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Macroalgas e poluentes marinhos: estudo dos extratos apolares de *Dichotomaria marginata*, *Acanthophora spicifera*, *Cladophora prolifera*, *Lobophora variegata*; identificação de sesquiterpenos em *Dictyopteris delicatula*

Tese apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares e Fungos em Análises Ambientais.

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"Autor desconhecido"

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RESUMO

As macroalgas marinhas eram apontadas como bioindicadoras de poluição com base apenas no aumento ou na diminuição de suas populações em ambientes contaminados. Contudo, nossos estudos atestam que podem acumular substâncias dissolvidas ou suspensas no ambiente aquático. Em estudo do extrato hexânico da alga vermelha *Dichotomatia marginata* (J. Ellis & Solander) Lamarck, coletada na Praia de Fortaleza, Ubatuba, São Paulo, foram identificados, por cromatografia gasosa/espectrometria de massas (CG/EM), 22 poluentes e por cromatografia líquida de alta eficiência/espectrometria de massas, 16 poluentes. Ambas as técnicas mostraram-se ferramentas úteis na identificação dos contaminantes dessorvidos do talo dessa alga, a qual pode ser considerada um valioso indicador de poluição do ambiente marinho. No indispensável levantamento dos poluentes aquáticos aqui apresentados, estão compilados as classes a que pertencem, fórmulas, massas moleculares e locais em que foram encontrados. Entre eles estão fármacos e drogas de abuso, produtos de higiene e cuidados pessoais e substâncias empregadas na indústria e agricultura. Muitos desses poluentes não constam em programas de monitoramento de água, normativas e legislações de controle ambiental. Também, pela técnica de CG/EM, foram identificados 13 sesquiterpenos, pertencentes a classe dos cadalanos, no extrato hexânico de *Dictyopteris delicatula* J.V. Lamouroux, estudo que contribuiu efetivamente para o conhecimento do terpenoma da família Dictyotaceae. Ainda utilizando CG/EM, foram investigadas as substâncias encontradas nos extratos hexânicos e em diclorometano das macroalgas *Acanthophora spicifera* (M.Vahl) Børgesen, *Cladophora prolifera* (Roth) Kützing e *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira. Esses organismos são pertencentes a diferentes taxa, foram coletadas em diferentes regiões litorâneas brasileiras e devido à natureza dos polímeros que compõem os seus talos, são capazes de reter e acumular poluentes dispersos nos ambientes aquáticos. Delas foram extraídos e identificados 51 contaminantes. Esses estudos embasam o conceito de que macroalgas são indicadoras da presença de poluentes no meio em que vivem. Também, pelo reconhecimento de muitos de seus constituintes, ampliam o conhecimento da diversidade química das macroalgas brasileiras.

Palavras-chave: bioindicadores de poluição, contaminantes aquáticos, adsorção, metabolitos algais.

ABSTRACT

Marine macroalgae so far were been considered pollution indicators only regarding their population increase or decrease, in polluted environments. However, our studies attest that they may accumulate dissolved or suspended substances in the aquatic environment. Twenty two pollutants have been identified, by gas chromatography/mass spectrometry (GC/MS), and 16, by high - performance liquid chromatography/mass spectrometry (HPLC/MS) in the hexane extract of the seaweed *Dichotomatia marginata* (J. Ellis & Solander) Lamarck, collected at Fortaleza Beach, Ubatuba, São Paulo. Both chromatographic techniques proved to be useful tools in the identification of contaminants desorbed from this algae tallus. which can be considered a valuable pollution indicator for the marine environment. The survey that was made regarding the aquatic pollutants presents their names, molecular masses, formulas, and sites where they were found. Among these contaminants are drugs of abuse, hygiene and personal care products, and substances used in industry and agriculture. Many them are not included in water monitoring programs, norms and environmental control legislation. Moreover, by the same technique (CG/MS), 13 sesquiterpenes, belonging to the cadalane class, were identified in the *Dictyopteris delicatula* J.V. Lamouroux hexane extract; this study has effectively contributed to the knowledge of the Dictyotaceae family terpenoma. The hexane and dichloromethane extract's constituents of the macroalgae *Acanthophora spicifera* (M.Vahl) Børgesen, *Cladophora prolifera* (Roth) Kützing, and *Lobophora variegate* (J.V.Lamouroux) Womersley ex E.C.Oliveira were investigated using GC/MS, as well. These three algae belong to different taxa, were collected in different Brazilian coastal regions and due to the nature of the polymers that make up their tallus, are able to retain and accumulate pollutants which are dispersed into the aquatic environments. Fifty-one contaminants were extracted from them and identified. The results of this study substantiate the potential application of macroalgae as pollutant indicators in the environment where they live. Also, by recognizing many of its constituents, they expand the knowledge of the chemical diversity of Brazilian macroalgae.

Key words: pollution bioindicators, aquatic contaminants, adsorption, algal metabolites.

PREFÁCIO

Esta tese é constituída por seis capítulos, sendo que quatro deles estão apresentados como artigos científicos. O primeiro capítulo, sob o título de "*Introdução geral*", reúne os conceitos, definições e classificações que norteiam os estudos sobre macroalgas marinhas. Também nele estão a justificativa e os objetivos deste estudo.

Os capítulos 2 a 5 são artigos, dois dos quais já foram submetidos à periódicos estrangeiros, motivo pelo qual estão em inglês. Todos os quatro são independentes entre si e estão constituídos por resumo, palavras-chave, introdução, material e métodos, resultados, discussão, conclusões e referências bibliográficas.

O capítulo 2 contém o artigo intitulado *"Dichotomaria marginata* (Rhodophyta) as a bioindicator for marine pollution: an overview about its metabolites and adsorbed pollutants", foi submetido ao periódico "Revista de Biología Marina y Oceanografía", desvenda os poluentes retidos pela macroalga "*Dichotomaria marginata*" e propõe o mecanismo pelo qual esses resíduos ligam-se aos talos algáceos.

O capítulo 3 apresenta uma extensa revisão de poluentes aquáticos denominada "Unregulated aquatic pollutants: a challenge for the future", cuja redação foi produto da necessidade que tivemos em identificar e listar os resíduos encontrados nos extratos das algas que selecionamos para este estudo. Este artigo foi submetido ao "Water, Air, & Soil Pollution".

O capítulo 4 consta de um estudo realizado no extrato hexânico da macroalga *Dictyopteris delicatula,* que nos surpreendeu com por apresentar expressiva quantidade de sesquiterpenos. O título de artigo resultante é: "Sesquiterpenos de *Dictyopteris delicatula* J.V. Lamouroux (Dictyotales, Ochrophyta)".

No capítulo 5, em estudo sobre os metabolitos naturais e contaminantes extraídos de três macroalgas coletadas em regiões distintas do Brasil, buscamos ampliar, além do conhecimento da biodiversidade química desses organismos, o conceito de algas como bioindicadores de poluição. No capítulo 6 apresentamos nossas conclusões.

Neste estudo, estabelecemos como parte de nossos objetivos, prospectar atividades biológicas em três macroalgas selecionadas. Iniciamos nossos trabalhos de laboratório realizando ensaios antifúngicos, anticolinesterásicos e antioxidantes nos extratos desses organismos. Nesses ensaios, todos os extratos hexânicos e em diclorometano apresentaram fortíssimas ações inibitórias sobre fungos e sobre a enzima acetilcolinesterase, além de atividade antioxidante. Selecionamos o extrato de *Dichotomaria marginata* para ser submetido a fracionamentos em primeiro lugar, devido à sua atividade frente ao fungo *Cladosporium cladosporioides* (Capítulo 2) porém, nesses procedimentos, encontramos numerosos poluentes que possuem essas atividades, como os ftalatos. Esse fato impediu-nos de continuar a prospecção nos extratos mencionados, pois suas ações biológicas não puderam ser consideradas e também nos levou a redirecionar o nosso trabalho.

Do extrato metanólico de *D. marginata,* foi isolada uma amida (m/z 277,089709) com atividade antifúngica (forte) e atividade anticolinesterásica (fraca). Entretanto, devido à baixa solubilidade desta substância, houve problemas na obtenção de seus espectros de ressonância de ^{1}H e de ^{13}C, o que postergou a determinação de sua estrutura. Estes resultados serão objeto de um artigo científico.

SUMÁRIO

Capítulo 1. ***Introdução geral***

Conceitos gerais

Os oceanos estendem-se sobre 70 % da superfície terrestre e nele habitam cerca de 200.000 espécies de plantas e invertebrados marinhos e milhões de micro-organismos (Wijesekara et al., 2011) porém, apenas pouco mais de 1% de todas essas espécies foram estudadas (Pinto et al., 2002; Cheney, 2016). Numerosas publicações têm descrito a descoberta de substâncias bioativas isoladas de organismos marinhos tais como tunicatos, esponjas, corais, lebres marinhas, micro-organismos e macroalgas (Donia & Hamann, 2003; Haefner, 2003; Stengel & Connan, 2015; Pye et al., 2017; Tripathi et al., 2018).

As macroalgas marinhas que têm, comparativamente, recebido menor atenção (Saravanahkumar, 2003) são organismos fotossintetizantes, não vasculares, multicelulares e eucarióticos. O comprimento do talo pode variar de apenas poucos milímetros a 60 m de comprimento. Apresentam estruturas reprodutivas simples. (Coppejans et al., 2009, Fleurence e Levine, 2016). Esse grupo é extremamente diversificado (existem aproximadamente 10.000 espécies descritas) e está subdividido em três grandes filos, segundo a pigmentação de seus talos, material de reserva, componentes da parede celular, e construção e orientação de flagelos: Chlorophyta (algas verdes), Ochrophyta (algas pardas) e Rhodophyta (algas vermelhas) (Kharkwal et al., 2012, Fleurence e Levine, 2016).

