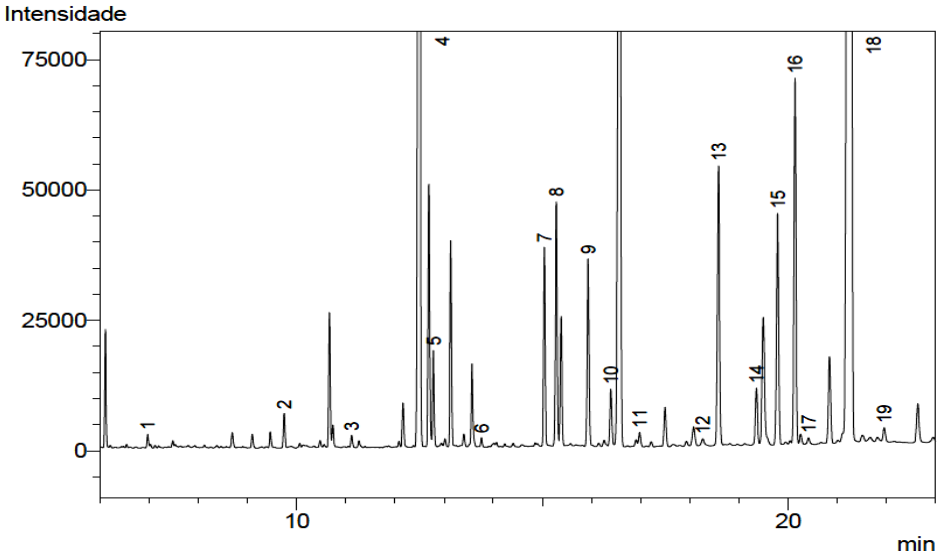

Figure 5. Chromatographic profile of the major fatty acids found in *P. endiviifolia*. Peaks **1.** C12:0; **2.** C14:0; **3.** C15:0; **4.** C16:0; **5.** C16:1; **6.** C17:0; **7.** C18:0; **8.** C18:1n9c; **9.** C18:2n6c; **10.** C18:3n6; **11.** C18:3n3; **12.** C20:0; **13.** C20:1n9; **14.** C20:2; **15.** C20:3n6; **16.** C20:4n6; **17.** C20:3n3; **18.** C20:5n3; **19.** C22:1n9

4.4 Manuscrito submetido no periódico “International Journal of Drug Discovery”

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Antitumor Activity of Biomolecules: A brief review

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Abstract: Cancer is one of the major public health problems at the world. The present review explores the cancer hallmarks and the basis of tumor progression, showing some biological characteristics, and how the immune system reacts to it. Half of drugs that are in clinical use today are of natural origin, indicating that natural products have a significant role

in the process of discovering and developing drugs as prototypes. From this understanding, the present review will show some biological molecules, including heterocyclic compounds that have potential activity against cancer.

Keywords: Active biologically molecules, Cancer, Drugs, Heterocyclic compounds, Immunological activity, Natural products.

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1. DEVELOPMENTS IN CANCER TREATMENT

Although the idea that cancer is a modern disease caused by current life style, there are references of this disease in ancient Greece and Egypt. Histological studies of mummies found in Chile and Egypt showed the presence of benign neoplasms. This theory is based in the concept that the genetic mutations accumulated throughout evolution may result in cell abnormalities and lead to tumor development (Greaves 2007; David & Zimmerman 2010).

Hippocrates, considered medicine's father (460-370 B.C.) introduced the terms carcinos and carcinoma to describe certain types of tumors, which in Greek mean crab. This name was given based on appearance of the tumor, since the projections and blood vessels around her remind the legs of the crustacean. Some centuries later, Galleno continued the Hippocrates's legacy and postulated cancer as an incurable and untreatable disease (Papavramidou et al. 2010).

For a long time the cancer treatment was restricted to the use of plants and therapeutic surgery (Nobili et al. 2009). In the early twentieth century, radiation therapy, which was based on the principle that the radiation was selective to tumor cells, while the surrounding normal cells were unaffected, was introduced to medical practice. However, the clinical data indicate that the radiation therapy is deleterious to normal tissues and high precision equipment to destroy the tumor without damaging the normal cells was developed to increase the treatment efficacy and reduce the side effects of radiation (Meesata et al. 2012).

Following World War II emerged chemotherapy drugs for cancer treatment, which has the function to impair cell mitosis, affecting the fast-growing cells. Thus, in addition to target cancer cells, this treatment also affects the cells responsible for hair growth and replacement of the epithelium gut wall (Morrison 2010).

Currently, depending on the tumor type and the severity of the situation, these treatments are combined to have greater effectiveness against cancers presented by the patient thereby allowing a greater survival and life quality after the development of the disease.

2. CHARACTERIZING TUMOR

Tumor cells or neoplastic cells, among other characteristics, accumulate mutations in several genes related to cell proliferation, death, migration which result in sustaining proliferative and angiogenesis signaling, resistance to cell death and to chemotherapy, activation of invasion and metastasis (Martens et al. 2011). Histopathological characteristics reveal that tumor mass architecture is less organized and structured when compared to normal tissues. This observation contributes to conception that tumors are composed by cells that have lost their ability to create tissues with normal form and function, arising from a complex cascade of phenotypic changes followed by uncontrolled growth (Huang & Ingber 2007).

The tumors were divided into two general categories according to its degree of aggressiveness: those located growth without invasion of adjacent tissues have been classified as benign while the tumors that invade nearby tissues and spread of metastasis were called malignant (Martens et al. 2011). Note that some benign tumors can cause clinical problems by releasing high levels of hormones that cause physiological imbalances in the body, including spinal and paraspinal tumors that releases catecholamine among others chemicals resulting in diarrhea and hypertension (Urata et al. 2013).

Hanahan & Weinberg (2000) outlined six essential characteristics acquired by mutation, which define a cell as neoplastic: self-sufficiency in growth signals, lack of response to external signaling antiproliferative, unlimited proliferative capacity, ability to promote angiogenesis, tissue invasiveness and metastasis and escape or resistance to apoptosis. New

enabling added two potential hallmarks to this list, those are reprogramming of energy metabolism and evading immune destruction. Another relevant discovery is that tumors exhibit other dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the ‘‘tumor microenvironment’’ (Hanahan & Weinberg 2011).

Oncogenes and anti-oncogenes are genes that participate in the formation of tumors. The anti-oncogenes or tumor suppressor genes are recessive, that is, the carcinogenic effect appears only when they are missing or defective in both chromosomes of the genome. In contrast, oncogenes are dominant and encode proteins related to disordered cell multiplication, becoming malignant. Just a copy of the oncogene in the genome promotes the transformation of normal cells into cancer cell. Proto-oncogenes are growth factor-related genes, which regulate normal cell differentiation and proliferation. They encode growth factors, membrane receptors and DNA binding proteins (Lopes et al. 2002).

Signaling pathways and growth factors

There is considerable interest in understanding how activated signaling pathways enhance tumor cell survival because this may lead to the introduction of more effective treatments to target the chemoresistant cell subpopulations (Filippi et al. 2011). As signals are propagated along the cancer pathways by a relay of protein kinases, developing specific protein kinase inhibitors to target particular pathways may constitute novel therapeutic strategies for cancer intervention (Cho 2012).

In general terms, the core of signaling pathways included those in which a single, frequently altered gene predominate, such as KRAS signaling and regulation of G1/S cell cycle transition; pathways in which a few altered genes predominated, such as TGF-β

signaling; and pathways in which many different genes were altered, such as integrin signaling, regulation of invasion, homophilic cell adhesion, and small guanine triphosphatase (GTPase)–dependent signaling. In addition, JNK (c-Jun N-terminal kinase) signaling, Wnt/Notch signaling, Hedgehog signaling, apoptosis and DNA damage control constitute pathways altered in cancer cells (Jones et al. 2008).

A genetic analysis of pancreatic cancer showed 12 cell signaling pathways that are genetically altered in 67-100% of these tumors (Jones et al. 2008). Mutations in the genome of cancer cells affect signaling pathways, which develop a crucial role in cell growth, proliferation, metastasis, angiogenesis, survival and apoptosis. Activation of these signaling pathways leads to up-regulation of a group of transcriptional factors that induce epithelial-to-mesenchymal transition in cells (Takebe et al. 2011). Some signaling pathways are essential for embryonic development that has critical roles in the variation in tumor progression and response to therapy in a variety of human cancers, such as Hedgehog and Wnt pathways. The activation of several pathways and their interactions also raise difficulties in overcoming chemoresistance (Cho 2012).

PI3K (phosphatidylinositol 3-kinase)/Akt signaling pathway is considered as the most important pathway involved in modulation of tumor survival and metastasis, that is activated by various growth factors and also by non-canonical pathways, including activation by various (proto-)oncogenes such as Ras, Her2/neu, cKIT (de Luca et al. 2012). A variety of reports have demonstrated in various cell types that the constitutive activation of Akt (protein kinase B) signaling is sufficient to block cell death induced by a variety of apoptotic stimuli and that the transduction of dominant-negative Akt inhibits growth factor-induced cell survival (Shin et al. 2009).

The PI3K/Akt signaling pathway may be associated with the motility and migration ability of metastatic tumor cells. Increasing PI3K signaling in cancer is triggered to some

mechanisms, among them tyrosine kinase receptors activation and somatic mutations or amplification of genes encoding key components of the signaling cascade (de Luca et al. 2012).

The embryonic signaling pathways, Hedgehog, Notch and Wnt, are critical for the regulation of normal stem cells and cellular development processes. They are also activated in the majority of cancers (Harris et al. 2012).

Hedgehog (Hh) signaling modulates tissue polarity, morphogenesis, proliferation and differentiation and is responsible for stem cell maintenance (Takebe et al. 2011). This pathway is activated in nearly every type of cancer, as glioblastoma, basal cell carcinoma, pancreatic cancer and breast cancer, and is important in maintaining the cancer stem cell in different hematological and solid malignancies, contributing to cancer proliferation, progression and invasiveness (Onishi & Katano 2011). The Hh pathway is deregulated differently depending on the tumor type, with abnormal activation occurring either in a ligand-independentor a ligand-dependent manner (Harris et al. 2012).

Notch signaling is important for cell-cell communication, cell fate specification, cell proliferation, and apoptosis both in embryonic development and in adult tissues. Notch deregulation has been implicated in oncogenesis (Razumilava & Gores 2013). Notch signaling is clearly context-dependent and its activation can result either in pro- or anti-oncogenic effects. The pro-tumorigenic outcome is based on anti-apoptotic, cell growth and angiogenesis induction, and EMT facilitating effects (Harris et al. 2012).

Wnt signaling plays a key role in several biological aspects, such as cell proliferation, tissue regeneration, embryonic development, and other systemic effects, such as angiogenesis and vascular disorders, bone biology, auto-immune diseases, neurological diseases, and neoplastic disorders, which were only recently discovered (Krishnan et al. 2006; Néstor et al. 2006; Chien et al. 2009; Maruotti et al. 2013). The Wnt pathway has been well studied in a

number of cancers that exhibit mutations on β-catenin (Rosenbluh et al. 2012; Gui et al. 2013). According to Rosenbluh and coworkers emerging evidence indicates that oncogenic β-catenin regulates several biological processes essential for cancer initiation and progression (Rosenbluh et al. 2012).

The Fas/FasL signaling plays a significant role in tumorigenesis. Fas is a membrane receptor belonging to the TNF receptor (TNFR) superfamily, which ligand is FasL. Since Fas/FasL play an important role in extrinsic pathway of apoptosis, alterations in this protein contribute to apoptosis resistance and consequent tumor progression (Villa-Morales & Fernández-Piqueras 2012).

TGF-β signaling also plays a significant role in EMT process, embryogenesis, and cancer pathogenesis. TGF-β can alter tight junction formation in mammary epithelium and controls a number of embryonic signaling pathways, including Wnt, Notch and Hh pathways. This signaling can inhibit or suppress transcription of E-cadherin, occludin and claudinin in order to initiate cancer growth and metastasis (Takebe et al. 2011).

Over two decades ago, VEGF (vascular endothelial growth factor), which was initially discovered as vascular permeability factor, was identified as the main regulator of tumor angiogenesis. The role VEGF receptor 1 (VEGFR1) in tumor angiogenesis is complex and still not completely understood. VEGFR1 is involved in the regulation of vascular development during embryogenesis and the recruitment of endothelial cell progenitors. Thus, the inhibition of VEGF results in the successful suppression of tumor growth (Waldner & Neurath 2012).

Epidermal growth factor receptors (EGFR) are a large family of receptor tyrosine kinases (RTK) expressed in several types of cancer, including breast, lung, esophageal, head and neck (Seshacharyulu et al. 2012). EGFR and its family members are the major contributors of a complex signaling cascade that modulates growth, signaling, differentiation,

adhesion, migration and survival of cancer cells (Renon et al. 2014). EGFR signaling activation can stimulate the synthesis and secretion of a number of angiogenic-regulating factors, such as VEGF, Interleukin-8 (IL-8) and basic fibroblast growth factor (bFGF). It is well known that phosphorylation of EGFR is essential for EGFR signaling, which results in phosphorylation at tyrosine residues on the intracellular domain of the receptor, thereby triggers the corresponding signaling pathways (Huang et al. 2013).

The insulin-like growth factor (IGF) system controls cell growth, differentiation, and development. IGF1 receptor (IGF1R) is a tyrosine kinase receptor with 60% homology to the insulin receptor (IR), which signaling is deregulated in many cancers (Tognon & Sorensen 2012). IGF1R exerts anti-apoptotic effects and plays a role in cancer cell proliferation and motility, supporting the mitogenic and metastatic role of such molecule (Trajkovic-Arsic et al. 2013).

The MAPK pathway includes some key signaling components and phosphorylation events that play important role in tumorigenesis. When activated, these kinases transmit extracellular signals that regulate cell growth, differentiation, proliferation, migration and apoptosis functions. Alteration of RAS-RAF-MEK-ERK-MAPK (RAS-MAPK) pathway was observed in human cancer as a result of abnormal activation of receptor tyrosine kinases or gain-of-function mutations mainly in the *RAS* or *RAF* genes (Santarpia et al. 2012).

Programmed cell death

In a multicellular organism, cell exposure to a set of environmental factors may activate specific intracellular programs that could lead to the cell morphological changes and ultimately to cell death (Peng et al. 2013). These cell-killing intracellular events constitute the programmed cell death phenomenon, which includes apoptosis, autophagy, necroptosis (programmed necrosis) and pyroptosis (Sanz et al. 2014)

Programmed cell death may balance cell death with survival of normal cells; when the equilibrium becomes disturbed, programmed cell death plays key roles in ultimate decisions of cancer cell destination (Hanahan & Weinberg 2011). It is well known that cancer cells have numerous strategies to overcome programmed cell death (Ouyang et al. 2012).

Apoptosis is characterized by retraction of pseudopodes, the rounding up of cells and the detachment from the basal membrane or cell culture substrate; a consistent decrease in cell volume (pyknosis); chromatin condensation and nuclear fragmentation (karyorrhexis); blebbing of the plasma membrane; shedding of vacuoles containing parts of the cytoplasm and apparently intact organelles (the so-called apoptotic bodies); and *in vivo* uptake of apoptotic corpses by neighboring cells or professional phagocytes (Galluzzi et al. 2007). The major mechanism that actually causes cell to die is associated with the organized degradation of cell organelles by activated members of caspase family of cysteine proteases (Hanahan & Weinberg 2011).

Apoptotic signals exhibit two main pathways that are interconnected. These are the extrinsic pathway, which is induced through the activation of cell-surface receptors, and the intrinsic pathway, which responds to stress signals (Hanahan & Weinberg 2011). The mitochondrial pathway is an intrinsic pathway to induce apoptosis in which the cell becomes initiated by either extracellular stimuli or intracellular signals, outer mitochondrial membranes become permeable to internal cytochrome c, which is then released into the cytosol (Ouyang et al. 2012). Cytochrome c recruits Apaf–1 and pro-caspase-9 to compose the apoptosome, which downstream triggers a caspase 9/3 signaling cascade, resulting in apoptosis (Bialik et al. 2010).

Transcriptionally independent activity of p53 can potentiate the apoptotic response in a direct interaction with members of the BCL2 family of proteins with structurally conserved domains, which have an important role in positively and negatively regulating mitochondrial

apoptotic pathway, allowing p53 to function as a so-called BCL2-homology domain-3 (BH3)-only protein. Two of these BH-domain proteins, BAX and BAK, function to promote apoptosis by regulating mitochondrial membrane potential (Vousden & Lane 2007).

According Vousden & Lane (2007), one of the key contributions of p53 to apoptosis is the induction of the expression of genes that encode apoptotic proteins, functioning in both extrinsic and intrinsic pathways. Many potential apoptotic target genes of p53 have been described, including those that encode the BH3-domain proteins NOXA and PUMA.

Autophagy mediates the turnover of long-lived proteins, the elimination of damaged organelles and misfolded proteins, and the recycling of cell building blocks following nutrient deprivation. Autophagy has a crucial pro-survival role in cellular homeostasis and during stress, but under certain circumstances may commit suicide by undergoing cell death (Bialik et al. 2010). Stress is a common feature of tumors, which have hypoxic regions lacking oxygen and probably also growth factors and nutrients due to abnormal or insufficient vasculature (Folkman 2007). So, autophagy can have either pro-survival or pro-death roles (Peng et al. 2013).

It is known that when a cell begins to starve, autophagy-related proteins are initiated (Sakurai et al. 2013). Among these proteins, microtubule associated process 1 light chain 3 (LC3), mammalian autophagy protein, is a key regulator involved in forming autophagosomes. Autophagosome marker (LC3) exists in three isoforms (LC3A, LC3B and LC3C) (Korpis et al. 2014). During autophagy LC3 is cleaved to its cytosolic form LC3-I. Lipidation leads to the LC3-II form, which becomes associated with the autophagosomes. Conversion of LC3-I to LC3-II is therefore a marker for the autophagosome formation, which fuses with the lysosome membrane, then their contents degraded by the lysosomal enzymes (Sakurai et al. 2013).

Studies have reported high levels of expression of LC3 in esophageal, gastric, colorectal and pancreatic cancers, suggesting that autophagy is closely associated with tumor (Fujii et al. 2008; Yoshioka et al. 2008; Sakurai et al. 2013). However, increased LC3 expression can't accurately reflect increased autophagic activity; it can also indicate a decrease in autophagic function as a result of a block in fusion after increases in the numbers of autophagosomes (Czaja 2011).

In addition, according Cui and colleagues autophagy promotes the growth and survival of cancer cells in response to cellular stress, and then facilitates tumorigenesis and leads to resistance to therapy (Cui et al. 2013). Inhibition of autophagy has been shown to sensitize tumor cells to the cytotoxic effects of chemotherapy and ionizing irradiation to enhance cancer treatments (Amaravadi et al. 2011).

In contrast to apoptosis, in which a dying cell contracts into an almost-invisible corpse that is soon consumed by neighbors, necrotic cells become bloated and explode, releasing their contents into the local tissue microenvironment. Thus, necrotic cell death releases pro-inflammatory signals into the surrounding tissue microenvironment, in contrast to apoptosis and autophagy, which do not (Ouyang et al. 2012).

Programmed necrosis or necroptosis is an alternative form of programmed cell death that depends on the serine/threonine kinase activity of RIP1 (Vandenabeele et al. 2010). The permeabilization of the plasma membrane can be the cause of later cell death due to ruptures caused by cytoplasmic swelling and consequent release of cytoplasmic contents. This feature is share with pyroptosis, another kind of cell death (Fink & Cookson 2006). In most cases, necroptosis is initiated by stimulation of the extrinsic apoptotic pathway when caspases are absentor inhibited, in other words, when apoptosisis blocked, necroptosis becomes the predominant form ofcell death. Increased ROS levels are a hallmark of necroptosis and may be one of the main causes of necroptotic cell death (Vandenabeele et al. 2010).

Altogether, self-destruction can occur not only as a response to signals originating from the outside environment of the cell, but also from its inside environment (Peng et al. 2013). Accordingly, genetic damage leads to p53 activation protein family, that induces either DNA repair and cell cycle arrest, or programmed cell death, a radical and extreme means preventing the emergence of genetic heterogeneity, and the progression towards cancer (Hanahan & Weinberg 2011). Similarly, alterations in endoplasmic reticulum integrity, induced, for example, by abnormal protein folding, or alterations in mitochondrial activity, such as respiratory chain dysfunction can induce signaling leading to programmed cell death (Vandenabeele et al. 2010). Thus, cell suicide plays anessential role in the maintenance of the genetic identity and the integrity of the body, by inducing the rapid elimination of altered cells.

