UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

DEPARTAMENTO DE BIOQUÍMICA

MARIANA LEIVAS MÜLLER HOFF

PERFIL REDOX-ATIVO *IN VITRO* DE EXTRATOS DE ESPONJAS MARINHAS DO LITORAL BRASILEIRO

PORTO ALEGRE

2008

MARIANA LEIVAS MÜLLER HOFF

PERFIL REDOX-ATIVO *IN VITRO* DE EXTRATOS DE ESPONJAS MARINHAS DO LITORAL BRASILEIRO

Dissertação de mestrado apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica como requisito à obtenção do grau de Mestre em Bioquímica.

Orientador: Prof. Dr. José Cláudio Fonseca Moreira

PORTO ALEGRE

2008

À minha mãe, minha maior incentivadora.

AGRADECIMENTOS

A minha mãe, pelo exemplo, amor, carinho e força na luta de cada dia.

Ao Prof. Dr. José Cláudio, pela orientação e pelo crédito.

Ao Guilherme, pela ajuda e troca de idéias.

Aos colegas de laboratório, pela partilha diária.

À Calhandra Pinter de Souza Santos, pela revisão de inglês.

À Prof. Dra. Amélia T. Henriques da Faculdade de Farmácia da UFRGS, pelos extratos.

Às Dras. Beatriz Mothes e Cléa Lerner da Fundação Zoobotânica do Rio Grande do Sul, pela coleta e identificação das esponjas marinhas.

RESUMO

A bioquímica farmacológica dos organismos marinhos tem emergido como uma interessante área a ser pesquisada. O Brasil com 8.000 km de litoral rico em espécies de esponjas representa um grande potencial de investigação de metabólitos secundários ativos. O *screening* de extratos de esponjas e de outros organismos marinhos é uma prática comum para identificar compostos de importância biomédica e reflete o comportamento do conjunto de compostos presentes numa dada espécie. Portanto, o objetivo deste trabalho foi realizar um *screening* de propriedades redox-ativas em vinte extratos de esponjas marinhas coletadas no litoral dos estados de Santa Catarina, Pernambuco e Paraíba. Os extratos foram avaliados através dos ensaios: TRAP, degradação da 2-deoxirribose via radical $^{\bullet}OH$, produção de nitritos via $^{\bullet}NO$, auto-oxidação da adrenalina por $O_2^{\bullet-}$, redução do NBT via $O_2^{\bullet-}$ e lipoperoxidação induzida por AAPH, Fe^{2+} e H_2O_2 pela técnica de TBARS. Este trabalho mostrou que extratos de esponjas marinhas possuem atividades de *scavenger* de $^{\bullet}NO$, H_2O_2 e $ROO^{\bullet}$ e de proteção de peroxidação lipídica induzida por H_2O_2, $ROO^{\bullet}$ e Fe^{2+}. Dada a importância e o envolvimento destas espécies reativas em várias patologias, os mecanismos bem como as moléculas responsáveis pelas ações antioxidantes observadas nestes extratos de esponjas marinhas merecem ser melhor entendidos. Este trabalho apresentou uma metodologia plausível para determinar potenciais redox-ativos de importância fisiopatológica em extratos de esponjas marinhas e forneceu dados estimulantes para o prosseguimento da pesquisa por moléculas antioxidantes nestes organismos. Conforme já descrito para outras bioatividades, é possível que as características redox destes extratos sejam reflexo da biologia e ecologia das esponjas marinhas.

ABSTRACT

The biochemistry of marine organisms is emerging as a promising research area. Brazil has a 8,000 km coastline and presents diversity of sponge species, which indicates a great potential of investigation of pharmacologically active secondary metabolites. Screening of crude extracts from marine sponge and other organisms is a powerfull tool to search for biomedical relevant compounds and also reflects the behavior of the metabolites mixture presented in a species. Hence, the aim of this study was perform a redox-activity screening of twenty marine sponge extracts collected off Santa Catarina, Pernambuco, and Paraíba States. Extracts were evaluated by: TRAP, 2-deoxiribose oxidative degradation by $^{\bullet}OH$ radical, $^{\bullet}NO$ production of nitrites, SOD-like activity, NBT reduction via $O_2^{\bullet-}$ and AAPH-, Fe^{2+}- and H_2O_2-induced lipid peroxidation by TBARS. It was observed that marine sponge extracts possess scavenging activity of $^{\bullet}NO$, H_2O_2 and $ROO^{\bullet}$, and are able to prevent lipid peroxidation induced by H_2O_2, $ROO^{\bullet}$ and Fe^{2+}. In the knowledge of the involvement of reactive species in relevant dysfunctions, the mechanisms and the molecules related to the antioxidant potentials observed in these marine sponge extracts must be better studied. This work presented a feasible approach to evaluate redox-active properties against reactive nitrogen and oxygen species of physiological and pathological relevance in marine sponge extracts. Furthermore, the data obtained estimulate the research for novel antioxidant prototypes in sponge extracts. It is possible that the redox features here observed be related to the biology and ecology of marine sponges, such it is for other bioactivities.

SUMÁRIO

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ANEXOS

LISTA DE ABREVIATURAS

AAPH: dihidrocloridrato de 2,2'-azobis 2-metilpropionamidina

AIDS: síndrome da imunodeficiência adquirida

ANOVA: análise de variância

AUC: *area under curve*, área abaixo da curva

CDKs: ciclinas dependentes de cinases

CK1: caseína cinase 1

DNA: ácido desoxirribonucléico

ERN: espécies reativas de nitrogênio

ERO: espécies reativas de oxigênio

GSH: glutationa reduzida

GSK-3β: glicogênio sintase quinase - 3β

H_2O_2: peróxido de hidrogênio

HD: hymenialdisine

HIV-1: vírus da imunodeficiência humana tipo 1

HOCl: ácido hipocloroso

LDL: *low-density lipoprotein*, lipoproteína de baixa densidade

MCNPOR: Museu de Ciências Naturais - Porifera

NBT: *nitroblue tetrazolium*

$(NO)^2$: monóxido de nitrogênio

$^{\bullet}NO$: óxido nítrico

NOS: *nitric oxide synthase*, óxido nítrico sintase

$^{\bullet}OH$: radical hidroxil

$O_2^{\bullet -}$: ânion superóxido

$ONOO^-$: peroxinitrito

PBS: Tampão fosfato tamponado com salina

SNP: nitroprussiato de sódio

TBARS: substâncias reativas ao ácido tiobarbitúrico

TRAP: *total radical-trapping antioxidant potential*, potencial total antioxidante de captura de radical

TAR: *total antioxidant reactivity*, reatividade antioxidante total

1. INTRODUÇÃO

1.1 Esponjas como Alvo de Estudo

Nas últimas décadas, os oceanos se revelaram provedores de um grande grupo de produtos naturais. A descrição de novos compostos de origem marinha, os quais muitas vezes são estruturalmente únicos, tem superado todas as expectativas (FAULKNER, 2000). Os organismos marinhos fontes de compostos com potencial bioativo e farmacológico compreendem bactérias, fungos, algas, tunicados e esponjas. Entre estes seres, as esponjas têm atraído a atenção de várias áreas da ciência. Um destes ramos de pesquisa corresponde à procura de bioatividades em extratos e metabólitos de poríferos. A descoberta de compostos químicos inéditos na literatura intensificou e animou a possibilidade de aplicação dos compostos de esponja visando à cura e ao tratamento de doenças.

O relacionamento entre esponjas e a medicina remete aos médicos alexandrinos e foi descrito pelo historiador romano Plínio. Médicos utilizavam esponjas saturadas com iodo para estimular a coagulação sanguínea ou com extratos de plantas para anestesiar pacientes. Esponjas embebidas em vinho puro eram colocadas no lado esquerdo do peito em casos de ataque cardíaco e utilizadas em compressas com urina para tratar picadas de animais venenosos. O uso de esponjas foi recomendado no tratamento de insolações, tonturas, fraturas ósseas, dores no estômago, doenças infecciosas e tumores testiculares, ou ainda como implantes depois de operações no peito (HOFRICHTER & SIDRI, 2001).

Desde o século 18, médicos russos, ucranianos e poloneses têm usado uma esponja de água doce por eles chamada Badiaga (figura 1). Seca, é empregada em massagens no peito ou costas de pacientes com doenças no pulmão, ou no local da dor em pacientes com

reumatismo (SCHRODER, 1942). Oficjalski (1937) descobriu que Badiaga não era realmente uma esponja, mas uma mistura de muitas esponjas de água doce que diferiam de acordo com a região. Na Polônia, correspondia a *Euspongilla lacustris*, *Ephydatia fluviatilis* e *Meyenia muelleri*. Enquanto na Rússia, Badiaga consistia na mistura de *Euspongilla lacustris*, *Ephydatia fluviatilis*, *Spongilla fragilis* e *Carterius stepanowi*. Atualmente, o xarope Stodal contendo *Spongia officinalis* é usado no Oriente para tratamento homeopático de tosse seca ou asmática (figura1).

Figura 1. Exemplos de drogas homeopáticas baseadas em extratos de esponja atualmente em uso (Badiaga e xarope Stodal).

Em 1951, foi isolado o primeiro arabino-nucleosídeo da esponja *Tectitethya crypta* (BERGMANN & FEENEY, 1950, 1951) utilizado como modelo para drogas anticâncer e antiinflamatória. O potencial deste novo ambiente a ser pesquisado chamou a atenção para a fauna marinha (PRADO *et al*., 2004; SIPKEMA *et al*., 2005). Contudo, a química de produtos naturais marinhos permaneceu incipiente até obter rápido desenvolvimento em 1980 e maturar nos anos 1990 (FAULKNER, 2000). Nos últimos anos, a ação farmacológica de compostos marinhos foi amplamente investigada. Estas moléculas abrangem atividades hemolíticas e de hemaglutinação (SEPCIĆ *et al*., 1997), antifúngicas e antibacterianas

(ZHENG *et al*., 2000), anticâncer, antiinflamatórias, de vesiculação do complexo de Golgi, de ação em proteínas motoras, como a actina, e de ação específica em proteínas e receptores (FAULKNER, 2000). Portanto, apresentam relevância não somente para a medicina, mas também no desenvolvimento de novas ferramentas para a biologia celular e molecular.

A justificativa para o interesse e os esforços na investigação de extratos e compostos biologicamente ativos provenientes de esponjas marinhas provém da biologia destes organismos.

1.2 Biologia das Esponjas

As esponjas pertencem ao Filo Porifera, importante grupo do Reino Animal por conter organismos modelo da transição entre unicelulares e pluricelulares. Os poríferos surgiram há mais de 550 milhões de anos, no período Pré-Cambriano, era Paleozóica. No Cambriano Inferior já existiam representantes de todos os grupos encontrados recentemente (BERGQUIST, 1978). O Filo Porifera está entre os maiores representantes do substrato duro bentônico, com respeito tanto ao número de espécies quanto à biomassa (SÀRA & VACELET, 1973).

O nome Porifera é justificado pela superfície perfurada por muitas aberturas pequenas, os poros. Cada poro é formado por um porócito, uma célula em forma de anel que se estende da superfície externa até a cavidade central, denominada átrio ou espongiocele. Internamente, a parede do corpo é revestida pelos coanócitos, as células flageladas típicas dos poríferos. Os coanócitos promovem a filtração da água capturando microorganismos e partículas alimentares nela presentes. Após a filtração, a água é expelida

para o meio externo através de uma abertura maior, chamada ósculo. A parede corporal é relativamente simples, com a superfície externa revestida de células achatadas, os pinacócitos, que constituem a pinacoderme. Estes pinacócitos secretam um material que fixa a esponja ao substrato. Por baixo da pinacoderme está o mesohilo, que consiste em uma matriz proteinácea gelatinosa que contém material esquelético e células amebóides. O mesohilo é equivalente ao tecido conjuntivo dos outros metazoários. O esqueleto é relativamente complexo e proporciona uma estrutura de sustentação para as células vivas do animal. A maioria das esponjas tem esqueleto formado por fibras de espongina juntamente com estruturas chamadas espículas, que se assemelham a pequenas agulhas cristalinas de sílica ou de carbonato de cálcio. O esqueleto que sustenta as esponjas é constituído por uma rede de espículas rígidas, fibras flexíveis e sedimentos externos, como areia. A combinação das dimensões, do tipo e da distribuição das espículas, bem como sua relação com o esqueleto fibroso, é a principal ferramenta utilizada para identificar esponjas (MOTHES *et al.*, 2003).

As esponjas são organismos filtradores capazes de ingerir partículas de tamanho entre 5 e 50 μm através de células do mesohilo e da pinacoderme, e micropartículas de 0,3 a 1 μm pelas câmaras de coanócitos (SIMPSON, 1984). Um espécime da esponja silicosa *Geodia cydonium* de 1 kg filtra 24.000 litros de água por dia (VOGEL, 1977). O alimento e o oxigênio para as esponjas são garantidos pela capacidade de bombeamento e filtração de grandes volumes de água (ZHANG *et al*., 2003). Portanto, não é surpreendente que possam viver em um ambiente pobre em nutrientes e que necessitem de um potente mecanismo de detoxificação.

Sésseis quando adultos, sua distribuição está condicionada à duração de seu curto período larval livre-natante, em geral de poucas horas. Devido a sua condição séssil e filtradora, tem sido freqüente o uso de esponjas como organismos-modelo no estudo de marcadores de potencial bioquímico (indução de oxidases de função múltipla) e níveis moleculares (expressão do gene codificador da proteína e do produto protéico) (MÜLLER, 1994; MÜLLER & MÜLLER, 1996), de resposta a choque térmico (MÜLLER *et al*., 1995; KOZIOL *et al*., 1995), a detergentes (ZAHN *et al*., 1977) e a metais pesados (BATEL *et al*., 1993). Recentemente, esponjas foram relatadas como um sistema capaz de detectar uma gama de poluentes de grupo desconhecido, os inativadores de bombas resistentes a multixenobióticos (KURELEC *et al.*, 1995; MÜLLER *et al*., 1996).

Como resultado de pressões evolutivas, muitos organismos, de ambientes terrestres e marinhos desenvolveram mecanismos de defesa química. A diversidade molecular dos organismos marinhos é atribuída a suas histórias evolutivas (BELARBI *et al*., 2003). O desenvolvimento de defesas químicas em organismos marinhos está atrelado e é direcionado pela intensa pressão de predação e competição (LIPPERT *et al*., 2004). A longa história de vida dos poríferos é bem sucedida porque as esponjas desenvolveram estratégias químicas para defesa contra predadores e competição por espaço (PROKSCH *et al.*, 2002). O metabolismo secundário das esponjas lhes confere uma capacidade adaptativa ao passo que aponta estes organismos como candidatos ideais para investigação farmacológica (FAULKNER, 2000). Adicionalmente, Porifera é um grupo monofilético (MÜLLER, 1995), característica que pode explicar a singularidade molecular encontrada nas esponjas.

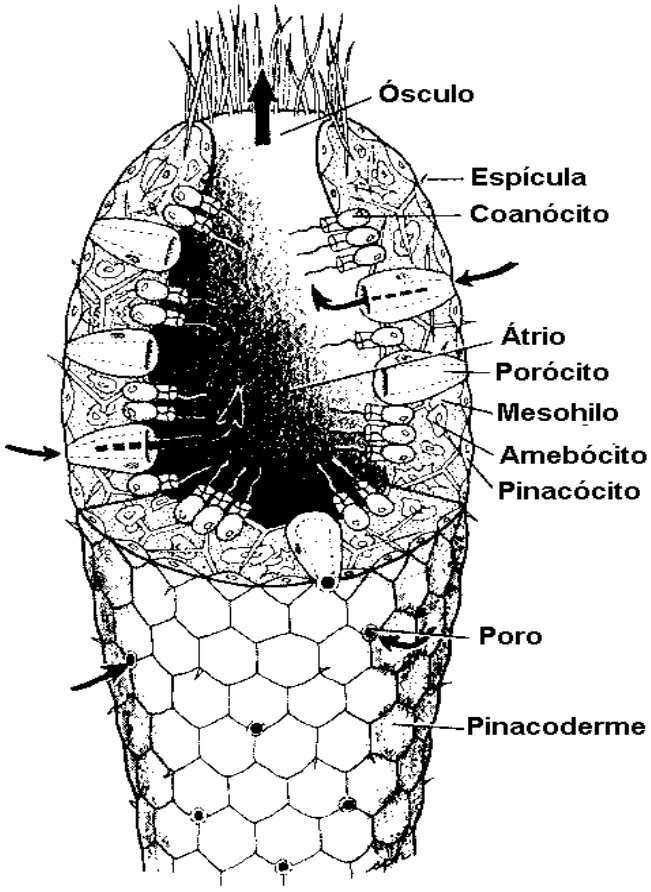


Figura 2. Estrutura básica de Porifera. Modificado de R. D. Barnes, 1991. As setas indicam o fluxo de água. Fonte: http://www.sfu.ca/~fankbone/v/lab02.html.

1.3. Esponjas e Bioatividades

Geralmente lembrada pela sua primitiva organização morfológica, a fauna existente é amplamente diversa em muitos outros aspectos, especialmente na sua constituição bioquímica (NEVALAINEN *et al*., 2004). Como são animais incapazes de movimento e vivem geralmente fixos ao fundo do mar, desenvolveram um sistema efetivo contra o estresse ambiental. As esponjas produzem toxinas e outros compostos para repelir e deter predadores (URIZ *et al*., 1996; PAWLIK *et al*., 2002), competir por espaços com outras

espécies sésseis (PORTER & TARGETT, 1988; DAVIS et al., 1991; BECERRO *et al*., 1997), para comunicação e proteção contra infecção.

Entre os animais marinhos, as esponjas têm a maior taxa de compostos citotóxicos descritos, presentes em mais de 10% das espécies investigadas (OSINGA *et al.*, 1998; ZHANG *et al*., 2003). Alguns destes compostos apontados em estudos recentes estão sob investigação ou mesmo sendo desenvolvidos como novos fármacos (FAULKNER, 2000; DA ROCHA *et al.*, 2001; SCHWARTSMANN *et al*., 2001). O princípio antiinflamatório manoalida de *Luffariella variabilis* já está disponível no mercado (MONKS *et al*., 2002). Em estudos pré-clínicos de fase I encontram-se os compostos KRN7000 de *Agelas mauritianus* e IPL 576092 de *Petrosia contignata* e, em fase II, o agente anticâncer halicondrina B de *Halichondria okadai*.

A cada ano centenas de novos compostos de origem marinha estão sendo descobertos. Dos 15.000 produtos marinhos descritos, as esponjas respondem por mais de 5.300 produtos diferentes. A considerável diversidade química das esponjas compreende além de nucleosídeos incomuns, terpenos, esteróis, peptídios cíclicos, alcalóides, ácidos graxos, peróxidos e derivados de aminoácidos halogenados (SIPKEMA *et al*., 2005). Entre os exemplos dessa singularidade molecular estão os alcalóides heteroaromáticos que bloqueam receptores adrenérgicos denomindados aaptaminas, isolados de esponjas marinhas do gênero *Aaptos* (GRANATO *et al*., 2000), e os sesterterpenos, originalmente encontrados em *Ircinia oros*, que apesar de constituírem o maior grupo molecular entre os metabólitos de esponjas, ocorrem menos freqüentemente nos demais grupos taxonômicos (FAULKNER, 2000).

O *screening* de extratos orgânicos de esponjas e organismos marinhos é uma prática comum para identificar compostos de importância biomédica (ELY *et al.*, 2004) e integra

respostas de todos os metabólitos presentes nestes organismos (KARBAN & MYERS, 1989; MARTÍN & URIZ, 1993; TURON *et al.*, 1996; BECERRO *et al.*, 1998), possibilitando uma visão mais holística da química ecológica das espécies que bioensaios usando metabólitos puros (HARPER *et al*., 2001). Recentes trabalhos com a utilização de extratos brutos de esponjas marinhas têm demonstrado propriedades antimicrobianas, antifúngicas e antiinflamatórias para algumas espécies do Caribe (NEWBOLD *et al*., 1999).

Entre os compostos isolados biologicamente ativos, a psammaplin A, por exemplo, possui propriedades anticâncer, através da inibição da replicação do DNA (THAKUR & MÜLLER, 2004). Uma relevante atividade antiviral contra HIV-1 foi observada em algumas avaronas e derivados avarol de *Dysidea cinerea* capazes de inibir a transcriptase reversa (HIRSCH *et al*., 1991) e em ácidos poliacetilênicos brominados inibidores de protease isolados de *Xestospongia muta* (PATIL *et al*., 1992). Além disso, foi demonstrada a inibição de CDKs, GSK-3β e CK1 por hymenialdisine (HD), um composto isolado de várias espécies de esponjas marinhas (MEIJER *et al*., 2000). Em particular, a fosforilação característica da proteína tau por GSK-3β foi completamente inibida por HD *in vivo*, sugerindo que este composto pode contribuir nos estudos de doenças neurodegenerativas como Alzheimer. Estudos realizados pelo Instituto Nacional do Câncer dos EUA retratam as esponjas como os organismos que mais produzem moléculas de alta singularidade com interesse farmacológico e potencial utilização em tratamentos de doenças como o câncer, AIDS e leucemias (KELECOM, 1991).