Os pigmentos presentes nas algas vermelhas são as clorofila **a** e **d**, as ficobilinas (aloficocianina, r-ficoeritrina e r-ficocianina), os carotenoides (α- e β- caroteno) e a xantofila (luteína). Nas algas pardas são as clorofilas **a, c1, c2** e **c3**, os carotenoides (α-, β- e ε-caroteno) e a xantofila (fucoxantina, violaxantina, diadinoxantina, heteroxantina e vaucheriaxantina). E nas algas verdes são as clorofilas **a** e **b**, os carotenoides (α-, β- e γ-caroteno) e a xantofila (luteina e prasinoxantina) (Sharma, 2011; Pereira e Neto, 2015; Aryee et al., 2018).

Esses organismos são notáveis produtores de incontáveis proteínas, peptídeos, polissacarídeos, aminoácidos, lectinas e ficobiliproteínas, que pertencem ao metabolismo primário. Seus numerosíssimos e estruturalmente interessantes metabolitos secundários - terpenos, acetogeninas, alcaloides e polifenóis - apresentam um abrangente leque de atividades (Blunt et al., 2018, e revisões anteriores). Muitas dessas substâncias vêm sendo utilizadas há séculos na alimentação e como agentes terapêuticos (Pei-Gen e Shan-Lin, 1986; Fitton, 2003; Fleurence e Levine, 2016).

Alguns de seus metabolitos têm larga aplicação na indústria: os alginatos extraídos das paredes celulares das algas pardas são polímeros compostos pelos ácidos D-manurônicos e Lglucorônicos que são utilizados como estabilizadores de emulsões e suspensões, na fabricação de alimentos e de medicamentos (Pulz e Gross, 2004; Szekalska et al., 2016). As carragenanas, retiradas das paredes celulares das algas vermelhas, além de serem empregadas nas indústrias farmacêuticas e de alimentos, também o são na indústria têxtil (Radmer, 1996; BeMiller, 2019).

De um modo geral, os polissacarídeos das algas vermelhas, verdes e pardas, especialmente os sulfatados como os fucanos, carragenanos e ulvanos são portadores de atividades antioxidante, antitumorais, imunoestimulatórias, anti-inflamatórias, anticoagulante/antitrombótica, inibidora da síntese de lipídeos, antivirais, bactericidas, antidiabéticas, regenerativas, etc. (Kraan, 2012; Patel, 2012; Xu et al., 2017).

Dois importantes grupos de proteínas bioativas - lectinas e ficobiliproteínas (encontrado nas algas vermelhas) - são substâncias de função mista proteína-carboidrato, cujas atividades englobam ações antibacterianas, anticancerígenas, anti-HIV e anti-inflamatória (Teixeira et al., 2012; Sharma e Sharma, 2017). Nas algas pardas, a presença de polifenóis impede a comprovação da presença de lectinas, pois polifenóis ligam-se também a proteínas, causando falsa aglutinação (Teixeira et al., 2012). As ficobiliproteinas de *Palmaria palmata* (L.) Kuntze exibem também as atividades antioxidante e redutora de colesterol (Teixeira et al., 2012; Sharma e Sharma, 2017).

As algas vermelhas, verdes e pardas são igualmente ricas em aminoácidos bioativos tais como os hipotensivos taurina e laminina (Girard et al., 1988; Wesseling et al., 2009), os anti-helmínticos ácidos caínico e domóico (Pei-Gen & Shan-Lin 1986; Maeno et al., 2018) e os antioxidantes tipo micosporinas (Yuan et al., 2009). O ácido caínico é também um composto neuroregenerativo que age sobre os receptores neuronais do glutamato (Hopkins et al., 2000; Sakai et al., 2005), assim como o ácido domóico.

Os metabolitos secundários de macroalgas marinhas são responsáveis por numerosas funções: defesa contra herbívoros, patógenos e organismos incrustantes; participam da reprodução, proteção contra a radiação ultravioleta e ainda atuam como agentes alelopáticos e inibidores da formação de biofilmes. Todas essas propriedades explicam o conjunto de atividades biológicas apresentadas pelas algas marinhas: as ações bactericida, anticoagulante, antifúngica, anti-inflamatória, imunomodulatória, anticancerígena, antiviral e muitas outras estão citadas nas muitas revisões existentes sobre os compostos sintetizados por organismos marinhos e sobre suas atividades farmacológicas (Garson, 2010; Schumacher et al. 2011;

Pereira e Costa-Lotufo, 2012; Mayer et al. 2013, 2011, 2009, 2005; Blunt et al., 2018, 2014 e revisões anteriores).

A lista destes compostos que exibem propriedades farmacêuticas seria, pelo menos, considerada excessivamente longa, porém mostraria a importância e as possibilidades inerentes aos bioativos de algas. Entre os mais promissores estariam o monoterpeno halogenado halomon, isolado de *Portieria hornemannii* (Lyngbye) P.C.Silva (Fuller et al., 1994; Clardy & Walsh, 2004) que possui atividade citotóxica seletiva para linhagens de células tumorais de cérebro, rins e de cólon e o laurenditerpenol, isolado de *Laurencia intricata* J.V.Lamouroux (Chittiboyina et al. 2007).

Dentre as substâncias de interesse, a ação fungitóxica foi encontrada, no Brasil, em *Ulva lactuca* Linnaeus, *Dictyota dichotoma* (Hudson) J. V. Lamouroux, *Padina gymnospora* (Kützing) Sonder, *Sargassum vulgare* C. Agardh, *Hypnea musciformis* (Wulfen) J. V. Lamouroux, *Digenea simplex* (Wulfen) C. Agardh, *Ochtodes secundiramea* (Montagne) M. Howe, e *Laurencia dendroidea* J. Agardh (Guedes et al., 2012). A atividade de *O. secundiramea* e de *L. dendroidea* está associada a terpenos halogenados (Machado et al., 2014).

A ação inibidora sobre a enzima acetilcolinesterase foi encontrada no extrato de *Gelidiella acerosa* (Forsskål) Feldmann & Hamel (Syad et al., 2012), de *Laminaria japonica* Areschoug (Sevevirathne et al., 2011) e de *O. secundiramea* (Machado et al., 2015)

A importância das substâncias com atividade frente à enzima acetilcolinesterase é devida ao fato de estarem entre os potenciais candidatos a serem utilizados no controle da Doença de Alzheimer. Esta é uma doença neurodegenerativa associada à deficiência de neurotransmissores, no cérebro, cujo tratamento sintomático é a restauração da função colinérgica, obtida pela inibição da enzima acetilcolinesterase (Francis et al., 1999; Trevisan et al., 2003; Nair & Hunter, 2004).

Além de possuirem todos esses metabolitos de interesse, as macroalgas são importantes ferramentas de monitoramento ambiental devido à sua capacidade de acumular poluentes presentes na água, principalmente por adsorção (Souza & Concetino, 2004).

Quanto aos poluentes aquáticos, além dos tradicionais, existem também os poluentes emergentes, que são os não regularizados pelas autoridades vigentes. Alguns desses contaminantes são substâncias consideravelmente tóxicas que, apesar de suas baixas concentrações no meio aquoso, podem causar problemas no ecossistema, por alterarem a formação e a composição da biodiversidade. Podem, ainda, afetar os seres humanos pois alguns desses poluentes são capazes de promover descontroles no sistema endócrino, que resultam ora em infertilidade masculina, ora em câncer mama, entre outros problemas. (Ribeiro et al., 2015, Sauvé e Desrosiers, 2014).

Justificativa

Foram estudados quimicamente os extratos apolares de cinco macroalgas marinhas; quatro delas foram avaliadas quanto à presença de poluentes aquáticos e a última, quanto à presença de terpenos. Houve, portanto, significativa contribuição aos conhecimentos do papel desses organismos como indicadores de poluição e também de suas diversidades químicas.

Objetivos

Geral

Identificar metabolitos de macroalgas marinhas brasileiras previamente selecionadas; investigar a presença de substâncias com atividades antifúngica e antioxidante, em seus extratos apolares; identificar nesses mesmos extratos, metabolitos algais e também poluentes, dessorvidos dos talos dessas macroalgas.

Específicos

Realizar a extração sequenciada das macroalgas marinhas brasileiras Dichotomaria marginata, Acanthophora spicifera, Cladophora prolifera, Lobophora variegata e Dictyopteris delicatula, com solventes apolares (hexano e em diclorometano); avaliar qualitativamente o potencial antifúngico e antioxidante desses extratos e submetê-los a análises por cromatografia/espectrometria de massas, para a detecção e identificação de metabolitos algais e de poluentes aquáticos.

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Capítulo 2. *Dichotomaria marginata (Rhodophyta) as a bioindicator for marine pollution: an overview about its metabolites and adsorbed pollutants*

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Dichotomaria marginata (Rhodophyta) as a bioindicator for marine pollution: an overview about its metabolites and adsorbed pollutants

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Abstract. Macroalgae are considered bioindicators for marine pollution, because they have the ability to quickly react to changes in their environment. In consequence, macroalgae populations fluctuate, according to species characteristics and adaptive strategies. Their cell wall polysaccharides contain sulfate groups that are capable of retaining and accumulating heavy metals. In addition to traditional contaminants, emerging pollutants are being recognized in aquatic environments. Herein, emerging pollutants have been identified after being desorbed from the macroalga *Dichotomaria marginata*, collected from Fortaleza Beach, Ubatuba, Brazil. Based on the fact that algal polysaccharide networks have the potential of forming hydrogen bonds with polar compounds, it was hypothesized that these pollutants would be bound to sugar polymers. Compounds present in the *D. marginata* samples were identified using both gas and liquid chromatography/mass spectrometry (GC/MS and HPLC/MS), assisted by computational methods. It was possible to unequivocally identify 22 emerging contaminants with GC/MS, and 16 substances with HPLC/MS.