Immune defense

One of the most important response mechanisms to DNA damage involves the p53 tumor suppressor, the so-called “guardian of the genome” (Vousden & Lane 2007). The p53 influences a lot of cellular processes and represents one of the most important and extensively studied tumor suppressors. Indeed, genomic and mutational analyses documenting inactivation of p53 in more than 50% of human cancers motivated drug development efforts to (re-) activate p53 in established tumors (Stegh 2012).

It is well known that the immune system is able to mount responses against tumors and that this effect can be enhanced using a number of strategies (Begley & Ribas 2008). The interplay between tumor cells and immune system is complex. The concept of cancer immune surveillance has been proposed more than one century ago, but has only recently been validated. According to this concept, immune cells repress tumor growth (Dunn et al. 2004).

Immune surveillance is the first step of the interactions between immune system and tumor cells, corresponding to the tumor elimination phase. If there are only few cancer cells, the immune system can eradicate the tumor at this early stage. If immunosurveillance fails to eradicate the tumor, a second step called the equilibrium phase can be observed. At this stage, tumors are not clinically detectable and the immune system only constrains tumor cell growth. Tumor cells that develop mechanisms to evade the immune system allow cancer to become immune resistant in a process known as cancer immune editing. Then, the tumor cell mass increases and becomes clinically detectable (Dunn et al. 2004).

Many elements are involved in the immune surveillance process. Tumor antigens can be presented by Major Histocompatibility Complex (MHC) molecules to the antigen-presenting cells (APCs). The anti-tumor immune response involves both innate effector cells such as Natural Killers cells (NK), Dendritic Cells (DCs), macrophages, granulocytes and proteins of complement, and adaptive immune responses mediated by B and T lymphocytes (Silva et al. 2013).

Immune cells can be activated by multiple mechanisms. Tumor antigens can be presented by MHC molecules, activating a tumor-specific response. NK cells can be activated by the decreased expression of MHC class I molecules on cancer cells. In addition, immune cells can be activated by the overexpression of activating ligands on cancer cells (Koudougou et al. 2013). Then, mucins, glycoproteins in mucus are frequently overexpressed in diverse cancer cells and are involved in chronic inflammation, oncogenesis, survival, tumor growth and invasion pathways. It has been shown that MUC1 mucin could inhibit tumor cell lysis by NK cells. This approach reveals mucins as a potential therapeutic target in oncology (Rachagani et al. 2009).

Tumor antigens can be presented to lymphocytes by APCs, mainly DCs, macrophages and $CD4^+$ lymphocytes. T-cell activation requires the recognition by the T-cell receptor of the

tumor antigens presented by MHC products. This activation also requires additional signals provided by the APC, e.g. generated through the interactions between B7 and CD40 ligand expressed on the T cell, with CD28 and CD40, respectively, expressed on the APC. CD28 stimulation induces the expression of CTLA-4 on the T-cell membrane. CTLA-4 will interact with B7 with a greater avidity than CD28, and will inhibit the activated T cell (Koudougou et al. 2013).

Tumor-infiltrating lymphocytes (TILs) are located both within the tumor and in the peritumoral stroma. Main subsets of TILs are NK lymphocytes, $CD8^+$ cytotoxic lymphocytes, $CD4^+$ helper lymphocytes and $CD45RO^+$ memory T cells. They can play a role in inhibition and control of the immune response ($CD25^+$ and $FOXP3^+$regulatory T cells) (Koudougou et al. 2013).

It has been demonstrated that many malignant tumors have high infiltration of macrophages. Macrophages at the tumor periphery can foster local invasion by supplying matrix-degrading enzymes such as metalloproteinases and cysteine cathepsin proteases (Kessenbrock et al. 2010); in one model system, the invasion promoting macrophages are activated by IL-4 produced by the cancer cells (Gocheva et al. 2010).

These macrophages, also known as tumor-associated macrophages (TAMs), have been implicated in stromal activation, invasion, and metastasis. TAMs have been shown to regulate angiogenesis and tumor growth by producing potent cytokines and growth factors (Gocheva et al. 2010). One of hallmarks of cancer allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells (Hanahan & Weinberg 2011).

Necrotic cells can release bioactive regulatory factors, such as IL-1α, which can directly stimulate neighboring viable cells to proliferate, with the potential, once again, to facilitate neoplastic progression (Grivennikov et al. 2010).

According cancer immunology, simplifying tumor-host immunological interactions, as highly immunogenic cancer cells may well evade immune destruction by disabling some components of the immune system that have been send to eliminate them (Hanahan & Weinberg 2011).

3. BIOMOLECULES WITH ANTITUMOR ACTIVITY

Natural products, especially those of terrestrial plants and microorganisms, have been exploited as a traditional source of molecules for pharmaceuticals; beyond its pharmacologically active compounds are important for further investigations (Butler 2008).

The first plant compound against cancer was discovered in the bark, and at low levels in the needles, of the relatively rare Pacific Yew, *Taxus brevifolia*. In the 1970s, taxol, recently named paclitaxel, was discovered (Figure 1). Taxol become one of the most effective drugs against breast and ovarian cancer and has been approved worldwide for the clinical treatment of cancer patients (Nobili et al. 2009). This finding represented significant advances in cancer therapy, paclitaxel has a well-known mechanism that blocks cell mitosis and kills tumor cells (Xu et al. 2012). In the 1990s, Robert A. Holton published the first total synthesis of paclitaxel (Holton et al. 1994).

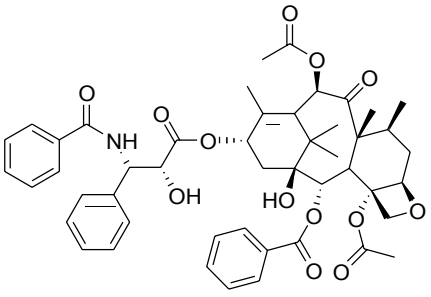


Figure 1. Structure of taxol.

Biosynthesis of carbohydrates is a basic process of life and quantitatively the most important. They are constituent parts of complex lipids (glycolipids) and proteins (glycoproteins) as well as nucleotide building blocks, consequently by nucleic acids and the chemical energy storage system of ADP/ATP (Sathisha et al. 2010). Due to the inherent properties of this class of molecules, carbohydrates have been used to prepare bioactive materials (Sears & Wong 1999), and drugs to specific targets (Rouquayrol et al. 2001).

The complexion of carbohydrates to metals was assessed by Sathisha and coworkers (2010) who associated N-glycosyl with chloride Co (II), Ni (II), Cu (II) and Zn (II) (Figure 2, 3 and 4) noting that the compounds significantly reversed ($p < 0.05$) in tumor-induced changes in the model of Ehrlich ascites carcinoma. All compounds showed significant cytotoxicity in cell viability test, 25% increase in the lifetime of the animals. By convention, a test compound showing 25% increase of the lifetime is considered possible with anticancer activity (Geren et al. 1972).

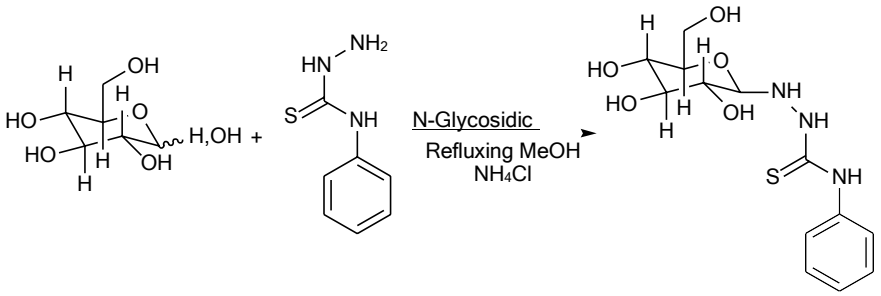


Figure 2. Synthesis of (D-glucopyranose)-phenylthiosemicarbazide LH.

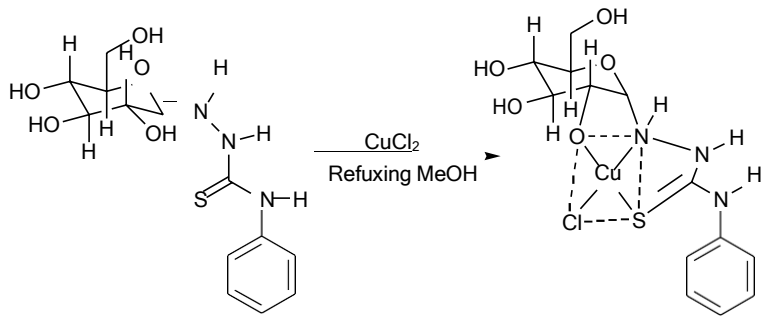


Figure 3. Synthesisof [CulCl].

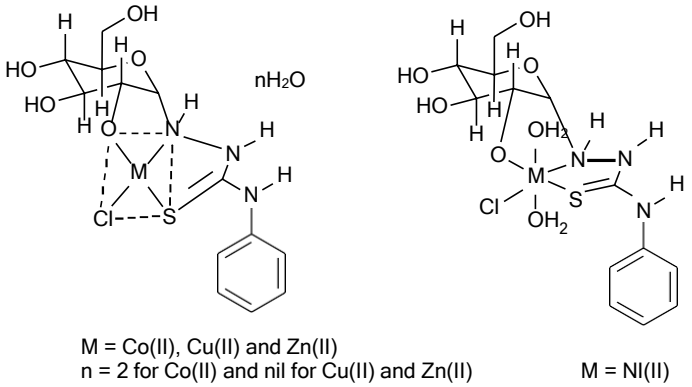


Figure 4. Synthesis of Cobalt(II), Nickel(II), Copper(II) and Zinc(II) complexes.

Polysaccharides are widely present in many plants and have numerous biological activities, being increasingly used in medicines (Wei et al. 2012). The biological activities of polysaccharides depend on the sugar moiety, glycosidic linkages in the main chain, the type and degree of polymerization of the branches, flexibility and configuration of the chains, which can be modified as a polymer by chemistry derivatization (Lu et al. 2008).

Modification in the structure of the polysaccharide is an effective way to increase the biological activities of the polysaccharide. Sulfated polysaccharides, including both naturally extracted from plants as derivatives synthesized are types of biomolecules with sulphated groups in their hydroxyl groups and have different or stronger biological activities, among them antitumoral (Nie et al. 2006).

Thus, Wei and coworkers (2012) extracted a polysaccharide from Radix hedysari (RHP), which is the dried root of Hedysarum polybotrys Hand.-Mazz., a popular phytotherapeutic medicine in China, that has attracted attention due to their antitumor activity (Shon et al. 2002; Song et al. 2004) and synthesized sulfated derivative (RHPS). They observed that the RHPS showed greater antitumor activity than the native polysaccharide ($p<0.05$) in assays *in vitro* with epithelial cell lines of human lung adenocarcinoma (A549) and human gastric cancer (BGC-823), confirming the action of grouping sulfate.

Antitumor substances have been identified in several species of mushrooms, the polysaccharides are better known and potent antitumor and immunomodulatory properties (Zhang et al. 2007). Compared with proteins and nucleic acids, polysaccharides offer greater capacity to carry biological information, because of its potential for structural variability (Ohno 2005). Such variability gives flexibility in accurate regulatory mechanisms multiple cell-cell interactions in complex organisms.

The bioactivity of Basidiomycetes mushrooms was confirmed first by Lucas in 1957, when he isolated a substance from *Boletus edulis* which showed a significant inhibitory effect against tumor cells of sarcoma (S-180) (Lucas 1957). The proposed mechanism by which mushroom polysaccharide exert antitumor effect include: 1) prevention of oncogenesis by oral administration of the polysaccharides isolated from medicinal mushrooms (cancer preventive activity); 2) enhancement of immunity against tumors bearing (activity immuno-enhancement), and 3) direct antitumor activity to induce apoptosis of tumor cells (direct inhibition of the activity of tumor) (Zhang et al. 2007).

Many polysaccharides and polysaccharide conjugates have been commercialized for the clinical treatment of patients in anticancer therapy, and they schizophyllan (Figure 5), lentinan (Figure 6), grifolan, krestin (polysaccharide-peptide complex) and PSK (polysaccharide-protein complex) (Zhang et al. 2007).

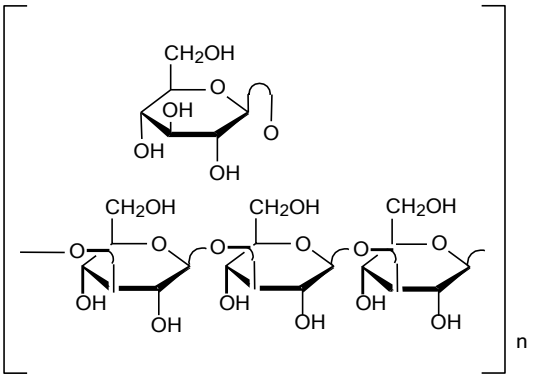


Figure 5. Structureofschizophyllan.

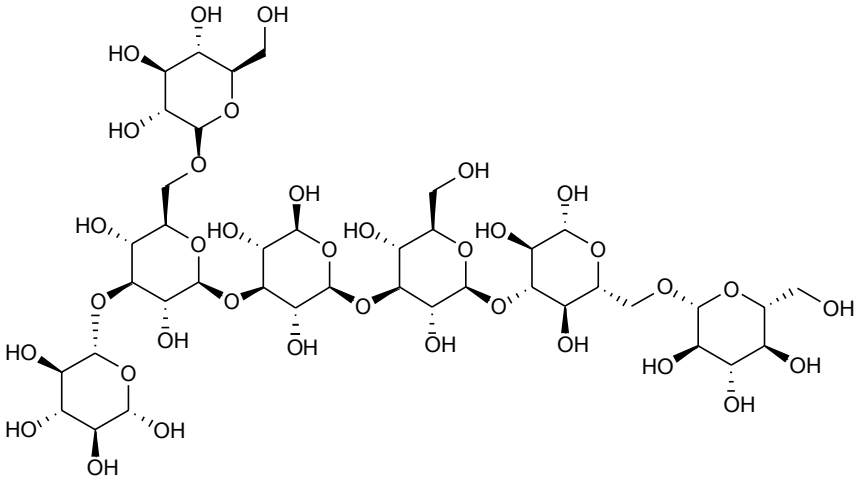


Figure 6. Structure of lentinan.

Recently, researchers have explored compounds of marine organisms, due to their remarkable physiological activities. Thus, Sasaki and coworkers (2011) isolated from the marine cyanobacterium *Lyngbya* sp. a new potent cytotoxic peptide bisebromoamide (Figure 7), which shows antiproliferative activity at nanomolar levels.

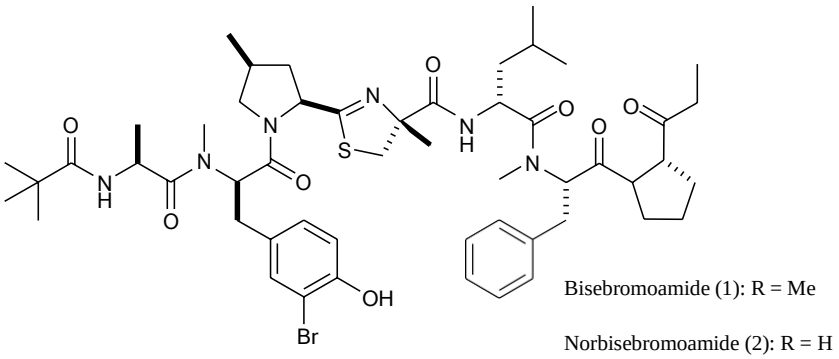


Figure 7. Structure of bis-bromoamide.

Yokosuka and coworkers (2007) isolated from the leaves of a Brazilian tree *Ateleia glazioviana* is a flavone glaziovianin A (Figure 8), which exhibited cytotoxicity against human promyelocytic leukemia cells (HL-60). The differential cytotoxicity standards have suggested that the mechanism of action involves the inhibition of tubulin polymerization

(Yokosuka et al. 2007). According Ikedo and associates (2010), this mechanism of action has become clinically important for drugs against breast cancer.

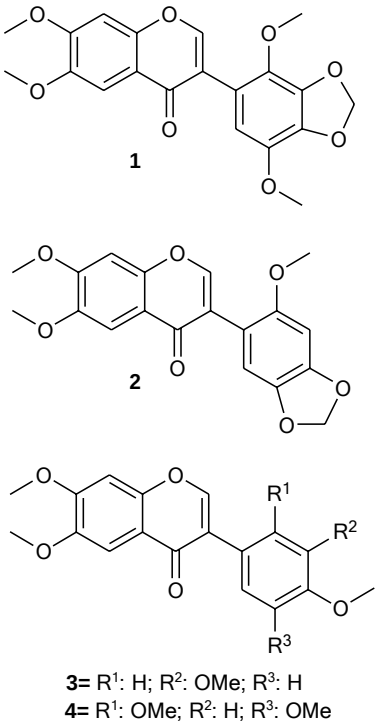


Figure 8. Glaziovianin A *(1)* 6,7,20,50-tetramethoxy-30,40-methylenedioxyisoflavone; *(2)* 6,7,20-trimethoxy-40,50-methylenedioxyisoflavone; *(3)* 6,7,30,40-tetramethoxyisoflavone; *(4)* 6,7,20,40,50-pentamethoxyisoflavone.

Smyrniotopoulos and coworkers (2010) isolated from the organic extract of the red alga *Sphaerococcus coronopifolius* brominated diterpenes (4-16) (Figure 9), with the most active compounds were 4, 6 and 8 through the test with the cell line glioblastoma (GBM) U373 resistant to apoptosis. Compounds 4 and 6 are cytostatic agents that retard the growth of GBM U373 cells by reducing the entry in mitosis (compound 4) and increasing the duration of mitosis (compound 6) with an average duration of 2-3 h in the control condition for over 15 h under experimental conditions where the cells were treated for three days. In contrast, compound 8 showed cytostatic effects through indirect effects: it does not increase the duration of mitosis but induces vacuolation process, which slows down cell proliferation. This process of vacuolation may be related to both the pro-autophagy (Lefranc et al. 2008), and

induction of permeability of lysosome membrane, which is related to cell death (Mijatovic et al. 2006).

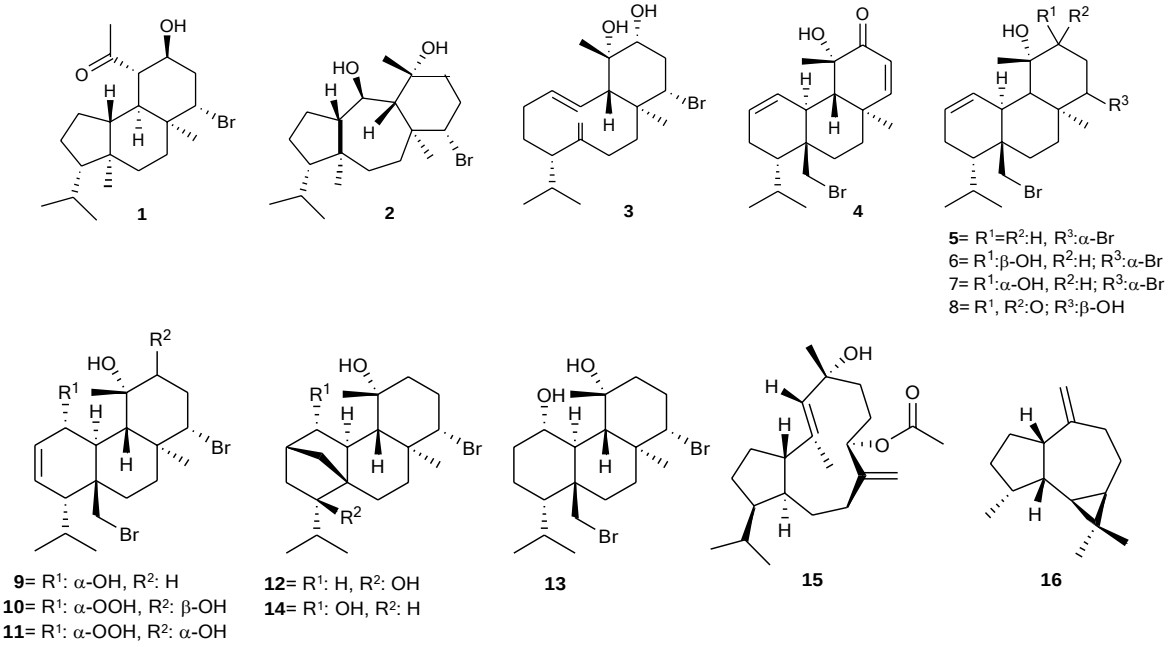


Figure 9. Structures of brominated diterpenes isolated from red alga *Sphaerococcus coronopifolius* and their relative configurations.