Em 2004, Berlinck e colaboradores publicaram uma revisão sobre os esforços na pesquisa de organismos marinhos. Neste trabalho foi ressaltada a existência de poucos estudos explorando a diversidade e a bioatividade de nossa fauna marinha. Os trabalhos

desenvolvidos com extratos de esponjas revelaram a existência de potencialidades: citotóxica e neurotóxica (RANGEL *et al.*, 2001), anticâncer, antiquimiotáxica e antimicrobianas (MONKS *et al*., 2002), de modulação de microtúbulos e ciclo celular (PRADO et al., 2004), genotoxicidade (AIUB *et al.*, 2006), antiviral contra herpes, adenovírus e rotavirus (SILVA *et al.*, 2006), antituberculose (AZEVEDO *et al.*, 2008a) and antiinflamatória e analgésica (AZEVEDO *et al.*, 2008b).

1.4 Esponjas e Propriedades Redox-Ativas

De todos os parâmetros bioquímicos estudados até agora, a investigação de propriedades antioxidantes de extratos de esponjas é insignificante. Mesmo apesar de Takamatsu e colaboradores (2003) já terem sugerido que produtos isolados de algas, cianobactérias e esponjas marinhas podem servir de protótipos para novos antioxidantes. Antioxidantes são compostos capazes de previnir, atrasar ou remover o dano causado por espécies reativas (HALLIWELL & GUTTERIDGE, 2007). O mecanismo da ação antioxidante é amplo, podendo ser devido tanto à captura (*scavenger*) ou à degradação de espécies reativas quanto à inibição do dano oxidativo.

Também são diversas as técnicas de determinação de capacidade antioxidante. As abordagens no estudo do potencial antioxidante em esponjas marinhas restringem-se à utilização do radical não-biológico $DPPH^{\bullet}$ (DUNLAP ET AL., 2003; TAKAMATSU *et al.*, 2003). Uma vez que se pode fazer quase qualquer composto químico exercer efeitos antioxidantes *in vitro* se escolhermos as condições apropriadas de ensaio (HALLIWELL & GUTTERIDGE, 2007), premissas para avaliação de capacidades antioxidantes foram

delineadas. Dentre as quais está convencionado que a avaliação do potencial antioxidante deve utilizar espécies reativas, radicais ou fontes de radicais biologicamente relevantes (PRIOR *et al.*, 2005).

As espécies reativas de oxigênio (ERO) são moléculas derivadas do oxigênio, e podem ter natureza radicalar ou não-radicalar. Um radical livre é uma molécula de existência independente, que tem elétrons desemparelhados e, por isso, é altamente reativa para oxidação ou redução de outras moléculas (SMITH *et al.*, 2005). O hidroxil ($^{\bullet}OH$) e o ânion superóxido ($O_2^{\bullet -}$) são exemplos de espécies radicalares, e o peróxido de hidrogênio (H_2O_2), bem como o ácido hipocloroso (HOCl), que são espécies não-radicalares. Um outro grupo denominado espécies reativas de nitrogênio (ERN) encerra os agentes oxidantes que possuem nitrogênio em sua composição. Nesta classe estão o radical óxido nítrico ($^{\bullet}NO$) e o peroxinitrito ($ONOO^-$).

Em organismos aeróbios, as ERO são subprodutos naturais do metabolismo celular, inclusive já relatadas em organismos aquáticos de vários níveis filogenéticos (GORDEEVA & LABAS, 2003), ou podem ser geradas em resposta a algum estresse (HENSLEY *et al*., 2000; CLEVELAND & KASTAN, 2000). Da mesma forma, algumas ERN, como monóxido de nitrogênio $(NO)^2$ e o $^{\bullet}NO$, são mensageiros essenciais participantes dos processos inflamatórios, regulatórios do tônus vascular, imunológicos e de neurotransmissão (BREDT & SNYDER, 1994; MICHEL & FERON, 1997). Em condições fisiológicas, há um equilíbrio entre a produção de tais moléculas e a defesa antioxidante celular. A defesa antioxidante celular compreende enzimas, como superóxido dismutase (SOD) e catalase (CAT), bem como compostos não-enzimáticos, como vitaminas e glutationa (GSH).

O estado de estresse oxidativo é caracterizado quando há um desbalanço entre a proporção de espécies reativas e a capacidade de defesa de um organismo. Devido a suas propriedades químicas, em geral, essas espécies possuem uma meia vida curta. Contudo, o seu potencial pode ser amplificado, uma vez que, ao aceitar elétrons para completar seu(s) orbital(is), as espécies reativas criam novos radicais e, por conseguinte, a instabilidade molecular é perpetuada em uma reação em cadeia.

ERO podem influenciar nos processos de carcinogênese e progressão do câncer através do dano a biomoléculas como proteínas, lipídios e DNA, e pela indução da expressão de uma gama de fatores envolvidos em transformação neoplásica (DAS, 2002). É sabido que células cancerosas estão em estresse oxidativo devido à superprodução de ERO (SATO *et al*., 1992). Muitos estudos associaram a malignidade tumoral à inflamação crônica, mas a sugestão de que $^{\bullet}$NO e seus produtos em tecidos inflamados podem contribuir ao processo de carcinogênese é recente e tem base em observações do potencial mutagênico endógeno dessa molécula (BOSCO, 1998). Por outro lado, embora muitas enzimas antioxidantes, como catalase e SOD, estejam negativamente reguladas em várias linhagens celulares, outras enzimas antioxidantes ou moléculas antioxidantes não-enzimáticas, como GSH, participam na defesa antioxidante das células cancerosas (SUN, 1990). Recentemente, a proteína supressora tumoral p53 foi apontada como reguladora da expressão de genes que têm como produtos enzimas ou moléculas antioxidantes, dado que acentua a importância do potencial de antioxidantes na profilaxia e tratamento do câncer (SABLINA *et al*., 2005).

Além do envolvimento do estresse oxidativo no câncer, sua participação em disfunções mitocondriais, como esclerose múltipla, na patogênese de doenças crônicas,

como *diabetes mellitus* (DURSUN *et al*., 2005), e na etiologia e progressão de doenças neurodegenerativas humanas, como Alzheimer e Parkinson (HALLIWELL, 2001; HALLIWELL, 2002; MOREIRA *et al.*, 2005; SULTANA *et al*., 2006), também já foi descrita. Danos oxidativos como a peroxidação de lipídios insaturados são associados à aterosclerose, inflamação, doença de Parkinson (MARZATICO *et al*., 1993), entre outras patologias.

A observação de que o estresse oxidativo esteja implicado em condições patológicas trouxe a idéia da utilização de antioxidantes como tratamento terapêutico. Algumas áreas da pesquisa médica evidenciaram em seus estudos algum efeito benéfico da intervenção com antioxidantes. Altas doses de α-tocoferol (2000U/dia) mostraram-se eficientes no atraso da deterioração de pacientes com doença de Alzheimer, embora não tenha afetado a progressão de estágios iniciais para tardios da doença (BLACKER, 2005). Portanto, a pesquisa de novos compostos antioxidantes para tratamento de doenças, como as neurodegenerativas, são áreas prioritárias (HALLIWELL, 2001; MOOSMANN & BEHL, 2002; MANDEL *et al*., 2005).

A sugestão de que esponjas marinhas possam modular as concentrações de espécies reativas advém de suas relações ecológicas. Foi observado que a esponja *Sycon* sp. produz altas taxas do radical superóxido sem necessidade de estímulo (PESKIN *et al*., 1998). Adicionalmente, o relato de que a esponja da Antártida *Haliclona dancoi* responde adaptativamente ao estresse pró-oxidante da simbiose com algas sugere a evolução de um mecanismo antioxidante em Porifera (REGOLI *et al*., 2004). A partir destas informações, a procura de propriedades antioxidantes nesses organismos torna-se pertinente.

2. OBJETIVOS

2.1 Objetivo geral

O objetivo do presente trabalho foi delinear um perfil de atividades redox *in vitro* de extratos aquosos de esponjas marinhas das espécies *Aaptos* sp., *Agelas* sp., *Chondrilla nucula*, *Cinachyrella alloclada*, *Cliona* sp., *Dragmacidon reticulatus*, *Guitarra sepia*, *Halichondria* sp. *Haliclona tubifera*, *Hyatella* sp., *Mycale arcuiris*, *Petromica citrina*, *Protosuberites* sp., *Raspailia elegans*, *Scopalina ruetzleri* amarela, *Scopalina ruetzleri* vermelha e *Tedania ignis*, e dos extratos orgânicos de esponjas marinhas das espécies *Axinella corrugata*, *Haliclona tubifera* e *Scopalina ruetzleri* vermelha, coletadas no litoral brasileiro.

2.2 Objetivos específicos

1) Verificar se os extratos possuem capacidade de captura (*scavenger*) de:

- radical peroxil ($ROO^{\bullet}$), através da técnica de Potencial Total de Atividade Antioxidante contra Radical (TRAP),
- radical hidroxil ($^{\bullet}OH$), pelo método de degradação oxidativa da 2-deoxirribose,
- radical óxido nítrico ($^{\bullet}NO$), gerado pelo nitroprussiato de sódio (SNP),
- radical ânion superóxido ($O_2^{\bullet -}$), através do ensaio de inibição da formação de adrenocromo, e

- H_2O_2, a partir da técnica do monitoramento da absorbância desta espécie reativa a 240nm.

2) avaliar a abilidade dos extratos em atuar contra a peroxidação lipídica induzida por:

- AAPH (dihidrocloridrato de 2,2'-azobis 2-metilpropionamidina),
- Fe^{2+}, e
- H_2O_2;

3) determinar o conteúdo de compostos fenólicos dos extratos de esponja marinha através do método de Folin-Ciocalteau; e

4) quantificar o conteúdo de tióis totais dos extratos pela reação de Ellman.

3 METODOLOGIA E RESULTADOS

Os resultados gerados nesta dissertação de mestrado, bem como a metodologia empregada para sua obtenção, estão dispostos no manuscrito entitulado *IN VITRO* ANTIOXIDANT PROFILE OF BRAZILIAN MARINE SPONGE EXTRACTS a ser submetido à publicação no periódico Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology.

IN VITRO ANTIOXIDANT PROFILE OF BRAZILIAN MARINE SPONGE EXTRACTS

Mariana Leivas Müller Hoff[1#], Guilherme Antônio Behr[1], Mario Luiz Conte da Frota Jr[1], Mirian Apel[2], Beatriz Mothes[3], Cléa Lerner[3], Amélia Terezinha Henriques[2], José Cláudio Fonseca Moreira[1]

[1]Centro de Estudos em Extresse Oxidativo, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul. [2]Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul. [3]Museu de Ciências Naturais-Porifera, Fundação Zoobotânica do Rio Grande do Sul. Brazil.

Correspondence author: Mariana L M Hoff, Centro de Estudos em Estresse Oxidativo (Lab. 32), Depto Bioquímica/UFRGS, Av. Ramiro Barcelos, 2600 Anexo, CEP 90035-003, Porto Alegre, RS, Brazil. Tel.: +55 51 33085578; FAX.: +55 51 33085535. E-mail address: hoffmlm@yahoo.com.br

Abstract

After a long time of absolute vegetal reign, marine sponges are reaching the top of bioactive natural providers. Despite several activities have been reported, just a few works have adressed the marine sponges antioxidant potentials. Here we describe the *in vitro* screening of 20 Brazilian marine sponges extracts for their capacity to scavenge reactive oxygen and nitrogen species and to protect lipids of oxidative damage induced by biologically relevant oxidants. The obtained data revealed that marine sponge extracts possess relevant antioxidant potential and also interesting redox properties, which need to be further studied. Crude extracts possess a metabolites mixture of the organisms and, therefore, may reflect their chemical ecology. For the first time, the *in vitro* antioxidant capacities evidenced may reflect marine sponge's biology and ecology. This study provided a set of feasible experimental trials to evaluate the *in vitro* redox activity of marine sponge extracts.

Introduction

Marine sponges are currently one of the most promising sources of bioactive compounds, which are secondary metabolites of incredibly diverse and unique chemical structures. Several reports

have compiled compounds hitherto isolated from marine sponges and other marine organisms (Faulkner, 2000; Zhang et al., 2003; Sipkema et al. 2005). The vast array of biological activities comprised in these reviews includes antifungic, antiviral, antibacterial, antimalarial, analgesic, antifouling, anti-inflammatory, and anticancer, as well as muscle relaxing and immunosuppressive effects. It has recently been published a review highlighting the efforts on the chemistry of marine organisms products in Brazil, in which researchers assure that Brazilian marine fauna remains practically unexplored (Berlinck et al., 2004).

General bioassays of crude extracts are a suitable approach to verify whether the species is active, when secondary chemistry of a given species is poorly known (Martí et al., 2003), and to guide the identification of biomedical important compounds in marine sponges and other organisms (Sepcić et al., 1997). Some crude extracts of Brazilian sponges have been assayed for cytotoxic and neurotoxic potential (Rangel et al., 2001), anticancer, antichemotactic and antimicrobial activities (Monks et al., 2002), microtubule and cell cycle modulation (Prado et al., 2004), genotoxicity (Aiub et al., 2006), antiviral action towards herpetic, adenovirus and rotavirus (Silva et al., 2006), anti-tuberculosis properties (Azevedo et al., 2008a) and anti-inflammatory and analgesic effects (Azevedo et al., 2008b). An overview in literature suggests that antioxidant potential in marine sponges was a neglected bioactivity.

Reactive species have physiological functions (Gruetter et al., 1981; Moncada et al., 1991; Rhee, 2006) and are also by-products of aerobic metabolism (Finkel, 2003; Balaban et al., 2005). Reactive species generation has also been reported in aquatic organisms (Gordeeva and Labas, 2003). ROS levels must be tightly and actively regulated in order to avoid increase in oxidative damaged biomolecules and subsequent impairment of physiological functions. Increased oxidative damage was observed in many organic dysfunctions, as increased levels of lipid peroxidation end-products, DNA and RNA base oxidation products and oxidative protein damage in neurodegenerative diseases (Halliwell, 2001; Halliwell, 2002; Moreira et al., 2005; Sultana et al., 2006) and high lipid TBARS and protein carbonyls parameters presented in septic shock patients (Goode et al. 1995; Andresen et al. 2008). Since the involvement of oxidative stress in pathologies has been reported, the suggestion that therapeutic antioxidant interventions might be beneficial has arisen. In this regard, the development of novel antioxidants, to treat neurodegenerative diseases, for example, is a major research area (Halliwell, 2001; Moosmann and Behl, 2002; Mandel et al., 2005) and studies which seek antioxidants prototypes are always welcome.

The few approaches on antioxidant potentials in extracts or compounds of marine organisms have only used DPPH$^{\bullet}$ radical assay (Dunlap et al., 2003; Takamatsu et al, 2003). Nevertheless, the *in vitro* assessment of antioxidant activity must resemble the scavenging capacity of a radical of biological relevance or originated from a biologically relevant source (Prior et al., 2005), as well as the ability to prevent or delay oxidative damage to biomolecules (Haliwell and Gutteridge, 2007). The aim of the present study was to perform a more complete and feasible antioxidant profile of 20 marine sponge extracts collected in Brazilian coastline. We looked for scavenging capacities of the reactive oxygen and nitrogen species peroxyl radical (ROO$^{\bullet}$), nitric oxide radical ($^{\bullet}$NO), hydroxyl radical ($^{\bullet}$OH), superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), and prevention of lipid oxidative damage induced by AAPH (2, 2-azobis[2-methylpropionamidine] dihydrochloride), Fe^{2+} and H_2O_2.

Materials and Methods

Sponge Material

Sponge samples were collected manually from exposed and semi-exposed habitats, at depths between 0.5 and 14 m, from the coasts of Brazilian states of Santa Catarina (SC), Paraíba (PB) or Pernambuco (PB). The taxonomic identification was based on analysis of skeletal slides and dissociated spicule by scanning electron microscope. The specimens were deposited in the collection of Museu de Ciências Naturais – Porifera (MCNPOR) of the Fundação Zoobotânica do Rio Grande do Sul, Brazil. Table 1 summarizes the Brazilian marine sponge extracts here studied.

Extracts Preparation

To obtain aqueous extracts, sponge materials were ground together with sand during 30 minutes for three times. The samples resulted of each procedure were placed together in order to form only one extract, which was subsequently filtered and freeze-dried. The remaining material followed an organic extraction consisting of five sequentially extractions with a methanol:toluene mixture (3:1) and maceration over five days. The resulting extract solution was then filtered and concentrated in vacuum at -40°C. Right before each assay, the organic or aqueous extracts were suspended in adequate buffer – never at concentrations higher than 4mg/ml. A curve of concentration of extracts in µg/ml range was performed in each assay, and the controls, containing only extracts in the respective buffers of each approach, were always carried out to evaluate whether colour could interfere.

Chemicals

AAPH (2,2-azobis[2-methylpropionamidine] dihydrochloride), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), 2-deoxyribose, glycine (aminoacetic acid), Folin-Ciocalteau (phenol reagent), Griess reagent, sodium nitroprusside, 2-thiobarbituric acid (4,6-dihydroxypyrimidine-2-thiol), hydrogen peroxide, tannic acid, NBT (nitrotetrazolium blue chloride), xanthine, xanthine oxidase, adrenaline, catalase and SOD (superoxide dismutase) were purchased from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid) was purchased from Aldrich Chemicals Co. (Milwaukee, WI, U.S.A.). Methanol, toluene and acetic acid were purchased from Merck Chemicals KGaA (Darmstadt, Germany).

Total Radical-Trapping Antioxidant Parameter (TRAP)

TRAP assay was used to determine the capacity of extracts to trap a flow of water-soluble peroxyl radical produced at constant rate, through thermal decomposition of AAPH (Lissi et al., 1992). Briefly, it was added 10µl of the test samples (sponge extracts or trolox) to 4ml of the free radical source (AAPH 10mM) in glycine buffer (100mM) pH 8.6 and 10µl luminol (4mM) as external probe to monitore radical production. The chemiluminescence generated was detected by (Wallac 1409 DSA Liquid Scintillation Counter, Wallac Oy, Turku, Finland) as counts per minute (CPM). The TRAP of extracts were evaluated for instantaneously inhibition of chemiluminescence by TAR index, and along 20 minutes as area under curve (AUC). Total antioxidant reactivity (TAR) was calculated as the ratio of light intensity in absence of samples (I^0)/ light intensity right after sample addition (I) (Lissi et al., 1995) and expressed as percent of inhibition. Area under curve (AUC) of 10µg/ml extracts, trolox 2µM, solvent (water), and radical basal production was achieved by software (GraphPad Software Inc., San Diego, CA, USA - version 5.00) as described by Dresch et al. (2009).

Scavenging Activity of Nitric Oxide

Nitric oxide ($^{\bullet}$NO) was generated from spontaneous decomposition of sodium nitroprusside in 20mM phosphate buffer (pH 7.4). Once generated, $^{\bullet}$NO interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction (Green et al., 1982). In a 96-well microplate, 20mM sodium nitroprusside in phosphate buffer and sponge extracts, at different concentrations, were incubated at 37°C for 1h, and then 20µl Griess reagent were added. After 15 minutes of reaction the absorbance of chromophore was determined at 540nm in an ELISA plate reader. The effect of 100µg/ml extracts was measured considering nitrites generation absorbance of system

(20mM sodium nitroprusside and solvent) as 100%, and results were presented as percent of system.

Hydroxyl Scavenging Activity

The $^{\bullet}OH$ formation from Fenton reaction was quantified using 2-deoxyribose oxidative degradation (Hermes-Lima et al., 1994). The principle of the assay is the quantification of a 2-deoxyribose degradation product, malondialdehyde, by its condensation with 2-thiobarbituric acid (TBA). Briefly, reaction was started by the addition of Fe^{2+} (6µM final concentration) to solutions containing 5mM 2-deoxyribose, 100mM H_2O_2 in 20mM phosphate buffer (pH 7.2). To measure the antioxidant activity against $^{\bullet}OH$, different concentrations of extracts were added to system before Fe^{2+} addition. Reactions were carried out for 15min at room temperature and were stopped by the addition of 4% phosphoric acid (v/v) followed by 1% TBA (w/v, in 50mM NaOH). Solutions were boiled for 15min at 95°C, and then cooled at room temperature. The absorbance was measured at 532nm.

Catalase-like Activity

Catalase-like activity was verified by the same method described for assaying the enzyme activity (Aebi, 1984). Hydrogen peroxide diluted in 0.02M phosphate buffer (pH 7.0), to obtain a 5mM final concentration, was added to the microtiter plate wells, in which solutions with different concentrations of extracts were already placed. The plate was then immediately scanned in an ELISA plate reader at 240 nm every 15 seconds for 5min at 37°C. Catalase activity was monitored based on the rate of decomposition of hydrogen peroxide, which is proportional to the reduction of the absorbance at 240nm. The catalase-like profile of 100µg/ml extracts was displayed and the respective AUC was calculated by software.

Superoxide Anion Radical ($O_2^{\bullet-}$) Assays

SOD-like activity Superoxide anion scavenging activity was evaluated by measuring the rate of inhibition of superoxide-mediated adrenaline auto-oxidation to adrenochrome as described previously (Misra and Fridovich, 1972). Fifty microliters of the tested fraction were mixed with 200µl of 50mM glycine buffer (pH 10.2) and 5µl of native catalase 100 U/ml. Superoxide generation was initiated by addition of 2mM adrenaline (5µl) and adrenochrome formation was monitored at 480nm for 5min at 32°C. Superoxide production was verified by monitoring the reaction curve of samples and measured as AUC.