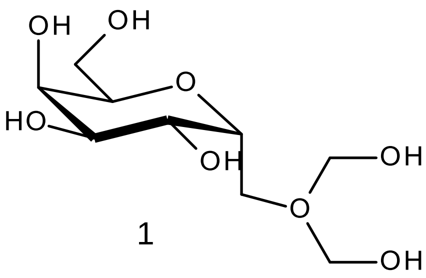
Key words: Emerging marine contaminants, algal polysaccharides, calcium carbonate, desorbance, macroalgae, bioindicators

Introduction

Over a century ago it was first proposed that there is a connection between the presence, absence or abundance of algae species and the properties of the marine environment (Kolkwitz & Marsson 1908). In fact, many species of algae have adapted to eutrophic conditions by undergoing morphological, physiological and/or ecological changes, which promote the growth of the population (Omar 2010), and the disappearance of others coincides with habitat eutrophication (Sousa & Cocetino 2004, Holt & Miller 2010, Omar 2010).

Previous studies have demonstrated that algae have the ability to adsorb heavy metals and nutrients (Omar 2010, Rajfur 2014, Rajfur & Kłos 2014). This characteristic is attributed to the high adsorption power of the thallus, whose cell walls are made up of polysaccharides, proteins, and lipids.

The polysaccharide composition into cell walls and the intercellular matrix can differ into different groups of red algae, but related to storage carbohydrates are basically two kinds floridoside (2-O-a-D-galactopyranosylglycerol, (**1**) - the most widely distributed substance of this series, two diastereoisomeric isofloridosides, 1-O-a-D-galactopyranosyl-D-glycerol (D-isofloridoside (**2**) and 1-O-a-D-galactopyranosyl-L-glycerol (L-isofloridoside, (**3**) and digeneaside (2-O-a-D-mannopyranosyl-D-glyceric acid (**4**) (Figure 1). *D. marginata* also biosynthesize specific water-soluble structural polysaccharides: sulfated xylomannans and neutral xylans (Usov 2011).



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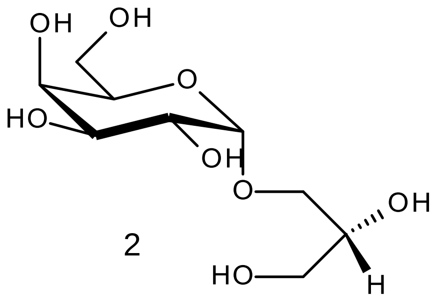

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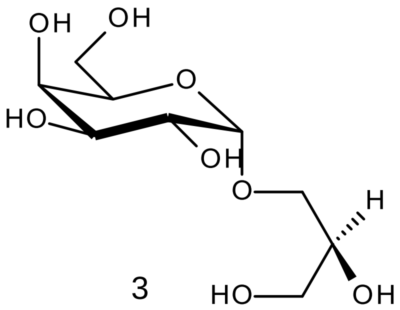

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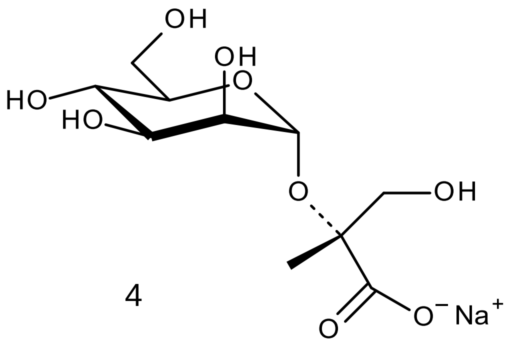

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Figure 1. Natural storage carbohydrates biosynthesized by red algae. Floridoside (1-3) and digeneaside (4) (Usov *et al.* 1981).

These polysaccharides contain a large amount of hydroxyl groups, which endows these carbohydrates with a hydrophilic character and the ability to form hydrogen bonds with environmental molecules. Hydrogen bonds occur when the hydrogen atom bound covalently to an electronegative atom (hydrogen donor group) electrostatically interacts with electronegative atoms (hydrogen receptor) forming non-covalent bonds. In the hydrogen donor group, usually the electronegative atom is either oxygen, nitrogen or sulfur. Hydrogen receptors are tipically O, N, S and also phenols (Schaeffer 2008).

The lipids contain carboxylate, sulfate and phosphate anionic groups, which function as metal-binding sites, as well as positively charged groups. Additionally, environmental pH and the presence of competing ions can impact the adsorption process (Gadd 2009, Chopra & Pathak 2010). Algae also adsorb pollutants through the use of phytochelatins, which are peptides that are capable of chelating heavy metals (Inouhe 2005).

According to Dokulil (2003), the pollutant adsorption process occurs in two steps. In the first step, the pollutant accumulates, by passive adsorption, on the outer surface of the seaweed thallus. In the second step, these compounds/substances are then slowly adsorbed, by metabolic processes. Interestingly, previous studies have demonstrated that these macroalgae can remove alkyl benzene sulfonates, phthalates, and textile dyes from aqueous solutions (Fernandez *et al*. 1995, El Maghraby 2013), thus emphasizing the adsorptive capabilities of these organisms.

In the marine environment, all organisms are in constant contact with dissolved or suspended substances. For example, in addition to common salts, bases, and organic or inorganic acids, marine organisms are also in contact with halogens, alkali metals, oxygen, nitrogen, silicon, trace elements, organic compounds (produced by aquatic organisms themselves) and pollutants (Bryan 1979, Millero *et al.* 2008).

Among these pollutants are emerging contaminants, which are a group of substances used extensively in everyday life. These contaminants are not currently included in routine water monitoring programs, norms or legislation for environmental control. This is primarily because the existing toxicity data are insufficient for establishing the respective reference doses. As a consequence, these unregulated pollutants pose a serious challenge to water quality regulators (Barceló 2003, Farré *et al.* 2008, Sousa *et al.* 2018). Furthermore, these agents have been detected in numerous aquatic organisms (Mearns *et al.* 2015).

With regards to macroalgae, compounds such as: surfactants, phthalates and polychlorinated biphenyls have been isolated from their extracts (Mackintosh *et al.* 2004, Gressler *et al.* 2012, Osman *et al.* 2013, Shobier *et al.* 2016, Stranska-Zachariasova *et al.* 2017). These three groups of compounds have antifungal activity (Uyanik *et al*. 2009, Memić *et al*. 2011, Lotfy *et al*. 2018, Paluch *et al.* 2018, Fait *et al*. 2019). In the late 1970s, phthalates were identified as algal components (Noguchi *et al.* 1979), and the bioconcentration, as well as the trophic magnification in the aquatic food web have been evaluated (Mackintosh *et al.* 2004).

In studies with algae, gas chromatography/mass spectrometry (GC/MS) has been utilized for the detection of phthalates, adipates and surfactants, due to the inherent thermal stability, volatility and of low polarity of these compounds (Mackintosh *et al.* 2004, Gressler *et al.* 2012, Osman *et al.* 2013, Shobier *et al.* 2016, Stranska-Zachariasova *et al.* 2017). On the other hand, high performance liquid chromatography coupled with mass spectrometry (HPLC/MS) has been successfully employed in research on non-volatile, thermally unstable and more polar contaminants (Goulitquer *et al.* 2012).

Both analytical platforms (GC/MS and HPLC/MS) become more valuable when assisted by computer tools such as the AMDIS (Automated Mass Spectrometry Deconvolution and Identification System) algorithm, which can separate co-eluting components (deconvolution), provide accurate molecular weights and generate both qualitative and quantitative data for each substance (Du & Zeisel 2013).

Our preliminary results showed that *D. marginata* hexane extracts exhibit a strong inhibitory activity against the fungi *Cladosporium cladosporioides*. And that result could be consequence of the synthesis of active secondary metabolites or the presence of emerging pollutants desorbed from the macroalgae. The main components of the hexane extracts are usually compounds of low molecular weight and low polarity, such as some hydrocarbons, fatty acids, terpenes and aromatics compounds. Antibiotic activities of any kind which were assigned to these extracts (essential oils) oftentimes are due to terpenes and aromatic (Nazzaro *et al*. 2017).

Thus, the present study was designed to identify metabolites from the algae and potential emerging pollutants, desorbed from the thallus of the benthic marine macroalgae *Dichotomaria marginata*, which may have antifungal activity, using a combination of GC/MS and HPLC/MS, followed by spectral deconvolution. The results will demonstrate the utility of *D. marginata* as a bioindicator for emerging contaminants and the interference of some of these contaminants in biological activity assays of crude extracts.

Material and methods

Sample collection and extraction

Dichotomaria marginata (J. Ellis & Solander) Lamarck, previously named as *Galaxaura marginata*, is a calcareous alga that belongs to the Rhodophyta (Johnson *et al.* 2014). Samples of this species were manually collected from Fortaleza Beach, in Ubatuba, São Paulo, Brazil (23º50'15"S / 40º17'40"W), during low tides. A voucher specimen (SP 428.540) was deposited at the Maria Eneyda P. Kauffman Fidalgo Herbarium, Instituto de Botânica, São Paulo.

Following collection, samples were washed, frozen and transported inside thermal box with ice to the laboratory. At the laboratory, they were shade dried at room temperature (20-25 °C), and ground into fine particles (Carvalho *et al.* 2003). The powdered biomass (419.63 g) was extracted with hexane (5× 3.45 L) until exhaustion; the pooled extracts were filtered through filter paper (Whatman Nº 5), the solvent was removed under reduced pressure and the final product was weighed (Bhagavathy *et al*. 2011).

Hexane extract fractionation

A portion (130 g) of the extract was subjected to column chromatography (designated as **C-I**) on silica gel (60, 0.2-0.5 mm, Vetec), and eluted with 400 mL of each of the following solutions: dichloromethane (DCM)/methanol (MeOH) 99.5:0.5 v/v, DCM/MeOH 97:3:2.7 v/v, DCM/MeOH 95:5 v/v and MeOH 100%. Each eluted 10 mL fraction was dried under an air stream, and subjected to Planar Chromatography (PC), using vanillin-sulfuric acid as visualization reagent. Similar fractions were pooled together.