Animal poisons are very rich sources of biomolecules with a wide range of activities on diverse physiological systems. Among these, the snake venoms are more concentrated secretory fluids that are known, and cause a variety of biological effects (Stocker 1991). Studies show that various substances found in snake venoms have some antitumor activity, as components belonging to the family of metalloproteinases, C-type lectin and disintegrins that inhibit cell migration *in vitro* and tumor progression *in vivo* by interacting specifically with some integrins in cells membranes (Kamiguti et al. 1996). More recently, it has been demonstrated that crotamine, protein isolated from the venom of the South American rattle snake *Crotalus durissusterrificus*, targets tumor tissue *in vivo* activating the executive enzyme caspase and triggers a lethal calcium-dependent pathway, changing mitochondrial membrane potentialin cultured cells, alters (Nascimento et al. 2011).

Lately, it has been reported in Cuba using an alternative drug for the treatment of cancer, from the venom of a scorpion endemic to that country *Rhopalurus junceus*, popularly called “blue scorpion”, which dilute the poison known by the brand “Escozul” is administered to patients (Garcia-Gomez et al. 2011).

The combination of natural products and commercialized drugs has also been explored recently. Sadzuka and coworkers (2012) extracted curcumin (diferuloylmethane), a phenolic compound from the root of the plant *Curcuma longa* Linn popularly known as Indian Saffron, which is used as a coloring and flavoring in foods (Figure 10). Curcumin was combined with chemotherapeutic drug doxorubicin (DOX), which alone did not reduce tumor weight and after the association was observed 56.5% reduction in tumor weight ($p<0.05$) compared to the group control. This combination enhanced apoptosis, decreased cell viability, and suppresses the activation of caspase-3, -8 and -9 compared with the action of chemotherapeutic alone.

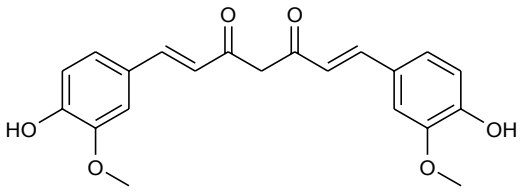


Figure 10. Structure of curcumin.

4. HETEROCYCLES IN CANCER TREATMENT

Heterocyclic molecules are cyclic molecules, aromatic or not, having in its constitution, in addition to carbon atoms, one or more atoms of oxygen, nitrogen or sulfur. Heretocycles have a long history in medicinal chemistry. Several compounds containing heterocyclic rings are being studied to treat different types of cancer, such as the monastrol and ispinesib. While others are used by patients as methotrexate used as anticancer agent, Gemzar® (gemcitabine)

for the treatment of lung and pancreas, and Temodal® (temozolomide) for the treatment of glioma and melanoma (Joule & Mills 2007).

Monastrol

Recent research involving dihydropyrimidinones (six-membered rings with two nitrogen atoms, in positions 3 and 4) that have a resemblance to the structure of nucleic acid bases found in RNA showed the monastrol (5-ethoxycarbonyl-6-methyl-4-(3-hydroxyphenyl)-3,4-dihiropirimidina-2(1H)-thione) as a promising anticancer agent, since it acts as an inhibitor of Eg5 kinesin (Soumyanarayanan et al. 2012). The kinesin Eg5 is a protein that plays a crucial role in the generation of bipolar spindle, which leads to disruption of mitosis and subsequently cell death by apoptosis (Debonis et al. 2004).

The monastrol had anticancer effect especially in mammalian cells, but similar dihydropyrimidinones being tested in order to find new functions in the body such heterocycles (Soumyanarayanan et al. 2012). Soumyanarayanan and coworkers (2012) synthesized a series of similar molecules to monastrol, starting Biginelli reaction to synthesis of dihydropyrimidinones, which was subsequently hydrolyzed and were finally subjected to a coupling reaction with cyclic amines according to Figure 11.

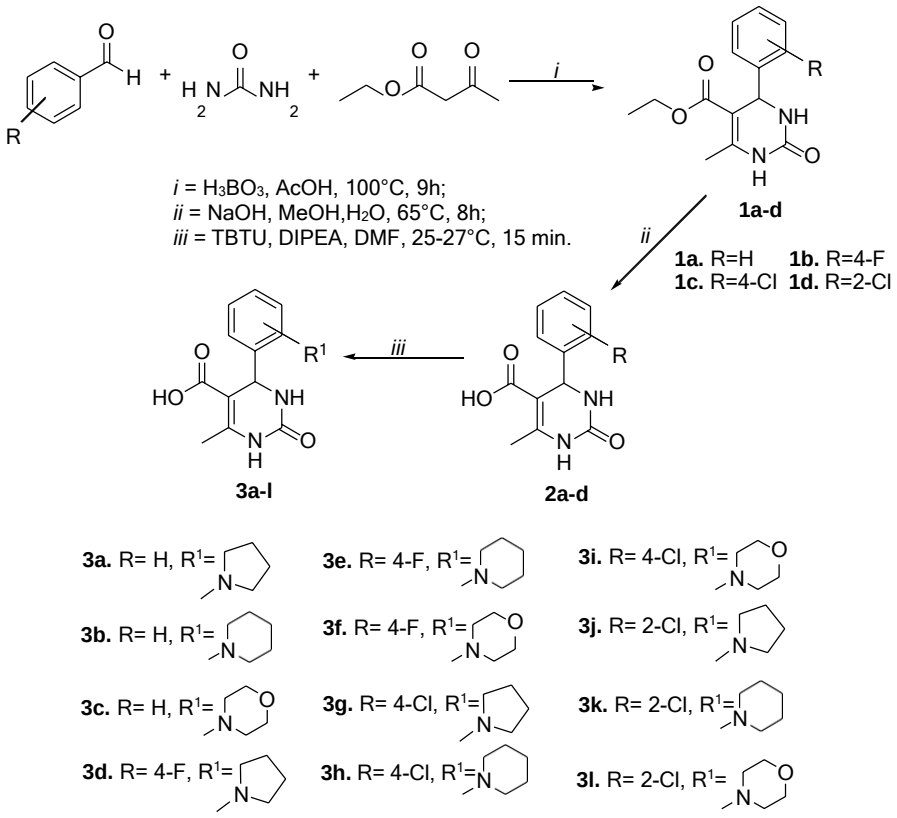
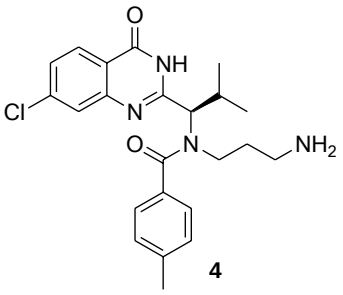


Figure 11. Synthesis of monastrol analogues.

Subsequently, these molecules were tested for anticancer activity in hepatocellular carcinoma cells (HepG2), and human epithelial carcinoma (HeLa). The best results for HepG2 were substances **3g** and **3h**, with IC_{50} 124.46 and 120.62 $mg.mL^{-1}$, respectively (Soumyanarayanan et al. 2012). Substances in these studies structure-activity relationships and molecular modeling were also performed.

Ispinesib

The Ispinesib, shown in Figure 12, is an inhibitor of KSP kinesin Eg5 as monastrol (Lad 2008).



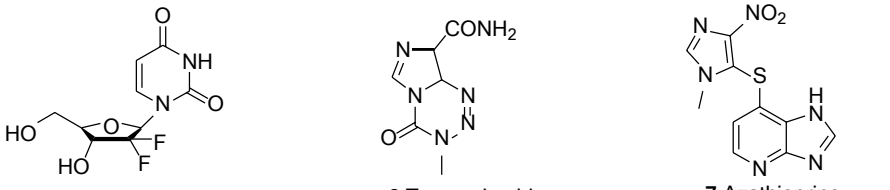
4

(R)-N-(3-aminopropyl)-N-(1-(7-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)-2-methylpropyl)-4-methylbenzamide

Figure 12. Ispinesib structure.

Purcell and coworkers (2010) studied the potential growth inhibition of breast cancer through the use of Ispinesib. Through experiments involving several cell lines of breast cancer in grafts in vitro and in vivo, was also tested the ability of Ispinesib to increase the antitumor activity of therapies approved. According to the observed results was perceived antiproliferative activity against 53 strains of cells in vitro and tumor regression in vivo as well as combination therapy, showing that this drug may help treat cancer (Purcell et al. 2010)

In Figure 13, structures of anticancer drugs that act during DNA synthesis are related.



5 Gemcitabine

1-((2*R*,4*S*,5*R*)-3,3-difluoro-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione

6 Temozolomide

3,4,8,8a-tetrahydro-3-methyl-4-oxoimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxamide

7 Azathioprine

7-(1-methyl-4-nitro-1*H*-imidazol-5-ylthio)-1*H*-imidazo[4,5-*b*]pyridine

Figure 13. Structures of drugs that interfere with DNA synthesis.

Gemcitabine

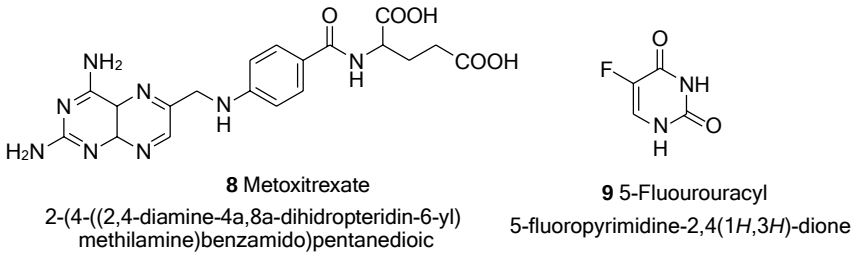
Gemcitabine (**5**) shown in Figure 13, is a nucleoside analogue that replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. The process contains tumor growth resulting in apoptose (Candelaria et al. 2012).

Temozolomide

Temozolomide (**6**), with the structure shown in Figure 13, is an agent imidazotetracenic alkylating with antitumor activity, which undergoes rapid chemical conversion at physiological pH in systemic circulation, that forms the active compound monomethyltriazeno-imidazole-carboxamide (MTIC). The cytotoxicity of MTIC is mainly due to DNA methylation at the O6 and N7 positions of guanine, resulting in inhibition of DNA replication and subsequently, cell death (Szeliga et al. 2012).

Azathioprine

Azathioprine (**7**) is employed to treat leukemia, metabolized to 6-mercaptopurine (6-MP). It is a purine analog, which has cytotoxic effects on lymphocytes, inhibits the synthesis of ribonucleotides and NK cells (Shih et al. 2012). In addition to these drugs, we can still highlight the methotrexate and 5-fluorouracyl with their structures shown in Figure 14.



8 Metoxitrexate
2-(4-((2,4-diamine-4a,8a-dihidropteridin-6-yl) methilamine)benzamido)pentanedioic

9 5-Fluourouracyl
5-fluoropyrimidine-2,4(1*H*,3*H*)-dione

Figure 14. Drugs used in cancer treatment.

Methotrexate

The methotrexate (**8**) exemplified in Figure 14 is largely established as anticancer drug in high doses and immunosuppressive agents when administered in smaller doses. This drug is an antimetabolite with similar structure to folic acid, which competitively inhibits the activity of the enzyme dihydrofolate reductase being considered specific chemotherapeutic the synthesis phase of the cell cycle, particularly exponentially growing cells, such as tumor (Balaji et al. 2012).

5-Fluourouracyl

Fluorouracyl (**9**), pyrimidine analogue which has the structure shown in Figure 14, is used for the treatment of solid tumors, acting as an uracil antimetabolite. In organism, prevents the biosynthesis of pyrimidine nucleotides interfering with vital cellular activity, and enzymatically converted to the active deoxynucleotide, then interfere with DNA synthesis (Ligabue et al. 2012).

5. Nanotechnology applied to drugs

Systemic administration of most chemotherapeutic drugs (e.g., Taxol®) can only deliver a limited amount of drug to the tumor site and commonly produces severe side effects at high doses, which limits its therapeutic index (Safavy 2008). Therefore, localized delivery of anti-tumor drugs can increase the long-term regional drug concentration and promote the absence of systemic toxicity (Lin et al. 2014). At this perspective, nanotechnology has received great attention due to its important clinical application on the treatment of various diseases, including cancer. Nanoparticles with diameters from 1 to100 nm are employed in nanomedicine to encapsulate drugs and target them to tumors (Valetti et al. 2013).

The nanoparticle enhances local drug delivery efficiency to the tumors via entrapment in leaky tumor vasculature, molecular targeting to cells expressing cancer biomarkers, and/or magnetic targeting. Another important feature is the improvement of localization by using triggered release in tumors via chemical, thermal, or optical signals. In order to optimize these nanoparticle drug delivery strategies, it is important to be able to image where the nanoparticles distribute and how rapidly they release their drug payloads (Moore et al. 2014).

Promising therapeutic paradigms are coming up as multifunctional or theranostic agents, those provide attractive vehicles for both image and therapeutic agents. Nanosystems are capable of diagnosis, specific targeted drug therapy and monitoring therapeutic response. Nanomolecules present well-developed surface nature, so they are easy to anchor with multifunctional groups (Rahman et al. 2012). Currently, biodegradable polymers or lipid-based colloids are the only drug vehicles approved for clinical use, among them micelles, nanoemulsions, nanosuspensions, solid lipid nanoparticles, lipid nanocapsules and lipoproteins which improve the *in vitro* dissolution velocity and *in vivo* efficiency of drugs with poor water solubility (Wang et al. 2014b).

Recent study showed that nano-sized thermo-sensitive amphiphilic micelles have the potential to be used as a drug carrier in the chemotherapy of cholangiocarcinoma, due to their passive targeting and thermo-induced active release mechanisms (Wang et al. 2014a). Nanoparticle generated heat melts the membrane of a complex nanocarrier or the linker between the nanoparticle and therapeutic molecule within minutes or less, and releases its therapeutic payload (Leung & Romanowski 2012). Wang and coworkers (2014c) reported that using cyclic peptide-based supramolecular structures as nanocarriers is a feasible and a potential solution for drug delivery to resistant tumor cells in tests with doxorubicin in human breast cancer (MCF-7/ADR).

Magnetic nanoparticles have been reported due to their peculiar characteristics and biomedical applications, acting as diagnostic molecular imaging agents and with therapeutic properties for different types of drug carriers (Sivakumar et al. 2014). Others nanoparticles such as iron oxide, quantum dots (QD), silica nanoparticles, carbon nanotubes (CNTs), gold, dendrimer and graphene have been investigated as multifunctional nanoparticles (Rahman et al. 2012).

The inhibition of cancer cell metastasis by graphene and graphene oxide might provide new insights into specific cancer treatment, once that exposure of cells to graphene led to the direct inhibition of the electron transfer chain complexes I, II, III and IV, specially by disrupting electron transfer between iron-sulfur centers, which is due to its stronger ability to accept electrons compared to iron-sulfur clusters. The decreased electron transfer chain activity results in low production of ATP and subsequent impairment of F-actin cytoskeleton assembly, which is crucial for the migration and invasion of metastatic cancer cells (Zhou et al. 2014).

6. Conclusion

The understanding of the complex regulatory system involved in tumorigenesis is fundamental to the elucidation of target pathways for action new anticancer drugs. Numerous drugs used today are synthesized from biomolecules discovered both plant and animal origin, as noted along the review. Ongoing research continues to discover new potentially active biomolecules in order to optimize the therapeutic effect mainly aimed at reducing side effects.

Acronyms and Definitions list

APCs: antigen-presenting cells – dendritic cells that play a critical role in the regulation of the adaptive immune response.

EGFR: Epidermal growth factor receptors

Hh: Hedgehog signaling

IGF: Insulin-like growth factor

JNK: c-Jun N-terminal kinase

KRAS: Protein involved primarily in regulating cell division

LC3: microtubule associated process 1 light chain 3 - mammalian autophagy protein

NK: natural killer

PI3K: Phosphatidylinositol 3-kinase

PUMA: protein that belongs to the BCL2 family and that promotes mitochondrial outer membrane permeabilization and apoptosis

TGF-β: Transforming growth factor-beta - potent immunosuppressor, which perturbation of its signaling is linked to autoimmunity, inflammation and cancer

TNF: Tumor necrosis factors - multifunctional group pro-inflammatory cytokines which activate signaling pathways for cell survival, apoptosis, inflammatory responses, and cellular differentiation.

VEGF: Vascular endothelial growth factor

References

Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N et al. Principles and current strategies for targeting autophagy for cancer treatment. *Clin. Cancer Res.,* 2011, 17(4), 654-66.

Balaji P, Thirumal M, Gowri R, Divya V, Vadivelan R. Design and evaluation of matrix type of transdermal patches of methotrexate. *Int. J. Pharm.Chem. Biol. Sci.*, 2012, 2(4), 464-71.

Begley J, Ribas A. Targeted therapies to improve tumor immunotherapy. *Clin. Cancer Res.*, 2008, 14(14), 4385–91.

Bialik S, Zalckvar E, Ber Y, Rubinstein AD, Kimchi A. Systems biology analysis of programmed cell death. *Trends Biochem. Sci.*, 2010, 35(10), 556-64.

Brustugun OT, Fladmark KE, Doskeland SO. Apoptosis induced by micro injection of cytochrome c is caspase-dependent and is inhibited by Bcl-2. *Cell Death Differ.*, 1998, 5(8), 660-68.

Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat. Prod. Rep.*, 2008, 25(3), 475-561.

Callahan MK, Williamson P, Schlegel RA. Surface expression of phosphatidylserine on macrophages is required for phagocytosis of apoptotic thymocytes. *Cell Death Differ.,* 2000, 7(7), 645-53.

Candelaria M, Hernandez ECH, Chayeb LT, Cardenas EP, Becerril CT et al. DNA Methylation-Independent Reversion of Gemcitabine Resistance by Hydralazine in Cervical Cancer Cells. *PLoSONE*, 2012, 7(3), e29181.

Chien AJ, Conrad WH, Moon RT. A Wnt survival guide: From flies to human disease. *J. Invest. Dermat.*, 2009, 129(7), 1614–27.

Cho WCS. Targeting the signaling pathways in cancer therapy. *Expert Opin. Ther. Targets*, 2012, 16(1), 1-3.

Counis MF, Torriglia A. Acid DNases and their interest among apoptotic endonucleases. *Biochimie*, 2006, 88(12), 1851-58.

Cui J, Gong Z, Shen HM. The role of autophagy in liver cancer: Molecular mechanisms and potential therapeutic targets. *Biochim. Biophys. Acta*, 2013, 1836(1), 15-26.

Czaja MJ. Functions of autophagy in hepatic and pancreatic physiology and disease. *Gastroenterology*, 2011, 140(7), 1895-1908.

David AR, Zimmerman MR. Cancer: an old disease, a new disease or something in between? *Nat. Rev.Cancer*, 2010, 10(10), 728-33.

de Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N. The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: Role in cancer pathogenesis and implications for therapeutic approaches. *Expert Opin. Ther. Targets*, 2012, 16(1), 17-27.

DeBonis S, Skoufias DA, Lebeau L. In vitro screening for inhibitors of the human mitotic kinesin Eg5 with antimitotic and antitumor activities. *Mol. Cancer Ther*, 2004, 3(9), 1079-90.

di Fillipo M, Bernardi G. The early apoptotic DNA fragmentation targets a small number of specific open chromatin regions. *PLoS*, 2009, 4(4), 5000-10.

Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*, 2004, 21(2), 137-48.

Fan X, Krahling S, Smith D. Macrophage surface expression of annexins I and II in the phagocytosis of apoptotic lymphocytes. *Mol. Biol. Cell*, 2004, 15(6), 2863-72.

Filippi I, Naldini A, Carraro, F. Role of the hypoxic microenvironment in the antitumor activity of tyrosine kinase inhibitors. *Curr. Med. Chem.*, 2011, 18(19), 2885-92.

Fink SL, Cookson BT. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell. Microbiol.*, 2006, 8(11), 1812-25.

Folkman J. Angiogenesis: an organizing principle for drug discovery? *Nat. Rev. Drug Discov.*, 2007, 6(4), 273-86.

Fujii S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G et al. Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. *Cancer Sci.*, 2008, 99(9), 1813-19.

Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L, Kroemer G. Cell death modalities: classification and pathophysiological implications. *Cell Death Differ.*, 2007, 14(7), 1237-43.

García-Gómez BI, Coronas FI, Restano-Cassulini R, Rodríguez RR, Possani LD. Biochemical and molecular characterization of the venom from the Cuban scorpion *Rhopalurus junceus*. *Toxicon*, 2011, 58(1), 18-27.

Geren RI, Greenberg NH, Mac Donald MM, Schumacher AM, Abbot BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemoth. Rep.*, 1972, 3(1), 1-102.

Gocheva V, Wang HW, Gadea BB, Shree T, Hunter KE et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev.*, 2010, 24(3), 241-55.

Greaves M. Darwinian medicine: a case for cancer. *Nat. Rev. Cancer*, 2007, 7(3), 213-21.