NBT Reducting Ability The effect of sponge extracts on NBT reduction to a blue formazan by $O_2^{\bullet-}$ was proceeded according to Beauchamp and Fridovich (1971), with a few modifications. Briefly, 50µl 0.4mM xanthine and XOD (0.1U/ml) (positive control) or 50µl of extracts were mixed with 200µl 0.24mM NBT (50mM glycine buffer, pH 10.2, 0.1mM EDTA) and incubated at 37°C for 30min. The NBT reducing activity of 100µg/ml extracts and negative (only NBT in buffer) and positive controls were displayed as curves and values of AUC.

TBARS Assay

A modified TBARS protocol from Esterbauer and Cheeseman (1990) was used to measure the antioxidant capacity of extracts to prevent lipid peroxidation induced by 5mM AAPH, 10µM $FeSO_4$, or 5mM H_2O_2. Oxidants and sponge extracts or Trolox in different concentrations were added to liposome preparations (egg yolk 1% w/v, 20mM phosphate buffer, pH 7.4, sonicated 10s in potency 4) incubated for 1h at 37°. Then 0.3ml samples were centrifuged with 0.6ml trichloroacetic acid (20%) at 1200***g*** for 10min. A 0.5ml of supernatant aliquot was mixed with 0.5ml TBA (0.67%) and heated at 95°C for 20min. After cooling, samples absorbance was measured using a spectrophotometer at 532nm. Results were expressed as the percent of damage inhibition of 100µg/ml extracts, considering the damage of each lipoperoxidative inducer as 100%.

Phenolic Content

Phenolic content was determined by an adapted colorimetric assay of Singleton and Rossi (1965). Solutions of extracts and tannic acid were prepared immediately before use and curves (10-100µl) were tested by Folin-Ciocalteau reaction, which has 1N Folin reagent and satured solution Na_2CO_3. Absorbance was read 10min later at 725nm with an ELISA microplate reader and the phenolic content was expressed as µg tannic acid equivalents/100µg extract (TAE).

Thiol Content

The levels of thiol (SH) content in samples were achieved by a procedure based on Ellman's publishing (1959). Briefly, 10µl of 10mM 5,5'-dithionitrobis 2-nitrobenzoic acid (DTNB) were added to a 200µl PBS 10mM mixture containing extract concentration curves. After 60min incubation at 25°C, absorbance was determined in spectrophotometer at 412nm. A cysteine curve was carried out as pattern and the thiol content of extracts was expressed as µg cysteine equivalents/100µg extract (CE).

Statistical analysis

Results were compiled in SPSS Data Editor (version 15.0, SPSS Incorporation, Chicago, USA). One way Analysis of Variance (ANOVA) was performed on data, and when differences were significant at $p<0.05$, a Duncan Test to compare groups was applied. SPSS software has also provided bivariate correlation analysis achieved by Pearson's coefficient, at levels of significance of $p<0.05$ or $p<0.01$.

Results

Total radical-trapping antioxidant potential (TRAP)

Total radical-trapping antioxidant potential (TRAP) assay consists of a peroxyl radical generating system, when reacting with Luminol, results in chemiluminescence detected by a liquid cintilator. If a sample possesses peroxyl scavenging capacity, the chemiluminescence decreases because there will be no or less radical to react with Luminol. TAR analysis showed that 10µg/ml of sponge extracts instantaneously decreased light emission, except *S. ruetzleri* amarela organic extract (table 2). Additionally, AUC parameter showed that the marine sponge extracts have also scavenged $ROO^{\bullet}$ during the elapsed time, except *M. arcuiris*, *P. citrina* and *R. elegans* aqueous extracts and *S. ruetzleri* amarela organic extract. TRAP analysis showed that the aqueous extract of *Agelas* sp. and the organic extract of *A. corrugata* were efficient in instantaneous scavenging of $ROO^{\bullet}$ and also they were more potent than 2µM Trolox (table 2).

Nitric Oxide Radical ($^{\bullet}$NO) Scavenging Capacity

The nitric oxide radical donor sodium nitroprusside (SNP) was employed to test whether the extracts could scavenge $^{\bullet}$NO and consequently decrease nitrite formation, which is generated by reacting nitric oxide and dissolved O_2. At 100µg/ml the aqueous extracts of *G. sepia*, *M. arcuiris*, *S. ruetzleri* vermelha, *T. ignis*, *S. ruetzleri* amarela, *C. nuculla*, *Agelas* sp., and the organic extracts of *H. tubifera*, *S. ruetzleri* amarela and *A. corrugata* were able to prevent nitrite formation suggesting a $^{\bullet}$NO scavenging activity (table 2).

Hydroxyl Radical ($^{\bullet}$OH) Scavenging Capacity

A Fenton reaction, using hydrogen peroxide and Fe^{+2} as substrates, was carried out to produce hydroxyl radical, which oxidizes 2-deoxyribose. None of the studied marine sponge extracts up to the concentration of 100µg/ml were able to significantly inhibit the oxidative damage induced by $^{\bullet}$OH to 2-deoxyribose (data not shown).

Catalase-like Activity

The extracts were submitted to an assay for H_2O_2 scavenging activity, in which the H_2O_2 absorbance at 240nm was monitored along the time. It was observed that *Protosuberites* sp., *S. ruetzleri* vermelha, *D. reticulatus*, *C. nucula*, *C. alloclada* and *Hyatella* sp. aqueous extracts have decreased the absorbance, which could indicate a catalase-like activity (fig. 1).

Superoxide Anion Radical ($O_2^{\bullet-}$) Assays

To evaluate whether the extracts could atenuate the adrenaline auto-oxidation mediated by superoxide anion radical, a reaction containing 100µg/ml extracts was monitored. If extracts were superoxide radical scavengers or if they had SOD-like activity, they would be able to delay adrenaline auto-oxidation, and consequently prevent adrenochrome formation and decrease absorbance at 480nm. Surprisingly, none of them could reduce adrenochrome generation. In fact, *S. ruetzleri* vermelha, *R. elegans*, *Cliona* sp., *T. ignis*, *G. sepia* and *Hyatella* sp. aqueous extracts increased absorbance, indicating enhancement in adrenaline auto-oxidation (fig. 2). To confirm whether extracts could generate $O_2^{\bullet-}$, they have been tested by a reaction with nitroblue tetrazolium (NBT), a yellow compound that, when reduced by superoxide radical, turns into blue chromogen monitored at 560nm. The aqueous extracts of *R. elegans*, *Cliona* sp., *T. ignis*, *G. sepia*, *Halichondria sp*., *Agelas* sp., and *Aaptos* sp., and the organic extract of *A. corrugata* have increased the absorbance at 560nm (fig. 3).

AAPH-induced Lipid Peroxidation

AAPH induced lipid peroxidation was prevented by *A. corrugata* organic extract and *Agelas* sp., *Hyattella* sp., *Halichondria* sp., *C. alloclada*, *Cliona* sp. and *R. elegans* aqueous extracts.

Fe^{2+}-induced Lipid Peroxidation

Fourteen of the twenty marine sponge extracts studied were able to prevent Fe^{2+} induced lipid peroxidation, nevertheless, around 85% (12/14) was weak preventers, protecting only at higher concentrations than 100µg/ml (table 2). *C. alloclada* aqueous extract had a more potent capacity, inhibiting 24.23% of lipid damage induced by Fe^{2+}.

H_2O_2-induced Lipid Peroxidation

In a co-incubation of H_2O_2 and 100µg/ml *Protosuberites* sp., *C. nucula* and *C. alloclada* aqueous extracts, the lipid damage inhibition observed was of 66.27, 59.41 and 52.57%, respectively (table 2). *T. ignis*, *S. ruetzleri* vermelha and *Aaptos* sp. aqueous extracts were able to protect against lipid peroxidation only at concentrations higher than 100µg/ml. Trolox-was not able to prevent lipid peroxidation induced by H_2O_2 up to 100µM the highest concentration tested.

Phenolic Content

The phenolic content of extracts was assessed by Folin-Ciocalteau reaction and it was expressed as tannic acid equivalents (TAE) (table 3). The marine sponge extracts that have higher µg of tannic acid per 100µg of extract were *Agelas* sp., *Guitarra sepia* and *Axinella corrugata*.

Thiols Content

The SH content values of extracts obtained by Ellman's reaction are displayed on table 3. The thiol content ranged between 0.007 to 0.4µg CE/100µg of extracts. The highest content was observed in the the aqueous extracts of *Agelas* sp., *Aaptos* sp., *C. alloclada*, and *S. ruetzleri* amarela. The lowest content was observed in the organic extract of *A. corrugata*, and S. ruetzleri amarela, and in the aqueous extracts of *C. nucula*, *D. reticulatus*, *G. sepia*, *Halichondria* sp., *Hyatella* sp., *Protosuberites* sp., and *R. elegans*.

Correlation Analysis

The statistically significant correlations that have been established amongst the results were displayed on table 4. Phenolic content and protective effect on Fe^{2+} induced lipid peroxidation correlation almost has reached significance level and, for this reason, was also included.

Discussion

Phenolic compounds are classically reported as antioxidants. Several works on plant extracts have attributed antioxidant activity assessed by TRAP to their phenolic content determined by Folin-Ciocalteau reaction (Netto Benetti et al., 2007; Silva et al., 2007; Kappel et al., 2008). A correlation analysis of both TRAP indexes, TAR and AUC, and phenolic content of marine sponge extracts corroborates the literature findings, which suggests the involvement of phenolic compounds in the noticed antioxidant activity against peroxyl radical. Despite being animals, phenolic compounds have already been reported in Porifera, and just a few articles have investigated these compounds antioxidant capacity (Takamatsu et al., 2003; Utkina et al., 2004). Additionally, the protection against AAPH-induced lipid peroxidation of extracts correlates to the phenolic content and to both TRAP indexes. As the mechanism of AAPH induced lipid damage relies on its property to decompose and form $ROO^{\bullet}$, we propose that the lipid peroxidation preventive mechanism of the extracts is their $ROO^{\bullet}$ scavenging activities, which are dependent on their phenolic compounds. Phenolics can also have a protective effect by acting as potent metal chelators (Hanasaki et al., 1994). Although this prevention could not be statistically significant

correlated with the phenolic content of extracts, phenolic compounds contribution to the prevention of Fe^{2+}-induced lipid damage can not be discarded.

In the other hand, it was outlined a correlation between thiol content and lipid prevention of oxidative damage induced by Fe^{2+}. Thiols are known to have critical role in life by maintanance of cellular redox potentials and protein thiol-disulfide ratios, as well as by the protection of cells from reactive oxygen species. Gamma-glutamylcysteine (GSH) is the predominant thiol in eukariotyc organisms (Newton and Fahey, 1995). Sponges pump large volume of water and therefore, face high expostition to xenobiotics (Reiswig, 1971; Verdenal et al., 1990), so they require GSH to metabolize these compounds. De Flora et al. (1995) have observed that GSH levels of sponges was higher than observed in rat liver. GSH can act as an antioxidant as well as react with adventitious occuring metals, despite GSH beeing auto-oxidized in the presence of heavy metals, such as copper and iron, to form disulfides and peroxides (Tsen and Tappel, 1958; Sundquist and Fahey, 1989).

In general, 100µg/ml sponge extracts have been able to avoid lipid peroxidation in a range between 8 to 25%. This protection can not be considered ineffective, although it seems weak. In marine environment, iron is mainly found as insoluble oxides of Fe(III), which might cycle to the obtaintion of soluble, bioavailable Fe(II) (Byrne and Kester, 1976), hence protection levels of extracts here evidenced may be sufficient and adequate to sponges deal with this metal in natural environment.

Thiols, mainly GSH, have the ability to react with $^{\bullet}NO$ to form S-nitrosothiols. Trapping $^{\bullet}NO$ from environment would decrease nitrites formation, representing a nitric oxide radical scavenging potential. We could not establish a relation between the thiol content and $^{\bullet}NO$ scavenging property of extracts. Even though it seems a contradiction, there are data asserting the S-nitrosothiols labile nature and easily dissociating feature in the presence of NO (Ignarro et al., 1981), which may explain the lack of correlation here evidenced. Nitric oxide sinthase activity has been detected in demosponges (Giovine et al., 2001). The same work has presented an increase in $^{\bullet}NO$ production followig heat stress. The rapid response NOS after heat stress stimulation suggests $^{\bullet}NO$ may act as a molecular messenger in Porifera. In these circumstances, $^{\bullet}NO$ scavenging potential could be beneficial to minimize oxidative damage or to modulate nitric oxide signalling pathways.

Furthermore, our screening failed in detecting $^{\bullet}OH$ scavenging capacity through 2-deoxyribose oxidative degradation method. Hydroxyl radical is a very reactive oxygen species which could interact fast with almost any adjacent biomolecule. In order to prevent an oxidative damage induced by $^{\bullet}OH$, the scavenger compound must be closer to the site of formation before hydroxyl generation and at high concentrations to compete with the surrounding biomolecules for a $^{\bullet}OH$ reaction (Huang et al., 2005). In this regard, some authors consider more relevant to evaluate the antioxidant scavenging activity against reactive species that could generate $^{\bullet}OH$, as $O_2^{\bullet-}$ and H_2O_2, than this radical itself.

The evaluation of hydrogen peroxide counteraction have revealed that marine sponge extracts have H_2O_2 scavenging capacities and these abilities have reflected a protective effect on lipids for *Protosuberites* sp., *S. ruetzleri* vermelha, *C. nucula* and *C. alloclada* aqueous extracts. It was possible to correlate both effects of H_2O_2 protection, which suggests that the mechanism of lipid oxidative damage prevention observed for these extracts may probably be due to their H_2O_2 scavenging actions. Endogenously produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase (Wang and Ballatori, 1998). At once only non-enzymatic properties were evaluated, it was not possible to establish a relation between thiols content and H_2O_2 scavenging activity of the studied extracts. Sponges often have associated microbial populations (Lee et al., 2001; Richelle-Maurer et al., 2003). Symbionts include archaea, bacteria, cyanobacteria, and microalgae (Bewley and Faulkner, 1998; Lee et al., 2001; Proksch et al., 2002). The success of a symbiotic relationship of sponges and cyanobacterias requires that sponges be able to deal with ROS generated by these photosyntetic bacteria. The activity of antioxidant enzymes SOD, catalase, glutathione S-transferase, and glutathione reductase of the Antartic sponge *Haliclona dancoi* were enhanced in the summer, when it was observed higher levels of simbiotic diatoms (Regoli et al., 2004). Environmental changes, such as elevated solar irradiation and temperature, can elicit bleaching process, and therefore, H_2O_2 leakage from the symbiont cell (Tchernov et al., 2004). In this regard, sponge abilities to scavenge and protect lipids from peroxidation induced by H_2O_2 are needed.

Adrenaline auto-oxidation and NBT reducing reaction are known to implicate an $O_2^{\bullet-}$ involving mechanism. *S. ruetzleri* vermelha, *R. elegans*, *Cliona* sp., *T. ignis*, *G. sepia*. aqueous extracts increased acceleration in adrenaline auto-oxidation rates and also were able to react with NBT, which is concerning with an $O_2^{\bullet-}$ involving mechanism. Molecular non-enzymatic generation of

superoxide radical has already been reported, as in the hydroquinone-quinone cycle. It is known that the most important reaction of quinones relies in their redox property to reversibly reduce to the correponding hydroquinones via semiquinone free radicals (Bentley and Campbell, 1974). Sesquiterpenoids quinones and hydroquinones are metabolites commonly found in Porifera from the families Dysideidae, Thorectidae, and Spongiidae of the order Dictyoceratidae (Rodriguez et al., 1992). Inumerous pharmacologically relevant activities were described for these compounds, as antileukemic (Müller et al., 1985) and anti-HIV (Loya et al., 1990). Additionally, the quinone ring seems to be essential to citotoxic and hemolytic activities (Prokof'eva et al., 2004). It has already been proposed an ecological pourpose for quinone cycle to marine sponges. The citotoxic metabolites aeroplysinin-1 and a related dienone, which have been presented a semiquinone radical eletron spin paramagnetic ressonance (EPR) spectrum, are assumed to be released as soon as the sponge is damage, repelling predators (Koulman et al., 1996).

Nonetheless, the data obtained by NBT and adrenaline approaches did not completely converged. The lack of correlation between them comes from the fact that most extracts reacted in only one of the assays. However, a correlation between NBT assay and phenolic content was observed. Phenol measurement occurs in alkaline pH, in which phenol deprotonate to anion phenolate and bind to Folin-Ciocalteau reagent (Huang et al., 2005). That is the reason why some authors refer to Folin-Ciocalteau method as a reducing capacity assay. Hydroquinones can deprotonate to a semiquinone in alkaline medium, in which NBT reducing reaction was conducted. Thus there is a possibility that the results here obtained demonstrate the reducing potential of extracts, which therefore, depends on phenolic content of the extracts, and it also supports the idea of hidroquinones and quinones presence in the extracts. Hydroquinones are probably able to donate electrons and, therefore, have antioxidant capacity (Tziveleka et al., 2002). Hydroquinones have already been isolated from *Axinella polipoides* (Cimino et al., 1974). The organic extract of *Axinella corrugata*, a sponge belonging to the same genus, has equally reduced NBT as xanthine+xanthine oxidase. These data support the suggestion of hydroquinones in the studied marine sponge extracts.

The marine sponge extracts studied posses abilities to scavenge $^{\bullet}NO$, H_2O_2, and $ROO^{\bullet}$, and to prevent lipid peroxidation induced by H_2O_2, $ROO^{\bullet}$ e Fe^{2+}. In some extent, these reactive oxygen and nitrogen species are involved in neurologic dysfunctions. The brain has particular susceptibility to oxidative damage due to (1) the throughout presence of iron in the brain (Burdo and Connor, 2003, Zecca et al., 2004), (2) the brain metabolism generates a lot of H_2O_2 by SOD

and flavoproteins, such as monoamine oxidases (MAO) A and B (Gal et al., 2005) and, probably, CAT cannot deal with all H_2O_2 generated (Halliwell, 2006), (3) the activated microglia produce ROS, interleukin-1 and -6, and tumor necrose factor α (TNF- α), (4) the citokines release can lead microglia and astrocytes to produce more ROS and inducible-NOS (iNOS), and hence excess $^{\bullet}NO$ (Duncan and Heales, 2005), and (5) the neuronal membrane lipids be rich in higly poliinsatured fatty acid side-chains, which are prone to lipid peroxidation. These reasons point out that marine sponge extracts are apparently an insteresting source for searching antioxidants, at least for the central nervous system. Hence, the mechanisms and compounds related to antioxidant potential in these marine sponge extracts must be better elucidated.

This article provides a feasible experimental design methodology to determine redox properties in sponge extracts, and also suggests a guide to further analysis in the Brazilian sponge extracts studied. The results of general bioassays of crude extracts integrate the response of all the metabolites present in the organisms (Karban and Mayers, 1989; Martín and Uriz, 1993; Turon et al., 1996; Becerro et al., 1998) and give a more holistic view of the chemical ecology of the species than bioassays using pure metabolites (Harper et al., 2001). These data provide stimulating results in the searching for novel antioxidants in marine sponges, at the same time, we have attempted to discuss the antioxidant capacities here reported in closer relation to, and as a reflect of, biological and ecological interactions of marine sponges.

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil). The authors thank all laboratory colleagues for technical assistance. We also thank Ms. Calhandra Pinter de Souza Santos for language revision.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol 105,121-126.

Aiub, C., Giannerini, A., Ferreira, F., Mazzei, J., Stankevicins, L., Lobo-Hadju, G., Guimarães, P., Hadju, E., Felzenswalb, I., 2006. Genotoxic evaluation of extracts from Aplysina fulva, a brazilian marine sponge. Mutat Res 611, 34-41.

Andresen, M., Regueira, T., Bruhn, A., Perez, D., Strobel, P., Dougnac, A., Marshall, G., Leighton, F., 2008. Lipoperoxidation and protein oxidative damage exhibit different kinetics during septic shock. Mediators Inflamm 2008,168652.

Azevedo, L.G., Muccillo-Baisch, A.L., Figueira, D.de M., Boyle, R.T., Ramos, D.F., Soares, A.D., Lerner, C., Silva, P.A., Trindade, G.S., 2008a. Comparative cytotoxic and anti-tuberculosis activity of *Aplysina caissara* marine sponge crude extracts. Comp Biochem Physiol C Toxicol Pharmacol 147, 1, 36-42.

Azevedo, L.G., Peraza, G.G., Lerner, C., Soares, A., Murcia, N., Muccillo-Baisch, A.L., 2008b. Investigation of the anti-inflammatory and analgesic effects from an extract of *Aplysina caissara*, a marine sponge. Fundam Clin Pharmacol 22, 5, 549-56.

Balaban, R. S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants and aging. Cell 120, 483–495.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to polyacrylamide gels. Anal Biochem 44, 276-287.