Re-fractionation of pooled fractions from C-I

Combined fractions from **C-I** (Fractions 4-9) (53.4 mg) were refractionated on a silica gel column (designated as **C-II**), using DCM/MeOH 99.5:0.5 v/v as the mobile phase, which yielded 55, 1 mL, fractions. After PC analysis, similar fractions were combined. The combined fractions from both columns (**C-I** and **C-II**) were then analyzed by GC/MS and HPLC/MS.

Gas chromatography and mass spectrometry

GC/MS analyses of all samples were performed using a mass spectrometer connected to a Shimadzu GCMS-QP2010 plus gas chromatograph (Kyoto, Japan), equipped with a HP-5MS column (5%-phenylmethylpolysiloxane, 30 m × 0.25 mm id, 0.25 μm film thickness). Helium gas was used as the carrier, at a flow rate of 1.0 mL/min. The injector temperature was 250 ºC and the oven temperature was initially set at 60 ºC and increased at 3 ºC per min, until reaching 260 ºC, at which time this temperature was held for 40 min.

The mass spectrometer was operated in full scan mode from 40 to 1000 m/z, at an interface temperature of 240 ºC, and the samples were ionized with an electron beam of 70 eV.

Identification of chemical constituents was performed by comparing the mass spectra with data from the NIST/EPA/NIH Mass Spectral Library (Version 2.0).

The linear retention indices were calculated according to the Kovats method (KI), using a mixture of C6-C28 n-alkanes as external references (Sigma-Aldrich, St. Louis, MO, USA).

Only compounds with retention times and fragmentation patterns that matched reference compounds or spectral data available in literature were considered reliable, and thus included in the text.

HPLC-ESI-EM

These analyses were carried out using a Bruker HPLC instrument (MicroTOFII) coupled to a diode-array detector and a mass spectrometer with electrospray ionization (ESI-MS). The mass spectrometer was operated in the positive ion mode and provided mass spectra data from m/z 50 to 1000.

Twenty microliters of each sample (5 mg mL^{-1}) were injected onto a Kinetex C18 column (150 mm × 3 mm, 2.6 µm; Phenomenex®), at room temperature. For elution, a mixture of methanol/ammonium acetate (1 mM) (70:30) (Eluent A) and acetonitrile/ ammonium acetate (10 mM) in methanol (98:2) (Eluent B) were used, at a -flow rate of 0.7 mL min^{-1}. The gradient was as follows: 0% B (0 to 1 min); 0 to 60% B (1-25 min); 60 to 100% B (25 to 27 min); 100% B (27 to 30 min) 100 to 0% B (30-31 min); 0% B (31 - 32 min). Substances were identified with the aid of the computational deconvolution process.

Thin-layer chromatography/bioautography for detection of antifungal activity

The assay was conducted using the microorganism *Cladosporium cladosporioides* Fresen (SPC 140), which have been maintained at the Instituto de Botânica, São Paulo, Brazil. This fungus was cultured on potato dextrose agar (Difco) for 12 days until sporulation. The spore suspension was then extracted in a solution containing glucose and salt (Homans & Fuchs 1970, Rahalison *et al*. 1994) to a final concentration of 108 spores mL^{-1} and used for qualitative assessment of the antifungal activity of the crude seaweed extract and fractions.

The test was carried out by applying 10 µL of a solution containing 100 µg of crude extract or fractions on silica gel GF 254 TLC plates (Merck, Germany) in DCM/MeOH (98.5:1.5 v/v), which were developed in tanks lined with solvent-saturated Whatman Nº 3M. After thorough air drying, the chromatograms were sprayed with fungal spore suspensions and incubated for 72 h at 28 °C. Antifungal compounds on the developed plates appeared as clear zones against the dark backgrounds of the TLC plates. Nystatin was used as positive controls.

Results

In Figure 2A, the inhibitory activity exerted by a *D. marginata* hexane extract against the fungi *Cladosporium cladosporioides* is shown. Likewise, the Figure 2B displays the antifungal bioautographic assay of all fractions of the hexane extract and shows that all of them were active.

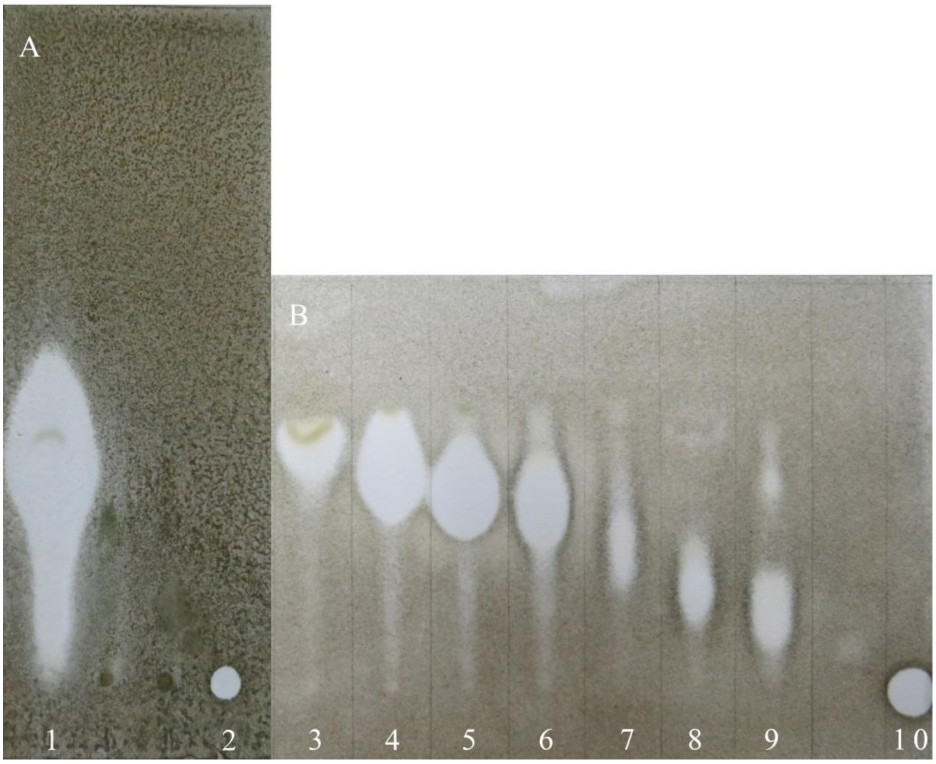


Figure 2. Antifungal TLC (thin layer chromatography) bioautographies of the *D. marginata* against *Cladosporium cladosporioides*. A- 1. Hexane extract and 2. Positive control (Nystatin). B- Combined *fractions* from (C-I): 3. (4-9), 4. (10-15), 5. (16-27), 6. (28-33), 7. (34-36), 8. (37-38), 9. (39-40) and 10. Positive control (Nystatin). Mobile phases (a and b) dichloromethane/methanol 98.5:1.5 v/v.

The combination of similar fractions from the C-I chromatographic process resulted in 12 fractions, five of which were analyzed by GC/MS. The refractionation (process C-II) of the pooled fractions (Fractions 4-9 from chromatographic process C-I) yielded 16 new fractions, 8 of which were analyzed by GC/MS.

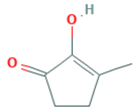
The characteristic algal metabolites identified by the GC/MS analyses are listed in Table 1. There were no terpenes or known aromatic substances in any of the analyzed fractions.

In Table 2, substances that are produced by algae, and also used in the production of toiletries and personal care products are listed, and include myristic, oleic and palmitoleic acids (Gressler *et al*. 2010, Zielinska & Nowak 2014).

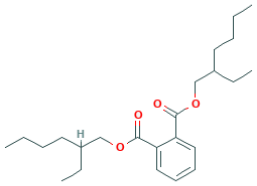
The emerging pollutants detected by GC/MS are shown in Table 3. In total, 23 compounds were identified, with industrial contaminants being the most prevalent, followed by personal care products. Pharmaceuticals, household cleaning products, food additives, endocrine disruptors and plasticizers were also identified.

The sixteen pollutants which were identified by HPLC/MS technique compounds are gathered in Table 4. Seven of these compounds are used for personal care, four as food additives, and two are pharmaceuticals. In fact, the drugs dipyrone and ethinylestradiol were only detected by HPLC/MS.

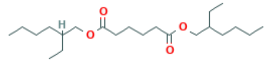
Figure 3 shows the structural formulas of some of these pollutants.



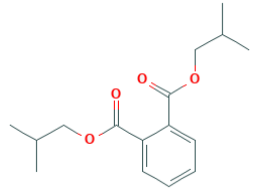
2-hydroxy-3-methyl-2-cyclopenten-1-one



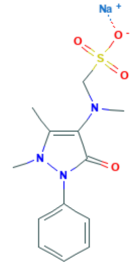
Bis(2-ethylhexyl) phthalate



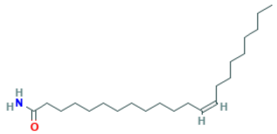
Diethylhexyl Adipate



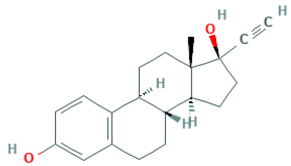
Diisobutyl Phthalate



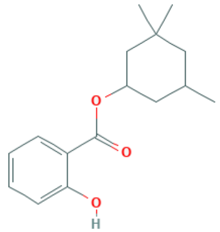
Dipyrone



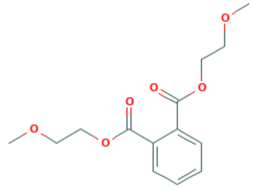
Erucylamide



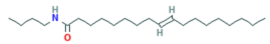
Ethinylestradiol



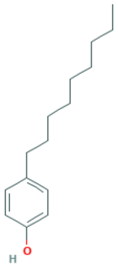
Homomenthyl salicylate



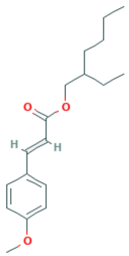
Methyl Glycol Phthalate



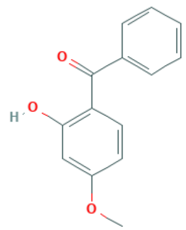
N-butyl-9-octadecenamide



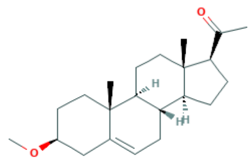
Nonylphenol



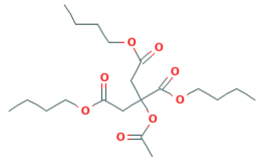
Octinoxate



Oxybenzone



Pregnenolone 3-methyl ether



Tributyl acetyl citrate

Figure 3. Chemical structure of some emergent pollutants identified in *D. marginata* (Pubchem, 2019).