Gui SY, Yuan G, Wang L, Zhou LL, Xue Y et al. Wnt3a regulates proliferation, apoptosis and function of pancreatic NIT-1 beta cells via activation of IRS2/PI3K signaling. *J Cell. Biochem.*, 2013, 114(7), 1488-97.

Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*, 2011, 144(5), 646-74.

Hanahan D, Weinberg RA. The Hallmarks of Cancer. *Cell*, 2000, 100(1), 57-70.

Harris PJ, Speranza G, Ullmann CD. Targeting embryonic signaling pathways in cancer therapy. *Expert Opin. Ther. Targets*, 2012, 16(1), 131-45.

Holton RA, Somoza C, Kim HB, Liang F, Biediger RJ et al. First Total Synthesis of Taxol.1.Functionalization of the B ring. *J. Am. Chem. Soc.*, 1994, 116(4), 1597-99.

Huang S, Ingber DE. A Non-Genetic Basis for Cancer Progression and Metastasis: Self-Organizing Attractors in Cell Regulatory Networks. *Breast Disease*, 2007, 26(1), 27-54.

Huang X, Li Y, Zhang J, Xu Y, Tian Y, Ma K. Ganglioside GM3 Inhibits Hepatoma Cell Motility via Down-Regulating Activity of EGFR and PI3K/AKT Signaling Pathway. *J. Cell. Biochem.*, 2013, 114(7), 1616-24.

Huerta S, Goulet BS, Huerta-Yepez S. Screening and Detection of Apoptosis. *J. Surg. Res.*, 2007, 139(1), 143-56.

Ikedo A, Hayakawa I, Usui T, Kazami S, Osada H, Kigoshi H. Structure–activity relationship study of glaziovianin A against cell cycle progression and spindle formation of HeLa S3 cells. *Bioorg. Med. Chem. Lett.*, 2010, 20(18), 5402-04.

Jones S, Zhang X, Parsons DW, Lin JCH, Leary RJ et al. Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses. *Science*, 2008, 321(5897), 1801-06.

Joule JA, Mills K. *Heterocyclic Chemistry at a Glance*, 2007. Oxford: Blackwell Publishing. Pp.160.

Kamiguti AS, Hay CRM, Theakston RGD, Zuzel M. Insights into the mechanism of hemorrhage caused by snake venom metalloproteinases. *Toxicon*, 1996, 34(6), 627-42.

Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Brit. J. Cancer*, 1972, 26(4), 239-57.

Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: Regulators of the tumor microenvironment. *Cell*, 2010, 141(1), 52-67.

Korpis K, Weber Fe, Brune S, Wünsch B, Bednarski PJ. Involvement of apoptosis and autophagy in the death of RPMI 8226 multiple myeloma cells by two enantiomeric sigma receptor ligands. *Bioorg. Med. Chem.*, 2014, 22(1), 221-33.

Koudougou C, Bonneville M, Matysiak-Budnik T, Touchefeu Y. Antitumoural immunity in colorectal cancer: current and potential future implications in clinical practice. *Aliment. Pharmacol. Ther.*, 2013, 38(1), 3-15.

Krishnan V, Bryant HU, MacDougald OA. Regulation of bone mass by Wnt signaling. *J. Clin. Invest.*, 2006, 116(5), 1202-09.

Lad L, Luo L, Carson JD, Wood KW, Hartman JJ, Copeland RA, Sakowicz R. Mechanism of inhibition of human KSP by ispinesib. *Biochemistry*, 2008, 47(11), 3576-3585.

Lefranc F, Mijatovic T, Kondo Y, Sauvage S, Roland I et al. Targeting the [alpha] 1 subunit of the sodium pump to combat glioblastoma cells. *Neurosurgery*, 2008, 62(1), 211-22.

Ligabue A, Marverti G, Liebl U, Myllykallio H. Transcriptional Activation and Cell Cycle Block Are the Keys for 5-Fluorouracil Induced Up-Regulation of Human Thymidylate Synthase Expression. *PLoSONE*, 2012, 7(10), e47318.

Lin, Z.; Gao, W.; Hu, H.; Ma, K.; He, B.; Dai, W.; Wang, X.; Wang, J.; Zhang, X.; Zhang, Q. Novel thermo-sensitive hydrogel system with paclitaxel nanocrystals: High drug-loading,

sustained drug release and extended local retention guaranteeing better efficacy and lower toxicity. *J. Control. Release*, 2014, 174, 161-170.

Lopes AA, Oliveira AM, Prado CBC. Principais genes que participam da formação de tumores. *Rev.Biol.Ciênc.Terra*, 2002, 2(2), 1-7.

Lu Y, Wang DY, Hu YL, Huang XY, Wang JM. Sulfated modification of epimedium polysaccharide and effects of the modifiers on cellular infectivity of IBDV. *Carbohydr. Polym.*, 2008, 71(2), 180-86.

Lucas EH. Tumor inhibition in *Boletus edulis* and other Holobasidiomycetes. *Antibiot. Chemother.*, 1957, 7(1), 1-15.

Luo X, Chen B, Zheng R. Hydrogen peroxide induces apoptosis through the mitochondrial pathway in rat Schwann cells. *Neurosci. Lett.*, 2010, 485(1), 60-64.

Martens EA, Kostadinov R, Maley CC, Hallatschek O. Spatial structure increases the waiting time for cancer. *New J. Phys.*, 2011, 13(1), 1-22.

Maruotti N, Corrado A, Neve A, Cantatore FP. Systemic Effects of Wnt Signaling. *J. Cell. Physiol.*, 2013, 228(7), 1428-32.

Meesata R, Belmouaddinea H, Allarda JF, Renauda CT, Lemaya R et al. Cancer radiotherapy based on femtosecond IR laser-beam filamentation yielding ultra-high dose rates and zero entrance dose. *Proc. Natl. Acad. Sci. U.S.A.*, 2012, 109(7), E2508-2513.

Mijatovic T, Mathieu V, Gaussin JF, de Neve N, Ribaucour F et al. Cardenolide-induced lysosomal membrane permeabilization demonstrates therapeutic benefits in experimental human non-small cell lung cancers. *Neoplasia*, 2006, 8(5), 402-12.

Moore T, Chen H, Morrison R, Wang F, Anker JN, Alexis F. Nanotechnologies for Noninvasive Measurement of Drug Release. *Mol. Pharmaceutics*, 2014, 11(1), 24-39.

Morrison Wb. Cancer Chemotherapy: An Annotated History. *J. Vet. Intern. Med.*, 2010, 24(6), 1249-62.

Nascimento FD, Sancey L, Pereira A, Rome C, Oliveira V et al. The natural cell-penetrating peptide crotamine targets tumor tissue in vivo and triggers a lethal calcium-dependent pathwayin cultured cells. *Mol. Pharmaceut.* , 2012, 9(2), 211-21.

Néstor T, Masckauchán H, Kitajewski J. Wnt/frizzled signaling in the vasculature: new angiogenic factors in sight. *Physiology*, 2006**,** 21(3), 181-88.

Nie XH, Shi BJ, Ding YT, Tao WY. Preparation of a chemically sulfated polysaccharide derived from *Grifola frondosa* and its potential biological activities. *Int. J. Biol. Macromol.*, 2006, 39(4-5), 228-233.

Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Minia E, Capaccioli S. Natural compounds for cancer treatment and prevention. *Pharmacol. Res.*, 2009, 59(6), 365-78.

Ohno, N. Structural diversity and physiological functions of b-glucans. *Int. J. Med. Mushrooms*, 2005, 7(1-2), 167-88.

Onishi H, Katano M. Hedgehog signaling pathway as a therapeutic target in various types of cancer. *Cancer Sci.*, 2011, 102(10), 1756-60.

Ouyang L, Shi Z, Zhao S, Wang F-T, Zhou T-T, Liu B, Bao J-K. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif.*, 2012, 45(6), 487-98.

Papavramidou N, Papavramidis T, Demetriou T. 1.Ancient Greek and Greco–Roman Methods in Modern Surgical Treatment of Cancer. *Ann. Surg. Oncol.*, 2010, 17(3), 665-67.

Peng Z, Xue B, Kurgan L, Uversky VN. Resilience of death: intrinsic disorder in proteins involved in the programmed cell death. *Cell Death Differ.* , 2013, 20(9), 1-11.

Purcell JW, Davis J, Reddy M, Martin S, Samayoa K et al. Activity of the Kinesin Spindle Protein Inhibitor Ispinesib (SB-715992) in Models of Breast Cancer. *Clin. Cancer Res.*, 2010, 16(2), 566-76.

Rachagani S, Torres MP, Moniaux N, Batra SK. Current status of mucins in the diagnosis and therapy of cancer. *BioFactors*, 2009, 35(6), 509-27.

Rahman M, Ahmad MZ, Kazmi I, Akhter S, Afzal M et al. Advancement in multifunctional nanoparticles for the effective treatment of cancer. *Expert Opin. Drug Deliv.*, 2012, 9(4), 367-381.

Razumilava N, Gores GJ. Notch-driven carcinogenesis: The merging of hepatocellular cancer and cholangiocarcinoma into a common molecular liver cancer subtypes. *J. Hepatol.*, 2013, 58(6), 1244-45.

Remon J, Morán T, Majem M, Reguart N, Dalmau E, Márquez-Medina D, Lianes P. Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer: A new era begins. *Cancer Treat. Rev.*, 2014, 40(1), 93-101.

Rosenbluh J, Nijhawan Cox AG, Li XN, Neal JT, Schafer EJ et al. beta-Catenin-Driven Cancers Require a YAP1 Transcriptional Complex for Survival and Tumorigenesis. *Cell*, 2012, 151(7), 1457-73.

Rouquayrol M, Gaucher W, Greiner J, Aubertin AM, Vierling P, Guedj R. Synthesis and anti-HIV activity of glucose-containing pro-drugs derived from saquinavir, indinavir and nelfinavir. *Carbohydr. Res.*, 2001, 336(3), 161-80.

Sadzuka Y, Nagamine M, Toyooka T, Ibuki Y, Sonobe T. Beneficial effects of curcumin on antitumor activity and adverse reactions of doxorubicin. *Int. J. Pharm.*, 2012, 432(1-2), 42-49.

Safavy, A. Recent developments in taxane drug delivery. *Curr. Drug Deliv.*, 2008, 5(1) 42-54.

Sakurai T, Okumura H, Matsumoto M, Uchikado Y, Setoyama T et al. The expression of LC-3 is related to tumor suppression through angiogenesis in esophageal cancer. *Med. Oncol*., 2013, 30(4), 701.

Santarpia L, Lippman SM, El-Naggar AK. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. *Expert Opin. Ther. Targets*, 2012, 16(1), 103-119.

Sanz AB, Sanchez-Niño MD, Izquierdo MC, Gonzalez-Espinoza L, Ucero AC et al. Macrophages and Recently Identified Forms of Cell Death. *Int. Rev. Immunol.*, 2014, 33(1), 9-22.

Sasaki H, Teruya T, Fukazawa H, Suenaga K. Revised structure and structure-activity relationship of bisebromoamide and structure of norbisebromoamide from the marine cyanobacterium *Lyngbya* sp. *Tetrahedron*, 2011, 67(5), 990-94.

Sathisha MP, Budagumpi S, Kulkarni NV, Kurdekar GS, Revankar VK, Pai KSR. Synthesis, structure, electrochemistry and spectral characterization of (D-glucopyranose)-4-phenylthiosemicarbazide metal complexes and their antitumor activity against Ehrlich Ascites Carcinoma in Swiss albino mice. *Eur. J. Med. Chem.*, 2010, 45(1), 106-13.

Sears, P.; Wong, C.H. Kohlenhydratmimetika: ein neuer Lösungsansatz für das Problem der kohlenhydratvermittelten biologischen Erkennung. *Angew. Chemie*, 1999, 111(16), 2446-71.

Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, Batra SK. Targeting the EGFR signaling pathway in cancer therapy. *Expert Opin. Ther. Targets*, 2012, 16(1), 15-31.

Shih DQ, Nguyen M, Zheng L, Ibanez P, Mei L et al. Split-dose administration of thiopurine drugs: a novel and effective strategy for managing preferential 6-MMP metabolism. *Aliment. Pharm. Therap.*, 2012, 36(5), 449-58.

Shin SW, Seo CY, Han H, Han JY, Jeong JS, Kwak JY, Park JI. 15d-PGJ2 induces apoptosis by reactive oxygen species-mediated inactivation of Akt in leukemia and colorectal cancer cells and shows in vivo antitumor activity. *Clin. Cancer Res.*, 2009, 15(17), 5414-25.

Shon YH, Kim JH, Nam KS. Effect of Astragali Radix Extract on Lipopolysaccharide-Induced Inflammation in Human Amnion. *Biol. Pharm. Bull.* , 2002, 25(1), 77-80.

Silva JM, Videira M, Gaspar R, Préat V, Florindo HF. Immune system targeting by biodegradable nanoparticles for cancer vaccines. *J. Controll. Release*, 2013, 168(2), 179-99.

Sivakumar B, Aswathy RG, Sreejith R, Nagaoka Y, Iwai S. Bacterial exopolysaccharide based magnetic nanoparticles: a versatile nanotool for cancer cell imaging, targeted drug delivery and synergistic effect of drug and hyperthermia mediated cancer therapy. *J. Biomed. Nanotechnol.*, 2014, 10(6), 885-899.

Smyrniotopoulos V, Vagias C, Bruyère C, Lamoral-Theys D, Kiss R, Roussis V. Structure and in vitro antitumor activity evaluation of brominated diterpenes from the red alga *Sphaerococcus coronopifolius*. *Bioorg. Med. Chem.*, 2010, 18(3), 1321-30.

Song ZH, Ji ZN, Lo CK. Chemical and Biological Assessment of a Traditional Chinese Herbal Decoction Prepared from Radix Astragali and Radix Angelicae Sinensis: Orthogonal Array Design to Optimize the Extraction of Chemical Constituents. *Planta Med.*, 2004, 70(12), 1222-27.

Soumyanarayanan U, Bhat VG, Kar SS, Mathew JA. Monastrol mimic Biginelli dihydropyrimidinone derivatives: synthesis, cytotoxicity screening against HepG2 and HeLa cell lines and molecular modeling study. *Org. Med. Chem. Lett.*, 2012, 2(1), 23.

Stegh A.H. Targeting the p53 signaling pathway in cancer therapy – the promises, challenges and perils. *Expert Opin. Ther. Targets*, 2012, 16(1), 67-83.

Stocker K.F. *Medical Use of Snake Venom Proteins*, 1990, 34-50. New York: CRC press. Pp.280.

Szeliga M, Zgrzywa A, Michelwska MO, Albrecht J. Transfection of a human glioblastoma cell line with liver-type glutaminase (LGA) down-regulates the expression of DNA-repair gene MGMT and sensitizes the cells to alkylating agents. *J. Neurochem.*, 2012, 123(3), 428-36.

Takebe N, Warren RQ, Ivy SP. Breast cancer growth and metastasis: interplay between cancer stem cells, embryonic signaling pathways and epithelial-to-mesenchymal transition. *Breast Cancer Res.*, 2011, 13(3), 211-22.

Tognon CE, Sorensen PHB. Targeting the insulin-like growth factor 1 receptor (IGF1R) signaling pathway for cancer therapy. *Expert Opin. Ther. Targets*, 2012, 16(1), 33-48.

Trajkovic-Arsic M, Kalideris E, Siveke JT. The role of insulin and IGF system in pancreatic cancer. *J. Mol. Endocrinol.*, 2013, 50(3), R67-R74.

Urata S, Yoshida M, Ebihara Y, Asakage T. Surgical management of a giant cervical ganglioneuroma. *Auris Nasus Larynx*, 2013, 40(6), 577-80.

Valetti S, Mura S, Stella B, Couvreur P. Rational design for multifunctional non-liposomal lipid-based nanocarriers for cancer management: theory to practice. *J. Nanobiotechnology*, 2013, 11(1-S6), 1-17.

Vandenabeele P, Galluzzi L, Vanden BT, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat. Rev. Mol. Cell Biol.*, 2010, 11(10), 700-14.

Villa-Morales M, Fernández-Piqueras J. Targeting the Fas/FasL signaling pathway in cancer therapy. *Expert Opin. Ther. Targets*, 2012, 16(1), 85-101.

Vousden KH, Lane DP. p53 in health and disease. *Nat. Rev. Mol. Cell Biol.*, 2007, 8(4), 275-83.

Waldner MJ, Neurath MF. Targeting the VEGF signaling pathway in cancer therapy. *Expert Opin.Ther. Targets*, 2012, 16(1), 5-13.

Wang X, Li S, Wan Z, Quan Z, Tan Q. Investigation of thermo-sensitive amphiphilic micelles as drug carriers for chemotherapy in cholangiocarcinoma in vitro and in vivo. *Int. J. Pharm.*, 2014a, 463(1), 81-88.

Wang Y, Zhang L, Wang Q, Zhang D. Recent Advances in the Nanotechnology-Based Drug Delivery of Silybin. *J. Biomed. Nanotechnol.*, 2014b, 10(4), 543-558.

Wang, Y.; Yi, S.; Sun, L.; Huang, Y.; L., Scott C.; Zhang, M. Doxorubicin-Loaded Cyclic Peptide Nanotube Bundles Overcome Chemoresistance in Breast Cancer Cells. *J. Biomed. Nanotechnol.*, 2014c, 10(3), 445-454.

Wei D, Wei Y, Cheng W, Zhang L. Sulfated modification, characterization and antitumor activities of Radix hedysari polysaccharide. *Int. J. Biol. Macromol.*, 2012, 51(4), 471-76.

Xu M, Takanashi M, Oikawa K, Nishi H, Isaka K et al. Identification of a novel role of Septin 10 in paclitaxel-resistance in cancers through a functionalgenomics screen. *Cancer Sci.*, 2012, 103(4), 821-27.

Yokosuka A, Haraguchi M, Usui T, Kazami S, Osada H, Yamori T, Mimaki Y. Glaziovianin A, a new isoflavone, from the leaves of *Ateleia glazioviana* and its cytotoxic activity against human cancer cells. *Bioorg. Med. Chem. Lett.* , 2007, 17(11), 3091-94.

Yoshioka A, Miyata H, Doki Y, Yamasaki M, Sohma I et al. LC-3, an autophagosome marker, is highly expressed in gastrointestinal cancers. *Int. J. Oncol*., 2008, 33(3), 461-68.

Zhang M, Cui SW, Cheung PCK, Wang Q. Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends Food Sci. Tech.*, 2007, 18(1), 4-19. Leung, S.; Romanowski, M. Light-activated content release from liposomes. *Theranostics*, 2012, 2(10), 1020-1036.

Zhou H, Zhang B, Zheng J, Yu M, Zhou T et al. The inhibition of migration and invasion of cancer cells by graphene via the impairment of mitochondrial respiration. *Biomaterials*, 2014, 35(5), 1597-1607.

5 Conclusão

A linhagem de adenocarcinoma de pulmão humano teve o IC_{50} na no intervalo de 45,7-93,0µg.mL^{-1}, sendo o melhor efeito antitumoral observado nos extratos apolares na sequência *Pyropia endiviifolia*, *D. anceps*, *I. cordata* e *Prasiola crispa*. Enquanto a linhagem de glioblastoma multiforme, por apresentar um maior grau de malignidade e agressividade, dessa forma tornando-se um complexo desafio para o tratamento, apresentou inibição de crescimento de 50% com o extrato da feófita *D. anceps* (250µg.mL^{-1}), 50% com o extrato da rodófita *Pyropia endiviifolia* (500µg.mL^{-1}) e 40% com o extrato clorofórmico da *I. cordata* (250µg.mL^{-1}). Devido à carência de estudos químicos dessas algas, a caracterização específica das moléculas bioativas com o potencial antitumoral avaliado ainda estão em curso.

Ao delinear os resultados obtidos nesse estudo, a rodófita *Pyropia endiviifolia* demonstrou-se a alga mais promissora dentre as demais analisadas, visto que foi capaz de inibir 40% do crescimento dos gliomas na menor concentração (10 µg.mL^{-1}) dos extratos hexânico e etanólico. Enquanto na avaliação da atividade antitumoral da mesma alga em relação ao adenocarcinoma de pulmão, observou-se que o extrato clorofórmico inibiu 50% do crescimento tumoral na concentração de 45,7 µg.mL^{-1}. Em ambas análises obteve-se seletividade dos extratos, necessitando-se de concentrações bem superiores àquelas das linhagens tumorais a fim de inibir significativamente o crescimento das células não tumorais.