Becerro, M.A., Paul, J.V., Starmer, J., 1998. Intracolonial variation in chemical defenses of the sponge *Cacospongia* sp. and its consequences on generalist fish predators and the specialist nudibranch predator *Glossodoris pallida*. Mar Ecol Prog Ser 168, 187-196.

Bentley, R., Campbell, I.M., 1974. In: Patai, S. (Ed.), The Chemistry of the Quinonoid Compounds. John Wiley & Sons, London, 683-735. Part 2.

Berlinck, G.S, Hadju, E., da Rocha, R.M., de Oliveira, J.H.H.L., Hernández, I.L.C., Seleghim, M.H.R., Granato, A.C., de Almeida, E.V.R., Nuñez, C.V., Muricy, G., Peixinho, S., Pessoa, C., Moraes, M.O., Cavalcanti, B.C., Nascimento, G.G.F., Thiemann, O., Silva, M., Souza, A.O., Silva, C.L., Manarini, P.R.R., 2004. Challenges and rewards of research in marine natural products chemistry in Brazil. J Nat Prod 67, 510-522.

Bewley, C.A., Faulkner, D.J., 1998. Lithistid sponges: star performers or hosts to the stars?. Angew Chem Int Ed Engl 37, 2162–2178.

Burdo, J.R., Connor, J.R., 2003. Brain iron uptake and homeostatic mechanisms: an overview. Biometals 16, 63-75.

Byrne, R.H., Kester, D.R., 1976. Solubility of hydrous ferric oxide and iron speciation in seawater. Mar Chem 4, 255-274.

Cimino, G., De Stefano, S., Minale, L., 1974. Occurrence of hydroxyhydroquinone and 2-aminoimidazole in sponges. Comp Biochem Physiol B Biochem Mol Biol 47, 4, 895-897.

de Flora, S., Bagnasco, M., Bennicelli, C., Camoirano, A., Bojnemirski, A., Kurelec, B., 1995. Biotransformation of genotoxic agents in marine sponges. Mechanisms and modulation. Mutagenesis 10, 357-364.

Dresch, M.T., Kappel, V.D., Rossato, S., Biegelmeier, R., Hoff, M.L.M., Mayorga, P., Zuanazzi, M., Henriques, A.T., Moreira, J.C.F., 2009. Optimization and validation of an alternative method to evaluate total reactive antioxidant potential. Anal Biochem doi:10.1016/j.ab.2008.10.036.

Duncan, A.J., Heales, S.J., 2005. Nitric oxide and neurological disorders. Mol Aspects Med 26, 67-96.

Dunlap, W., Llewellyn, L., Doyle, J., Yamamoto, Y., 2003. A microtiter plate assay for screening antioxidant activity in extracts of marine organisms. Mar Biotechnol 5, 294-301.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch Biochem Biophys 82, 70-77.

Esterbauer H., Cheeseman K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 186, 407-421.

Faulkner, D.J., 2000. Highlights of marine natural products chemistry (1972-1999). Nat Prod Rep 17, 1-6.

Finkel, T., 2003. Oxidant signals and oxidative stress. Curr Opin Cell Biol 15, 247-254.

Gal, S., Zheng, H., Fridkin, M., Youdim, M.B., 2005. Novel multifunctional neuroprotective iron chelating monoamine oxidase inhibitor drugs for neurodegenerative diseases. *In vivo* selective brain monoamine oxidase inhibition and prevention of MPTP-induced striatal dopamine depletion. J Neurochem 95, 79-88.

Goode, H.F., Cowley, H.C., Walker, B.E., Howdle, P.D., Webster, N.R., 1995. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Critical Care Medicine 23, 4, 646–651.

Gordeeva, A.V., Labas, I., 2003. Reactive oxygen species generation by external surfaces of aquatic organisms. Tsitologiia 45, 3, 284-289.

Giovine, M., Pozzolini, M., Favre, A., Bavestrello, G., Cerrano, C., Ottaviani, F., Chiaramtimi, L., Cerasi, A., Cangiotti, M., Zocchi, E., Scarfi, S., Sarà, M., Benatti, U., 2001. Heat stress-activated, calcium-dependent nitric oxide synthase in sponges. Nitric Oxide 5, 5, 427-431.

Green L.C., Wagner D.A., Glogowski J., Skipper P.L., Wishnok J.S., Tannenbaum S.R., 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 126, 131-138.

Gruetter, C.A., Gruetter, D.Y., Lyon, J.E., Kadowitz, P.J., and Ignarro, L.J., 1981. Relationship between cyclic guanosine 3':5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 219, 181-186.

Halliwell, B., 2002. Hypothesis: proteasomal dysfunction: a primary event in neurodegeneration that leads to nitrative and oxidative stress and subsequent cell death. Ann N Y Acad Sci 962, 182-194.

Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we know? J Neurochem 97, 1634-1658.

Halliwell, B., 2001. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 18, 685-716.

Halliwell, B., Gutteridge, J.M.C., 2007. Free Radical biology and medicine. 4th Ed. Oxford University Press Inc., New Iork, 871 p.

Hanasaki Y., Ogawa S., Fukui S., 1994. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. Free Radic Biol Med 16, 845-850.

Harper, M.K., Bugni, T.S., Copp, B.R., James, R.D., Lindsay, B.S., Richardson, A.D., Schnabel, P.C., Tasdemir, D., Vanwagoner, R.M., Verbitski, S.M. Ireland, C.M., 2001. Introduction to the chemical ecology of marine natural products, pp. 3-69, In: McClintock, J.B., Baker, B.J. (eds.) Marine Chemical Ecology. CRC Press, Boca Raton, Florida.

Hermes-Lima, M., Wang, E. M., Schulman, H. M., Storey, K. B. and Ponka, P., 1994. Deoxyribose degradation catalyzed by Fe(III)-EDTA: kinetic aspects and potential usefulness for submicromolar iron measurements. Mol Cell Biochem 17, 137, 1, 65-73.

Huang, D., Ol, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. J Agric Food Chem 53, 1841-1856.

Ignarro, L.J., Lippton, H., Edwards, J.C., Baricos, W.H., Hyman, A.L., Kadowitz, P.J., Gruetter, C.A., 1981. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. J Pharmacol Exp Ther 218, 3, 739-749.

Kappel, V.D., Costa, G.M., Scola, G., Silva, F.A., Landell, M.F., Valente, P., Souza, D.G., Vanz, D.C., Reginatto, F.H., Moreira, J.C.F., 2008. Phenolic Content and Antioxidant and Antimicrobial Properties of Fruits of *Capsicum baccatum* L. var. *pendulum* at Different Maturity Stages. J Med Food 11, 2, 267–274.

Karban, R., Myers, J.H., 1989. Induced plant responses to herbivory. Annu Rev Ecol Syst 20, 331-348.

Koulman, A., Proksch, P., Ebel,R., Beekman, A.C., Uden, W., Konings, A.W.T., Pedersen, J.A., Pras, N., Woerdenbag, H.J., 1996. Cytoxicity and mode of action of Aeroplysinin-1 and a related dienone from the sponge *Aplysina aerophoba*. J Nat Prod 59, 591-594.

Lee, Y.K., Lee, J.H., Lee, H.K., 2001. Microbial symbiosis in marine sponges. J Microbiol 39, 254-264.

Lissi, E., Pascual, C., Del Castillo, M.D., 1992. Luminol luminiscence induced by AAPH thermolysis. Free Rad Res Comms 17, 5, 299-311.

Lissi, E., Salim-Hanna, M., Pascual, C., Del Castillo, M.D., 1995. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements. Free Rad Biol Med 18, 2, 153-158.

Loya, S., Tal, R., Kashman, Y., Hizi, A., 1990. Ilimaquinone, a selective inhibitor of the Rnase-H activity of human-immunodeficiency-virus type-I reverse-transcriptase. Antimicrob Agents Chemother 34, 2009-2012.

Mandel, S.A., Avramovich-Tirosh, Y., Reznichenko, L., Zheng, H., Weinreb, O., Amit, T., Youdim, M.B., 2005. Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. Neurosignals 14, 46-60.

Martí, R., Fontana, A., Uriz, M.J., Cimino, G., 2003. Quantitative assessment of natural toxicity in sponges: toxicity bioassay versus compound quantification. J Chem Ecol 29, 6, 1307-1318.

Martín, D., Uriz, M.J., 1993. Chemical bioactivity of Mediterranean benthic organisms against embryos and larvae of marine invertebrates. J Exp Mar Biol Ecol 173, 11-27.

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple sssay for superoxide dismutase. J Biol Chem 247,10, 3170-3175.

Moncada, S., Palmer, R.M.J., Higgs, E.A.,1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 43, 109–142.

Monks, N.R., Lerner, C., Henriques, A.T., Farias, F.M., Schapoval, E.E.S., Suyenaga, E.S., da Rocha, A.B., Schwartsmann, G., Mothes, B., 2002. Anticancer, antichemotactic and antimicrobial activities of marine sponges collected off the coast of Santa Catarina, southern Brazil. J Exp Mar Biol Ecol 281, 1-12.

Moosmann, B., Behl, C., 2002. Antioxidants as treatment for neurodegenerative disorders. Expert Opin Invest Drugs 11, 1407-1435.

Moreira, P.I., Honda, K., Liu, Q., Santos, M.S., Oliveira, C.R., Aliev, G., Nomura, A., Zhu, X, Smith, M.A., Perry, G., 2005. Oxidative stress: the old enemy in Alzheimer's disease pathophysiology. Curr Alzheimer Res 2, 403-408.

Müller, W.E.G., Maidhof, A., Zahn, R.K., Schröder, H.C., Gacic, M.J., Heidemann, D., Bernd, A., Kurelec, B., Eich, E., Seibert, G., 1985. Potent antileukemic activity of the novel cytostatic agent avarone and its analogues *in vitro* and *in vivo*. Cancer Res 45, 4822-4826.

Netto Benetti, M.R., Rudnicki, M., Zanotto-Filho, A., de Oliveira, M.R., Kurek, A.G., Coitinho, A., Schwartsmann, G., Moreira, J.C.F., 2007. Evaluation of antioxidant effect of extracts of *Symphyopappus casarettoi*. Fitoterapia. 78, 232–234.

Newton, G.L., Fahey, R.C., 1995. Determination of biothiols by bromobimane labeling and high-performance liquid chromatography. Methods Enzymol 251, 148-166.

Prado, M.P., Torres, Y.R., Berlinck, R.G.S., Desiderá, C., Sanchez, M.A., Craveiro, M.V., Hadju, E., da Rocha, R.M., Machado-Santelli, G.M., 2004. Effects of marine organisms extracts on microtubule integrity and cell cycle progression in cultures cells. J Exp Mar Biol Ecol 313, 125-137.

Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determintion of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53, 4290-4302.

Prokof'eva, N.G., Utkina, N., Chaikina, E.L., Makarchenko, A.E., 2004. Biological activities of marine sesquiterpenoid quinones: structure-activity relationships in cytotoxic and hemolytic assays. Comp Biochem Physiol B Biochem Mol Biol 139, 169-173.

Proksch, P., Edrada, R.A., Ebel, R., 2002. Drugs from the seas - current status and microbiological implications. Appl Microbiol Biotechnol 59, 125-134.

Rangel, M., Sanctis, B., Freitas, J.C., Polatto, J.M., Granato, A.C., Berlinck, R.G.S., Hadju, E., 2001. Cytotoxic and neurotoxic activities in extracts of marine sponges (Porifera) from southeastern brazilian coast. J Exp Mar Biol Ecol 262, 31-40.

Regoli, F., Nigro, M., Chierici, E., Cerrano, C., Schiapparelli, S., Totti, C., Bavestrello, G., 2004. Variations of antioxidant efficiency and presence of endosymbiotic diatoms in the Antarctic porifera *Haliclona dancoi*. Mar Environ Res 58, 2-5, 637-640.

Reiswig, H.M., 1971. *In situ* pumping activities of tropical Demospongiae. Mar Biol 9, 38-50.

Rhee, S.G., 2006. H_2O_2, a necessary evil for cell signalling. Science 312, 1882-1883.

Richelle-Maurer, E., Gomez, R., Braekman, J.-C., van de Vyver, G., van Soest, R.W.M. and Devijver, C., 2003. Primary cultures from the marine sponge *Xestospongia muta* (Petrosiidae, Haplosclerida). J Biotechnol 100,169–176.

Rodriguez, J., Quiñoa, E., Riguera, R., Peters, B.M., Abrell, L.M., Crews, P., 1992. The structure and stereochemistry of cytotoxic sesquiterpene quinones from *Dactilospongia elegans*. Tetrahedron 32, 6667-6680.

Sepcić, K., Batista, U., Vacelet, J., Macek, P., Turk, T., 1997. Biological activities of aqueous extracts from marine sponges and cytotoxic effects of 3-alkylpiridinium polymers from *Reniera sarai*. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 117, 1, 47-53.

Silva, A.C.da, Kratz, J.M., Farias, F.M., Henriques, A.T., Santos, J. dos, Leonel, R.M., Lerner, C., Mothes, B, Barardi, C.R.M., Simões, C.M.O., 2006. *In vitro* antiviral activity of marine sponge collected off Brazilian coast. Biol Pharm Bull 29, 1, 135-140.

Silva, E.G.da, Behr, G.A., Zanotto-Filho, A., Lorenzi, R., Pasquali,M.A.B., Ravazolo, L.G., Bordignon, C.L.J., Silva, F.A., Aboy, A.L., Bassani, V.L., Henriques, A.T., Reginatto, F.H., Dal-Pizzol, F., Moreira, J.C.F., 2007. Antioxidant Activities and Free Radical Scavenging Potential of *Bauhinia microstachya* (RADDI) MACBR. (Caesalpinaceae) Extracts Linked to Their Polyphenol Content. Biol Pharm Bull 30, 8, 1488-1496.

Singleton, V.L., Rossi, J.A., 1965. Colorimetric of total phenolics with phosphomolybidic-phosphotungstic acid reagents. Am J Enol Vitic 16, 144-158.

Sipkema, D., Franssen, M.C.R., Osinga, R., Tramper, J., Wijffels, R.H., 2005. Marine sponges as pharmacy. Mar Biotechnol 7, 142-162.

Sultana, R., Perluigi, M., Butterfield., D.A., 2006. Redox proteomics identification of oxidatively modified proteins in Alzheimer's disease brain and in vivo and in vitro models of AD centered around Abeta (1-42). J Chromtogr B 833, 3-11.

Sundquist, A.R., Fahey, R.C., 1989. The function of gamma-glutamylcysteine and bis-gamma-glutamylcystine reductase in *Halobacterium halobium*. J Biol Chem 264, 719-725.

Takamatsu, S., Hodges, T.W., Rajbhandari, I., Gerwick, W.H., Hamann, M.T., Nagle, D.G., 2003. Marine natural products as novel antioxidant prototypes. J Nat Prod 66, 605-608.

Tchernov, D., Gorbunov, M.Y., Vargas, C.D., Yadav, S.N., Milligan, A.J., Haggblom, M., Falkowsi, P.G., 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc Natl Acad Sci U S A 101, 13531–13535.

Tsen, C.C., Tappel, A.L., 1958. Catalytic Oxidation of Glutathione and Other Sulfhydryl Compounds by Selenite. J Biol Chem 233, 1230-1232.

Turon, X., Becerro, M.A., Uriz, M.J., 1996. Seasonal patterns of toxicity in benthic invertebrates: The incrusting sponge *Crambe crambe* (Poecilosclerida). Oikos 75, 33-40.

Tziveleka, L.A., Kouronakis, A.P., Kouronakis, P.N., Roussis, V., Vagias, C., 2002. Antioxidant potential of natural and sinthesised polyprenylated hydroquinones. Bioorg Med Chem 10, 935-939.

Utkina, N.K., Makarchenko, A.E., Shchelokova, O.V., Virovaya, M.V., 2004. Antioxidant activity of phenolic metabolites from marine sponges. Chem Nat Compd 40, 4, 373-377.

Verdenal, B., Diana, C., Arnous, A., Vacelet, J., 1990. Pollutants levels in Mediterranean commercial sponges. In: Rutzler K., (Ed.), New Perspectives in Sponge Biology Smithsonian Institution Press, Washington, DC.

Wang, W., Ballatori, N., 1998. Endogenous glutathione conjugates: occurrence and biological functions. Pharmacol Rev 50, 3, 335-356.

Zecca, L., Youdim, M.B., Riederer, P., Connor, J.R., Crichton, R.R., 2004. Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5, 863-873.

Zhang, W., Xue, S., Zhao, Q., Zhang, X., Li, J., Jin, M., Yu, X., Yuan, Q., 2003. Biopotentials of marine sponges from China oceans: past and future. Biomol Eng 20, 413-419.

Table 1. Brazilian Marine Sponge Extracts Studied

Species	Collection number	Family	Order	Collected off from	Extracts tested
Aaptos sp.	MCN 5144	Suberitidae	Hadromerida	PE	A
Agelas sp.	MCN 4269	Agelasidae	Agelida	PE	A
Axinella corrugata	MCN 3772	Axinellidae	Halichondrida	SC	O
Chondrilla nucula	MCN 5141	Chondrillidae	Chondrosida	PB	A
Cinachyrella alloclada	MCN 5157	Tetillidae	Spirophorida	PE	A
Cliona sp.	MCN 5070	Clionaidae	Hadromerida	SC	A
Dragmacidon reticulatus	MCN 3425	Axinellidae	Halichondrida	SC	A
Guitarra sepia	MCN 3413	Guitarridae	Poecilosclerida	SC	A
Halichondria sp.	MCN 5140	Halichondriidae	Halichondrida	PE	A
Haliclona tubifera	MCN 3771	Chalinidae	Haplosclerida	SC	A+O
Hyatella sp.	MCN 4654	Spongiidae	Dictyoceratida	PE	A
Mycale arcuiris	MCN 3984	Mycalidae	Poecilosclerida	SC	A
Petromica citrina	MCN 3395	Halichondriidae	Halichondrida	SC	A
Protosuberites sp.	MCN 4660	Suberitidae	Hadromerida	PE	A
Raspailia elegans	MCN 5058	Raspaillidae	Poecilosclerida	SC	A
Scopalina ruetzeri amarela	MCN 3976	Dictyonellidae	Halichondrida	SC	A+O
Scopalina ruetzeri vermelha	MCN 3976	Dictyonellidae	Halichondrida	SC	A
Tedania ignis	MCN 3397	Tedaniidae	Poecilosclerida	SC	A

MCN=Natural Science Museum; A=aqueous; O=organic; SC,PE, PB=Santa Catarina, Pernambuco and Paraíba States, respectively.

Table 2. Reactive Oxygen and Nitrogen Scavenging Capacity and Lipid Peroxidation Inhibitory Profile of Brazilian Marine Sponge Extracts

Sample tested	TRAP		NO Scavenging Activity (%S)	Inhibition of Lipid Peroxidation Induced by		
	TAR (% I)	AUC (% I)		AAPH (%I)	Fe^{2+} (%I)	H_2O_2 (%I)
Aaptos sp.	63.4±4.1^{b}	12.4±1.3^{h,i}	95.9±5.0	n.d.	> 100 µg/ml	> 100 µg/ml
Agelas sp.	94.5±0.9^{a}	81.2±4.7^{b}	81.3±0.6^{d}	65.2±7.3^{a}	9.6±2.8^{c}	n.d.
Axinella corrugata	83.3±3.9^{a}	96.7±0.4^{a}	75.8±1.9^{c,d}	40.6±3.8^{b}	8.7±1.8^{c}	n.d.
Chondrilla nucula	49.3±4.7^{c,d,e}	20.4±2.0^{f,g,h}	70.9±1.3^{d}	n.d.	n.d.	59.4±1.4^{a}
Cinachyrella alloclada	25.8±3.2^{g,h}	16.6±3.1^{g,h,i}	96.2±4.8	> 100 µg/ml	24.2±2.3^{b}	52.6±7.0^{a}
Cliona sp.	53.3±4.5^{b,c,d}	9.9±2.9^{i,j}	167.7±3.1^{f}	25.9±5.3^{b}	> 100 µg/ml	n.d.
Dragmacidon reticulatus	45.7±2.3^{d,e,f}	19.6±1.1^{f,g,h}	119.3±3.3^{e}	n.d.	> 100 µg/ml	n.d.
Guitarra sepia	50.9±3.8^{c,d,e}	26.5±1.7^{d,e,f}	49.6±1.7^{a}	n.d.	> 100 µg/ml	n.d.
Halichondria sp.	62.9±8.1^{b}	31.3±4.3^{d}	101.1±7.4	22.1±4.5^{b}	10.0±3.1^{c}	n.d.
Haliclona tubifera A	18.6±2.0^{h,i}	10.0±1.1^{i,j}	78.7±5.2^{d}	n.d.	> 100 µg/ml	n.d.
Haliclona tubifera O	27.5±2.6^{g,h}	18.0±1.6^{g,h,i}	64.8±0.5^{b,c}	n.d.	> 100 µg/ml	n.d.
Hyatella sp.	53.0±3.7^{b,c,d}	34.4±1.2^{d}	105.3±3.9	> 100 µg/ml	n.d.	n.d.
Mycale arcuiris	10.2±1.9^{i}	0.1±1.7	55.5±3.2^{a,b}	n.d.	n.d.	n.d.
Petromica citrina	45.9±3.0^{d,e,f}	0±1.4	124.4±10.5^{e}	n.d.	n.d.	n.d.
Protosuberites sp.	61.9±3.7^{b,c}	21.6±0.5^{e,f}	4.7±5.1	n.d.	n.d.	66.3±9.6^{a}
Raspailia elegans	37.6±4.4^{e,f,g}	2.8±0.9	110.4±2.2	> 100 µg/ml	> 100 µg/ml	n.d.
Scopalina ruetzeri amarela A	36.2±4.0^{f,g}	12.6±1.6^{h,i}	78.4±1.4^{d}	n.d.	> 100 µg/ml	n.d.
Scopalina ruetzeri amarela O	0±1.2	2.5±1.3	73.8±8.2^{c,d}	n.d.	n.d.	n.d.
Scopalina ruetzeri vermelha	60.2±4.1^{b,c}	28.5±1.4^{d,e}	56.0±1.9^{a,b}	n.d.	> 100 µg/ml	> 100 µg/ml
Tedania ignis	55.7±5.6^{b,c,d}	16.9±2.1^{g,h,i}	63.2±5.8^{b,c}	n.d.	> 100 µg/ml	> 100 µg/ml
Trolox	94.5±1.4^{a}	58.5±6.3^{c}	n.t.	75.5±5.6^{a}	90.5±2.0^{a}	n.d.