Discussion

Among the primary metabolites herein identified (Table 1) only one, eicosane, has antifungal activity (Ahsan *et al.* 2017).

Special mention should be made of fatty acids C_{12}, C_{14} and C_{16} (Table 2), since they are valuable components of the human diet, precursors of the eicosanoid biosynthesis and bioregulators of several cellular processes (Gressler *et al*. 2010). Additionally, these compounds are considered to be a useful tool in chemotaxonomy, since they help to distinguish classes, families and genera of macroalgae (Khotimchenko 2005).

Among the compounds listed in Table 3, which are all pollutants, phthalates deserve special attention since they are very often isolated from macroalgae and sometimes mistakenly regarded as the natural components of these organisms (Chan *et al.* 2004, Gressler *et al.* 2012, Avio *et al.* 2016). Phathalates are not only antifungal compounds but also they may have toxic effects on humans and animals (Rowdhwal & Chen 2018). Still in Table 3, various other substances which have adverse effects on living beings are listed (references cited in Table 3).

Apart from four variants of phthalates, the pollutants nonyphenol (Karley *et al*. 1997), and cyclohexasiloxane (Moustafa *et al*. 2013) have antifungal activity as well. The fungitoxic action of these compounds could justify much of the observed fungal inhibition, since only one algal metabolite showed this activity.

The adsorption of these pollutants relied on the ability of the structural polysaccharides of the macroalgae to sequester and accumulate substances, with which these compounds can electrostatically bond.

On the other hand, the molecules of these substances must have oxygen atoms (hydrogen receptors) which are the ones that make up carbonyl (from acids, esters and ketones) and phenolic hydroxyl groups as well, nitrogen atoms which make up amines and amides and sulfur atoms, for sulfate groups (Paoloni *et al*. 1975, Hay *et al*. 2004, Schaeffer 2008, Ouellette & Rawn 2015).

The molecules of the sixteen pollutants herein identified have groups which can act as hydrogen receptors, by bonding with the numerous hydroxyl groups of sugar polymers, forming hydrogen bonds. The pollutants octinoxate, diisobutyl phthalate, homomenthyl salicylate, methyl glycol phthalate, pregnenolone 3-methyl ether, erucylamide, n-butyl-9-octadecenamide, oxybenzone, tributyl acetyl citrate, dipyrone, and 2-hydroxy-3-methyl-2-cyclopenten-1-one have a caboxyl group. Ethinylestradiol, oxybenzone, nonylphenol, and homomenthyl salicylate are phenolic substances; erucylamide and n-butyl-9-octadecenamide are amides and dipyrone is a sulfated substance (Figure 3).

While the GC/MS method reliably identified emerging pollutants desorbed from the thallus of *D. marginata*, the HPLC/MS procedure, assisted by spectral deconvolution, proved to be a valuable tool for identifying non-metallic, polar, non-volatile and thermally unstable environmental contaminants. These types of compounds are often present in minute quantities in samples with a profuse mixture of compounds (Magi & Di Carro 2016, Martín *et al*. 2017).

In conclusion, the significant number of pollutants detected by both chromatographic methods (GC and HPLC) indicate that macroalgae have the ability to adsorb a variety of substances dissolved or suspended in the aquatic environment. Due to the fact that some of the identified compounds were from pharmaceuticals residues and industrial debris, the present study has demonstrated that the red alga *Dichotomaria marginata* could be a reliable bioindicator for water pollution. Early detection and subsequent remediation will minimize the harmful effects associated with these pollutants, and benefit the human population, in general.

Table 1. Substances synthesized by algae and identified in *D. marginata* by GC/MS

Nº	Material analyzed	Rt (min)	SI*	KI*	Substance	Other names	Molecular formula	Molecular mass (g mol^{-1})	Reference
1	A; B; C	48.850	97	2114.6	Phytol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.539	Souza & Nes 1969
2	A	54.200	96	2300.5	Triacontane	N-triacontane	$C_{30}H_{62}$	422.826	Moustafa *et al*. 2008
3	A; C; D; E	78.117	91	3134.8	Demosterol	Cholesta-5,24-dien-3-ol	$C_{27}H_{44}O$	384.648	Burn *et al*. 1957
4	C; D	74.775	94	3057.5	Cholesta-5, 22-dien-3-β-ol	22-dehydrocholesterol	$C_{27}H_{44}O$	384.648	Burn *et al*. 1957
5	A; C; D	76.317	87	3097.5	Epicholesterol	Cholest-5-en-3-ol	$C_{27}H_{46}O$	386.664	Govindan *et al*. 1993, Plouguerné *et al*. 2006

* IS- similarity index. ** KI - Kovats Index
A: total hexane extract; B: C-I (Fr 10-15); C: C-I (Fr 16-27); D: C-I (Fr 28-33) and E: C-I (Fr 34-37)

Table 2. Substances synthesized by algae and produced for various industrial uses, identified in *D. marginata* by GC/MS

Nº	Material analyzed	Rt (min)	SI*	KI*	Substance	Other names	Molecular formula	Molecular mass (g mol^{-1})	Substance information	Reference/ Patent
1	A	15.183	85	1200.1	Dodecane	Dihexyl	$C_{12}H_{26}$	170.340	I; II	EFSA 2008, EP0002349 B1
2	A	23.650	92	1400.9	Tetradecane	N-tetradecane	$C_{14}H_{30}$	198.394	II; VIII	Fujimura *et al.* 1990, Kalhor *et al*. 2017, EP0002349 B1

3	A; B; C; G; H	28.817	93	1529.6	Dihydroactinidiolide	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	$C_{11}H_{16}O_2$	180.247	V	Yao *et al*. 1998, Xian *et al*. 2006, Borik 2014, US20130014771 A1
4	A; C; G; H	31.583	97	1601.0	N-hexadecane	Cetane	$C_{16}H_{34}$	226.448	I	Islam *et al*. 2013
5	A; G	35.033	97	1694.4	Heptadecene	1-heptadecene	$C_{17}H_{34}$	238.459	I; V; VIII	Rezanka *et al*. 1982, Yamamoto *et al*. 2014, US20150376544 A1
6	A; G; H	35.433	97	1705.5	Heptadecane	N-heptadecane	$C_{17}H_{36}$	240.475	V	McInnes *et al*. 1979, Sugisawa *et al*. 1990, Oliveira *et al*. 2012, US8933334 B2
7	C	35.808	94	1716.1	Tetradecene	1-Tetradecene	$C_{14}H_{28}$	196.378	I; V	Yamamoto *et al*. 2014
8	A	37.575	94	1766.2	Myristic acid	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.376	I; II; V	Widianingsih *et al*. 2012, Zielińska A & I Nowak 2014, El Maghraby & Fakhry 2015
9	A; G; H	38.783	96	1800.5	Octadecane	N-octadecane	$C_{18}H_{38}$	254.502	I	Borik 2014, EP0002349 B1, US8961622 B2
10	A	39.675	93	1827.0	Pentadecanoic acid	Pentadecylic acid	$C_{15}H_{30}O_2$	242.403	I	Plaza *et al.* 2010, EP0003032 B1, EP0022871 B1

11	A; B; C; G; H	40.317	94	1846.1	Phytone	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268.485	I	Eltz *et al*. 2010, Adegoke *et al*. 2015, Hatem *et al*. 2016
12	A; H	42.142	95	1900.5	Nonadecane	N-nonadecane	$C_{19}H_{40}$	268.529	V	Abou-El-Wafa *et al*. 2011, US8859471 B2
13	G; H	42.983	92	1926.7	Methyl palmitate	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.457	I; III; IV; V; VI	Knaggs *et al*. 1965, Parris *et al*. 1973, Lee *et al*. 2010, El-Demerdash 2011, US20160089464 B1, US20160089317 B1
14	A; F; G; H	43.808	95	1952.3	Palmitoleic acid	9-hexadecenoic acid	$C_{16}H_{30}O_2$	254.414	V	Pereira *et al*. 2012
15	A; F; G; H	44.300	92	1967.7	Palmitic acid	N-hexadecanoic acid	$C_{16}H_{32}O_2$	256.430	V	Kamenarska *et al*. 2006, Pereira *et al*. 2012, Sahu *et al*. 2013, US20150191607 A1, US4537782 A
16	G; H	45.175	95	1994.9	Ethyl palmitate	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284.484	V	Cortés *et al*. 2014, US20160089311 A1, US20160089317 A1
17	A; G; H	45.367	96	2000.9	Eicosane	Icosane	$C_{20}H_{42}$	282.556	I	Karabay-Yavasoglu *et al*.