Portanto, foi possível corroborar com a hipótese do presente estudo, segundo a qual as macroalgas do continente Antártico apresentariam classes de moléculas com potencial atividade antitumoral. Além disso, foi possível visualizar por meio dos cromatogramas a variação das moléculas presentes em cada espécie analisada, conforme cada tipo de extração realizada, o que pode justificar as diferentes atividades mediante as distintas linhagens testadas. Cabe considerar, que pela primeira vez está sendo demonstrada a atividade antiglioma a partir de macroalgas da Antártica. Apesar de mais estudos serem necessários, é inegável o potencial dessas algas como protótipos para a síntese de novos fármacos e/ou exploração dessas biomoléculas por meio da engenharia genética.

Referências

ABIDA, H.; RUCHAUD, S.; RIOS, L.; HUMEAU, A.; PROBERT, I.; DE VARGAS, C.; BACH, S.; BOWLER, C. Bioprospecting Marine Plankton. **Marine Drugs**, v.11, n.11, p.4594-4611, 2013.

ABRAHAM, I.; EL SAYED, K.; CHEN, Z.S.; GUO, H. Current status on marine products with reversal effect on cancer multidrug resistance. **Marine Drugs**, v.10, n.10, p.2312-2321, 2012.

ALVES, A.; SOUSA, R.A.; REIS, R.L. A practical perspective on ulvan extracted from green algae. **Journal of Applied Phycology**, v.25, n.2, p.407–424, 2013.

AMSLER, C.D.; FAIRHEAD, V.A. Defensive and sensory chemical ecology of brown algae. **Advances in Botanical Research**, v.43, n.1, p.1-91, 2006.

AMSLER, C.D.; ROWLEY, R.J.; LAUR, D.R.; QUETIN, L.B.; ROSS, R.M. Vertical distribution of Antarctic Peninsular macroalgae: cover, biomass, and species composition. **Phycologia**, v.34, n.5, p.424-430, 1995.

ANK, G.; GAMA, B.A.P.; PEREIRA, R.C. Polyphenols from Stypopodium zonale (Phaeophyceae): Intrapopulational variation, induction by simulated herbivory, and epibiosis effects. **Aquatic Botany**, v.111, p.125-129, 2013.

ANTUNES, E.M.; AFOLAYAN, A.F.; CHIWAKATA, M.T.; FAKEE, J.; KNOTT, M.G.; WHIBLEY, C.E.; HENDRICKS, D.T.; BOLTON, J.J.; BEUKES, D.R. Identification and *in vitro* anti-esophageal cancer activity of a series of halogenated monoterpenes isolated from the South African seaweeds *Plocamium suhrii* and *Plocamium cornutum*. **Phytochemistry**, v.72, n.8, p.769-772, 2011.

ARAVINDAN, S.; DELMA, C.R.; THIRUGNANASAMBANDAN, S.S.; HERMAN, T.S.; ARAVINDAN, N. Anti-Pancreatic Cancer Deliverables from Sea: First-Hand Evidence on the Efficacy, Molecular Targets and Mode of Action for Multifarious Polyphenols from Five Different Brown-Algae. **Plos One**, v.8, n.4, p.e61977, 2013.

ARNOLD, T.M. To grow and defend: lack of tradeoffs for brown algal phlorotannins. **Oikos**, v.100, n.2, p.406-408, 2003.

BALAKRISHNAN, B.; PRASAD, B.; RAI, A.K.; VELAPPAN, S.P.; SUBBANNA, M.N.; NARAYAN, B. *In vitro* antioxidant and antibacterial properties of hydrolysed proteins

of delimed tannery fleshings: comparison of acid hydrolysis and fermentation methods. **Biodegradation**, v.22, n.2, p.287-295, 2011.

BARGAGLI, R. Environmental contamination in Antarctic ecosystems**. Science of the Total Environment**, v.400, n.21, p.212-226, 2008.

BATISTA, J.A.; DIAS, E.G.N.; BRITO, T.V.; PRUDÊNCIO, R.S.; SILVA, R.O.; RIBEIRO, R.A.; SOUZA, M.H.L.P.; PAULA, R.C.M.; FEITOSA, J.P.A.; CHAVES, L.S.; MELO, M.R.S.; FREITAS, A.L.P.; MEDEIROS, J.-V.R.; BARBOSA, A.L.R. Polysaccharide isolated from *Agardhiella ramosissima*: Chemical structure and anti-inflammation activity. **Carbohydrate Polymers**, v.99, p.59-67, 2014.

BAUCHET, L.; MATHIEU-DAUDE, H.; FABBRO-PERAY, P.; RIGAU, V.; FABBRO, M.; CHINOT, O.; PALLUSSEAU, L.; CARNIN, C.; LAINE, K.; SCHLAMA, A.; THIEBAUT, A.; PATRU, M.C.; BAUCHET, F.; LIONNET, M.; WAGER, M.; FAILLOT, T.; TAILLANDIER, L.; FIGARELLA-BRANGER, D.; CAPELLE, L.; LOISEAU, H.; FRAPPAZ, D.; CAMPELLO, C.; KERR, C.; DUFFAU, H.; REME-SAUMON, M.; TRETARRE, B.; DAURES, J.P.; HENIN, D.; LABROUSSE, F.; MENEI, P.; HONNORAT, J. Oncological patterns of care and outcome for 952 patients with newly diagnosed glioblastoma in 2004. **Neuro-Oncology**, v.12, n.7, p.725-735, 2010.

BECKER, S.; GRAEVE, M.; BISCHOF, K. Photosynthesis and lipid composition of the Antarctic endemic rhodophyte *Palmaria decipiens*: effects of changing light and temperature levels. **Polar Biology**, v.33, n.7, p.945–955, 2010.

BECKER, S.; QUARTINO, M. L.; CAMPANA, G. L.; BUCOLO P.; WIENCKE, C.; BISCHOF K. The biology of an antarctic rhodophyte, *Palmaria decipiens*: recent advances. **Antarctic Science**. v.23, n.5, p.419–430, 2011.

BECKER, S.; WALTER, B.; BISCHOF, K. Freezing tolerance and photosynthetic performance of polar seaweeds at low temperatures. In: WIENCKE, C. (ed.) **Biology of polar benthic algae**. Berlin: De Gruyter, 2011. p. 23–52.

BISCHOF, K.; GÓMEZ, I.; MOLIS, M.; KARSTEN, U.; LÜDER, U.; ROLEDA, M.Y.; ZACHER, K.; WIENCKE, C. Ultraviolet radiation shapes seaweed communities. **Reviews in Environmental Science and Biotechnology**, v.5, n.2-3, p.141-166, 2006.

BLUNT, J.W.; COPP, B.R.; KEYZERS, R.A.; MUNRO, M.H.G.; PRINSEP, M.R. Marine natural products, Natural Product Reports, v.31, n.2, p.160-258, 2014.

BLUNT, J.W.; COPP, B.R.; MUNRO, M.H.G.; NORTHCOTE, P.T.; PRINSEP, M.R. Marine natural products. **Natural Product Reports**, v.28, n.2, p.196-268, 2011.

BLUNT, J.W.; COPP, B.R.; MUNRO, M.H.G.; NORTHCOTE, P.T.; PRINSEP, M.R. Marine Natural Products. **Natural Product Reports**, v.20, n.1, p.1-48, 2003.

BLUNT, J.W.; COPP, B.R.; MUNRO, M.H.G.; NORTHCOTE, P.T.; PRINSEP, M.R. Marine Natural Products. **Natural Product Reports**, v.28, n.2, p.196-268, 2011.

BORJA, Á.; FONTÁN, A.; MUXIKA, I. Interactions between climatic variables and human pressures upon a macroalgae population: Implications for management. **Ocean & Coastal Management**, v.76, n., p.85-95, 2013.

BRANDT, A. Biodiversity, Marine. In: RIFFENBURGH, B. (ed.). **Encyclopedia of the Antarctic.** v.1. New York: A-K. Routledge, 2007. p.144-149.

CABRITA, M.T.; VALE, C.; RAUTER, A.P. Halogenated Compounds from Marine Algae. **Marine Drugs**, v.8, n.8, p.2301-2317, 2010.

CAMPOS, A.; SOUZA, C.B.; LHULLIER, C.; FALKENBERG, M.; SCHENKEL, E.P.; RIBEIRO-DO-VALLE, R.M.; SIQUEIRA, J.M. Anti-tumour effects of elatol, a marine derivative compound obtained from red algae *Laurencia microcladia*. **Journal of Pharmacy and Pharmacology**, v.64, n.8, p.1146-1154, 2012.

CATTANEO-VIETTI, R.; CHIANTORE, M.; GAMBI, M.C.; ALBERTELLI, G.; CORMACI, M.; DI GERONIMO, I. Spatial and vertical distribution of benthic littoral communities in Terra Nova Bay. In: FARANDA, F.M., GUGLIELMO, L.; IANORA, A. (eds) **Ross sea ecology: Italiantartide expeditions (1985–1995).** Berlin: Springer-Verlag, 2000. p.503-514

CHOWN, S.L. Biodiversity, Terrestrial. In: RIFFENBURGH, B. (ed.). **Encyclopedia of the Antarctic.** v.1. New York: A-K. Routledge, 2007. p.149-153.

CHUNG, I.K.; OAK, J.H.; LEE, J.A.; SHIN, J.A.; KIM, J.G.; PARK, K.-S. Installing kelp forests/seaweed beds for mitigation and adaptation against global warming: Korean Project Overview. **ICES Journal of Marine Science**, v.70, n.5, p.1038-1044, 2013.

CIAN, R.E.; ALAIZ, M.; VIOQUE, J.; DRAGO, S.R. Enzyme proteolysis enhanced extraction of ACE inhibitory and antioxidant compounds (peptides and polyphenols) from *Porphyra columbina* residual cake. **Journal of Applied Phycology**, v.25, n.4, p.1197-1206, 2013.

CIAN, R.E.; MARTÍNEZ-AUGUSTIN, O.; DRAGO, S.R. Bioactive properties of peptides obtained by enzymatic hydrolysis from protein byproducts of *Porphyra columbina*. **Food Research International**, v.49, n.1, p.364-372, 2012.

CLAYTON, M.N. Evolution of the Antarctic benthic algal flora. **Journal of Phycology**, v.30, n.6, p.897-904, 1994.

CONVEY, P. Antarctic terrestrial biodiversity in a changing world. **Polar Biology**, v.34, n.11, p.1629-1641, 2011.

CONVEY, P. Biogeography. In: RIFFENBURGH, B. (ed.). **Encyclopedia of the Antarctic.** v.1. New York: A-K. Routledge, 2007. p.154-160.

CONVEY, P.; GIBSON, J.A.E.; HILLENBRAND, C.-D.; HODGSON, D.A. PUGH, P.J.A.; SMELLIE, J.L.; STEVENS, M.I. Antarctic terrestrial life – challenging the history of the frozen continent? **Biological Reviews**, v.83, n.2, p.103-117, 2008.

CRONIN, G. Resource allocation is seaweed and marine invertebrates: chemical defenses patterns in relation to defense theories. In: MCCLINTOCK, J.M.; BAKER, B.J. (eds.). **Marine Chemical Ecology**. Florida: CRC Press, 2001. p.325-353.

CRUCES, E.; HUOVINEN, P.; GÓMEZ, I. Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae. **Marine Biology**, v.160, n.1, p.1-13, 2013.

CRUCES, E.; HUOVINEN, P.; GÓMEZ, I. Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three South Pacific kelps. **Photochemistry and Photobiology**, v.88, n.1, p.58-66, 2012.

DE LA MARE, J.; LAWSON, J.C.; CHIWAKATA, M.T.; BEUKES, D.R.; EDKINS, A.L.; BLATCH, G.L. Quinones and Halogenated Monoterpenes of Algal Origin show Anti-proliferative Effects against Breast Cancer Cells *in vitro*. **Investigational New Drugs**, v.30, n.6, p.2187-2200, 2012.

DESOTI, V.C.; LAZARIN-BIDÓIA, D.; SUDATTI, D.B.; PEREIRA, R.C; ALONSO, A.; UEDA-NAKAMURA, T.; DIAS FILHO, B.P.; NAKAMURA, C.V.; SILVA, S.O. Trypanocidal Action of (−)-Elatol Involves an Oxidative Stress Triggered by Mitochondria Dysfunction. **Marine Drugs**, v.10, n.8, p.1631-1646, 2012.

DÍEZ, I.; MUGUERZA, N.; SANTOLARIA, A.; GANZEDO, U.; GOROSTIAGA, J.M. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. **Estuarine, Coastal and Shelf Science**, v.99, p.108-120, 2012.

DORE, C.M.P.G.; ALVES, M.G.C.F.; WILL, L.S.E.P.; COSTA, T.G.; SABRY, D.A.; RÊGO, L.A.R.S.; ACCARDO, C.M.; ROCHA, H.A.O.; FILGUEIRA, L.G.A.; LEITE, E.L. A sulfated polysaccharide, fucans, isolated from brown algae *Sargassum vulgare* with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. **Carbohydrate Polymers**, v.91, n.1, p.467– 475, 2013.

FAIRHEAD, V.A.; AMSLER, D.A.; MCCLINTOCK, J.B. Lack of defence or phlorotannin induction by UV radiation or mesograzers in *Desmarestia anceps* and D. menziesii (Phaeophyceae). **Journal of Phycology**, v.42, n.6, p.1174-1183, 2006.

FAULKNER, D. J. Marine Natural Products. **Natural Product Reports**, v.18, n.1, p.1-49, 2001.

FENICAL, W. Halogenation in the Rhodophyta, a Review. **Journal of Phycology**, v.11, n.3, p.245-259, 1975.

FITZGERALD, C.; MORA-SOLER, L.; GALLAGHER, E.; O'CONNOR, P.; PRIETO, J.; SOLER-VILA, A.; HAYES, M. Isolation and Characterization of Bioactive Pro-Peptides with *in vitro* Renin Inhibitory Activities from the Macroalga *Palmaria palmata*. **Journal of Agricultural and Food Chemistry**, v.60, n.30, p.7421-7427, 2012.

FLEURENCE, J.; MORANÇAIS, M.; DUMAY, J.; DECOTTIGNIES, P.; TURPIN, V.; MUNIER, M.; GARCIA-BUENO, N.; JAOUEN, P. What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? **Trends in Food Science & Technology**, v.27, n.1, p.57-61, 2012.

FOLMER, F.; JAPARS, M.; DICTATO, M.; DIEDERICH, M. Photosynthetic marine organisms as a source of anticancer compounds. **Phytochemistry Reviews**, v.9, n.4, p.557-579, 2010.

FRENOT, Y.; CHOWN, S.L.; WHINAM, J.; SELKIRK, P.M.; CONVEY, P.; SKOTNICKI, M.; BERGSTROM, D.M. Biological invasions in the Antarctic: extent, impacts and implications. **Biological Reviews**, v.80, n.1, p.45-72, 2005.

GLASER, K.B.; MAYER, A.M.S. A renaissance in marine pharmacology: From preclinical curiosity to clinical reality. **Biochemical Pharmacology**, v.78, n.5, p.440-448, 2009.

GÓMEZ, I.; HUOVINEN, P. Induction of phlorotannins during UV exposure mitigates inhibition of photosynthesis and DNA damage in the kelp *Lessonia nigrescens*. **Photochemistry and Photobiology**, v.86, n.5, p.1056-1063, 2010.

GRAHAM, L.E.; GRAHAM, J.M.; WILCOX, L.W. **Algae**. 2.ed. São Francisco: Pearson Education, 2009. 616p.

HAMDY, A.-H.A.; ABOUTABL, E.A.; SAMEER, S.; HUSSEIN, A.A.; DIAZ-MARRERO, A.R.; DARIAS, J.; CUETO, M. 3-Keto-22-epi-28-nor-cathasterone, a brassinosteroid-related metabolite from *Cystoseira myrica*. **Steroids**, v.74, n.12, p.927-930, 2009.

HANAHAN, D.; WEINBERG, R.A. Hallmarks of cancer: the next generation. **Cell**, v.144, n.5, p.646-674, 2011.

HANELT, D. Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. **Marine Biology**, v.131, n.2, p.361-369, 1998.

HARLEY, C.D.G.; ANDERSON, K.M.; DEMES, K.W.; JORVE, J.P.; KORDAS, R.L.; COYLE, T. A. Effects of climate change on global seaweed communities. **Journal of Phycology**, v.48, n.5, p.1064-1078, 2012.

HARNEDY, P.A.; FITZGERALD, R.J. Bioactive proteins, peptides and amino acids from macroalgae. **Journal of Phycology**, v.47, n.2, p.218-232, 2011.

HDEIB, A.; SLOAN, A.E. Convection-enhanced delivery of (131)IchTNT- 1/B mAB for treatment of high-grade adult gliomas. Expert Opinion on Biological Therapy, v.11, n.6, p.799-806, 2011.

HOLZINGER, A.; KARSTEN, U.; LÜTZ, C.; WIENCKE, C. Ultrastructure and photosynthesis in the supralittoral green macroalga *Prasiola crispa* from Spitsbergen (Norway) under UV exposure. **Phycologia**, v.45, n.2, p.168-177, 2006.

HOMMERSAND, M.H.; MOE, R.L.; AMSLER, C.D.; FREDERICQ, S. Notes on the systematics and biogeographical relationships of Antarctic and sub-Antarctic Rhodophyta with descriptions of four new genera and five new species. In: WIENCKE, C. (ed.). **Biology of Polar Benthic Algae.** Berlin: De Gruyter, 2011. p.53-100.

HU, G-P.; YUAN, J.; SUN, L.; SHE, Z-G.; WU, J-H.; LAN, X-J.; ZHU, X.; LIN, Y-C.; CHEN, S-P. Statistical Research on Marine Natural Products Based on Data Obtained between 1985 and 2008. **Marine Drugs**, v.9, n.4, p.514-525, 2011.

HUOVINEN, P.; GÓMEZ, I. Photosynthetic characteristics and UV stress tolerance of Antarctic seaweeds along the depth gradient. **Polar Biology**, v.36, n.9, p.1319-1332, 2013.

IBAÑEZ, E.; CIFUENTES, A. Benefits of using algae as natural sources of functional ingredients. **Journal of the Science of Food and Agriculture**, v.93, n.4, p.703-709, 2013.

INDUMATHY, S.; DASS, C.R. Finding chemo: the search for marine-based pharmaceutical drugs active against cancer. **Journal of Pharmacy and Pharmacology**, v.65, n.9, p.1280-1301, 2013.

INSTITUTO NACIONAL DE CÂNCER (Brasil). **Estimativa 2012. Incidência do Câncer no Brasil.** Rio de Janeiro: INCA, 2011.

JACKSON, A.E.; SEPPELT, R.D. Physiological adaptations to freezing and UV radiation exposure in *Prasiola crispa*, an Antarctic terrestrial alga. In: BATTAGLIA, B.; VALENCIA, J.; WALTON, D.W.H. (eds.). **Antarctic Communities:** Species, Structure, and Survival. Cambridge: Cambridge University Press, 1997. p.226-233.

JIN, W.; WANG, J.; JIANG, H.; SONG, N.; ZHANG, W.; ZHANG, Q. The neuroprotective activities of heteropolysaccharides extracted from *Saccharina japonica*. **Carbohydrate Polymers**, v.97, n.1, p.116-120, 2013.

KAMEI, Y.; SUEYOSHI, M.; HAYASHI, K.-I.; TERADA, R.; NOZAKI, H. The novel anti-Propionibacterium acnes compound, Sargafuran, found in the marine brown alga *Sargassum macrocarpum*. **The Journal of Antibiotics**, v.62, n.5, p.259-263, 2009.

KARSTEN, U.; FRIEDL, T.; SCHUMMAN, R.; HOYER, K.; LEMBCKE, S. Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). **Journal of Phycology**, v.41, n.3, p.557-566, 2005.

KARSTEN, U.; WULFF, A.; ROLEDA, M.Y.; MÜLLER, R.; STEINHOFF, F.S.; FREDERSDORF, J.; WIENCKE, C. Physiological responses of polar benthic algae to ultraviolet radiation. In: WIENCKE, C. (ed.). **Biology of polar benthic algae**. Berlin: De Gruyter, 2011. p.271-298.

KIM, Y.M.; KIM, I.-H.; NAM, T.-J. Inhibition of AGS human gastric cancer cell invasion and proliferation by *Capsosiphon fulvescens* glycoprotein. **Molecular Medicine Reports**, v.8, n.1, p.11-16, 2013.

KLEIHUES, P; LOUIS, D.N.; SCHEITHAUER, B.W.; RORKE, L.B.; REIFENBERGER, G.; BURGER, P.C.; CAVENEE, W.K. The WHO classification of tumors of the nervous system. **Journal of Neuropathology & Experimental Neurology**, v.61, n.3, p.215-225, 2002.

KLÖSER, H.; QUARTINO, M.L.; WIENCKE, C. Distribution of macroalgae and macroalgal communities in gradients of physical conditions in Potter Cove, King George Island, Antarctica. **Hydrobiologia**, v.333, n.1, p.1-17, 1996.