Results as mean±standard error of three independent experiments; same letters indicate no statistical differences (ANOVA, $p<0.05$, Duncan test). n=3; n.d.=not detected; n.t.=not tested; %I=Inhibiton percent of 10 µg/ml extracts in TAR and AUC and 100µg/ml in Lipid Peroxidation assay; %S=percent of nitrites formed in 100µg/ml extracts incubation considering the NO producting system as 100%; >100µg/ml= extracts which statystically inhibited lipid peroxidation only in concentrations higher than 100µg/ml.

Table 3. Brazilian Marine Sponge Extracts Phenols and Thiols Content

Extracts	Phenols Content (µg TAE/100µg extract)	Thiols Content (µg CE/100µg extract)
Aaptos sp.	1.52 ± 0.13^{c}	$0.41\pm0.05^{f,g}$
Agelas sp.	3.36 ± 0.28^{a}	0.47 ± 0.04^{g}
Axinella corrugata	2.57 ± 0.22^{b}	$0.068\pm0.01^{a,b}$
Chondrilla nucula	$0.93\pm0.17^{d,e,f,g}$	$0.104\pm0.023^{a,b,c}$
Cinachyrella alloclada	$1.23\pm0.10^{c,d,e}$	$0.41\pm0.035^{f,g}$
Cliona sp.	$0.73\pm0.04^{d,e,f,g}$	$0.21\pm0.02^{d,e}$
Dragmacidon reticulatus	0.41 ± 0.04^{g}	0.013 ± 0.002^{a}
Guitarra sepia	$3.01\pm0.27^{a,b}$	$0.055\pm0.006^{a,b}$
Halichondria sp.	1.50 ± 0.08^{c}	$0.086\pm0.01^{a,b,c}$
Haliclona tubifera A	$0.75\pm0.06^{e,f,g}$	0.25 ± 0.02^{e}
Haliclona tubifera O	$1.15\pm0.05^{c,d,e,f}$	$0.17\pm0.005^{c,d,e}$
Hyatella sp.	$0.77\pm0.04^{e,f,g}$	0.011 ± 0.003^{a}
Mycale arcuiris	$0.61\pm0.03^{f,g}$	$0.185\pm0.05^{c,d,e}$
Petromica citrina	0.041 ± 0.02^{g}	$0.13\pm0.04^{b,c,d}$
Protosuberites sp.	$1.33\pm0.14^{c,d}$	$0.047\pm0.01^{a,b}$
Raspailia elegans	0.48 ± 0.07^{g}	0.029 ± 0.006^{a}
Scopalina ruetzeri amarela A	$1.28\pm0.10^{c,d,e}$	0.37 ± 0.05^{f}
Scopalina ruetzeri amarela O	0.56 ± 0.11^{g}	$0.103\pm0.02^{a,b,c}$
Scopalina ruetzeri vermelha	$1.11\pm0.08^{c,d,e,f}$	$0.14\pm0.03^{b,c,d}$
Tedania ignis	$1.39\pm0.21^{c,d}$	0.26 ± 0.04^{e}

TAE=tannic acid equivalents; CE=cysteine equivalents. Same letters indicate no statistical differences (ANOVA, $p<0.05$, Duncan test).

Table 4. Established Correlations Between the Studied Parameters.

Correlated Parameters	Pearson's Coefficient	P
Phenol X TAR	0.646	0.02
Phenol X AUC/TRAP	0.765	<0.000
Phenol X LPO/AAPH	0.774	<0.000
Phenol X LPO/Fe^{2+}	0.433	0.56
Phenol X NBT	0.630	<0.000
Thiol X LPO/Fe^{2+}	0.448	0.48
AbsH_2O_2 X LPO/H_2O_2	0.582	0.007

Phenol=phenolic content, TAR=total antioxidant reactivity, AUC/TRAP=area under curve of total radical antioxidant potential, LPO/AAPH=lipid peroxidation induced by AAPH, LPO/Fe^{2+}=lipid peroxidation induced by Fe^{2+}, NBT=Nitroblue tetrazolium reduction,Thiol=thiol content, AbsH_2O_2=H_2O_2 absorbance, LPO/H_2O_2=lipid peroxidation induced by H_2O_2.

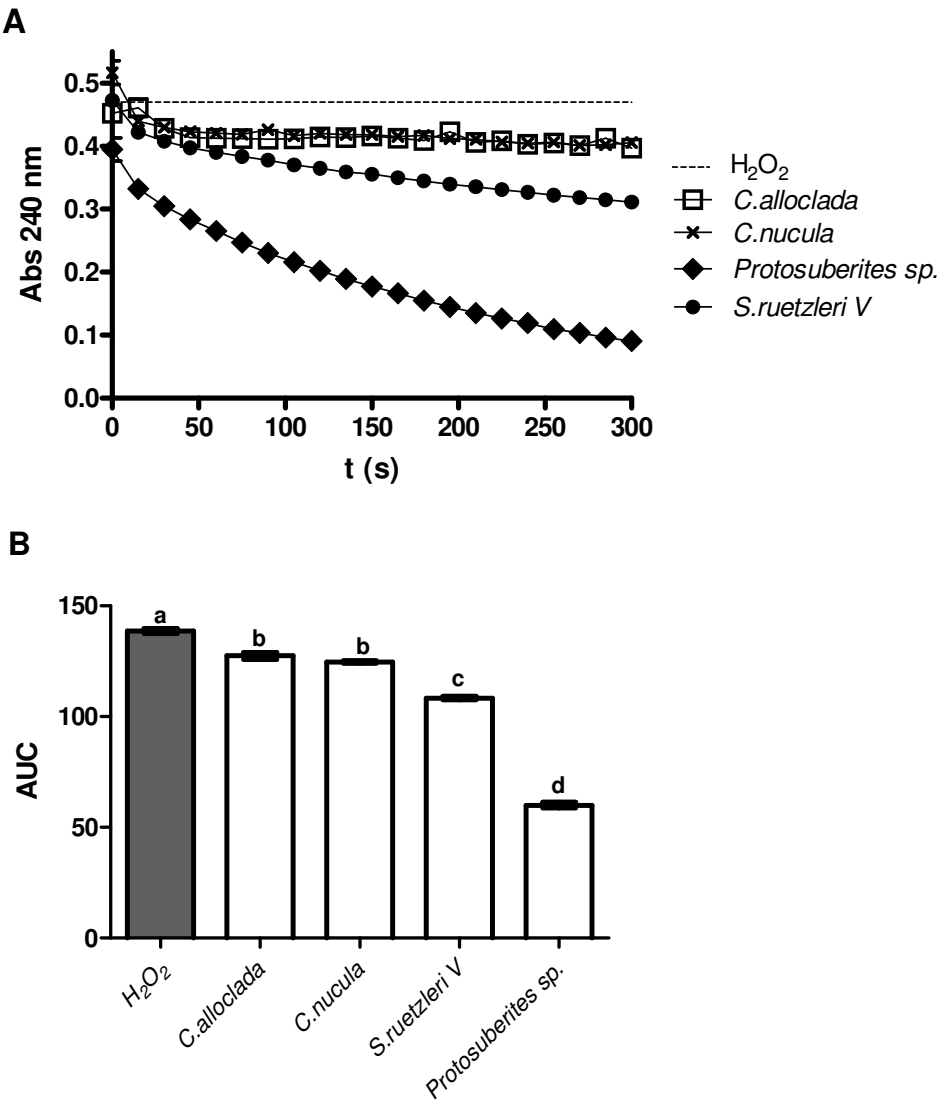


Figure 1. Decreasing Effect of Brazilian Marine Sponge Extracts in H_2O_2 Absorbance. Decrease of H_2O_2 absorbance along the time elapsed (A), and quantification of each respective area under curve (AUC) (B). Data expressed as mean ± standard error of three different experiments (n=3). Same letters indicate no significant differences (ANOVA, $p<0.05$, Duncan Test).

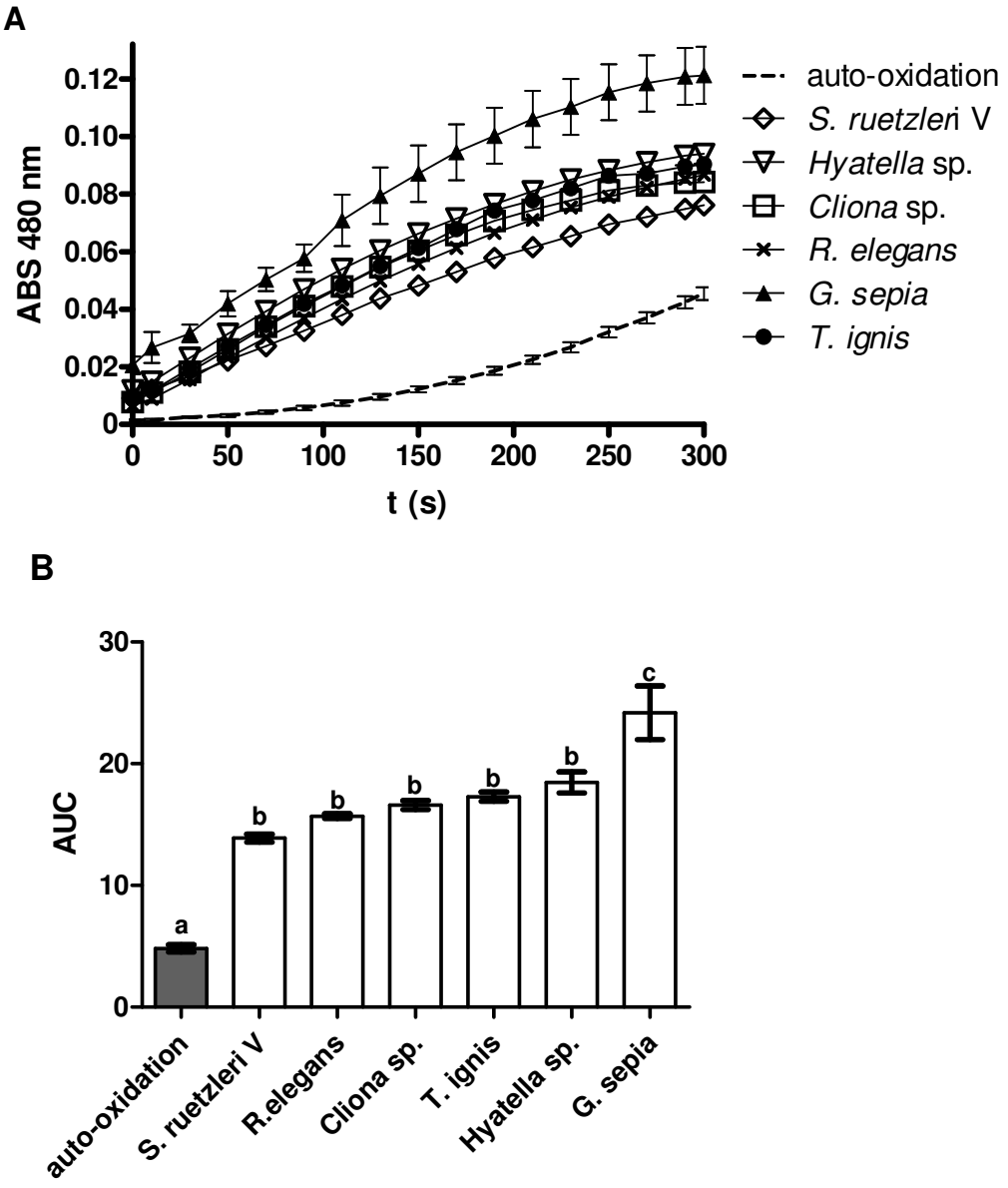


Figure 2. Enhancing Effect of Brazilian Marine Sponge Extracts in Adrenaline Auto-Oxidation. Enhance in adrenochrome formationalong time (A), and quantification of each respective area under curve (AUC) (B). Data expressed as mean ± standard error of three different experiments (n=3). Same letters indicate no statistically significant differences (ANOVA, $p<0.05$, Duncan Test).

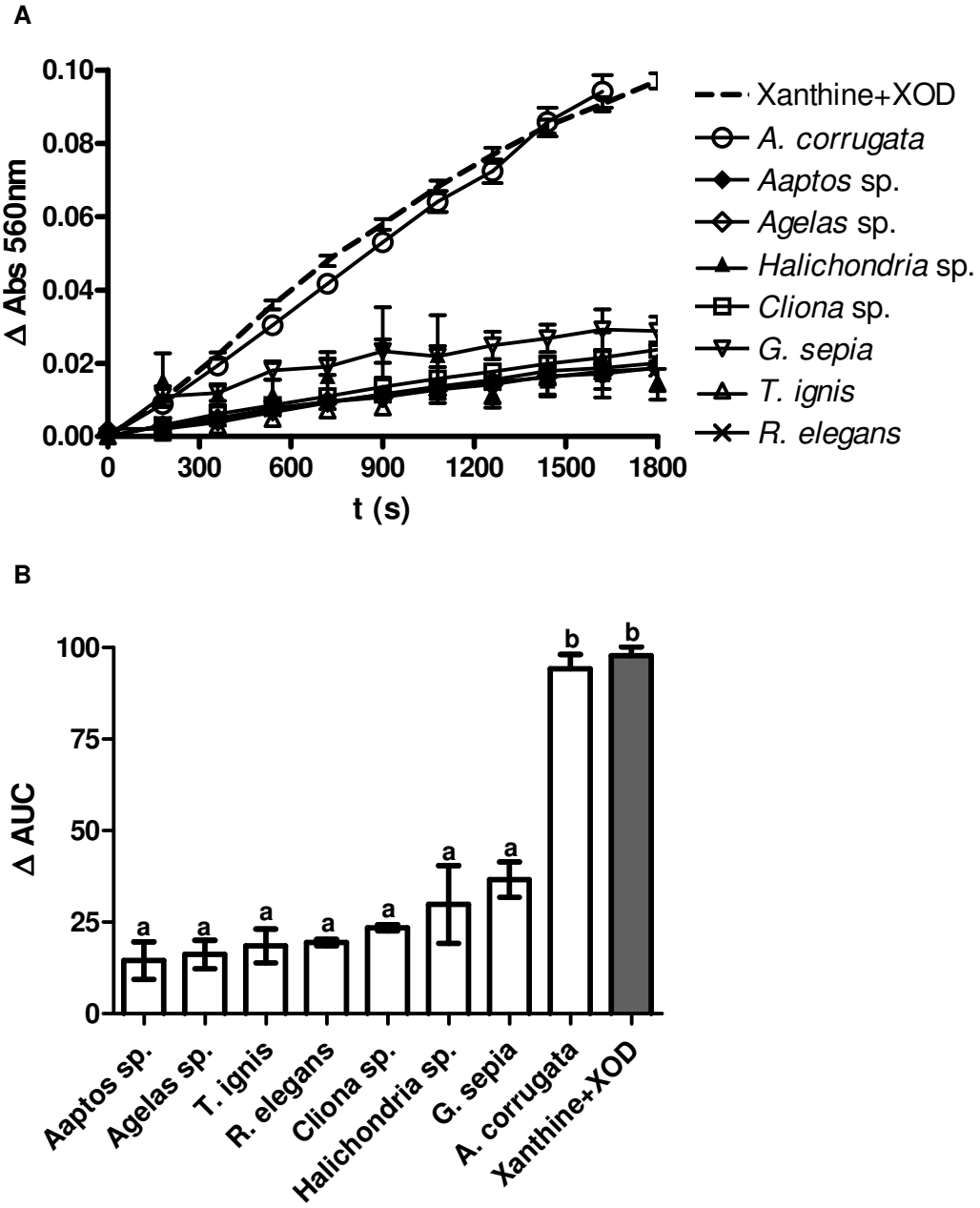


Figure 3. Nitroblue Tetrazolium (NBT) Reducing Ability of Brazilian Marine Sponge Extracts. NBT reducing profile of marine sponge extracts and xanthine+xanthine oxidase (XOD) expressed as variation of absorbance at 560nm (A). Quantification of each area under curve (AUC) disposed in (A) (B). Data expressed as mean ± standard error of three different experiments (n=3). Same letters indicate no statistically significant differences (ANOVA, $p<0.05$, Duncan Test).

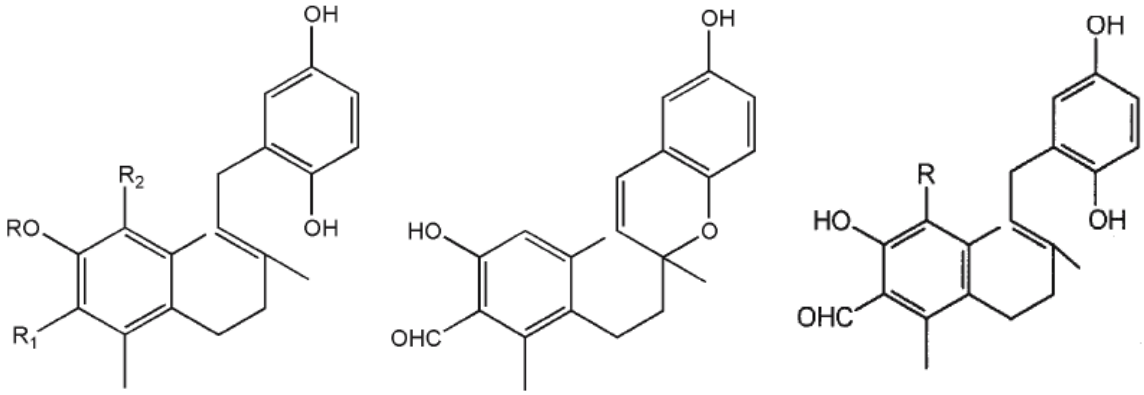
4. DISCUSSÃO

Polifenóis são compostos onipresentes no Reino Vegetal. Muitos compostos fenólicos são conhecidos por suas propriedades antioxidantes. O resveratrol, encontrado em uvas e em muitas outras plantas, possui capacidade de prevenir a lipoperoxidação da lipoproteína de baixa densidade (LDL, *low-density lipoprotein*) (TADOLINI *et al.*, 2000), bem como de proteger o coração de ratos do processo de isquemia-reperfusão (HUNG *et al.*, 2000). Outro flavonóide amplamente distribuído em vegetais, a quercitina, também é capaz de inibir a peroxidação lipídica (GRYGLEWSKI *et al.*, 1987; LAUGHTON *et al.*, 1991). Muitos trabalhos com extratos de plantas atribuem a atividade antioxidante obtida pelo ensaio TRAP ao conteúdo de fenólicos determinado pelo método de Folin-Ciocalteau (NETTO BENETTI *et al*., 2007; SILVA *et al.*, 2007; KAPPEL *et al*., 2008). Neste trabalho encontramos uma correlação entre ambos índices de TRAP, TAR e AUC, e o conteúdo fenólico dos extratos de esponjas marinhas. Este resultado sugere o envolvimento dos compostos fenólicos dos extratos em suas atividades de *scavenger* de radical peroxil.

Apesar de serem animais, compostos fenólicos também foram encontrados em esponjas marinhas. Um grupo interessante de triprenilfenóis isolados de esponjas marinhas inclui panicein A (1), B1 (2), B2 (3), B3 (4) e C (5) (CIMINO *et al.*, 1973; CASAPULLO *et al*., 1993; JASPARS *et al*., 1995) (figura 3). Porém poucos trabalhos investigaram a capacidade antioxidante de compostos fenólicos em esponjas (TAKAMATSU *et al.*, 2003; UTKINA *et al*., 2004).

A proteção contra lipoperoxidação induzida por AAPH também foi correlacionada ao TRAP e ao conteúdo de fenóis. O mecanismo de dano induzido por AAPH consiste na sua propriedade de se decompor e formar $ROO^{\bullet}$ (NIKI, 1990). Possivelmente, a correlação

observada indique que o mecanismo protetor dos extratos é a capacidade de captura de radical peroxil, o qual é dependente do conteúdo de fenóis dos extratos.