										2007, US8859471 B2
18	G; H	48.158	81	2091.5	Propyl palmitate	Propyl hexadecanoate	$C_{19}H_{38}O_2$	298.511	V	Lui *et al*. 2008, US20160051455 A1
19	A; G	48.425	94	2100.2	Heneicosane	Methyl-eicosane	$C_{21}H_{44}$	296.583	I	Abou-El-Wafa *et al*. 2011, US8598098 B2
20	A; G; H	50.021	89	2154.4	Neophyitadiene	2-(4,8,12-Trimethyltrydecyl) buta -1,3-diene	$C_{20}H_{38}$	278.524	V	Plaza *et al*. 2010, Oliveira *et al*. 2012, Peres *et al*. 2012, US2012211015 A1
21	G; H	50.417	86	2167.9	Ethyl oleate	Oleic acid ethyl ester	$C_{20}H_{38}O_2$	310.522	II; III; V; VI	Bookstaff *et al*. 2003, Phaechamud & Savedkairop 2012, Castillo *et al*. 2012, Abdel-Aal *et al*. 2015
22	A; G; H	51.383	95	2200.8	Docosane	N-docosane	$C_{22}H_{46}$	310.610	II	Karabay-Yavasoglu *et al*. 2007, US8980346 B2
23	A; F; G; H	59.967	89	2518.1	Oleic acid	Cis-9-octadecenoic acid	$C_{18}H_{34}O_2$	282.468	I; V; VI	Khotimchenko *et al*. 2002, Huang *et al*. 2010, Sahu *et al*. 2013

* IS- similarity index. ** KI - Kovats Index
A: total hexane extract; B: C-I (Fr 10-15); C: C-I (Fr 16-27); F: C-I (Fr 38); G: C-II (Fr 26-29) e H: C-II (Fr30-33).
I: industrial inputs; II: food additives, III: drugs; IV: household products; V: products for personal use; VI: plasticizers and VIII: flame retardants.

Table 3. Emergent pollutants identified in *D. marginata* by GC/MS

Nº	Material analyzed	Rt (min)	SI *	KI*	Substance	Other name	Molecular formula	Molecular mass (g mol^{-1})	Substance information	Reference/ Patent
1	C; D; E; H	5.267	97	913.8	Tetrachloroethane	1,1,2,2-tetrachloroethane	$C_2H_2Cl_4$	167.838	I	ATSDR 2008
2	A	10.933	73	1095.3	2-hydroxy-3-methyl-2-cyclopenten-1-one	Ciclotene	$C_6H_8O_2$	112.128	II	EP0080600 B1, EP0413368 B1
3	A	16.175	76	1223.5	Cyclohexylpiperidine	1-piperidinocyclohexane	$C_{11}H_{21}N$	167.296	III	EP0392317 B1, EP0406111 B1
4	A	19.442	95	1300.5	Tridecane	N-tridecane	$C_{13}H_{28}$	184.367	I	EP0002002 B1
5	A; C; D; E; F; H	20.825	90	1333.5	Dodecamethylcyclohexasiloxane	Cyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	444.924	IV; V	Horii & Kannan 2008, EP0285364 B1, EP0246007
6	A; H	24.792	93	1429.1	α-ionone	Iraldeine	$C_{13}H_{20}O$	192.302	V	Adams *et al*. 1996, Lalko *et al*. 2007, Brechbill 2009, 2012; CA2813334 A1
7	A; B	27.150	88	1487.2	β-ionone	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	$C_{13}H_{20}O$	192.302	II; I	Lalko *et al*. 2007, EP0012246 B1
8	A; G; H	27.708	95	1501.0	Pentadecane	N-pentadecane	$C_{15}H_{32}$	212.421	I	El-Boujaddaini *et al*. 2010, EP0000819 B1

9	C	33.840	94	1662.1	1-octadecanol	Stearyl alcohol	$C_{18}H_{38}O$	270.501	I; V; VI	Bingham & Cohrssen 2012, Shore & Shelley 2015, US20160089321 A1, US20160089323 A1
10	F	35.108	89	1696.4	Nonylphenol	N-nonylphenol	$C_{15}H_{24}O$	220.356	IV; VII	Duan *et al*. 2016, He *et al*. 2016, Sieppi *et al*. 2016, EP0004108 B1
11	G; H	37.139	92	1753.9	Octinoxate	2-ethylhexyl 4-methoxycinnamate	$C_{18}H_{26}O_3$	290.403	V	Schlumpf *et al*. 2004, Axelstad *et al*. 2011, US20160089317 A1
12	A; B; C; D; G; H	41.092	97	1869.2	Diisobutyl Phthalate	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.348	VI	Poon *et al*. 1997, Koch *et al*. 2006, Gao & Wen 2016
13	C	41.433	96	1879.4	N-hexadecanol-1-ol	Cetilic alcohol	$C_{16}H_{34}O$	242.447	II; III; V	US8865144 A1
14	G; H	41.617	86	1884.8	Homomenthyl salicylate	3,3,5-trimethylcyclohexyl salicylate	$C_{16}H_{22}O_3$	262.349	V	Rietschel & Lewis, 1978, Sambandan & Ratner 2011, Jiménez-Díaz *et al*. 2013
15	A	44.150	91	1963.0	Methyl Glycol Phthalate	1,2-benzenedicarboxylic acid, bis (2-methoxyethyl) ester	$C_{14}H_{18}O_6$	282.292	I; VII	Poon *et al*. 1997, Koch *et al*. 2006, Gao & Wen 2016
16	A	51.033	93	2188.8	Butyl hexadecanoate	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	312.538	II; V	Khan *et al*. 2016

17	B; G; H	56.883	95	2399.3	Diethylhexyl Adipate	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.574	I; V; VI	Jobling *et al*. 1995, Fasano *et al*. 2012, US20160089321 A1
18	G; H; I	60.717	95	2548.0	Bis(2-ethylhexyl)phthalate	1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.564	I; VII	Poon *et al*. 1997, Koch *et al*. 2006, Gao *&* Wen 2016, US20160075671 A1
19	A; C; D; F; G	60.775	97	2550.3	Monoetylhexyl phthalate	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.344	I; VI	Koch *et al*. 2006, Poon *et al*. 1997, Gao *&* Wen 2016
20	A; D	64.458	95	2699.9	Tetracontane	N-tetracontane	$C_{40}H_{82}$	563.096	I; III	Mortimer & Luke 1966, Vazquez & Mansoori 2000, US8013091 B2
21	C; D	64.650	75	2708.1	Pregnenolone methyl ether	Pregnenolone 3-methyl ether	$C_{22}H_{34}O_2$	330.512	III	Marx *et al*. 2011
22	C; D; E; G	66.267	91	2777.0	Erucylamide	13-docosenamide	$C_{22}H_{43}NO$	337.592	I	US20160083586 A1, US20130177703 A1

* IS- similarity index. ** KI- Kovats Index
A: total hexane extract; B: C-I (Fr 10-15); C: C-I (Fr 16-27); D: C-I (Fr 28-33); E: C-I (Fr 34-37); F: C-I (Fr 38); G: C-II (Fr 26-29), H: C-II (Fr 30-33) e I: C-II (Fr 34-38).
I: industrial inputs; II: food additives, III: drugs; IV: household products; V: products for personal use; VI: plasticizers and VII: endocrine disruptors

Table 4. Substances identified in *D. marginata* by HPLC/MS

Nº	Substance	Molecular formula	Molecular mass (g mol^{-1})	[M+H]$^+$		Substance information
				Theoretical m/z	Observed m/z	
1	3-methylcyclopenta-2-enone	C_6H_8O	96.0575	97.0647	97.0644	I; II; V
2	α-ionone	$C_{13}H_{20}O$	192.302	193.1587	193.1562	V
3	Dihydroactinidiolide	$C_{11}H_{16}O_2$	180.247	181.1223	181.1228	V
4	Diisobutyl Phthalate	$C_{18}H_{36}O$	278.348	279.1590	279.1590	VI
5	n-butyl-9-octadecenamide	$C_{22}H_{43}NO$	337.592	338.3417	338.3416	I
6	Palmitoleic acid	$C_{16}H_{30}O_2$	254.414	255.2240	255.2276	V
7	Homomenthyl salicylate	$C_{16}H_{22}O_3$	262.349	263.1641	263.1645	V
8	Oxybenzone	$C_{14}H_{12}O_3$	228.247	229.0859	229.0844	V
9	2-propenoic acid 3- (4-methoxyphenyl) -2-ethylhexyl ester (octinoxate)	$C_{18}H_{26}O_3$	290.403	291.1954	291.1954	V
10	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.564	391.2842	391.2853	VI
11	β-ionone	$C_{13}H_{20}O$	192.302	193.1586	193.1555	I; II
12	7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6-9-diene-1,8-dione	$C_{17}H_{24}O_3$	276.376	277.1798	277.1787	I
13	Tributyl acetyl citrate	$C_{20}H_{34}O_8$	402.484	403.2326	403.2344	VI
15	Dipyrone	$C_{13}H_{16}N_3NaO_4S$	333.076	334.0831	334.0884	III
16	Ethinylestradiol	$C_{20}H_{24}O_2$	296.403	297.1849	297.1832	III

I: industrial inputs; II: food additives, III: drugs; V: products for personal use and VI: plasticizers

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Capítulo 3. ***Unregulated aquatic pollutants: a challenge for the future***

Unregulated aquatic pollutants: a challenge for the future

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Abstract: The oceans are continuously receiving water contributions from atmosphere, inland reservoirs, and glaciers, which carry some pollutants such as inorganic salts. However, the rivers also transport an abundant quantity of pollutants that are mainly constituted by salts, metallic ions and plastic debris that are daily disposed to sewage systems. Nowadays, along with these pollutants, which are called the traditional ones, residues of pharmaceuticals, personal care products, agricultural and industrial inputs, and abuse drugs and their main urinary metabolites are seen as well. Herein, we present a broad collection of these contaminants which are still not included in the guidelines for bathing, surface, and drinking water, and effluent control in any country of the world. To the list of these emerging contaminants we added data which may help the search for them in samples of water and human and animal tissues and fluids by chromatographic techniques coupled to mass spectrometry.