KOLLÁR, P.; RAJCHARD, J.; BALOUNOVÁ, Z.; PAZOUREK, J. Marine natural products: Bryostatins in preclinical and clinical studies. **Pharmaceutical Biology**, v.52, n.2, p.237-242, 2014.

KOSUGI, M.; KATASHIMA, Y.; AIKAWA, S.; TANABE, Y.; KUDOH, S.; KASHINO, Y.; KOIKE, H.; SATOH, K. Comparative study on the photosynthetic properties of *Prasiola* (Chlorophyceae) and *Nostoc* (Cyanophyceae) from Antarctic and non-Antarctic sites. **Journal of Phycology**, v.46, n.3, p.466-476, 2010.

KOTB, E. Activity assessment of microbial fibrinolytic enzymes. **Applied Microbiology and Biotechnology**, v.97, n.15, p.6647-6665, 2013.

KOVÁČIK, L.; PEREIRA, A.B. Green alga *Prasiola crispa* and its lichenized form *Mastodia tessellata* in Antartic environment: general aspects. **Beihefte zur Nova Hedwigia**, v.123, p.465-478, 2001.

KUMARI, P., BIJO, A. J., MANTRI, V. A., REDDY, C. R. K., JHA, B. Fatty acid profiling of tropical marine macroalgae: An analysis from chemotaxonomic and nutritional perspectives. **Phytochemistry**, v.86, n., p.44-56, 2013.

KWON, M.-J.; NAM, T.-J. Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines. **Life Sciences**, v.79, n.20, p.1956–1962, 2006.

KWON, T.-H.; KIM, T.-W.; KIM, C.-G.; PARK, N.-H. Antioxidant Activity of Various Solvent Fractions from Edible Brown Alga, *Eisenia bicyclis* and Its Active Compounds. **Journal of Food Science**, v.78, n.5, p.C679-C684, 2013.

LAWVER, L.A.; SCLATER, J.G.; MEINKE, M. Mesozoic and Cenozoic Reconstructions of the South Atlantic. **Tectonophysics**, v.114, n.1-4, p.233-254, 1985.

LEE, S.-H.; KIM, J.K.; KIM, D.W.; HWANG, H.S.; EUM,W.S.; PARK, J.; HAN, K.H.; OH, J.S.; CHOI, S.Y. Antitumor activity of methyl gallate by inhibition of focal adhesion formation and Akt phosphorylation in glioma cells. Biochimica et Biophysica Acta, v.1830, n.8, p.4017–4029, 2013.

LI, Y.; GONG, Y.; LI, L.; ABDOLMALEKY, H.M.; ZHOU, J.R. Bioactive Tanshinone I inhibits the growth of lung cancer in part via down regulation of Aurora A function. **Molecular Carcinogenesis**, v.52, n.7, p.535-543, 2013.

LIANG, W.; MAO, X.; PENG, X.; TANG, S. Effects of sulfate group in red seaweed polysaccharides on anticoagulant activity and cytotoxicity. **Carbohydrate Polymers**, v.101, p.776-785, 2014.

LÜDER, U.; KNOETZEL, J.; WIENCKE, C. Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. **Polar Biology**, v.24, n.8, p.598–603, 2001.

MACDONALD, T.J.; AGUILERA, D.; KRAMM, C.M. Treatment of a high-grade glioma in children and adolescents. Neuro-Oncology, v.13, n.10, p.1049–1058, 2011.

MARSH, J.C.; GOLDFARB, J.; SHAFMAN, T.D.; DIAZ, A.Z. Current Status of Immunotherapy and Gene Therapy for High-Grade Gliomas. Cancer Control, v.20, n.1, p.43-48, 2013.

MASCHEK, J.A.; BAKER, B.J. The chemistry of algal secondary metabolism. In: AMSLER, C.D. (ed.). **Algal Chemical Ecology**. Berlin: Springer-Verlag, 2008. p.1-24.

MAYER, A.M.S.; GLASER, K.B.; CUEVAS, C.; JACOBS, R.S.; KEM, W.; LITTLE, R.D.; MCINTOSH, J.M.; NEWMAN, D.J.; POTTS, B.C.; SHUSTER, D.E. The odyssey of marine pharmaceuticals: A current pipeline perspective. **Trends in Pharmacological Sciences**, v.31, n.6, p.255-265, 2010.

MICHALAK, I.; CHOJNACKA, K. Algal compost - toward sustainable fertilization. **Reviews in Inorganic Chemistry**, v.33, n.4, p.161-172, 2013.

MOGHADAMTOUSI, S.Z.; KARIMIAN, H.; KHANABDALI, R.; RAZAVI, M.; FIROOZINIA, M.; ZANDI, K.; KADIR, H.A. Anticancer and Antitumor Potential of Fucoidan and Fucoxanthin, Two Main Metabolites Isolated from Brown Algae. **The Scientific World Journal**, v.2014, p.1-10, 2014.

MOHAMED, S.; HASIM, S.N.; RAHMAN, H.A. Seaweeds: a sustainable functional food for complementary and alternative therapy. **Trends in Food Science & Technology**, v.23, n.2, p.83-96, 2012.

MONIZ, M.B.J.; RINDI, F.; NOVIS, P.M.; BROADY, P.A.; GUIRY, M.D. Molecular phylogeny of Antarctic *Prasiola* (Prasiolales, Trebouxiophyceae) reveals extensive cryptic diversity. **Journal of Phycology**, v.48, n.4, p.940-955, 2012.

MORGAN-KISS, R.M.; PRISCU, J.C.; POCOCK, T.; GUDYNAITE-SAVITCH, L.; HUNER, N.P.A. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. **Microbiology and Molecular Biology Reviews**, v.70, n.1, p.222-252, 2006.

MULLINS, C.S.; SCHUBERT, J.; SCHNEIDER, B.; LINNEBACHER, M.; CLASSEN, C.F. Cilengitide response in ultra-low passage glioblastoma cell lines: relation to molecular markers. Journal of Cancer Research and Clinical Oncology, v.139, p.1425–1431, 2013.

MURUGAN, K.; IYER, V.V. Differential growth inhibition of cancer cell lines and antioxidant activity of extracts of red, brown, and green marine algae. **In Vitro Cellular & Developmental Biology - Animal**, v.49, n.5, p.324-334, 2013.

NAKADA, M.; NAKADA, S.; DEMUTH, T.; TRAN, N.L.; HOELZINGER, D.B.; BERENS, M.E. Molecular targets of glioma invasion. Cellular and Molecular Life Sciences, v.64, p.458- 478, 2007.

NISHINO, T.; AIZU, Y.; NAGUMO, T. The influence of sulfate content and molecular weight of a fucan sulfate from the brown seaweed *Ecklonia kurome* on its antithrombin activity. **Thrombosis Research**, v.64, n.6, p.723–731, 1991.

PAN, Q.W.; CHEN, M.Z.; LI, J.; WU, Y.; ZHEN, C.; LIANG, B. Antitumor function and mechanism of phycoerythrin from *Porphyra haitanensis*. **Biological Research**, v.46, n.1, p.87-95, 2013.

PARADAS, W.C.; SALGADO, L.T.; SUDATTI, D.B.; CRAPEZ, M.A.; FUJII, M.T.; COUTINHO, R.; PEREIRA, R.C.; AMADO-FILHO, G.M. Induction of halogenated vesicle transport in cells of the red seaweed *Laurencia obtusa*. **Biofouling**, v.26, n.3, p.277-286, 2010.

PARK, H.S.; HWANG, H.J.; KIM, G.-Y.; CHA, H.-J.; KIM, W.-J.; KIM, N.D.; YOO, Y.H.; CHOI, Y.H. Induction of Apoptosis by Fucoidan in Human Leukemia U937 Cells through Activation of p38 MAPK and Modulation of Bcl-2 Family. **Marine Drugs**, v.11, n.7, p.2347-2364, 2013.

PELLIZZARI, F.; REIS, R.P. Seaweed cultivation on the Southern and Southeastern Brazilian Coast. **Brazilian Journal of Pharmacognosy**, v.21, n.2, p.305-312, 2011.

PENG , Y.; XIE, E.; ZHENG, K.; FREDIMOSES, M.; YANG, X.; ZHOU, X.; WANG, Y.; YANG, B.; LIN, X.; LIU, J.; LIU, Y. Nutritional and Chemical Composition and Antiviral Activity of Cultivated Seaweed *Sargassum naozhouense* Tseng et Lu. **Marine Drugs**, v.11, n.1, p.20-32, 2013.

PENG, J.; YUAN, J.P.. WU, C.F.; WANG, J.H. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. **Marine Drugs**, v.9, n.10, p.1806-1828, 2011.

PEREIRA, R.C.; DA GAMA, B.A.P.; TEIXEIRA, V.L.; YONESHIGUE-VALENTIN, Y. Ecological roles of natural products from the Brazilian red seaweed *Laurencia obtusa*. **Brazilian Journal of Biology**, v.63, n.4 , p.665-672, 2003.

PROGRAMA ANTÁRTICO BRASILEIRO (PROANTAR): Responsável: CMG José Corrêa Filho. Disponível em:< http://www.mar.mil.br/secirm/proantar.htm> Acesso em: 29 jan. 2014.

QUARTINO, M.L.; BORASO DE ZAIXSO, A.L. Summer macroalgal biomass in Potter Cove, South Shetland Islands. Antarctica: its production and flux to the ecosystem. **Polar Biology**, v.31, n.3, p.281-294, 2008.

RAGAN, M.A.; GLOMBITZKA, K.W. Phlorotannins, brown algal polyphenols. **Progress in Phycological Research**, v.4, p.129-241, 1986.

RAYBAUD, V.; BEAUGRAND, G.; GOBERVILLE, E.; DELEBECQ, G.; DESTOMBE, C.; VALERO, M.; DAVOULT, D.; MORIN, P.; GEVAERT, F. Decline in Kelp in West Europe and Climate. **Plos One**, v.8, n.6, p.e66044, 2013.

RESEARCH CORP. **Porous material for prostheses or bone implantation - hydroxy-apatite or whitlockite having skeleton structure of marine organisms e.g. corals.** JP82033057-B, 07 nov. 1974, 14 jul. 1982.

REZENDE, B.M.; BERNARDES, P.T.T.; RESENDE, C.B.; ARANTES, R.M.E.; SOUZA, D.G.; BRAGA, F.C.; CASTOR, M.G.M.; TEIXEIRA, M.M.; PINHO, V. *Lithothamnion muelleri* Controls Inflammatory Responses, Target Organ Injury and

Lethality Associated with Graft-versus-Host Disease in Mice. **Marine Drugs**, v.11, n.7, p.2595-2615, 2013.

RIBEIRO, A. P.; FIGUEIRA, B. C. L.; MARTINS, C.C.; SILVA, C. R. A..; FRANÇA, E. J.; BÍCEGO, M. C.; MAHIQUES, M. M.; MONTONE, R. C. Arsenic and trace metal contents in sediment profiles from the Admiralty Bay, King George Island, Antarctica. **Marine Pollution Bulletin**, v.62, p.192–196, 2011.

RÍOS-MARCO, P.; MARTÍN-FERNÁNDEZ, M.; SORIA-BRETONES, I.; RÍOS, A.; CARRASCO, M.P.; MARCO, C. Alkylphospholipids deregulate cholesterol metabolism and induce cell-cycle arrest and autophagy in U-87 MG glioblastoma cells. Biochimica et Biophysica Acta, v.1831, n.8, p.1322–1334, 2013.

RODRIGUEZ-JASSO, R.M.; MUSSATTO, S.I.; PASTRANA, L.; AGUILAR, C.N.; TEIXEIRA, J.A. Chemical composition and antioxidant activity of sulphated polysaccharides extracted from *Fucus vesiculosus* using different hydrothermal processes. **Chemical Papers**, v.68, n.2, p.203-209, 2014.

ROLEDA, M.Y.; HANELT, D.; WIENCKE, C. Exposure to ultraviolet radiation delays photosynthetic recovery in Arctic kelp zoospores. **Photosynthesis Research**, v.88, n.3, p.311-322, 2006.

RWEHUMBIZA, V.M.; VENNAPUSA, R.R.; GAVARA, P.R.; FERNÁNDEZ-LAHORE, H.M.; AL-KARABLIEH, N.; ULLRICH, M.S.; Thomsen, C. Potential of fibrous adsorbents for the binding and characterization of *Porphyridium purpureum* bioactive polysaccharides. **Journal of Chemical Technology and Biotechnology**, v.89, n.1, p.65-72, 2013.

RYAN, K. G.; MCMINN, A.; HEGSETH, E. N.; DAVY, S. K. The effects of ultraviolet-b radiation on antarctic sea-ice algae. **Journal of Phycology**, v. 48, 74–84, 2012.

SANTOS, A.O.; VEIGA-SANTOS, P.; UEDA-NAKAMURA, T.; DIAS FILHO, B.P.; SUDATTI, D.B.; BIANCO, E.M.; PEREIRA, R.C.; NAKAMURA, C.V. Effect of elatol, isolated from red seaweed *Laurencia dendroidea* on *Leishmania amazonensis*. **Marine Drugs**, v.8, n.11, p.2733-2743, 2010.

SCHIEL, D.R. Rivets or bolts? When single species count in the function of temperate rocky reef communities. **Journal of Experimental Marine Biology and Ecology**, v.338, n.2, p.233-252, 2006.

SCHIEL, D.R.; FOSTER, M.S. The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. **Annual Review of Ecology, Evolution, and Systematics**, v.37, p.343-372, 2006.

SCHIEL, D.R.; LILLEY, S. Gradients of disturbance to an algal canopy and the modification of an intertidal community. **Marine Ecology Progress Series**, v.339, n.1, p.1-11, 2007.

SERENO, M.; RODRÍGUEZ-ESTEBAN, I.; GÓMEZ-RAPOSO, C.; MERINO, M.; LÓPEZ-GÓMEZ, M.; ZAMBRANA, F.; CASADO, E. Lung cancer and peritoneal carcinomatosis. **Oncology Letters**, v.6, n.3, p.705-708, 2013.

SHAH, A.H.; SNELLING, B.; BREGY, A.; PATEL, P.R.; TEMEME, D.; BHATIA, R.; SKLAR, E.; KOMOTAR, R.J. Discriminating radiation necrosis from tumor progression in gliomas: a systematic review what is the best imaging modality? Journal of Neuro-Oncology, v.112, n.2, p.141-152, 2013.

SIEGEL, R.; NAISHADHAM, D.; JEMAL, A. Cancer statistics, 2012. **CA: A Cancer Journal for Clinicians**, v.62, n.1, p.10-29, 2012.

SIEGERT, M.J.; BARRETT, P.; DECONTO, R.; DUNBAR, R.; COFAIGH, C.; PASSCHIER, S.; NAISH, T. Recent advances in understanding Antarctic climate evolution. **Antarctic Science**, v.20, n.4, p.313-325, 2008.

SMITH, D.; KUMAR, M.M.K.; RAMANA, H.; RAO, D.V. Rubrolide R: a new furanone metabolite from the ascidian *Synoicum* of the Indian Ocean. **Natural Product Research**, v.28, n.1, p.12-17, 2014.

SOUZA, R.M.C.; MARQUES, C. T.; DORE, C.M.G.; SILVA, F.R.F.; ROCHA, H.A.O.; LEITE, E.L. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. **Journal of Applied Phycology**, v.19, n.2, p.153-160, 2007.

STEFENON, V.M.; ROESCH, L.F.W.; PEREIRA, A.B. Thirty years of Brazilian research in Antarctica: ups, downs and perspectives. **Scientometrics**, v.95, n.1, p.325-331, 2013.

STOREY, B.C.; VAUGHAN, A.P.M.; RILEY, T.R. The links between large igneous provinces, continental break-up and environmental change: evidence reviewed from Antarctica. **Earth and Environmental Science Transactions of the Royal Society of Edinburgh**, v.104, n.1, p.1-14, 2013.

STUPP, R.; MASON, W.P.; VAN DEN BENT, M.J.; WELLER, M.; FISHER, B.; TAPHOORN, M.J.; BELANGER, K.; BRANDES, A.A.; MAROSI, C.; BOGDAHN, U.; CURSCHMANN, J.; JANZER, R.C.; LUDWIN, S.K.; GORLIA, T.; ALLGEIER, A.; LACOMBE, D.; CAIRNCROSS, J.G.; EISENHAUER, E.; MIRIMANOFF, R.O. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. **The New England Journal of Medicine**, v.352, n.10, p.987-996, 2005.

TABOURET, E.; BARRIE, M.; THIEBAUT, A.; MATTA, M.; BOUCARD, C.; AUTRAN, D.; LOUNDOU, A.; CHINOT, O. Limited impact of prognostic factors in patients with recurrent glioblastoma multiforme treated with a bevacizumab-based regimen. Journal of Neuro-Oncology, v.114, n.2, p.191-198, 2013.

TENG, Z.; QIAN, L.; ZHOU, Y. Hypolipidemic activity of the polysaccharides from *Enteromorpha prolifera*. **International Journal of Biological Macromolecules**, v.62, p.254-256, 2013.

UNIV GEORGIA SYSTEM; UNIV VALDOSTA STATE. T.J. MANNING. **Producing marine natural products (e.g. bryostatin, dolastatin, halichondrin, aplidine and spongistatin) and terrestrial natural product (Taxol), comprises immersing a substrate in a marine environment using principles of green technology**. US2013004993-A1, 28 jun. 2011, 03 jan. 2013.

VEIGA-SANTOS, P.; PELIZZARO-ROCHA, K.J.; SANTOS, A.O.; UEDA-NAKAMURA, T.; DIAS FILHO, B.P.; SILVA, S.O.; SUDATTI, D.B.; BIANCO, E.M.;

PEREIRA, R.C.; NAKAMURA, C.V. In vitro antitrypanosomal activity of elatol isolated from red seaweed *Laurencia dendroidea*. **Parasitology**, v.137, n.11, p.1661-1670, 2010.

VISHCHUK, O.S.; ERMAKOVA, S.P.; ZVYAGINTSEVA, T.N. Sulfated polysaccharides from brown seaweeds Saccharina japonica and *Undaria pinnatifida*: isolation, structural characteristics, and antitumor activity. **Carbohydrate Research**, v.346, n. 17, p.2769-2776, 2011.

VISHCHUK, O.S.; ERMAKOVA, S.P.; ZVYAGINTSEVA, T.N. The fucoidans from brown algae of Far-Eastern seas: Anti-tumor activity and structure–function relationship. **Food Chemistry**, v.141, n.2, p.1211-1217, 2013.

WANG, R.; PAUL, V.J.; LUESCH, H. Seaweed extracts and unsaturated fatty acid constituents from the green alga *Ulva lactuca* as activators of the cytoprotective Nrf2–ARE pathway. **Free Radical Biology and Medicine**, v.57, p.141–153, 2013.

WIENCKE, C.; AMSLER, C.D. Seaweeds and their communities in polar regions. In: WIENCKE, C.; BISCHOF, K. (eds.). **Seaweed biology: novel insights into ecophysiology, ecology and utilization**. Berlin: Springer-Verlag, 2012. p.265-291.

WIENCKE, C.; CLAYTON, M.N. **Antarctic Seaweeds**. Ruggell: ARG Gantner Verlag, 2002. 239p.

WIENCKE, C.; CLAYTON, M.N. The life history of *Porphyra endiviifolium* from the South Shetland Islands, Antarctica. **Polar Biology**, v.19, n.4, p.257-263, 1998.

WIJESEKARA, I.; QIAN, Z.J.; RYU, B.; NGO, D.H.; KIM, S.K. Purification and identification of antihypertensive peptides from seaweed pipefish (*Syngnathus schlegeli*) muscle protein hydrolysate. **Food Research International**, v.44, n.3, p.703-707, 2011.

WIJESINGHE, W.A.J.P.; JEON, Y.J. Exploiting biological activities of brown seaweed *Ecklonia cava* for potential industrial applications: a review. **International Journal of Food Sciences and Nutrition**, v.63, n.2, p.225-235, 2012.

WU, X.; HU, A.; ZHANG, M.; CHEN, Z. Effects of Rab27a on proliferation, invasion, and anti-apoptosis in human glioma cell. Tumor Biology, v.34, n.4, p.2195–2203, 2013.

WULFF, A.; IKEN, K.; QUARTINO, M.L.; AL-HANDAL, A.; WIENCKE, C.; CLAYTON, M.N. Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. In: WIENCKE, C. (ed.). **Biology of polar benthic algae**. Berlin: De Gruyter, 2011. p.23-52.

YANG, C.; CHUNG, D.; SHIN, I. S.; LEE, H.; KIM, J.; LEE, Y.; YOU, S. Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of *Undaria pinnatifida*. **International Journal of Biological Macromolecules**, v.43, n.5, p.433-437, 2008.