1 R = R_1 = Me, R_2 = H
2 R = R_2 = H, R_1 = CHO

3

4 R = H
5 R = OH

Figura 3. Compostos fenólicos isolados de esponjas marinhas. Retirado e modificado de Bakhuni & Rawat, 2005.

Compostos fenólicos também podem atuar como potentes quelantes de metal (HANASAKI *et al.*, 1994). Entretanto, não foi evidenciada correlação entre o conteúdo de fenóis e a proteção lipídica contra dano oxidativo induzido por Fe^{2+}. A falta de correlação indica que o conteúdo de fenóis não está relacionado à proteção lipídica contra Fe^{2+}, mas não exclui a participação dos compostos fenólicos nesta capacidade antioxidante. Estudos de relação estrutura e atividade (SAR, *structure-activity relationship*) mostram que, dependendo de sua estrutura, moléculas pertencentes à mesma função química diferindo em apenas um átomo ou um grupo funcional ligante podem possuir diferentes graus de determinada bioatividade. Portanto, esta proteção contra ferro pode estar relacionada à qualidade dos compostos fenólicos presentes nos extratos. Um estudo qualitativo dos compostos fenólicos poderia ajudar a esclarecer qual a contribuição destas moléculas para a atividade antioxidante contra Fe^{2+}.

Por outro lado, foi evidenciada uma correlação entre o conteúdo de tióis e o efeito protetor contra peroxidação lipídica induzida por Fe^{2+}. Tióis desempenham papéis vitais na manutenção dos potenciais redox celulares e das razões protéicas tiol-dissulfeto, assim como na proteção das células contra espécies reativas de oxigênio. Glutationa (GSH) é o tiol predominante em organismos eucarióticos (NEWTON & FAHEY, 1995). GSH pode agir como antioxidante bem como reagir com metais, apesar de ser auto-oxidada na presença de metais pesados, formando dissulfetos e peróxidos (TSEN & TAPPEL, 1958; SUNDQUIST & FAHEY, 1989). Esponjas filtram grandes volumes de água e, por isso, estão extremamente expostas a xenobióticos presentes no ambiente (REISWIG, 1971) e, portanto, GSH é essencial para que desempenhem esta função metabólica. De Flora e colaboradores (1995) demonstraram que os níveis de GSH em esponjas das espécies *Geodia cydonium* e *Tethya aurantium* são duas vezes maiores que os níveis encontrados em fígado de ratos.

No geral, concentrações maiores que 100µg/ml dos extratos de esponja foram capazes de evitar a peroxidação lipídica induzida por Fe^{2+} e quatro extratos na concentração de 100µg/ml inibiram entre 8 a 25% do dano lipídico. Embora a proteção possa parecer fraca, não pode ser considerada ineficiente. No ambiente marinho, o ferro é um elemento crítico para a produção primária (DE BAAR *et al*., 1995; CHISHOLM, 2000; WATSON *et al.*, 2000). O suprimento de ferro até pesquenas profundidades nos oceanos tem origem na poeira do solo que entra em contato com a superfície dos mares (TURNER *et al*., 2001; JICKELLS *et al*., 2005) e é limitado pelo pH mar, que favorece a insolubilidade deste metal. Na água do mar, ferro é encontrado principalmente como óxidos de Fe(III), mas sua insolubilidade resulta na deposição como colóides de ferro no fundo do mar (BYRNE & KESTER, 1976). Para que o ferro esteja biodisponível, esta matéria deve ser convertida a compostos solúveis, através de dissolução térmica (WELLS *et al.*, 1983; RICH & MOREL, 1990), redução de Fe(III) por

redutores orgânicos (FINDEN *et al.*, 1984) ou dissolução foto-redutora de óxidos de Fe(III) com ou sem ligantes orgânicos (FINDEN *et al.*,1984; WELLS *et al.*, 1987, 1991; RICH & MOREL, 1990). Por todos estes motivos, os níveis de proteção não-enzimática evidenciados neste trabalho podem ser suficientes e adequados para esponjas marinhas lidarem com o ferro existente em seu ambiente natural.

Tióis, principalmente GSH, têm a abilidade de reagir com $^{\bullet}$NO e formar S-nitrosotióis. A formação de S-nitrosotióis poderia retirar $^{\bullet}$NO do meio e, portanto, os tióis poderiam atuar como *scavenger* de $^{\bullet}$NO. Contudo, não foi estabelecida correlação entre conteúdo tiólico e a propriedade de captura de radical $^{\bullet}$NO. Existem dados publicados acerca da natureza lábil e do caráter de pronta dissociação dos S-nitrosotióis quando expostos ao radical óxido nítrico (IGNARRO *et al.*, 1981). Tal fato pode explicar a falta de correlação evidenciada neste estudo. A atividade de óxido nítrico sintase (NOS) foi detectada em esponjas do grupo Demospongiae (GIOVINE *et al.*, 2001). Giovine e colaboradores (2001) observaram um aumento na produção de $^{\bullet}$NO subsequente ao estresse térmico. A rápida resposta da NOS ao estímulo térmico sugeriu a atuação de $^{\bullet}$NO como uma molécula sinalizadora. Muitos invertebrados marinhos possuem sinalização por óxido nítrico, como moluscos e celenterados (JACKLET, 1997; COLASANTI & VENTURINI, 1998). O óxido nítrico é tanto essencial quanto tóxico para a célula. Em baixas concentrações, difunde-se através do citosol e membranas lipídicas e para dentro das células como um gás, onde se liga a compostos de elétrons simples e atua fisiologicamente como neurotransmissor e hormônio vasodilatador. Entretanto, em altas concentrações, combina-se com O_2 ou com o radical superóxido formando espécies reativas como o peroxinitrito ($ONOO^-$) (SMITH *et al.*, 2005). Essas espécies reativas estão envolvidas em doenças

neurodegenerativas, como Parkinson, e em doenças inflamatórias crônicas, como artrite reumatóide. Nestas circunstâncias, a atividade de *scavenger* de $^{\bullet}NO$ evidenciada pode ser benéfica tanto para evitar danos oxidativos quanto para ajudar a modular sinais exacerbados das vias de sinalização.

Nenhum extrato apresentou atividade de scavenger de $^{\bullet}OH$ através do método de degradação oxidativa da 2-desoxirribose. O radical hidroxil é uma espécie reativa de oxigênio capaz de interagir rapidamente com qualquer biomolécula adjacente. A fim de prevenir um dano oxidativo induzido por $^{\bullet}OH$, o composto *scavenger* deve estar próximo do sítio de formação antes do hidroxil ser formado e em altas concentrações para competir com as moléculas por uma reação com o $^{\bullet}OH$ (HUANG *et al*., 2005). Então, torna-se relevante avaliar a capacidade antioxidante de scavenger de espécies reativas de oxigênio que podem gerar $^{\bullet}OH$, como $O_2^{\bullet-}$ e H_2O_2.

Neste sentido, as atividades peroxidásica dos extratos de esponjas foram testadas. Os extratos aquosos de *Protosuberites* sp., *S. ruetzleri* vermelha, *C. nucula* e *C. alloclada* revelaram-se *scavengers* de peróxido de hidrogênio. Estes mesmos extratos foram capazes de proteger os lipídios do dano oxidativo contra peróxido de hidrogênio. Este dado sugere que o mecanismo de prevenção ao dano lipídico induzido por H_2O_2 possa ser a atividade de *scavenger* destes extratos, uma vez que foi evidenciada uma correlação entre ambas atividades antioxidantes contra H_2O_2. É importante salientar que trolox até 100µM, a máxima concentração testada, não foi capaz de proteger os lipídios da peroxidação induzida por H_2O_2. A glutationa protege as células do dano oxidativo através da atividade de *scavenger* não-enzimática e pela neutralização de peróxido de hidrogênio e hidroperóxidos lipídicos pela ação de peroxidases dependentes de GSH (WANG & BALLATORI, 1998;

POMPELLA *et al*., 2003). Entretanto, não foi possível estabelecer uma correlação entre conteúdo de tióis e nenhuma das capacidades antioxidantes contra peróxido de hidrogênio.

A associação com populações microbianas é freqüente em Porifera (LEE *et al*., 2001; RICHELLE-MAURER *et al*., 2003). Os organismos simbiontes compreendem grupos de archaea, bactérias, cianobactérias e microalgas (BEWLEY & FAULKNER, 1998; LEE *et al.*, 2001; PROKSCH *et al*., 2002). O sucesso da relação de simbiose entre os organismos depende das capacidades de adaptação do hospedeiro e do simbionte. Esponjas em simbiose com cianobactérias e microalgas fotossintetizantes respondem adaptativamente ao aporte de O_2 e de espécies reativas geradas a partir da fotossíntese. O aumento de diatomáceas no período do verão na esponja da Antártica *Haliclona dancoi* ocasionou o aumento de atividade das enzimas de defesa antioxidante SOD e catalase, e das enzimas que recicla glutationa, glutationa redutase (REGOLI *et al.*, 2004). Mudanças ambientais, como irradiação solar e temperaturas elevadas, também podem alterar a eficiência fotossintética do simbionte e gerar mais espécies reativas de oxigênio. Nestas condições, um processo chamado *bleaching* (clareamento) pode ocorrer e permitir o vazamento de H_2O_2 da célula do simbionte para o hospedeiro (TCHERNOV *et al*., 2004). A fotorredução de ferro também pode gerar peróxido de hidrogênio no ambiente marinho (MILLER & KESTER, 1994). Neste contexo, as habilidades de capturar o H_2O_2 a fim de proteger às biomoléculas da esponja são fundamentais.

Recentemente, o peróxido de hidrogênio vem sendo tratado como um mensageiro celular envolvido em respostas de replicação celular, regulação do metabolismo, indução de genes específicos e apoptose nas mais diversas formas de vida (LAMBETH, 2004; CAI, 2005; LIU & FINKEL, 2006; RHEE, 2006). Entretanto, a formação de H_2O_2 e aldeídos tóxicos a partir da degradação de dopamina na região nigro-estriatal do cérebro através da

monoamina oxidase (MAO) B pode estar relacionada à patogênese da doença de Parkinson (NAGATSU & SAWADA, 2006). Adicionalmente, o H_2O_2 pode ser formado endogenamente por óxido-redutases como a glicose oxidase (MASSEY *et al.*, 1969), e principalmente pela dismutação de superóxido produzido a partir de: NADPH oxidases (LAMBETH, 2002), vazamento da cadeia de transporte de elétrons mitocondrial (LOSCHEN *et al*., 1974), ciclo redox de quinonas (MCCORD & FRIDOVICH, 1970) e flavoproteínas xenobióticas (MASSEY *et al.*, 1969). Portanto, mais estudos são necessários para entender as promissoras atividades protetoras dos extratos de esponja contra dano oxidativo induzido por peróxido de hidrogênio.

Com relação à capacidade de *scavenger* o radical ânion superóxido, as reações de auto-oxidação da adrenalina e de redução do NBT foram utilizadas. Os extratos aquosos de *S. ruetzleri* vermelha, *R. elegans*, *Cliona* sp., *T. ignis*, *G. sepia* aceleraram as taxas de auto-oxidação de adrenalina e também foram capazes de reagir com o NBT. Estes dados indicam que há possibilidade do mecanismo de ação destes extratos ser através da geração de $O_2^{\bullet-}$. A geração não-enzimática do radical ânion superóxido já foi relatada. Um exemplo é o ciclo hidroquinona-quinona. A mais importante reação das quinonas consiste nas suas propriedades redox de redução reversível a hidroquinonas correspondentes via radicais semiquinonas (BENTLEY & CAMPBELL, 1974). A fauna marinha é capaz de sintetizar quinonas (BAKHUNI & RAWAT, 2005). Muitas benzoquinonas foram isoladas de organismos marinhos e predominantemente possuem estruturas relacionadas a naftazarina (1) e juglona (2) (figura 4). Além destas, 2,5-dihidroxi-3-etilbenzoquinona (3) e antraquinonas, especialmente análogos de rodocomatulina (4) forma isolados (figura 4). Sesquiterpenóides

quinonas e hidroquinonas são metabólitos comumente encontradas em esponjas da família Dysideidae, Thorectidae e Spongiidae da ordem Dictyoceratidae (RODRIGUEZ *et al.*, 1992).

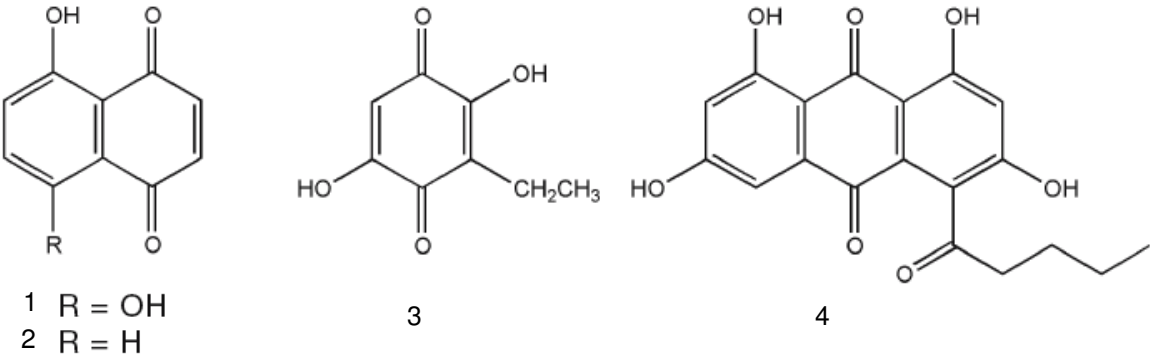


Figura 4. Quinonas isoladas de esponjas marinhas. Retirado e modificado de Bakhuni e Rawat, 2005.

Quinonas possuem inúmeras atividades farmacologicamente relevantes. São exemplos, os agentes antileucêmicos avarol e avarone (MÜLLER *et al.*, 1985) e o composto anti-HIV ilimaquinona (LOYA *et al*., 1990). Além disso, o anel quinona parece ser essencial às atividades citotóxicas e hemolíticas (PROKOF'EVA *et al*., 2004). Um propósito ecológico para o ciclo das quinonas em esponjas marinhas já foi proposto. Os metabólitos citotóxicos aeroplysinina-1 e uma dienona relacionada apresentaram espectros de ressonância paramagnética de radical. E acredita-se que sua formação ocorra assim que a esponja é danificada, repelindo predadores (KOULMAN *et al*., 1996).

Os resultados obtidos pelas técnicas do NBT e da adrenalina não convergiram completamente, o que se refletiu na ausência de correlação entre o resultado destas técnicas. Esta discrepância pode ser atribuída aos diferentes períodos de monitoramento das técnicas. A faixa de linearidade da auto-oxidação da adrenalina ocorre até 5 minutos do início da reação, não sendo possível e correto analisar tempos maiores que este. Enquanto

que nos cinco primeiros minutos de reação com NBT, o controle positivo de geração de superóxido, xantina+xantina oxidase, foi incapaz de reduzir o NBT de forma estatisticamente significante, o que nos fez optar por avaliar um tempo maior de reação. Entretanto, foi estabelecida uma correlação entre a capacidade de reduzir o NBT e o conteúdo de fenóis dos extratos. A quantificação de compostos fenólicos ocorre em pH alcalino, no qual o fenol é deprotonado ao ânion fenolato e liga-se ao núcleo fosfo-molibdato do reagente de Folin-Ciocalteau (HUANG *et al*., 2005). Como há liberação de H^+ para o meio, a reação de Folin-Ciocalteau pode servir como indicadora da capacidade redutora das amostras. A redução do NBT também foi conduzida em meio alcalino, no qual compostos fenólicos podem dissociar a ânion fenolato e próton. Então, é possível que, no geral, os resultados da reação com NBT demonstrem o potencial redutor dos extratos e, por isso, sejam correlacionados ao conteúdo de fenólicos obtido pelo método de Folin-Ciocalteau. Hidroquinonas são provavelmente capazes de reduzir fortes espécies reativas e oxidantes pela doação de elétrons (TZIVELEKA *et al*., 2002). Hidroquinonas livres já foram isoladas de *Axinella polipoides* (CIMINO *et al*., 1974). Neste estudo, o extrato de uma esponja pertencente a este mesmo gênero, o extrato de *Axinella corrugata*, apresentou capacidade de reduzir o NBT igual ao controle positivo xantina+xantina oxidase. Este conjunto de dados reforça a sugestão da presença de hidroquinonas nos extratos de esponjas marinhas.

5. Conclusão

Este trabalho mostrou que extratos de esponjas marinhas possuem capacidades de *scavenger* de $^{\bullet}NO$, H_2O_2 e $ROO^{\bullet}$ e de proteger da peroxidação lipídica induzida por H_2O_2, $ROO^{\bullet}$ e Fe^{2+} (tabela 1). Entretanto, nenhum dos extratos foi capaz de prevenir o dano oxidativo a 2-desoxirribose induzido por $^{\bullet}OH$ até 100µg/ml. Além disso, o conteúdo de fenóis dos extratos parece estar relacionado à capacidade antioxidante contra a $ROO^{\bullet}$, evidenciada nas técnicas de TRAP e TBARS, enquanto o conteúdo de tióis parece estar envolvido com a proteção lipídica contra Fe^{2+}. A capacidade de *scavenger* de H_2O_2 se refletiu na proteção dos lipídios contra a peroxidação induzida por peróxido de hidrogênio, sugerindo ser esta habilidade o mecanismo de prevenção de lipoperoxidação ocasionada por tal agente oxidante. Dada a importância e o envolvimento destas espécies reativas em várias disfunções, os mecanismos bem como as moléculas responsáveis pelas ações antioxidantes observadas nestes extratos de esponjas marinhas merecem ser melhor entendidas.

Este trabalho apresentou uma metodologia plausível para determinar potenciais redox-ativos de importância fisiopatológica em extratos de esponjas marinhas. Além disso, levantou sugestões para futuras análises das propriedades redox observadas nos extratos de esponjas, bem como forneceu dados estimulantes para o prosseguimento da pesquisa por moléculas antioxidantes em esponjas marinhas.

Conforme já descrito para outras bioatividades, é possível que as características redox encontradas nestes extratos de esponjas marinhas possam ser reflexo da biologia e ecologia destes animais.

Tabela 1. Sumário das Propriedades Redox-Ativas dos Extratos de Esponjas Marinhas Estudados

	TRAP		Capacidade *Scavenger*	Capacidade *Scavenger*	Capacidade *Scavenger* de $O_2^{\bullet-}$		Capacidade *Scavenger*	Inibição da Lipoperoxidação induzida por		
	TAR	AUC	de $^{\bullet}NO$	de $^{\bullet}OH$	AAO	NBT	de H_2O_2	AAPH	Fe^{2+}	H_2O_2
Aaptos sp.	+	+	n.d.	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.
Agelas sp.	+	+	+	n.d.	n.d.	-	n.d.	+	+	n.d.
Axinella corrugata	+	+	+	n.d.	n.d.	-	n.d.	+	+	n.d.
Chondrilla nucula	+	+	+	n.d.	n.d.	n.d.	+	n.d.	n.d.	+
Cinachyrella alloclada	+	+	n.d.	n.d.	n.d.	n.d.	+	n.d.	+	+
Cliona sp.	+	+	-	n.d.	-	-	n.d.	+	n.d.	n.d.
Dragmacidon reticulatus	+	+	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Guitarra sepia	+	+	+	n.d.	-	-	n.d.	n.d.	n.d.	n.d.
Halichondria sp.	+	+	n.d.	n.d.	n.d.	-	n.d.	+	+	n.d.
Haliclona tubifera A	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Haliclona tubifera O	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hyatella sp.	+	+	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.
Mycale arcuiris	+	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Petromica citrina	+	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Protosuberites sp.	+	+	n.d.	n.d.	n.d.	n.d.	+	n.d.	n.d.	+
Raspailia elegans	+	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.
Scopalina ruetzeri amarela A	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Scopalina ruetzeri amarela O	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Scopalina ruetzeri vermelha	+	+	+	n.d.	-	n.d.	+	n.d.	n.d.	n.d.
Tedania ignis	+	+	+	n.d.	-	-	n.d.	n.d.	n.d.	n.d.
Trolox	+	+	n.t.	n.t.	n.t.	n.t.	n.t.	+	+	n.d.

Resultados expressos como média±erro padrão de um experimento representativo de três experimentos independentes (ANOVA, $p<0.05$, teste Duncan); n.d.=não detectada; n.t.=não testada; +=efeito antioxidante ou -=pró-oxidante de10µg/ml de extratos no TRAP e 100µg/ml de extrato nos demais ensaios.

6. REFERÊNCIAS

AIUB, C., GIANNERINI, A.M., FERREIRA, F., MAZZEI, J., STANKEVICINS, L., LOBO-HADJU, G., GUIMARÃES, P., HADJU, E., FELZENSWALB, I. Genotoxic evaluation of extracts from *Aplysina fulva*, a brazilian marine sponge. Mutat Res 611, 34-41, 2006.

AZEVEDO, L.G., MUCCILLO-BAISCH, A.L., FILGUEIRA, D..DE M., BOYLE, R.T., RAMOS, D.F., SOARES, A.D., LERNER, C., SILVA, P.A., TRINDADE, G.S. Comparative cytotoxic and anti-tuberculosis activity of *Aplysina caissara* marine sponge crude extracts. Comp Biochem Physiol C Toxicol Pharmacol 147, 1, 36-42, 2008a.