Keywords: aquatic pollutants review, emerging contaminants, pollutants molecular masses, pollutants molecular formulas

Review

The water mass, which constitutes the oceans, constantly receives contributions from atmosphere, inland reservoirs, and glaciers (Ngo-Duc et al. 2005). However, rainwater may bring with it some pollutants such as sulphuric acid (H_2SO_4), ammonium nitrate (NH_4NO_3)

and nitric acid (HNO_3), compounds that result from the reaction of acid particles and vapors released into the atmosphere with air humidity (Welburn 1988; Mellanby 1991).

Meltwaters can carry dissolved inorganic and organic carbon, and nitrate, phosphate, and silicate, nutrients that sometimes may negatively influence the marine environment (Sommaruga 2015). The rivers also transport to the oceans a large amount of pollutants which are mainly constituted by salts, metallic ions and plastic debris that are daily released to domestic and industrial sewage (Middelkoop 2000; Moiseenko et al. 2011; Bernardes et al. 2012; ; Gasparotti 2014; Hongmei et al. 2014; Orlandi et al. 2014; Montuori et al. 2016; Shafeek et al. 2017).

Along with these pollutants, the so-called traditional ones, current worldwide research into water quality has pointed, as well, at the presence of countless others xenobiotic compounds from the most diverse origins: trace of pharmaceuticals and personal care products (Boxall et al. 2012; Brausch et al. 2012; Daughton 2013), and agricultural and industrial inputs (Scott et al. 2017) are the most frequent ones. Abuse drugs and their main urinary metabolites are found in aquatic environments as well (Zuccato et al. 2005). All of these contaminants have as final destination the coastal oceans (Orlandi et al. 2014).

Therefore, foreign compounds have been continuously pouring out into the aquatic environments, which compels biota to live immersed in an adverse habitat. Many of these contaminants were already been detected in animal tissues' (Vrhovnik et al. 2013; Bergmann et al. 2015) as also evidences from their deleterious effects on their organisms (McLeese et al. 1981; Long et al. 1995; Dubinsky and Stambler 1996; Tanabe, 2002; van der Oost et al. 2003; Scott and Sloman 2004; Wen et al. 2006; Turner 2010).

Numerous records document on the accumulation of petroleum hydrocarbons, polycyclic aromatic, plastic and metal residues in marine sediments, as well (Filipkowska et al. 2005, Abrahim and Parker 2008, Claessens 2011; Zaghden 2014; Mearns et al. 2015).

Weis (2015) compiled a comprehensive list of these contaminants, describing them in terms of chemical properties, uses and effects on marine organisms. It brings together petroleum hydrocarbons, surfactants, dioxins, plastic components, insecticides, personal care products, cosmetics, therapeutic agents and some of its degradation products in the human body, domestic sewage, etc.

We have assembled an extensive collection of these aquatic pollutants, which are, in fact, essential components of every day-use products (Barceló 2013, Sauvé and Desrosiers, 2014; Sousa et al. 2018). Likewise, industrial products and byproducts, additives for fuel and pesticides are in this collection (Table 1). Our intention here is to provide an overview of the pollution in aquatic environments and some information about these pollutants, organizing the valuable data which are spread throughout the literature, and thus enabling further researches.

All data were collected from world's peer-reviewed literature through an extensive survey; in the resulting substantive list of contaminants, in addition to updated references, each compound is supplemented with its molecular formula and weight, information about its use, and location of the spot analyzed.

Table 1. Substances identified as emerging pollutants in aquatic environments.

DRUGS	Name	Molecular formula	Molecular mass (g/mol)	Localization		Reference
ANTIBIOTICS FOR VETERINARY AND HUMAN USE	Albendazole	$C_{12}H_{15}N_3O_2S$	265.331	Sea	Spain	Moreno-González et al. 2015
	Amoxycillin	$C_{16}H_{19}N_3O_5S$	365.404	Water for consumption/ Wastewaters /Surface waters/ Sea	Brazil/ Greece/ Italy/ Portugal/ UK	Petrovic et al. 2005; Gros et al. 2008; Locatelli et al. 2011; Gaffney et al. 2014; Petrie et al. 2015; Alygizakis et al. 2016
	Ampicillin	$C_{16}H_{19}N_3O_4S$	349.405	River/ Wastewaters/ Surface waters	Brazil	Petrovic et al. 2005; Silva et al. 2016
	Azithromycin	$C_{38}H_{72}N_2O_{12}$	748.984	Wastewaters/ Sea	Spain	Rivera-Utrilla et al. 2013; Moreno-González et al. 2014
	Cefaclor	$C_{15}H_{14}ClN_3O_4S$	367.804	-	-	Rivera-Utrilla et al. 2013
	Cefalexin	$C_{16}H_{17}N_3O_4S$	347.389	River	Brazil	Locatelli et al. 2011; Rivera-Utrilla et al. 2013
	Chloramphenicol	$C_{15}H_{18}Cl_2N_2O_9$	441.217	Wastewaters/ Surface waters	UK	Petrovic et al. 2005; Gros et al. 2008; Petrie et al. 2015
	Chlortetracycline	$C_{22}H_{23}ClN_2O_8$	478.880	-	-	Petrovic et al. 2005; Gros et al. 2008; Rivera-Utrilla et al. 2013
	Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.341	Water for consumption/ River	Brazil/ France/ Greece/ Italy/ Portugal/ Sweden/ USA	Petrovic et al. 2005; Gros et al. 2008; Melo et al. 2009; Locatelli et al. 2011; Gaffney et al. 2014
	Clarithromycin	$C_{38}H_{69}NO_{13}$	747.953	Wasterwater/ Surface waters/ Sea	Germany/ Greece/ Italy/ Turkey/ Spain/ USA	Petrovic et al. 2005; Gros et al. 2008; Ferrer et al. 2010; Ferrer and Thurman 2012; Moreno-González et al. 2014; Nödler et al. 2014
	Cloxacillin	$C_{19}H_{18}ClN_3O_5S$	435.879	-	-	Petrovic et al. 2005
	Dicloxacillin	$C_{19}H_{17}Cl_2N_3O_5S$	470.326	-	-	Petrovic et al. 2005; Gros et al. 2008
	Dimetridazole	$C_5H_7N_3O_2$	141.130	Sea	Spain	Moreno-González et al. 2015
	Doxycycline	$C_{22}H_{26}N_2O_9$	462.450	Water for consumption	Portugal	Petrovic et al. 2005; Gros et al. 2008; Gaffney et al. 2014
	Enrofloxacin	$C_{19}H_{22}FN_3O_3$	359.395	-	-	Petrovic et al. 2005; Gros et al. 2008

Erythromicin – dihydratade	$C_{37}H_{71}NO_{15}$	769.957	Water for consumption	EUA	Gros et al. 2008
Erythromycin	$C_{37}H_{67}NO_{13}$	733.927	Water for consumption/ Wastewaters/ Surface waters/ Sea/ Groundwater	Canada/ France/ Germany/ Italy/ Portugal/ Spain UK/ USA	Barceló 2003; Petrovic et al. 2005; Ferrer et al. 2010; Ferrer and Thurman 2012; Gaffney et al. 2014; Nödler et al. 2014; Lopez et al. 2015; Moreno-González et al. 2015; Petrie et al. 2015
Fluconazole	$C_{13}H_{12}F_2N_6O$	306.271	-	-	Loos et al. 2013
Flumequine	$C_{14}H_{12}FNO_3$	261.252	Sea	Greece	Alygizakis et al. 2016
Ivermectin	$C_{48}H_{74}O_{14}$	875.106	-	-	Petrovic et al. 2005
Levamisole	$C_{11}H_{12}N_2S$	204.291	Sea	Spain	Moreno-González et al. 2015
Levofloxacin	$C_{18}H_{20}FN_3O_4$	361.367	-	-	Rivera-Utrilla et al. 2013
Lincomycin	$C_{18}H_{34}N_2O_6S$	406.537	-	-	Barceló 2003; Rivera-Utrilla et al. 2013
Methicillin	$C_{17}H_{20}N_2O_6S$	380.415	-	-	Petrovic et al. 2005
Metronidazole	$C_6H_9N_3O_3$	171.154	Wastewaters/ Surface waters/ Sea/ Groundwater	France/ Greece/ UK	Rivera-Utrilla et al. 2013; Lopez et al. 2015; Petrie et al. 2015; Alygizakis et al. 2016
Nafcillin	$C_{21}H_{22}N_2O_5S$	414.475	-	-	Petrovic et al. 2005; Gros et al. 2008
Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.331	River	Brazil	Petrovic et al. 2005; Gros et al. 2008; Locatelli et al. 2011
Ofloxacin	$C_{18}H_{20}FN_3O_4$	361.367	Wastewaters/ Surface waters/ Sea	Greece/ UK	Petrovic et al. 2005; Gros et al. 2008; Petrie et al. 2015; Alygizakis et al. 2016
Oleandomycin	$C_{35}H_{61}NO_{12}$	687.858	-	-	Petrovic et al. 2005; Gros et al. 2008
Oxacillin	$C_{19}H_{19}N_3O_5S$	401.436	-	-	Petrovic et al. 2005; Gros et al. 2008
Oxolinic acid	$C_{13}H_{11}NO_5$	261.233	Sea	Greece	Alygizakis et al. 2016
Oxytetracycline	$C_{22}H_{24}N_2O_9$	460.434	Water for consumption/ Wastewaters/ Surface waters	Brazil/ Portugal/ UK	Petrovic et al. 2005; Gros et al. 2008; Gaffney et al. 2014; Petrie et al. 2015; Silva et al. 2016
Penicillin G	$C_{16}H_{18}N_2O_4S$	334.390	-	-	Petrovic et al. 2005; Gros et al. 2008
Penicillin V	$C_{16}H_{18}N_2O_5S$	350.389			Petrovic et al. 2005; Gros et al. 2008