YANG, L.; WANG, P.; WANG, H.; LI, Q.; TENG, H.; LIU, Z.; YANG, W.; HOU, L.; ZOU, X. Fucoidan Derived from Undaria pinnatifida Induces Apoptosis in Human

Hepatocellular Carcinoma SMMC-7721 Cells via the ROS-Mediated Mitochondrial Pathway. **Marine Drugs**, v.11, n.6, p.1961-1976, 2013.

YANIK, J.; STAHL, R.; TROEGER, N.; SINAG, A. Pyrolysis of algal biomass. **Journal of Analytical and Applied Pyrolysis**, v.103, p.134-141, 2013.

YE, H.; WANG, K.; ZHOU, C.; LIU, J.; ZENG, X. Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed *Sargassum pallidum*. **Food Chemistry**, v.111, n.2, p.428-432, 2008.

YONESHIGUE-VALENTIN, Y.; SILVA, I.B.; FUJII, M.T.; YOKOYA, N.S.; PUPO, D.; GUIMARÃES, S.M.P.B.; MARTINS, A.P.; SANCHES, P.F.; PEREIRA, D.C.; SOUZA, J.M.C.; PEREIRA, C.M.P.; PELLIZZARI, F.M.; COLEPICOLO, P. Marine macroalgal diversity in Admiralty Bay, King George Island, South Shetlands Islands, Antarctica. **Annual Activity Report**, v.4, p.1-9, 2013.

YUVARAJ, N.; KANMANI, P.; SATISHKUMAR, R.; PAARI, A.; PATTUKUMAR, V.; ARUL, V. Antinociceptive and Anti-Inflammatory Activities of *Sargassum wightii* and *Halophila ovalis* Sulfated Polysaccharides in Experimental Animal Models. **Journal of Medicinal Food**, v.16, n.8, p.740-748, 2013.

ZACHER, K.; RAUTENBERGER, R.; HANELT, D.; WULFF, A.; WIENCKE, C. The abiotic environment of polar benthic algae. In: WIENCKE, C. (ed.) **Biology of polar benthic algae**. Berlin: De Gruyter, 2011. p.9-22.

ZACHER, K.; ROLEDA, M. Y.; WULFF, A.; HANELT, D.; WIENCKE, C. Responses of Antarctic Iridaea cordata (Rhodophyta) tetraspores exposed to ultraviolet radiation. **Phycological Research**, v.57, p.186–193, 2009.

ZHANG, L.X.; CAI, C.E.; GUO, T.T.; GU, J.W.; XU, H.L.; ZHOU, Y.; WANG, Y.; LIU, C.C.; HE, P.M. Anti-cancer effects of polysaccharide and phycocyanin from *Porphyra yezoensis*. **Journal of Marine Science and Technology**, v.19, n.4, p.377-382, 2011.

ZHANG, Y.; HAN, J.Y.; MU, J.; FENG, Y.; GU, X.J.; JI, Y.X. Bioactivity and constituents of several common seaweeds. **Chinese Science Bulletin**, v.58, n.19, p.2282-2289, 2013.

ZHANG, Z.Y.; ZHANG, P.J.; HAMADA, M.; TAKAHASHI, S.; XING, G.Q.; LIU, J.Q.; SUGIURA, N. Potential chemoprevention effect of dietary fucoxanthin on urinary bladder cancer EJ-1 cell line. **Oncology Reports**, v.20, n.5, p.1099-1103, 2008.

ZHOU, X.; SUN, J.; MA, W.; FANG, W.; CHEN, Z.; YANG, B.; LIU, Y. Bioactivities of six sterols isolated from marine invertebrates. **Pharmaceutical Biology**, v.52, n.2, p.187-190, 2014.

ZIELINSKI, K. Bottom macroalgae of the Admiralty Bay (King George Island, South Shetlands, Antarctica). **Polish Polar Research**, v.11, n.1-2, p.95-131, 1990.

ANEXOS

ANEXO A

Normas para submissão no periódico **European Journal of Lipid Science and Technology**

RESEARCH ARTICLES

Research articles consist of experimental and theoretical work with new results in the topics given in section 1. Research articles should be as concise as possible, typically up to 8 printed pages including up to 8 figures and tables (about 7500 words including figure and table legends and references); longer manuscripts will be considered if the increased length is justified by the amount of data and information presented. Please include large datasets, supporting chromatograms, etc. in the Supplementary material. Sections and subdivisions of sections should be indicated by numbered headings. The manuscript should be organized as described in section

MANUSCRIPT ORGANIZATION

First page (all manuscripts)

The first page of the manuscript must contain:

1. Title of the paper. Titles should be concise and informative

2. Authors' full names (including first name spelled out) and the name of the institution or company. If the publication originates from authors of different affiliations, they should be clearly stated by using superscript numbers

3. A running title not exceeding 60 letters

4. Name and full postal address, including phone, fax and e-mail numbers, of the author to whom all correspondence (including galley proofs) should be sent

5. Up to 5 key words, which will be used for compiling the subject index

6. A list of abbreviations used

7. Any details regarding joint first authors, additional addresses, etc. These should be linked to the author's name by asterisks (single, double etc. as appropriate). Please note that funding sources should be included in the Acknowledgements section.

Manuscript sections - Research Articles

Research articles should be divided into the following sections:

Abstract (summary)

The abstract should be structured to contain the main body and practical applications:

1. The main body can be up to 200 words long; must be self-explanatory and intelligible without reference to the text, and should concisely describe the scope and objectives of the work, methods, and results. Any citations should be written in full.

2. Practical applications – considered a subsection of the abstract (start on a new line, with the words Practical applications: …). Please describe how the results of your research can be applied. This text should highlight the (potential) uses. Please do not repeat the information given in the body of the abstract. Practical applications should not exceed 150 words.

Introduction

The Introduction should contain a description of the problem under investigation including objectives and a brief and up-to-date survey of the existing literature on the subject.

Materials and methods

For special materials and equipment, the manufacturer's name and, if possible, location should be provided. The methods including statistical analysis should be written in a manner that enables the reader to follow in detail and reproduce the experiments. It is sufficient to cite the corresponding reference for exact description of a method. An additional short description is advisable if the references are not easily accessible or are given in unfamiliar languages.

Experiments using live vertebrates and/or higher invertebrates must be performed in accordance with relevant guidelines and regulations and with the permission of national or local authorities. Please include a statement identifying the institutional and/or licensing committee approving the experiments, including any relevant details (accreditation number of the laboratory and of the investigator), in the Materials and Methods. If no such rules or permission are stipulated in the particular country, this must also be mentioned in the paper.

Human studies require obtaining appropriate ethical committee approval and informed written consent from the subjects; please include a statement that informed consent was obtained from all subjects and indicate whether the experiments conformed to the principles set out in the WMA Declaration of Helsinki, and include a statement identifying the institutional committee that approved the experiments in the Materials and Methods.

Results

The contents of tables and figures should not be repeated in the text, but should be elucidated if necessary. Experimental data should be evaluated by suitable statistical methods. Asterisks are reserved to indicate statistics in tables and figures. Full statistical analysis should be performed. The name of each test used and its outcome

should be detailed, as should the number of samples, replicates and data presentation (SD vs SEM). Charts should have error bars where appropriate.

Discussion

The results should be discussed in relation to present knowledge and the aim of the work. The discussion should not repeat the introduction or results.

Conclusions

A short paragraph summarizing the most important results.

Acknowledgements

Funding for the research presented in the article should be detailed in this section.

Conflict of interest

All financial and commercial conflicts of interest must be disclosed. If there are none, this should be stated.

References

1. Author 1, A.B.; Author 2, C.D. Title of the cited article. *Journal Title* **2007**, *6*, 100–110.
2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, Editor 2, Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
3. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Journal Abbreviation* Year, phrase indicating stage of publication.
5. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).
6. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.
7. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, (Page range), DOI or other identification number. Available online: URL (accessed on Day Month Year).
8. Title of Site. URL (accessed on Day Month Year).

Tables

Tables should be included at the end of the main manuscript file along with the captions. It is not necessary to submit tables as separate files. Tables should consist of the Arabic number of the table in order of its mention (indicated as, e.g, Table 1), an explanatory headline (following the number of the table), and the table itself. All Tables must be cited in the text.

Figures and figure legends

You may include figures directly in the main manuscript file for the convenience of reviewers.

SUPPORTING INFORMATION

Supporting information is permitted and will be published online.

Supporting Information can be a useful way for an author to include important but ancillary information with the online version of an article. Examples of Supporting Information include additional tables, data sets, figures, movie files, audio clips, 3D structures, and other related nonessential multimedia files. Supporting Information should be cited within the article text, and a descriptive legend should be included. It is published as supplied by the author, and a proof is not made available prior to publication; for these reasons, authors should provide any Supporting Information in the desired final format.

ANEXO B

Normas para submissão no periódico **Polar Biology**

Instructions for Authors

Original papers report on original research in all fields of polar biology and conform to the accepted standards of scientific quality. They must present scientific results which are essentially new. They should not be divided into several parts to make them appear shorter, but no more material should be included in one paper than can be treated in one coherent discussion. "Polar Biology" is not narrow regarding the geographical limits of polar regions. Contributions linking polar and non-polar phenomena are welcome. Highly methodological and taxonomic papers are not suited for the broad audience of "Polar Biology", unless the authors address explicitly the relevance to polar issues.

The length of Original papers should not exceed 20 printed pages including tables and illustrations. Please note that one printed page corresponds to approximately 850 words text, or 3 illustrations with their legends, or 55 references.

TITLE PAGE

The title page should include:

1. The name(s) of the author(s)
2. A concise and informative title
3. The affiliation(s) and address(es) of the author(s)
4. The e-mail address, telephone and fax numbers of the corresponding author

ABSTRACT

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

KEYWORDS

Please provide 4 to 6 keywords which can be used for indexing purposes.

TEXT FORMATTING

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

ABBREVIATIONS

Abbreviations should be defined at first mention and used consistently thereafter.

ACKNOWLEDGMENTS

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

CITATION

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

REFERENCE LIST

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Tables

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

ARTWORK AND ILLUSTRATIONS GUIDELINES

For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,

"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

ELECTRONIC SUPPLEMENTARY MATERIAL

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

ANEXO C

Normas para submissão no periódico **Polar Research**

AUTHOR GUIDELINES

Manuscript submission

Submission of a manuscript implies that: the work has not been published before; it is not being considered for publication elsewhere; and its submission has been approved by all co-authors.

Style

Manuscripts should be double-spaced.

The abstract, not to exceed 250 words, must be specific regarding aims, methods, results and conclusions. Common problems in abstracts include: too much introductory/background material; too little information about methods and actual results; and vague conclusions. Be concise and specific. Use no more than three grades of headings. Do not include foot- or endnotes.

Polar Research generally treats recent editions of the Concise Oxford Dictionary as its spelling authority. When there are alternatives, choose the spelling indicated by the COD as the preferred British spelling. Some examples: organize rather than organise; behaviour rather than behavior; centre rather than center; palaeo- rather than paleo-.

Use metric units. Abbreviate units like kilometre, metre and centimetre to km, m and cm. Use these abbreviations to refer to events that took place in the past: Kya (thousands of years ago) and Mya (millions of years ago). Use these abbreviations to refer to the age of geological material: Ky (thousands of years) and My (millions of years).

Scientific names are italicized and in parentheses following the first mention of the common name of the species. Except where this might cause confusion, abbreviate genus names to the first initial when these are repeated within a few paragraphs. Do not capitalize common names of species unless these are derived from personal or place names. Dates should be given like this: 16 November 2006. In figures and tables, they may be abbreviated: 16/11/06. Use the 24 hour clock for times, e.g., 16:30.

References

Ensure that all entries in the reference list are cited in the manuscript and that all sources cited in the manuscript are listed in the references.

Citations are mentioned chronologically in the main body of the text, using this style:

…(Smith et al. 1968; Jones 1992a, 2001; Hansen & Smith 1999).

In the reference list sources are ordered alphabetically. Non-English letters (e.g., Ø, Å and) are alphabetized like their nearest English equivalents (e.g., O, A and Ae).

Make each reference as complete as possible; superfluous information will be weeded out during copyediting.

JOURNAL ARTICLE

Uchida M., Nakatsubo T., Kanda H. & Koizumi H. 2006. Estimation of the annual primary production of the lichen Cetrariella delisei in a glacier foreland in the High Arctic, Ny-Ålesund, Svalbard. Polar Research 25, 39–49.

Give the full name of the journal. If a DOI is provided instead of a page span, place it where the page span would have been.

BOOK

Revkin A.C. 2006. The North Pole was here. Boston: Kingfisher.

Do not include the page lengths of books.

BOOK CHAPTER

Green T.G.A, Schroeter B. & Sancho L.G. 1999: Plant life in Antarctica. In F.I. Pugnaire & F. Valladares (eds.): Handbook of functional plant ecology. Pp. 495-543. New York: Marcel Dekker.

NON-ENGLISH TITLES

Non-English titles of articles, chapters, books and reports are followed by the English translation in parentheses. Translations are not included for non-English serial (journal) titles. Examples:

Magnus M. H. 1983. Norge og den sovjetiske polarforskning i nord og sør. (Norway and Soviet polar research in the north and in the south.) Farmand 6, 84–87.

Figures and tables

In the manuscript, figures and tables should be referred to in the order in which they are numbered.

Because figures may be reduced in size for printing, avoid the use of very small letters, numbers and other symbols. Labels on maps, other figures and tables should not consist entirely of upper case letters; use capital letters sparingly, e.g., 'Annual primary production' rather than 'Annual Primary Production' or 'ANNUAL PRIMARY PRODUCTION'. Fonts like Helvetica and Arial are preferred in figures. Label parts of composite figures (a), (b) and (c), preferably in an upper corner. Figures should be submitted in colour or greyscale depending on how authors would like them to appear in the final print version.

Illustrations can be submitted in the following digital formats: ai, eps, ps, tif and jpg. Tif and jpg images should be at a high enough resolution for good-quality printing: 300 dots per inch (dpi) at whatever dimensions the figure will be printed, minimally 8 cm wide. Very large files may prove difficult to upload to the journal’s submission website. It is not recommended to upload files larger than 6 MB each.

ANEXO D

Normas para submissão no periódico **International Journal of Drug Discovery**

MANUSCRIPT PREPARATION – General

STYLE

The style manuals to which we refer at Discovery publication include Webster's Dictionary, the Chicago Manual of Style (University of Chicago Press), and Council of Science Editors' Scientific Style and Format.

Proofreading

Please proofread carefully for both errors and inconsistencies in the following: spelling (especially of scientific terminology, proper names, and foreign words), mathematical notation, numerical values in tables and text, and accuracy of quotations. **Be sure all references are cited and all tables and figures are called out in the text.**

Footnotes

We discourage the use of footnotes, as these tend to interrupt the flow of the text. If footnotes are used, number all text footnotes consecutively in order of appearance throughout the article. Use a superscript number to key each footnote to the word or statement annotated (e.g., "The term operator1 is used").

Designate footnotes to tables by superscript lowercase letters; begin lettering anew for each table.

Unpublished observations and personal communications should not be included in the footnote.

Italics

Indicate italics using an italic type style. If you wish something to be printed with an underline,
use an underline type style.

Use italics for

1. Scientific names of bacteria and protozoa
2. Genera, species, and subspecific taxa
3. In chemical names, *p*, *o*, *m*, *n*, *cis*, *sec*, *sic*, *trans*, *syn*
4. Genes, genotypes, loci, markers, mutants, alleles, operons
5. Mathematical variables

Do NOT use italics for

1. Emphasis
2. Common foreign words such as ad hoc, a priori, in vivo, in vitro
3. Abbreviations such as sp., spp., var.
4. Names of taxa of rank higher than genus
5. Generic names used as adjectives
6. Names of microorganisms used colloquially (e.g., actinomycetes)
7. Strain designations
8. Names of cells, phages, hosts, phenotypes
9. Abbreviations for subatomic particles
10. R, X, M, B, A, etc. in formulas and equations where they represent chemical elements or groups

LENGTH

Each Discovery publication volume has an assigned length. Likewise, each article has a length assigned
by the editors. Please keep to this length, which includes any figures and tables submitted. Generally we
are allowed the length at any extent for innovative research and reviews.

FONT AND SAPCE

Times New Roman, 12 size, double space, single column

CONTENT TYPES

A **Review** is an authoritative, balanced and scholarly survey of recent developments in a research field. The requirement for balance need not prevent authors from proposing a specific viewpoint, but if there are controversies in the field, the authors must treat them in an evenhanded way. Reviews are normally 3,000–7,000 words, and illustrations are strongly encouraged. References are limited to 100, with exceptions possible in special cases. Citations should be selective and, in the case of particularly important studies (≤10% of all the references), we encourage authors to provide short annotations explaining why these are key contributions. The scope of a Review should be broad enough that it is not dominated by the work of a single laboratory, and particularly not by the authors' own work. Review authors must provide a competing financial interests statement before publication. Received/accepted dates are not included. Reviews are always peer reviewed to ensure factual accuracy, appropriate citations and scholarly balance.

ARTICLE COMPONENTS

REQUIRED ELEMENTS

To help readers better find and understand what they seek, we ask authors to provide the following:

1. Title Page: full article title, author(s) name(s) and affiliation(s) including email(s),
2. Corresponding Author contact information
3. Article Table of Contents listing all 1st- and 2nd-level headings
4. Keywords: as many as 6, not already in title
5. Abstract: 300 words maximum
6. Graphical Abstract: not more than 80 words with suitable figure, should be available as in image format (for online only)
7. Headings: clearly formatted throughout text (1st, 2nd, 3rd and 4th level headings are allowed)
8. Figures (color (Preferable) and black & white): submit each with its own caption clearly labeled; separate file for each figure, do not integrate within text. Number figures consecutively in text (i.e.,Figure 2 should not come before Figure 1). In addition to individual figure files, provide a PDF file containing all figures. Obtain any necessary permission for use.

9. Tables: either all at end of article, following Literature Cited, or submitted together in a separate file

10. Literature Cited: formatted per series' specifications

11. Acronyms and Definitions list (glossary): provide definitions for as many as 25 of the most important acronyms or key terms, limited to 25 words maximum; insert below Literature Cited section

12. Summary Points list: highlight the central points of your review (as many as 8), in complete sentences; insert above the Acknowledgments and/or Literature Cited section

13. Future Issues list: note where research may be headed (as many as 8), in complete sentences; insert above the Acknowledgments and/or Literature Cited section

14. Annotated References: brief (15 words maximum) explanation of citations' importance (as many as 10); insert below the Literature Cited section

15. Related Resources list: up to 10 references, not listed in Literature Cited, to materials (Web sites, articles, animations) that may be of interest to readers; insert below the Literature Cited section

16. Sidebar (50 words minimum, 200 words maximum) briefly discussing a fascinating adjacent topic; please give the sidebar a title and insert it below the Literature Cited section, but indicate near which section in text the sidebar should be typeset; the sidebar cannot contain figures or tables.

17. Give list of abbreviation (Abbreviate required terms).

HARVARD-STYLE REFERENCES

Discovery publication use the unnumbered, name and year (Harvard) bibliographic style.

CITATIONS IN TEXT

1. Use the name-and-year system.
2. Use ampersand to indicate authorship for two authors. For three or more authors, use "et al.," "and coworkers," or "and associates" in text. Use no comma before ampersand. White & Gray (2004) experimented... Smith et al. (1999) tested the theory.
3. Distinguish between references with the same author(s) and year by indicating 1987a, 1987b, etc. Byron et al. (1986; 1987a,b) determined...
4. In multiple citations, references should appear in either chronological or alphabetical sequence throughout. If inconsistent, alpha order will be applied in copyediting.
5. Use semicolons to separate unlike elements within the parentheses. (Moorehouse 2006; JS Smith, unpublished information).
6. Do not use author's initials for published references in text unless necessary to distinguish two authors of the same surname.
7. Italicize titles of books and journals.
8. References to unpublished observations, personal communications, papers in preparation, etc., should be enclosed in parentheses in text (Jones RS, unpublished observations). List all authors—do not use et al.—and include all their initials (as well as your own) in these citations.