AZEVEDO, L.G., PERAZA, G.G., LERNER, C., SOARES, A., MURCIA, N., MUCCILLO-BAISCH, A.L. Investigation of the anti-inflammatory and analgesic effects from an extract of *Aplysina caissara*, a marine sponge. Fundam Clin Pharmacol 22, 5, 549-56, 2008b.

BATEL, R., BIHARI, N., RINKEVIC, B., DAPPER, J., SCHÄCKE, H., SCHRÖDER, H.C., MÜLLER, W.E.G. Modulation of organotin-induced apoptosis by the water pollutant methyl mercury in a human lymphoblastoid tumor cell line and a marine sponge. Marine Ecol Prog Ser 93, 245-251, 1993.

BHAKUNI, D.S & RAWAT, D.S. Bioactive Marine Natural Products. Springer, Now Iork, USA & Anamaya Publishers, Nova Delhi, Índia, 382 p., 2005.

BECERRO, M.A., PAUL, J.V., STARMER, J. Intracolonial variation in chemical defenses of the sponge *Cacospongia* sp. and its consequences on generalist fish predators and the specialist nudibranch predator *Glossodoris pallida*. Mar Ecol Prog Ser 168, 187-196, 1998.

BECERRO, M. A., TURON, X., URIZ, M. J. Multiple functions for secondary metabolites in encrusting marine invertebrates. J Chem Ecol 23, 1527-1547, 1997.

BELARBI, H.E., GÓMEZ, A.C., CHISTI,Y., CAMACHO, F.G., GRIMA, E.M. Producing drugs from marine sponges. Biotechnol Adv 21, 585-598, 2003.

BENTLEY, R. & CAMPBELL, I.M. In: Patai, S. (Ed.), The Chemistry of the Quinonoid Compounds. John Wiley & Sons, London, pp.683-735. Part 2, 1974.

BERGMANN, W. & FEENEY, R. J. Contributions to the study of marine products: XXXII. The nucleosides of sponges I. J Org Chem 16, 981- 987, 1951.

BERGMANN, W. & FEENEY, R. J. The isolation of a new thymine pentoside from sponges. J Am Chem Soc 72, 2809-2810, 1950.

BERGQUIST, P. R. Sponges. London, Hutchinson, 268 p., 1978.

BERLINCK, R.G., HADJU, E., DA ROCHA, R.M., DE OLIVEIRA, J.H.H.L., HERNÁNDEZ, I.L.C., SELEGHIM, M.H.R., GRANATO, A.C., DE ALMEIDA, E.V.R., NUÑEZ, C.V., MURICY, G.,

PEIXINHO, S., PESSOA, C., MORAES, M.O., CAVALCANTI, B.C., NASCIMENTO, G.G.F., THIEMANN, O., SILVA, M., SOUZA, A.O., SILVA, C.L., MANARINI, P.R.R. Challenges and rewards of research in marine natural products chemistry in Brazil. J Nat Prod 67, 3, 510-22, 2004.

BEWLEY, C.A. & FAULKNER, D.J. Lithistid sponges: star performers or hosts to the stars?. Angew Chem Int 37, 2162–2178, 1998.

BLACKER, D. Mild cognitive impairment – no benefit from vitamin E, little for donepezil. N Engl J Med 352, 2439-2441, 2005.

BOSCO, E. F. Role of nitric oxide in genotoxicity: Implications for carcinogenesis. Cancer Metastasis Rev. 17, 25-37, 1998.

BREDT, D.S. & SNYDER, S. Nitric oxide: A physiologic messenger molecule. Annu Ver Biochem 63, 175-195, 1994.

BYRNE, R.H., KESTER, D.R. Solubility of hydrous ferric oxide and iron speciation in seawater. Mar Chem 4, 255-274, 1976.

CAI, H. Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences, Cardiovasc Res 68, 26–36, 2005.

CASAPULLO, A., MINALE, L., ZOLLO, F. Paniceins and related sesquiterpenoids from the Mediterranean sponge *Reniera fulva*. J Nat Prod 56, 4, 527-533, 1993.

CHISHOLM, S. W. Stirring times in the Southern Ocean. Nature 407, 685-687, 2000.

CIMINO, G., DE STEFANO, S., MINALE, L. Occurrence of hydroxyhydroquinone and 2-aminoimidazole in sponges. Comp Biochem Physiol B Biochem Mol Biol 47, 4, 895-897, 1974.

CIMINO, G., DE STEFANO, S., MINALE, L. Paniceins, unusual aromatic sesquiterpenoids linked to a quinol or quinone system from the marine sponge *Halichondria panicea* Tetrahedron 29, 17, 2565-2570, 1973.

CLEVELAND, J.L. & KASTAN, M.B. Cancer: A radical approach to treatment. Nature 21, 407, 6802, 309-11, 2000.

COLASANTI, M. & VENTURINI, G. Nitric oxide in invertebrates. Mol Neurobiol 17, 157-74, 1998.

DA ROCHA, A.B., LOPES, R.M.; SCHWARTSMANN, G. Natural products in anticancer therapy. Curr Opin Pharmacol 1, 364– 369, 2001.

DAS, U.N. A radical approach to cancer. Med Sci Monit 8, 79-92. 2002.

DAVIS, A.R., BUTLER, A., ALTENA, I. Settlement behaviour of ascidian laral: perliminary evidence for inhibition by sponge allelochemicals. Mar Ecol Prog Ser 72, 117-123, 1991.

DE BAAR, H.J., DE JONG, J.T. M., BAKKER, D.C. E., LÖSCHER, B.M., VETH, C., BATHMANN, U., SMETACEK, V. Importance of iron for plankton blooms and carbon dioxide drawdown in the Southern Ocean. Nature 373, 412- 415, 1995.

DE FLORA, S., BAGNASCO, M., BENNICELLI, C., CAMOIRANO, A., BOJNEMIRSKI, A., KURELEC, B. Biotransformation of genotoxic agents in marine sponges. Mechanisms and modulation. Mutagenesis 10, 357-364, 1995.

DUNLAP, W., LLEWELLYN, L., DOYLE, J., YAMAMOTO, Y. A microtiter plate assay for screening antioxidant activity in extracts of marine organisms. Mar Biotechnol 5, 294-301, 2003.

DURSUN, E., DURSUN, B., SULEYMANLAR, G., OZBEN, T. Effect of haemodialysis on the oxidative stress and antioxidants in diabetes mellitus. Acta Diabetol 42, 123–128, 2005.

ELY, R., SUPRIYA, T., NAIK, C.G. Antimicrobial activity of marine organisms collected off the coast of South East India. J Exp Mar Biol Ecol 309, 121–127, 2004.

FAULKNER, D. J. Highlights of marine natural products chemistry (1972– 1999). Nat Prod Rep 17, 1–6, 2000.

FINDEN, D.A.S., TIPPING, E., JAWORSKI, G.H.M., REYNOLDS, L.S. Light-induced reduction of natural iron (III) oxide and its relevance to phytoplankton. Nature 309, 783-784, 1984.

GIOVINE, M., POZZOLINI, M., FAVRE, A., BAVESTRELLO, G., CERRANO, C., OTTAVIANI, F., CHIARAMTIMI, L., CERASI, A., CANGIOTTI, M., ZOCCHI, E., SCARFI, S., SARÀ, M., BENATTI, U. Heat stress-activated, calcium-dependent nitric oxide synthase in sponges. Nitric Oxide 5, 5, 427-431, 2001.

GORDEEVA, A.V. & LABAS, I. Reactive oxygen species generation by external surfaces of aquatic organisms. Tsitologiia 45, 3, 284-289, 2003.

GRANATO, A.C., BERLINCK, R.G.S., MAGALHÃES, A., SCHEFER, A.B., FERREIRA, A.G., SANCTIS, B., FREITAS, J.C., HAJDU, E., MIGOT, A.E. Produtos naturais das esponjas marinhas *Aaptos* sp., *Hymeniacidon* aff. *heliophila* e do nudibrânquio *Doris* aff. *verrucosa*. Quim Nova, 23, 5, 594-599, 2000.

GRYGLEWSKI, R.J., KOROUT, R., ROBAK, J., SWIES, J. On the mechanism of anti-thrombic action of flavonoids. Biochem Pharmacol 36, 317-322. 1987

HALLIWELL, B. Hypothesis: proteasomal dysfunction: a primary event in neurodegeneration that leads to nitrative and oxidative stress and subsequent cell death. Ann N Y Acad Sci 962, 182-194, 2002.

HALLIWELL, B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 18, 685-716, 2001.

HALLIWELL, B. & GUTTERIDGE, J.M.C. Free Radical biology and medicine. 4ª Ed. Oxford University Press Inc., Nova Iorque, 871 p., 2007.

HANASAKI Y., OGAWA S., FUKUI S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. Free Radic Biol Med 16, 845-850, 1994.

HARPER, M.K., BUGNI, T.S., COPP, B.R., JAMES, R.D., LINDSAY, B.S., RICHARDSON, A.D., SCHNABEL, P.C., TASDEMIR, D., VANWAGONER, R.M., VERBITSKI, S.M. IRELAND, C.M. Introduction to the chemical ecology of marine natural products. In: McClintock, J.B., Baker, B.J. (eds.), Marine Chemical Ecology CRC Press, Boca Raton, Florida., 3-69, 2001.

HENSLEY, K., ROBINSON, K.A., GABBITA, S.P., SALSMAN, S., FLOYD, R.A. Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 15, 28, 10, 1456-62, 2000.

HIRSCH, S., RUDI, A., KASHMAN, Y., LOYA, Y. New avarone and avarol derivatives from the marine sponge *Dysidea cinerea.* J Nat Prod 54, 92-97, 1991.

HOFRICHTER, R & SIDRI, M. ‘‘Ein Mittel fur jeden Zweck: der Badeschwamm’’. In: Das Mittelmeer Flora, Fauna, Ökologie, Hofrichter R (ed.) Spektrum Verlag, Bd 1, 608–809, 2001.

HOOPER, J.N.A. & LÉVI, C. Biogeography of Indo-west ´Pacific sponges: microcionidae, raspailiidae, axinellidae. In: van Soest, R.W.M., van Kempen, T.M.G., Braekman, J.C. (Eds.), Sponges in Time and Space. Balkema, Rotterdam, 191–212, 1994.

HUANG, D., OL, B., PRIOR, R.L. The chemistry behind antioxidant capacity assays. J Agric Food Chem 53, 1841-1856, 2005.

HUNG, L.M., CHEN, J.K., HUANG, S.S., LEE, R.S., SU, M.J. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res 47, 549–555, 2000.

IGNARRO, L.J., LIPPTON, H., EDWARDS, J.C., BARICOS, W.H., HYMAN, A.L., KADOWITZ, P.J., GRUETTER, C.A. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. J Pharmacol Exp Ther 218, 3, 739-749, 1981.

JACKLET, J.W. Nitric oxide signaling in invertebrates. Invert Neurosci 3, 1, 1-14, 1997.

JASPARS, M.; HORTON, P. A.; MADRID, L. H.; CREWS, P. The cyclorenierins, sesquiterpenoid quinols from the sponge *Haliclona* sp. collected in Vanuatu. J Nat Prod 58, 609-612, 1995.

JICKELLS T.D., AN, Z.S., ANDERSEN, K.K., BAKER, A.R., BERGAMETTI, G., BROOKS, N., CAO, J.J., BOYD, P.W., DUCE, R.A., HUNTER, K.A., KAWAHATA, H., KUBILAY, N., LA ROCHE, J., LISS, P.S., MAHOWALD, N., PROSPERO, J.M., RIDGWELL, A.J., TEGEN, I., TORRES,

R. Global Iron Connections Between Desert Dust, Ocean Biogeochemistry, and Climate. Science 208, 5718, 65-71, 2005.

KAPPEL, V.D., COSTA, G.M., SCOLA, G., SILVA, F.A., LANDELL, M.F., VALENTE, P., SOUZA, D.G., VANZ, D.C., REGINATTO, F.H., MOREIRA, J.C.F. Phenolic Content and Antioxidant and Antimicrobial Properties of Fruits of *Capsicum baccatum* L. var. *pendulum* at Different Maturity Stages. J Med Food 11, 2, 267–274, 2008.

KARBAN, R. & MYERS, J.H. Induced plant responses to herbivory. Annu Rev Ecol Syst 20, 331-348, 1989.

KELECOM, A. Marine organisms: na alternative source of potentially valuable natural products. Mem Inst Oswaldo Cruz 86, 2, 99-106, 1991.

KOULMAN, A., PROKSCH, P., EBEL, R., BEEKMAN, A.C., UDEN, W., KONINGS, A.W.T., PEDERSEN, J.A., PRAS, N., WOERDENBAG, H.J. Cytoxicity and mode of action of Aeroplysinin-1 and a related dienone from the sponge *Aplysina aerophoba*. J Nat Prod 59, 591-594, 1996.

KOZIOL, C., KRUSE, M., WAGNER-HÜLSMANN, C., PANCER, Z., BATEL, R., MÜLLER, W.E.G. Cloning of the heat-inducible stress protein (HSP70) from the marine sponge *Geodia cydonium.* Can Techn Rep Fisheries Aquat Sci 2093, 104-109, 1995.

KURELEC, B., PIVCEVIC, B., MÜLLER, W.E.G. Determination of pollutants with multixenobiotic-resistance inhibiting properties. Marine Env Res 39, 261-265, 1995.

LAMBETH, J.D. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. Curr Opin Hematol 9, 11–17, 2002.

LAMBETH, J.D. NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4, 181–189, 2004.

LAUGHTON, M.J., EVANS, P.A., MORONEY, M.A., HOULT J.R.S., HALLIWELL, B. Inhibition of mammalian 5-1ipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Biochem Pharmacol 42, 1673-1681, 1991.

LEE, Y.K., LEE, J.H., LEE, H.K. Microbial symbiosis in marine sponges. J Microbiol 39, 254–264, 2001.

LIPPERT, H., IKEN, K., VOLK, C., KOCK, M., RACHOR, E. Chemical defence against predators in a sub-Arctic fjord. J Exp Mar Biol Ecol 310, 131-146, 2004.

LIU, J., FINKEL, T. Stem cell aging: what bleach can teach, Nat Med 12, 383–384, 2006.

LOSCHEN, G., AZZI, A., RICHTER, C., FLOHE, L. Superoxide radicals as precursors of mitochondrial hydrogen peroxide, FEBS Lett 42, 68-72, 1974.

LOYA, S., TAL, R., KASHMAN, Y., HIZI, A. Ilimaquinone, a selective inhibitor of the Rnase-H activity of human-immunodeficiency-virus tipe-I reverse-trascriptase. Antimicrob Agents Chemother 34, 2009-2012, 1990.

MANDEL, S.A., AVRAMOVICH-TIROSH, Y., REZNICHENKO, L., ZHENG, H., WEINREB, O., AMIT, T., YOUDIM, M.B. Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. Neurosignals 14, 46-60, 2005.

MARTÍN, D. & URIZ, M.J. Chemical bioactivity of Mediterranean benthic organisms against embryos and larvae of marine invertebrates. J Exp Mar Biol Ecol 173, 11-27, 1993.

MARZATICO, F., CAFÉ, C., TABORELLI, M., BENZI, G. Experimental Parkinson's disease in monkeys. Effect of ergot alkaloid derivative on lipid peroxidation in different brain areas. Neurochem Res 18, 10, 1101-1106, 1993.

MASSEY, V., STRICKLAND, S., MAYHEW, S.G., HOWELL, L.G., ENGEL, P.C., MATTHEWS, R.G., SCHULMAN, M., SULLIVAN, P.A. The production of superoxide anion radicals in the reaction of reduced flavins and flavoproteins with molecular oxygen. Biochem Biophys Res Commun 36, 891-897, 1969.

MCCORD, J.M. & FRIDOVICH, I. The utility of superoxide dismutase in studying free radical reactions. II. The mechanism of the mediation of cytochrome c reduction by a variety of electron carriers. J Biol Chem 245, 1374–1377, 1970.

MEIJER, L., THUNNISSEN, A-M.W.H., WHITE, A.W., GARNIER, M., NIKOLIC, M., TSAI, L-H., WALTER, J., CLEVERLEY, K.E., SALINAS, P.C., WU, Y-Z., BIERNAT, J., MANDELKOW, E-M., KIM, S-H., PETTIT, G.R. Inhibition of cyclin-dependent kinases, GSK-3β and CK1 by hymenialdisine, a marine sponge constituent. Chem Biol 7, 51-63, 2000.

MICHEL, T. & FERON, O. Nitric oxide synthases: Which, where, how and why. J Clin Invest 100, 2146-2152, 1997.

MILLER, W. & KESTER, D. Photochemical iron reduction and iron bioavailability in Seawater. J Mar Res 52, 32, 343,1994.

MONKS, N.R., LERNER, C., HENRIQUES, A.T., FARIAS, F.M., SCHAPOVAL, E.E.S., SUYENAGA, E.S., DA ROCHA, A.B., SCHWARTSMANN, G., MOTHES, B. Anticancer, antichemotactic and antimicrobial activities of marine sponges collected off the coast of Santa Catarina, southern Brazil. J Exp Mar Biol Ecol 281, 1-12, 2002.

MOREIRA, P.I., HONDA, K., LIU, Q., SANTOS, M.S., OLIVEIRA, C.R., ALIEV, G., NOMURA, A., ZHU, X, SMITH, M.A., PERRY, G. Oxidative stress: the old enemy in Alzheimer's disease pathophysiology. Curr Alzheimer Res 2, 403-408, 2005.

MOOSMANN, B. & BEHL, C. Antioxidants as treatment for neurodegenerative disorders. Expert Opin Invest Drugs 11, 1407-1435, 2002.

MOTHES, B., LERNER, C., SILVA, M.M. da. Guia ilustrado – Esponjas Marinhas – Costa Sul-brasileira. Pelotas/RS: USEB, 82p., 2003.

MÜLLER, W.E.G. (ed.). Use of Aquatic Invertebrates as Tools for Monitoring of Environmental Hazards. Gustav Fischer Verlag, Stuttgart, New York, 1994.

MÜLLER, W.E.G. Molecular phylogeny of metazoa (animals): Monophyletic origin. Naturwiss 82, 321-329, 1995.

MÜLLER, W. E. G., KOZIOL, C., KURELEC, B., DAPPER, J., BATEL, R., & RINKEVICH, B. Combinatory efects of temperature stress and nonionic organic pollutants on stress protein (hsp70) gene expression in the fresh water sponge *Ephydatia fluviatilis*. Arch Environm Contam Toxicol 14, 1203- 1208, 1995.

MÜLLER, W. E. G. & MÜLLER, I. Sponge cells and tissue *in vitro*: Useful biomarkers of aquatic pollution. In: Wells, P.G.; Lee K.; Blaise, C. (eds) Microscale Aquatic Toxicology Advances, Techniques and Practice, CRC Lewis Publishers, 1996.

MÜLLER, W.E.G., SLADIC, D., ZAHN, R.K., SCHRÖDER, H.C., GACIC, M.J., HEIDEMANN, D., BERND, A., KURELEC, B, EICH, E., SEIBERT, G. Potent antileukemic activity of the novel cytostatic agent avarone and its analogues *in vitro* and *in vivo*. Cancer Res 45, 4822-4826, 1985.

MÜLLER, W.E.G., STEFFEN, R., RINKEVICH, B., MATRANGA, V., & KURELEC, B. The multixenobiotic resistance mechanism in the marine sponge *Suberites domuncula:* Its potential application in assessment of environmental pollution. Marine Biol 125, 165-170, 1996.

NAGATSU, T. & SAWADA, M. Molecular mechanism of the relation of monoamine oxidase B and its inhibitors to Parkinson's disease: possible implications of glial cells. J Neural Transm Suppl 71, 53-65, 2006.

NETTO BENETTI, M.R., RUDNICKI, M., ZANOTTO-FILHO, A., DE OLIVEIRA, M.R., KUREK, A.G., COITINHO, A., SCHWARTSMANN, G., MOREIRA, J.C.F. Evaluation of antioxidant effect of extracts of Symphyopappus casarettoi. Fitoterapia 78, 232–234, 2007.

NEVALAINEN, T.J., QUINN, R.J., HOOPER, J.N.A. Phospholipase A2 in porifera. Comp Biochem Physiol B Biochem Mol Biol 137, 413–420, 2004.

NEWBOLD, R.W., JENSEN, P.R., FENICAL, W., PAELIK, J.R. Antimicrobial activity of Caribbean sponge extracts. Aquat Microb Ecol 9, 279-284, 1999.

NEWTON, G.L. & FAHEY, R.C. Determination of biothiols by bromobimane labeling and high-performance liquid chromatography. Methods Enzymol 251, 148-166, 1995.

NIKI, E. Free radical initiators as source of water- or lipid-soluble peroxyl radicals. Methods Enzymol 185, 100-108, 1990.

OFICJALSKI, P. Spongia fluviatilis (Badiaga). Pharm Zentralhalle Dtschl 78, 173–175, 1937.

OSINGA, R., TRAMPER, J., WIJIFFELS, R.H. Cultivation of marines sponges for metabolites production: applications for biotechnology? TIBTECH 16, 130– 134, 1998.

PATIL, A.D., KOKKE, W.C., COCHRAN, S., FRANCIS, T.A., TOMSZEK, T. WESTLEY, J.W. Brominated polyacetylenic acids from the marine sponge *Xestospongia muta*: Inhibitors of HIV protease. J Nat Prod 55, 1170-1177, 1992.

PAWLIK, J.R., MCFALL, G., ZEA, S. Does the odor from sponges of the genus *Ircinia* protect them from fish predators? J Chem Ecol 28, 6, 1103-15, 2002.

PESKIN, A.V., YULII, A.L., TIKHONOV, A.N. Superoxide radical production by sponges *Sycon* sp. FEBS Lett 434, 201-204, 1998.

POMPELLA, A., VISVIKIS, A., PAOLICCHI, A., DE TATA, V., CASINI, A.F. Advanced oxidation protein products in serum of patients with myotonic disease type I: association with serum γ-glutamyltransferase and disease severity. Biochem Pharmacol 66, 1499-1503, 2003.

PORTER, J.M. & TARGETT, W.M. Allelochemical interactions between sponges and corals. Biol Bull 175, 230-239, 1988.

PRADO, M.P., TORRES, Y.R., BERLINCK, R.G.S., DESIDERÁ, C., SANCHEZ, M.A., CRAVEIRO, M.V., HADJU, E., DA ROCHA, R.M., MACHADO-SANTELLI, G.M. Effects of marine organisms extracts on microtubule integrity and cell cycle progression in cultured cells. J Exp Mar Biol Ecol 313, 125– 137, 2004.

PRIOR, R.L., WU, X., SCHAICH, K. Standardized methods for the determintion of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53, 4290-4302, 2005.

PROKOF'EVA, N.G., UTKINA, N., CHAIKINA, E.L., MAKARCHENKO, A.E. Biological activities of marine sesquiterpenoid quinones: structure-activity relationships in cytotoxic and hemolytic assays. Comp Biochem Physiol B Biochem Mol Biol 139, 169-173, 2004.

PROKSCH, P.; EDRADA, R.A.; EBEL, R. Drugs from the seas - current status and microbiological implications. Appl Microbiol Biotechnol 59, 125-134, 2002.

RANGEL, M., SANCTIS, B., FREITAS, J.C., POLATTO, J.M., GRANATO, A.C., BERLINCK, R.G.S., HADJU, E. Cytotoxic and neurotoxic activities in extracts of marine sponges (Porifera) from southeastern Brazilian coast. J Exp Mar Biol Ecol 262, 31-40, 2001.

REGOLI, F., NIGRO, M., CHIERICI, E., CERRANO, C., SCHIAPPARELLI, S., TOTTI, C., BAVESTRELLO, G. Variations of antioxidant e.ciency and presence of endosymbiotic diatoms in the Antarctic porifera *Haliclona dancoi*. Mar Environ Res 58, 637–640, 2004.

REISWIG, H.M. *In situ* pumping activities of tropical Demospongiae. Mar Biol 9, 38-50, 1971.

RHEE, S.G. Cell signaling: H_2O_2, a necessary evil for cell signaling, Science 312, 1882–1883, 2006.

RICH, H.W., MOREL, F.M.M. Availability of well-defined colloids to the marine diatom *Thalassiosira weissflogii*. Limnol Oceanogr 35, 652-662, 1990.

RICHELLE-MAURER, E., GOMEZ, R., BRAEKMAN, J.-C., VAN DE VYVER, G., VAN SOEST, R.W.M., DEVIJVER, C. Primary cultures from the marine sponge *Xestospongia muta* (Petrosiidae, Haplosclerida). J Biotechnol 100, 169–176, 2003.

RODRIGUEZ, J., QUIÑOA, E., RIGUERA, R., PETERS, B.M., ABRELL, L.M., CREWS, P. The structure and stereochemistry of cytotoxic sesquiterpene quinones from *Dactilospongia elegans*. Tetrahedron 32, 6667-6680, 1992.

SABLINA, A.A., ILYINSKAYA, G.V., AGAPOVA, L.S., KRAVCHENKO, J.E., CHUMAKOV, P.M. The antioxidant function of the p53 tumor suppressor. Nat Med 11, 12,1306-13, 2005.

SÀRA, M. & VACELET, J. Ecologic des Dimosponges, Traire' de Zoologie: Spongiaires. Tome III. P.P. Gras & Masson, Paris. pp. 462-576, 1973.

SATO, K., ITO, K., KOHARA, H., YAMAGUCHI, Y., ADACHI, K., ENDO, H. Negative regulation of catalase gene expression in hepatoma cells. Mol Cell Biol 12, 2525-33, 1992.

SCHRODER, K. Die Verwendung der Susswasserschwamme in der Ukraine. Umsch Wiss Tech 46, 507–509, 1942.

SCHWARTSMANN, G., BRONDANI DA ROCHA, A., BERLINCK, R.G.S., JIMENO, J. Marine organisms as a source of new anticancer agents. Lancet Oncol 2, 221– 225, 2001.

SEPCIĆ, K., BATISTA, U., VACELET, J., MACEK, P., TURK, T. Biological activities of Aqueous extracts from marine sponges and cytotoxic effects of 3-alkylpyridinium polymers from *Reniera sarai*. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 117, 1, 47-53, 1997.

SILVA, A.C. DA, KRATZ, J.M., FARIAS, F.M., HENRIQUES, A.T., SANTOS, J. DOS, LEONEL, R.M., LERNER, C., MOTHES, B, BARARDI, C.R.M., SIMÕES, C.M.O. *In vitro* antiviral activity of marine sponge collected off Brazilian coast. Biol Pharm Bull 29, 1, 135-140, 2006.

SILVA, E.G. DA, BEHR, G.A., ZANOTTO-FILHO, A., LORENZI, R., PASQUALI,M.A.B., RAVAZOLO, L.G., BORDIGNON, C.L.J., SILVA, F.A., ABOY, A.L., BASSANI, V.L., HENRIQUES, A.T., REGINATTO, F.H., DAL-PIZZOL, F., MOREIRA, J.C.F. Antioxidant Activities and Free Radical Scavenging Potential of *Bauhinia microstachya* (RADDI) MACBR. (Caesalpinaceae) Extracts Linked to Their Polyphenol Content. Biol Pharm Bull 30, 8, 1488-1496, 2007.

SIMPSON, T. L. The Cell Biology of Sponges. Springer-Verlag, New York-Berlin, 681p., 1984.

SIPKEMA, D., FRANSSEN, M.C.R., OSINGA, R., TRAMPER, J., WIJFFELS, R.H. Marine Sponges as Pharmacy. Mar Biotechnol 7, 142–162, 2005.

SMITH, C. M.; MARKS, A.; LIEBERMAN, M. Marks' basic medical bichemistry: A clinical Approach. 2ª Edição. Lippincott Williams & Wilkins, Baltimore, EUA, 439-457, 2005.

SULTANA, R., PERLUIGI, M., BUTTERFIELD., D.A. Redox proteomics identification of oxidatively modified proteins in Alzheimer's disease brain and *in vivo* and *in vitro* models of AD centered around Abeta (1-42). J Chromtogr B 833, 3-11, 2006.

SUN, Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med 8, 6, 583-599, 1990.

SUNDQUIST, A.R. & FAHEY, R.C. The function of gamma-glutamylcysteine and bis-gamma-glutamylcystine reductase in *Halobacterium halobium*. J Biol Chem 264, 719-725, 1989.

TADOLINI, B., JULIAN, C., PIU, L., FRANCONI, F., CABRINI, L., Resveratrol inhibition of lipid peroxidation. Free Radic Res 33, 105–114, 2000.

TAKAMATSU, S., HODGES, T.W., RAJBHANDARI, I., GERWICK, W.H., HAMANN, M.T., NAGLE, D.G. Marine natural products as novel antioxidant prototypes. J Na. Prod 66, 605-608, 2003.

TCHERNOV, D., GORBUNOV, M.Y., VARGAS, C.D., YADAV, S.N., MILLIGAN, A.J., HAGGBLOM, M., FALKOWSI, P.G. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc Natl Acad Sci USA 101, 13531–13535, 2004.

THAKUR, N. L. & MÜLLER W. E. G. Biotechnological potential of marine sponges. Curr Sci 86, 11, 1506-1512, 2004.

TSEN, C.C. & TAPPEL, A.L. Catalytic Oxidation of Glutathione and Other Sulfhydryl Compounds by Selenite. J Biol Chem 233, 1230-1232, 1958.

TURNER, D.R. & HUNTER, K.A. The Biogeochemistry of Iron in Seawater; John Wiley & Sons, Ltd.: Chicester, 2001.

TURON, X., BECERRO, M.A., URIZ, M.J. Seasonal patterns of toxicity in benthic invertebrates: The incrusting sponge *Crambe crambe* (Poecilosclerida). Oikos 75, 33-40, 1996.

TZIVELEKA, L.A., KOURONAKIS, A.P., KOURONAKIS, P.N., ROUSSIS, V., VAGIAS, C. Antioxidant potential of natural and sinthesised polyprenylated hydroquinones. Bioorg Med Chem 10, 935-939, 2002.

URIZ, M.J., TURON, X., BECERRO, M.A., GALERA, J. Feeding deterrence in sponges. The role of toxicity, physical defenses, energetics contents, and life-history stage. J Exp Mar Biol Ecol 205, 187-204, 1996.

UTKINA, N.K., MAKARCHENKO, A.E., SHCHELOKOVA, O.V., VIROVAYA, M.V. Antioxidant activity of phenolic metabolites from marine sponges. Chem Nat Compd 40, 4, 373-377, 2004.

VOGEL, S. Current-induced flow through living sponges in nature. Proc Natl Acad Sci USA 74, 2069-2071, 1977.

WANG, W. & BALLATORI, N. Endogenous glutathione conjugates: occurrence and biological functions. Pharmacol Rev 50, 3, 335-356, 1998.

WATSON, A. J.; BAKKER, D.C. E.; RIDGWELL, A.J.; BOYD, P. W.; LAW, C. S. Effect of iron supply on Southern ocean CO_2 uptake and implications of glacial atmospheric CO_2. Nature 407, 730-733, 2000.

WELLS, M.L., MAYER, L.M., GUILLARD, R.R.L. A chemical method for estimating the availability of iron to phytoplankton in seawater. Mar Chem 33, 23-40, 1991.

WELLS, M.L., MAYER, L.M., GUILLARD, R.R.L. Photoinduced changes in the lability of iron in seawater and its significance to phytoplankton. Trans Amer Geophys Union 68, 1762, 1987.

WELLS, M.L., ZORKIN, N.G., LEWIS, A.G. The role of colloid chemistry in providing a source of iron to phytoplankton. J Mar Res 41, 731-746, 1983.

ZAHN, R.K., ZAHN, G., MÜLLER, W.E., MÜLLER, I., BEYER, R., MÜLLER-BERGER, U., KUPELEC, B., RIJAVEC, M., BRITVIĆ, S. Consequences of detergent pollution of the sea: Effects on regenerating sponge cubes of *Geodia cydonium.* Sci Total Environ 8, 109-151, 1977.

ZHANG, W., XUE, S., ZHAO, Q., ZHANG, X., LI, J., JIN, M., YU, X., YUAN, Q. Biopotentials on marine sponges from China oceans: past and future. Biomol Eng 20, 413-419, 2003.

ZHENG, Z., ZENG, W., HUANG, Y., YANG, Z., LI, J., CAI, H., SU, W. Detection of antitumor and antimicrobial activities in marine organisms associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol Lett 188, 87–91, 2000.

ANEXO 1: Regras para submissão de manuscrito para o periódico Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology

Guide for Authors

An International Journal
Please bookmark this URL: http://www.elsevier.com/locate/cbpc

Guide for Authors

The journal publishes original articles emphasizing comparative and environmental aspects of the physiology, biochemistry, molecular biology, pharmacology, toxicology and endocrinology of animals. Adaptation and evolution as organizing principles are encouraged. Studies on other organisms will be considered if approached in a comparative context.

Part A. Molecular and Integrative Physiology deals with molecular, cellular, integrative, and ecological physiology. Topics include bioenergetics, circulation, development, excretion, ion regulation, endocrinology, neurobiology, nutrition, respiration, and thermal biology. Studies on regulatory mechanisms at any level or organization such as signal transduction and cellular interactions and control of behaviour are encouraged.

Part B. Biochemistry and Molecular Biology covers biochemical and molecular biological aspects of metabolism, enzymology, regulation, nutrition, signal transduction, promoters, gene structure and regulation, metabolite and cell constituents, macromolecular structures, adaptational mechanisms and evolutionary principles.

Part C. Toxicology and Pharmacology is concerned with chemical and drug action at different levels of organization, biotransformation of xenobiotics, mechanisms of toxicity, including reactive oxygen species and carcinogenesis, endocrine disruptors, natural products chemistry, and signal transduction. A molecular approach to these fields is encouraged.

Part D. Genomics and Proteomics covers the broader comprehensive approaches to comparative biochemistry and physiology that can be generally termed as "-omics", e.g., genomics, functional genomics (transcriptomics), proteomics, metabolomics, and underlying bioinformatics. Papers dealing with fundamental aspects and hypotheses in comparative physiology and biohcemistry are encouraged rather than studies whose main focus is purely technical of methodological.

Naturally, a certain degree of overlap exists between the different sections, and the final decision as to where a particular manuscript will be published after passing the rigorous review process lies with the editorial office.

Submission and review of manuscripts

Online submission of papers

Manuscripts are to be submitted to the CBP Editorial Office electronically at

http://gemini.econ.umd.edu/cbp_c .
After registration, authors are asked to upload their article and associated artwork, preferably in a single file. If necessary, the CBP Editorial Office will generate a PDF file to be used for the reviewing proces.

Full instructions on how to use the online submission tool will be available at the above web address or can be requested by e-mail from the CBP Editorial Office (CBPjrnl@interchange.UBC.ca).

During the submission proces, authors are asked to select an appropriate section of CBP and to provide names and address (including e-mail) of at least five researchers of recognized competence who may be considered as reviewers.

Review articles
Before writing their manuscripts, potential authors of review articles should contact one of the Editors who, after consultations with the other editor and/or members of the Editorial Board, will provide feedback on suitability of the topic. Reviews should be topical, and serve as critical appraisals of areas of research. They should provide an up-to-date analysis of concepts and point out future directions. For manuscript preparation, follow the instructions below.

Revision of manuscripts: Revised manuscripts must be submitted within two months of the authors' receipt of the referees' reports. Otherwise they will be considered as new submissions.

Proofs: The corresponding author will receive proofs by e-mail or post. Proofs must be checked immediately and returned to Elsevier. Corrections to the proofs should be restricted to printer's errors only. Substantial alterations may be charged to the author. Elsevier will do everything possible to get your article corrected and published as quickly and accurately as possible. *Therefore, it is important to ensure that all of your corrections are sent back to us in one communication.* Subsequent corrections will not be possible, so please ensure your first sending is complete.

Reprints: The corresponding author will receive **twenty five offprints free of charge.** Additional offprints may be purchased using the order form accompanying the proofs.

Page charges: **CBP has no page charges.**

Preparation of manuscripts

Please note: Investigators are encouraged to report measured experimental/exposure levels of toxicants whenever possible, rather than simply stating nominal values.

Sections: Manuscripts should be subdivided into the following sections: Title page, abstract, introduction, materials and methods, results, discussion, acknowledgements, references, captions to figures, tables.

Format: All sections of the manuscript must be 1.5-spaced with 2.5 cm (1 inch) margins. Pages should be numbered consecutively. Avoid footnotes. Underline only words or letters that will be printed in italics. Mark the position of each figure and table in the margin. The full Latin name of all species used in the study must be supplied.

Title page: The title should be short, concise and informative. Consult a recent issue of CBP for author format (⇨ http://www/elsevier.com/locate/cbp). The author's name should be followed by his/her department, institution, city, and country. Indicate the author to whom correspondence and proofs should be addressed, and supply full postal address as well as phone and fax numbers, and an e-mail address. If submitting a review article, write "REVIEW" at the top of the title page.

Abstract: The second page of the manuscript must contain only the abstract and the key words. The abstract should be a single paragraph not exceeding 200 words. Non-standard abbreviations and reference citations should be avoided.

Key words: Up to eight key words, which may or may not appear in the title, should be listed in alphabetical order after the abstract. Only these key words, together with the title, will be used to compile the subject index.

References:
1. All publications cited in the text should be presented in alphabetical order in a list following the text of the manuscript.
2. In the text refer to the author's name and year of publication.
3. If reference is made in the text to a publication written by more than two authors the name of the first author should be used followed by "et al.". In this list names of first authors and all co-authors should be mentioned.
4. References cited together in the text should be arranged chronologically.
5. The List of references should be arranged alphabetically on authors' names, and chronologically per author. Names of all authors must be included. *Do not use et al*. Publications by the same author(s) in the same year should be listed as 2000a, 2000b, etc. Follow the relevant examples below.

Axelsson, M., Farrell, A.P., 1993. Coronary blood flow in vivo in the coho salmon *(Oncorhynchus kisutch).* Am. J. Physiol. 264, R963 - 971.
Hiramatsu, N., Cheek, A.O., Sullivan, C.V., Matsubara, T., Hara, A., 2005. Vitellogenesis and endocrine disruption. In: Mommsen, T.P., Moon, T.W. (Eds.), Biochemistry and Molecular Biology of Fishes, vol. 6. Environmental Toxicology, Elsevier, Amsterdam, pp. 431-471.
Lindsley, J.E., Rutter, J., 2004. Nutrient sensing and metabolic decisions. Comp. Biochem. Physiol. B 139, 543-559.
Moyle, P.B., Cech, J.J., 2004. Fishes. An introduction to ichthyology. 5th ed. Prentice Hall, Upper Saddle River, NJ.

Tables: Tables should be prepared as follows:
(a) Refer to current tables in the journal, for required spatial layout. If possible, a Times Roman font should be used.

(b) Each table, including heading and legend should be typed on a separate sheet. If possible, a Times Roman font should be used.
(c) Insert heavy rules at the head and foot of each table, and fine rules below column headings.

Italics: Genus and species names, and other words normally italicized, should be typed in italics or underlined.

Illustrations: Photographs, charts and diagrams are to be referred to as "figs" and should be ordered consecutively.

Colour Illustrations
If, together with your accepted article, you submit usable colour figures then Elsevier will publish these figures in colour on the web (e.g., ScienceDirect and other sites) free of charge. For colour reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions .

Please note: Because of technical complications which can arise by converting colour figures to 'grey scale' (for the printed version should you not opt for colour in print) please submit in addition usable black and white versions of the file(s) corresponding to all the colour illustrations.

As only one figure caption is used for both colour and black and white versions of figures, please ensure that the figure captions are meaningful for both versions, if applicable.

Instructions regarding GenBank/DNA Sequence Linking:

DNA sequences and GenBank Accesion numbers: Many Elsevier journals cite "gene accession numbers" in their running text and footnotes. Gene accession numbers refer to genes or DNA sequences about which further information can be found in the database at the National Center for Biotechnical Information (NCBI) at the National Library of Medicine. Elsevier authors wishing to enable other scientists to use the accession numbers cited in their papers via links to these sources, should type this information in the following manner:

For each and every accession number cited in an article, authors should type the accession number in **bold, underlined text.** Letters in the accession number should always be capitalised. (See Example 1 below). This combination of letters and format will enable Elsevier's typesetters to recognize the relevant texts as accession numbers and add the required link to GenBank's sequences.

Example 1: "B-cell tumor from a chronic lymphatic leukemia (GenBank accession no. **BE675048**), and a T-cell lymphoma (GenBank accession no. **AA361117**)".

Authors must check accession numbers very carefully. **An error in a letter or number can result in a dead link.**

In the final version of the ***printed*** **article**, the accession number text will not appear bold or underlined (see Example 2 below).

Example 2: "B-cell tumor from a chronic lymphatic leukemia (GenBank accession no. BE675048), and a T-cell lymphoma (GenBank accession no. AA361117)".

In the final ***electronic*** **copy**, the accession number text will be linked to the appropriate source in the NCBI databases enabling readers to go directly to that source from the article (see Example 3 below).

Example 3: "B-cell tumor from a chronic lymphatic leukemia (GenBank accession no. BE675048), and a T-cell lymphoma (GenBank accession no. AA361117)".

Summary of requirements

1. Designate the corresponding author and provide telephone and fax numbers, and an e-mail address.
2. Provide an abstract of less than 200 words; append up to eight key words to the abstract page.
3. Check the style in which references are cited; unpublished work will not be listed in this section unless it is "in press".
4. If referencing manuscripts "in press", these must be uploaded as supplementary material during the manuscript submission process.
5. Provide names and addresses (including phone and fax numbers & e-mail addresses) of at least five researchers of recognized competence who may be considered as referees.

Author enquiries

Visit Elsevier's Quickguide http://www.elsevier.com for the facility to track accepted articles and set up e-mail alerts to inform you of when an article's status has changed. The Elsevier Quickguide also provides detailed artwork guidelines, copyright information, frequently asked questions and more.

Contact details for questions arising after acceptance of an article, especially those relating to proofs, are provided when an article is accepted for publication, by Elsevier.

For additional information, visit our website at http://www.elsevier.com/locate/cbp

March 2007 version