BIBLIOGRAPHIC STYLE

Most cited sources can be formatted using the general guidelines below. For exceptions or special cases (Web sites, conference papers, errata, abstracts, etc.), see Appendix A at the end of this handbook. List numbered references in the Literature Cited with numerals and period, without parentheses. Include the following information (in this order):

1. Name(s) of author(s), last name first, followed by initials without periods. Include both (or all) initials for each author whenever they were included in the original article or book. Do not leave space between initials. Do not use a comma between surnames and initials—use commas only to separate different authors' names. If a given reference has seven or more authors, list the first five, then type "et al." in the bibliography. (But in text, use et al. for three or more authors.) If a reference has six or fewer authors, list them all.
2. Year of publication of the article or book, followed by a period, with no parentheses. If the article has recently been accepted for publication and is actually in press, list it in the Literature Cited section. Provide journal title and expected year of publication, plus volume and pages when known.
3. Title of article or chapter
4. Title of journal (abbreviated unless only one word) or book (not abbreviated unless part of a periodical series), e.g., J. Psychol.
5. For a book reference, name(s) of editor(s).
6. Volume, issue or number (if any) in parentheses, then a colon and inclusive page numbers; if there is no volume number, inclusive page numbers preceded by a comma and "pp." Do not repeat hundreds digit unless, e.g., 3–10, 71–77, 100–9, 331–35, 1002–3, 1198–202, 1536–38. For example: 10(4), 123–30
7. For a book reference, place of publication, name of publisher, total number of pages (optional), and edition, if necessary. For example: New York: Sage (do not put a period at the end of the reference).

Curriculum Vitae

Formação acadêmica/titulação

2012 - 2014 Mestrado em Bioquímica e Bioprospecção.
Universidade Federal de Pelotas, UFPEL, Pelotas, Brasil
Título: Macroalgas da Antártica: Propriedades Químicas e Avaliação Biológica, Ano de obtenção: 2014
Orientador: Claudio Martin Pereira de Pereira
Co-orientador: Elizandra Braganhol
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2007 - 2011 Graduação em Ciências Biológicas - Bacharelado.
Universidade Federal de Pelotas, UFPEL, Pelotas, Brasil
Título: Cultivo de Cianofíceas e aplicação na biorremediação de águas residuais dos laboratórios da Química
Orientador: Claudio Martin Pereira de Pereira
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico

Atuação profissional

1. Universidade Federal de Pelotas - UFPEL

Vínculo institucional

2012 - 2014 Vínculo: Bolsista , Enquadramento funcional: Mestranda , Carga horária: 20, Regime: Dedicação exclusiva
2009 - 2012 Vínculo: Livre , Enquadramento funcional: Aluna de graduação e bolsista de IC , Carga horária: 20, Regime: Parcial

Atividades

03/2012 - 03/2014 Estágio, Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Estágio no Laboratório de Lipidômica e BioOrgânica com avaliação química e biológica de macroalgas da Antártica

03/2009 - 02/2012 Estágio, Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Estágio no Laboratório de Heterociclos Bioativos e Bioprospecção com microalgas (biodiesel e biorremediação)

03/2008 - 03/2009 Conselhos, Comissões e Consultoria, Instituto de Biologia - Diretório Acadêmico do Curso de Ciências Biológicas
Especificação: Colaboradora

11/2007 - 01/2008 Outra atividade técnico-científica, Universidade Federal de

Pelotas
Especificação: Monitora da disciplina de Morfologia e Sistemática Vegetal, do Departamento de Botânica, do Instituto de Biologia, UFPel.

08/2007 - 02/2009 Estágio, Universidade Federal de Pelotas
Estágio no Laboratório de Biologia Celular e Molecular Vegetal, do Centro de Biotecnologia

04/2007 - 02/2009 Estágio, Universidade Federal de Pelotas
Estágio no Departamento de Botânica, avaliando o banco de sementes de Lolium multiflorum

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Projetos

Projetos de pesquisa

2014 - Atual Macroalgas Vermelhas: Validação de ácidos graxos poliinsaturados por cromatografia gasosa

2012 - Atual Macroalgas da Antártica: propriedades químicas e avaliação biológica

2009 - 2011 Cultivos de Microalgas destinadas à produção de Biodiesel

2008 - 2013 Utilização de Plantas para Produção de Antígenos Vacinais

2007 - 2008 Utilização de plantas como reatores biológicos para expressão de antígenos vacinais: produção de um antígeno vacinal contra pneumonia micoplásmica suína

---

Prêmios e títulos

2013 ISAP Award - Third Place, Category Postgraduate Study, IV Latin American Congress for Algae Biotechnology and IV Workshop of Brazilian Network of Marine AT

2012 Menção honrosa, Universidade Federal de Pelotas

2011 2° lugar das Ciências Biológicas no XX Congresso de Iniciação Científica da UFPel com o trabalho "Avaliação do Potencial das Cianofíceas na Biorremediação de Águas Residuais contendo Pb", Universidade Federal de Pelotas

Producão

Produção bibliográfica
Artigos completos publicados em periódicos

1. **Souza, Priscila O.**, Ferreira, Lizângela R., Pires, Natanael R. X., S. Filho, Pedro J., Duarte, Fabio A., Pereira, Claudio M. P., Mesko, Márcia F.
Algae of economic importance that accumulate cadmium and lead: a review. Revista Brasileira de Farmacognosia (Impresso). , v.22, p.825 - 837, 2012.

2. Hobuss, Cristiane B., Rosales, Pauline F., Venzke, Dalila, SOUZA, P. O., Gobbi, Priscilla C., Gouvea, Lidiane P., Santos, Marco A. Z., Pinto, Ernani, Jacob-Lopes, Eduardo, Pereira, Claudio M. P.
Cultivation of algae in photobioreator and obtention of biodiesel. Revista Brasileira de Farmacognosia (Impresso). , v.21, p.361 - 364, 2011.

Trabalhos publicados em anais de eventos (resumo)

1. POSSIGNOLO, J., **SOUZA, P. O.**, SINHOR, V., SANTOS, M. A. Z., FREITAG, R. A., NETO, P.C., FUJII, M. T., SANTOS, D. P., PEREIRA, C. M. P.
Análise de ácidos graxos da macroalga da Antártica *Desmarestia anceps* In: IV Latin American Congress of Algae Biotechnology & Workshop of the National Network of Marine Algae Biotechnology, 2013, Florianópolis.
Anais CLABA & WSRedealgas. , 2013.

2. **SOUZA, P. O.**, SINHOR, V., PEREIRA, C. M. P., PIRES, N., SANCHES FILHO, P. J., NETO, P.C., MESKO, M. F.
Cultiv de Cianofíceas e avaliação da capacidade de biorremediação de águas residuais contendo chumbo e cromo In: III Workshop da Redealgas: biodiversidade, aplicação tecnológica e sustentabilidade, 2011, Paty do Alferes.
Resumos dos trabalhos. , 2010.

3. Santos, Marco A. Z., **SOUZA, P. O.**, NUNES, C. F. P., FARIAS, M. D., MESKO, M. F., SINHOR, V., PEREIRA, C. M. P.
Cultivo de microalga *Scenedesmus obliquus* em fotobioreator e glicerol In: III Workshop da Redealgas: biodiversidade, aplicação tecnológica e sustentabilidade, 2011, Paty do Alferes.
Resumo dos trabalhos. , 2010.

4. **SOUZA, P. O.**, CORRÊA, B.F., MALDANER, C., SILVA, J. M., HOLLAS, R., MORAES, D. M.
Avaliação do crescimento de sementes de *Raphanus sativus* L. submetidas a reguladores de crescimento In: XVIII Congresso de Iniciação Científica/XI ENPOS/I Mostra Científica, 2009, Pelotas.
Evoluir sem extinguir: por uma ciência do devir. Pelotas: Editora Universitária, 2009.

Trabalhos publicados em anais de eventos (resumo expandido)

1. **SOUZA, P. O.**, PIRES, N., SANCHES FILHO, P. J., SINHOR, V., MESKO, M. F.
Avaliação do potencial das Cianofíceas na biorremediação de águas residuais contendo chumbo dos laboratórios da Química In: XX Congresso de Inicação Científica/III Mostra Científica, 2011, Pelotas.
CIC 2011. , 2011.

2. **SOUZA, P. O.**, GOBBI, P. C., GOUVEA, L., LOPES, E. J., PEREIRA, C. M. P.
Crescimento de biomassa de Chlorella vulgaris em fotobiorreator In: XIX Congresso de Iniciação Científica/XII ENPOS/II Mostra Científica, 2010, Pelotas.
Anais do Congresso de Iniciação Científica 2010. Pelotas: Editora Universitária, 2010.

3. Rosales, Pauline F., HOBUSS, C. B., **SOUZA, P. O.**, PACHECO, B.S., PEREIRA, C. M. P.
Estudo comparativo do teor lipídico de quatro diferentes microalgas para produção de biodiesel In: XIX Congresso de Iniciação Científica/XII ENPOS/II Mostra Científica, 2010, Pelotas.
Anais do Congresso de Iniciação Científica 2010. Pelotas: Editora Uniersitária, 2010.

4. **SOUZA, P. O.**, ROSALES, P., HOBUSS, C. B., PEREIRA, C. M. P.
Teor de lipídios em *Phormidium* sp. cultivada em meio com redução de nitrogênio In: V Congresso Sul-Riograndense de Biociências, 2010, Pelotas.
Anais do V Congresso Sul-Riograndense. Pelotas: , 2010.

5. **SOUZA, P. O.**, BRAGA, M. R., MITTELMANN, A., GARCIA, E. N.
Banco de sementes do solo de *Lolium multiflorum* Lam. (azevém) In: XVIII Congresso de Iniciação Científica/XI ENPOS/ I Mostra Científica, 2009, Pelotas.
Evoluir sem extinguir: por uma ciência do devir. Pelotas: Editora Universitária, 2009.

6. SILVA, J. M., MALDANER, C., CAMPESATO, C.B.M., HOLLAS, R., **SOUZA, P. O.**, LIMA, M.G.S., MENDES, C.R., MORAES, D. M.
Qualidade fisiológica de sementes de *Raphanus sativus* L. submetidas a reguladores de crescimento In: XVIII Congresso de Iniciação Científica/XI ENPOS/I Mostra Científica, 2009, Pelotas.
Evoluir sem extinguir: por uma ciência do devir. Pelotas: Editora Universitária, 2009.

Apresentação de trabalho e palestra

1. SANTOS, M. A. Z., **SOUZA, P. O.**, SILVA, I. B., NETO, P.C., FUJII, M. T., SANTOS, D. P., PEREIRA, C. M. P.
Detecção de omegas em macroalas da Antártica por Espectrometria de Massas, 2012. (Simpósio,Apresentação de Trabalho)

2. **SOUZA, P. O.**, SANTOS, M. A. Z., SILVA, I. B., NETO, P.C., FUJII, M. T., SANTOS, D. P., FREITAG, R. A., PEREIRA, C. M. P.
Detection of omegas macroalgae Antartic in gas chromatography, 2012. (Simpósio,Apresentação de Trabalho)

3. SANTOS, M. A. Z., ROCKEMBACH, C. T., **SOUZA, P. O.**, NETO, P.C., FUJII, M. T., SANTOS, D. P., PEREIRA, C. M. P.
Identificação e análise semiquantitativa de ômegas na macroalga parda Cystosphaera jacquinotii Montagne da região Antártica por Espectrometria de Massas, 2012. (Congresso,Apresentação de Trabalho)

4. **SOUZA, P. O.**, PEREIRA, C. M. P., BRAGANHOL, E.
Macroalgae from Antartica: chemistry properties and biological evaluation, 2012. (Outra,Apresentação de Trabalho)

5. SOUZA, P. O., SINHOR, V., PEREIRA, C. M. P., PIRES, N., SANCHES FILHO, P. J., NETO, P.C., MESKO, M. F.
Cultivo de cianofíceas e avaliação da capacidade de biorremediação de águas residuais contendo chumbo e cromo, 2011. (Congresso,Apresentação de Trabalho)

6. Santos, Marco A. Z., **SOUZA, P. O.**, NUNES, C. F. P., FARIAS, M. D., MESKO, M. F., NETO, P.C., SINHOR, V., PEREIRA, C. M. P.
Cultivo de microalga Scenedesmus obliquus em fotobioreator e glicerol, 2011. (Outra,Apresentação de Trabalho)

7. GOUVEA, L., **SOUZA, P. O.**, GOBBI, P. C., HOBUSS, C. B., Jacob-Lopes, Eduardo, PEREIRA, C. M. P.
Cultivo das microalgas Phormidium sp. e Chlorella vulgaris e perspectivas de aplicações, 2010. (Congresso,Apresentação de Trabalho)

8. SOUZA, P. O., GOBBI, P. C., GOUVEA, L., FREITAG, R., LOPES, E. J., PEREIRA, C. M. P.
Cultivo de microalgas em fotobioreatores, 2010. (Outra,Apresentação de Trabalho)

9. HOBUSS, C. B., VENZKE, D., SOUZA, P. O., ROSALES, P., GOBBI, P. C., PEREIRA, C. M. P.
Efeito da variação de nutrientes no teor lipídico da microalga Phormidium sp., 2010. (Outra,Apresentação de Trabalho)

10. HOBUSS, C. B., VENZKE, D., SOUZA, P. O., GOUVEA, L., GOBBI, P. C., PEREIRA, C. M. P.
Estudo comparativo do crescimento da microalga Chlorella vulgaris: método convencional versus fotobiorreator, 2010. (Simpósio,Apresentação de Trabalho)

11. GOUVEA, L., PEREIRA, C. M. P., DOURADO, M. T., MESKO, M. F., **SOUZA, P. O.**, GOBBI, P. C.
Manufatura de sabão derivado de óleo residual: uma proposta de reciclagem e sustentabilidade, 2010. (Congresso,Apresentação de Trabalho)

12. GOBBI, P. C., SOUZA, P. O., ROSALES, P., FREITAG, R., LOPES, E. J., BRETANHA, L.C., ULTRAMARI, M. A., PINTO, E., PEREIRA, C. M. P.
Seleção de Algas: Matéria-prima para produção de Biodiesel, 2009. (Outra,Apresentação de Trabalho)

13. SOUZA, P. O., NORA, F. R., Osório, Marina Borges, Klafke, Gabriel Baracy
Expressão da subunidade B da enterotoxina de E.coli (LTB) fusionada à região R1 da adesina P97 de Mycoplasma hyopneumoniae em plantas transgênicas de alface (Lactuca sativa L.), 2008. (Congresso,Apresentação de Trabalho)

Patentes e registros

Patente

1. MESKO, M. F., PEREIRA, C. M. P., NETO, P.C., STREIT, N. M., VIEIRA, B. M., RITTER, M., ELICKER, C., SINHOR, V., TUCHTENHAGEN, C. P., POSSIGNOLO, J., **SOUZA, P. O.**, FERREIRA, L. R., SANTOS, M. A. Z., PIRES, N. R. X.
Aplicação de microalgas para biorremediação do glicerol oriundo da obtenção de biodiesel, 2012. Categoria: Processo. Instituição onde foi depositada: INPI - Instituto Nacional da Propriedade Industrial. País: Brasil. Natureza: Patente de Invenção. Número do registro: BR1020120279630. Data de depósito: 31/10/2012. Depositante/Titular: Mesko, Márcia F..

Participação em eventos

1. **IV Latin American Congress of Algae Biotechnology & Workshop of the National Network of Marine Algae Biotechnology**, 2013. (Congresso)
Atividade Antineoplásica da macroalga da Antártica *Pyropia endiviifolia*.

2. Apresentação de Poster / Painel no(a) **Stem Cells in developmental biology and cancer**, 2012. (Outra)
Macroalgae from Antartica: chemistry properties and biological evaluation.

3. Apresentação Oral no(a) **XX Congresso de Iniciação Científica/III Mostra Científica**, 2011. (Congresso)
Avaliação do potencial das cianofíceas na biorremediação de águas residuais contendo chumbo dos laboratórios da Química.

4. Apresentação Oral no(a) **III Workshop da Redealgas: Biodiversidade, aplicação tecnológica e sustentabilidade**, 2011. (Outra)
Cultivo de cianofíceas e avaliação da capacidade de biorremediação de águas residuais contendo chumbo e cromo.

5. **Jornada de Toxicologia**, 2011. (Congresso)
.
6. **Jornada de Toxicologia**, 2011. (Outra)
.
7. Apresentação Oral no(a) **XIX Congresso de Iniciação Científica**, 2010. (Congresso)
Crescimento de biomassa de *Chlorella vulgaris* em fotobiorreator.

8. Apresentação de Poster / Painel no(a) **XI Semana Acadêmica da Biologia**, 2010. (Outra)
Cultivo de microalgas em fotobioreatores.

9. Apresentação de Poster / Painel no(a) **V Congresso Sul-Riograndense de Biociências**, 2010. (Congresso)

Teor de lipídios em *Phormidium* sp. cultivada em meio com redução de nitrogênio.

10. **A Histologia e suas interrelações**, 2010. (Seminário)
.
11. **3° BIOURCAMP**, 2010. (Encontro)
.
12. **I Ciclo de debates sobre direito social à educação: educação básica e o acesso ao ensino superior**, 2010. (Outra)
.
13. Apresentação de Poster / Painel no(a) **XVIII Congresso de Iniciação Científica**, 2009. (Congresso)
Avaliação do Crescimento de Sementes de *Raphanus sativus* L. submetidas a Reguladores do Crescimento.

14. Apresentação de Poster / Painel no(a) **XVIII Congresso de Iniciação Científica**, 2009. (Congresso)
Banco de Sementes do Solo de *Lolium multiflorum* Lam. (azevém).

15. Apresentação de Poster / Painel no(a) **II Workshop: Novos Bioativos de Macroalgas - Manejo e Cultivo, Conservação, Biotecnologia e Técnicas de Bioatividade**, 2009. (Outra)
Seleção de Algas: Matéria-prima para produção de Biodiesel.

16. **Nicho do Conhecimento Pedagógico**, 2009. (Outra)
.
17. **IV Ciclo de Palestras de Biologia Molecular e Biotecnologia**, 2009. (Outra)
.
18. **Charles Darwin - Teoria, Vida e Repercussão**, 2009. (Seminário)
.
19. **Semana Acadêmica da Biologia - UCPel**, 2009. (Outra)
.
20. Apresentação de Poster / Painel no(a) **IV Congresso Sul-Riograndense Biociências**, 2008. (Congresso)
Expressão da subunidade B da enterotoxina de *E.coli* (LTB) fusionada à região R1 da adesina P97 de *Mycoplasma hyopneumoniae* em plantas transgênicas de alface (Lactuca sativa L.).

21. **3° Ciclo de Palestras de Biologia Molecular - Aplicação Contemporânea**, 2008. (Seminário)
.
22. **Diálogos Culturais e Ambientais: Crédito de Carbono**, 2008. (Outra)
.
23. **X Semana Acadêmica da Biologia – Reflexões sobre conservação**, 2008. (Outra)
.
24. **IV Congresso Sul-Riograndense Biociências**, 2008. (Congresso)
.
25. **IX Jornada Biológica - "Uma Reflexão sobre o Sul do Rio Grande do Sul"**, 2007. (Outra)
.

26. **Ciências Micromorfológicas em Discussão**, 2007. (Outra)
.
27. **IX Semana Acadêmica de Biologia**, 2007. (Outra)
.
28. **Seminário sobre o papel do eucalipto no Rio Grande do Sul**, 2007. (Seminário)
.

Organização de evento

1. GARCIA, L. E., ALVES, M.S., PAZINATO, P. G., CASTRO, P. E. E., CORRÊA, L., DIAS, V. B., **SOUZA, P. O.**, SILVA, J. M., CUNHA, S. K., MOLINA, S., TEIXEIRA, C., ISLAS, C. A.
XII Semana Acadêmica da Biologia, 2011. (Outro, Organização de evento)

2. GIL, R. L., **SOUZA, P. O.**
Ressignificando os Cursos de Ciências Biológicas da UFPEL, 2010. (Outro, Organização de evento)

3. SOUZA, P. O., VALENTE, C. B., MEDRONHA, M.A., STONE, S.C., BRAGA, M. R., MORAES, V. S., BERNARDON, F. F., ZANCHETTA, L. N., ROSA, P., SOUZA, E. C., BONILHA, C. L.
A Biologia em Debate, 2008. (Outro, Organização de evento)

4. BONILHA, C. L., VALENTE, C. B., ZANCHETTA, L. N., MEDRONHA, M.A., STONE, S.C., SOUZA, P. O., SOUZA, E. C., ROSA, P., MORAES, V. S., BERNARDON, F. F., BRAGA, M. R.
X Semana Acadêmica da Biologia: "Reflexões sobre conservação", 2008. (Outro, Organização de evento)

Totais de produção

Produção bibliográfica
Artigos completos publicados em periódico 2
Trabalhos publicados em anais de eventos. 10
Apresentações de trabalhos (Congresso). 5
Apresentações de trabalhos (Simpósio)..... 3
Apresentações de trabalhos (Outra). 5

Patentes e Registros
Patente..... 1

Eventos
Participações em eventos (congresso). 9
Participações em eventos (seminário). 4
Participações em eventos (encontro). 1
Participações em eventos (outra). 14
Organização de evento (outro). 4