Ronidazole	$C_6H_8N_4O_4$	200.154	Sea	Greece/ Spain	Moreno-González et al. 2015; Alygizakis et al. 2016
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	837.047	Sea	Germany/ USA	Petrovic et al. 2005; Gros et al. 2008; Nödler et al. 2014
Salicylic acid	$C_7H_6O_3$	138.122	Wastewaters/ Surface waters/ Sea	Belgium/ Germany/ Greece/ Spain/ UK	Ternes 1998; Claessens et al. 2013; Moreno-González et al. 2014; Petrie et al. 2015; Alygizakis et al. 2016
Sulphadiazine	$C_{10}H_{10}N_4O_2S$	250.277	Water for consumption / Sea/ Groundwater	Greece/ Portugal/ Spain	Petrovic et al. 2005; Gros et al. 2008; Jurado et al. 2012; Gaffney et al. 2014; Alygizakis et al. 2016
Sulphadimethoxine	$C_{12}H_{14}N_4O_4S$	310.328	Groundwater	Spain	Jurado et al. 2012
Sulphamethazine	$C_{12}H_{14}N_4O_2S$	278.330	Water for consumption/ Groundwater	Portugal/ Spain	Petrovic et al. 2005; Gros et al. 2008; Jurado et al. 2012; Gaffney et al. 2014;
Sulphamethizole	$C_9H_{10}N_4O_2S_2$	270.325	Sea/ Groundwater	Greece/ Spain	Jurado et al. 2012; Alygizakis et al. 2016
Sulphamethoxazole	$C_{10}H_{11}N_3O_3S$	253.278	Water for consumption / Wastewaters/ Surface waters/ Sea/ River/ Groundwater	Canada/ Brazil/ France/ Germany/ Greece/ Italy/ Netherlands/ Portugal/ Spain/ Sweden/ Turkey/ UK/ USA	Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Ferrer et al. 2010; Locatelli et al. 2011; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014; Petrie et al. 2015
Sulphapyridine	$C_{11}H_{11}N_3O_2S$	249.288	River/ Groundwater/ Wastewaters/ Surface waters	Portugal/ Spain/ UK	Jurado et al. 2012; Gaffney et al. 2014; Petrie et al. 2015
Sulphasalazine	$C_{18}H_{14}N_4O_5S$	398.393	Wastewaters/ Surface waters	UK	Petrovic et al. 2005; Petrie et al. 2015
Sulphathiazole	$C_9H_9N_3O_2S_2$	255.310	Groundwater	Spain	Jurado et al. 2012
Tetracycline	$C_{22}H_{24}N_2O_8$	444.435	river	Brazil/ Italy/ USA	Petrovic et al. 2005; Gros et al. 2008; Melo et al. 2009; Locatelli et al. 2011; Rivera-Utrilla et al. 2013
Thiabendazole	$C_{10}H_7N_3S$	201.247	Sea	Spain	Ferrer and Thurman 2012; Moreno-González et al. 2015
Trimethopim	$C_{14}H_{18}N_4O_3$	290.323	Wastewaters/ Surface waters/ Sea/ River	Belgium/ Brazil/ Canada/ England/ France/ Germany/ Greece/ Italy/ Spain/ Sweden/ UK/ USA	Petrovic et al. 2005; Gros et al. 2008; Ferrer et al. 2010; Locatelli et al. 2011; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Moreno-González et al. 2014; Petrie et al. 2015; Alygizakis et al. 2016

	Tylosin	$C_{46}H_{77}NO_{17}$	916.100	Water for consumption/ Sea	Greece/ Italy	Petrovic et al. 2005; Gros et al. 2008; Gaffney et al. 2014; Alygizakis et al. 2016
ANALGESIC, ANTI-INFLAMMATORY AND THEIR METABOLITES	7-aminonitrazepam	$C_{15}H_{13}N_3O$	251.283	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Acetaminofen (paracetamol)	$C_8H_9NO_2$	151.163	Water for consumption/ River/ Wastewaters/ Surface waters/ Sea	Australia/ Belgium/ Brazil/ France/ Germany/ Greece/ Israel/ Italy/ Portugal/ Spain/ Turkey/ UK/ USA	Ternes 1998; Barceló 2003; Gros et al. 2008; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014; Birch et al. 2015; Petrie et al. 2015; Pereira et al. 2016
	Acetylsalicylic acid	$C_9H_8O_4$	180.157	Water for consumption/ River	Germany/ Portugal	Ternes 1998; Barceló 2003; Gaffney et al. 2014
	Azaperol	$C_{19}H_{24}FN_3O$	329.419	Sea	Spain	Moreno-González et al. 2015
	Azaperone	$C_{19}H_{22}FN_3O$	327.403	Sea	Spain	Moreno-González et al. 2015
	Buprenorphine	$C_{29}H_{41}NO_4$	467.640	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Loos et al. 2013; Petrie et al. 2015
	Codeine	$C_{18}H_{21}NO_3$	299.364	Water for consumption/ Wastewaters/ Surface waters/ Sea/ Groundwater	Australia/ France/ Spain/ UK/ USA	Barceló 2003; Baker and Kasprzyk-Hordern 2011; Gaffney et al. 2014; Moreno-González et al. 2014; Birch et al. 2015; Lopez et al. 2015; Petrie et al. 2015
	Dexamethasone	$C_{22}H_{29}FO_5$	392.467	Sea	Spain	Moreno-González et al. 2015
	Diacetylmorphine (Heroin)	$C_{21}H_{23}NO_5$	369.417	Wastewaters/ Surface waters	UK	Loos et al. 2013; Petrie et al. 2015
	Diclofenac	$C_{14}H_{11}Cl_2NO_2$	296.149	Water for consumption/ River/ Wastewaters/ Surface waters/ Sea	Brazil/ Costa Rica/ France/ Finland/ Germany/ Greece/ Israel/ Italy/ Spain Sweden/ Turkey/ UK	Ternes 1998; Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Nödler et al. 2014; Moreno-González et al. 2015; Petrie et al. 2015; Pereira et al. 2016
	Dihydrocodeine	$C_{18}H_{23}NO_3$	301.380	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Fenoprofen	$C_{15}H_{14}O_3$	242.270	River	Germany	Ternes 1998; Barceló 2003; Gros et al. 2008
	Fentanyl	$C_{22}H_{28}N_2O$	336.470	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Loos et al. 2013; Petrie et al. 2015

Ibuprofen	$C_{13}H_{18}O_2$	206.281	Water for consumption/ Lakes/ Canal and rivers/ Wastewaters/ Surface waters/ Sea	Australia/ Brazil/ Canada/ China/ France/ Finland/ Germany/ Greece/ Israel/ Italy/ Spain/ Sweden/ Switzerland/ Turkey/ UK/ USA	Ternes 1998; Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Nödler et al. 2014; Petrie et al. 2015; Pereira et al. 2016
Indomethacine	$C_{19}H_{16}ClNO_4$	357.788	River/ Sea	Germany/ Spain	Ternes 1998; Petrovic et al. 2005; Gros et al. 2008; Moreno-González et al. 2015
Ketamine	$C_{13}H_{16}ClNO$	237.725	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Ketoprofen	$C_{16}H_{14}O_3$	254.281	Water for consumption/ River/ Wastewaters/ Surface waters/ Wastewater treatment plants/ Sea	Brazil/ France/ Finland/ Germany/ Spain/ Sweden/ UK	Gros et al. 2008; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Moreno-González et al. 2014; Petrie et al. 2015
Lidocaine	$C_{14}H_{22}N_2O$	234.343	Sea	Greece	Alygizakis et al. 2016
Mefenamic acid	$C_{15}H_{15}NO_2$	241.285	Sea	England/ Greece	Gros et al. 2008; Stuart et al. 2012; Rivera-Utrilla et al. 2013; Alygizakis et al. 2016
Methadone	$C_{21}H_{27}NO$	309.445	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Methaqualone	$C_{16}H_{14}N_2O$	250.301	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
Morphine	$C_{17}H_{19}NO_3$	285.338	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Naproxen	$C_{14}H_{14}O_3$	230.263	Water for consumption/ River/ Wastewaters/ Surface waters/ Wastewater treatment plants/ Sea	Australia/ Brazil/ Canada/ France/ Finland/ Germany/ Greece/ Italy/ Portugal/ Spain/ Sweden	Ternes 1998; Petrovic et al. 2005; Gros et al. 2008; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014; Petrie et al. 2015
Niflumic acid	$C_{13}H_9F_3N_2O_2$	282.222	Sea	Greece	Alygizakis et al. 2016
Norbuprenorphine	$C_{25}H_{35}NO_4$	413.550	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Norcodeine	$C_{17}H_{19}NO_3$	285.338	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Norfentanyl	$C_{14}H_{20}N_2O$	232.327	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015

	Norketamine	$C_{12}H_{14}ClNO$	223.699	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Normorphine	$C_{16}H_{17}NO_3$	271.311	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Norpropoxiphene	$C_{21}H_{27}NO_2$	325.445	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Nortramadol	$C_{15}H_{24}ClNO_2$	288.830	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Oxazepam	$C_{15}H_{11}ClN_2O_2$	286.713	Wastewaters/ Surface waters/ Groundwater	France/ UK	Baker and Kasprzyk-Hordern 2011; Stuart et al. 2012; Lopez et al. 2015; Petrie et al. 2015
	Oxycodone	$C_{18}H_{21}NO_4$	315.364	Wastewaters/ Surface waters/ Sea	Spain/ UK	Baker and Kasprzyk-Hordern 2011; Moreno-González et al. 2014; Petrie et al. 2015
	Oxymorphone	$C_{17}H_{19}NO_4$	301.337	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Phenazone	$C_{11}H_{12}N_2O$	188.226	Water for consumption/ River/ Sea	Germany/ Greece/ Spain/ Turkey	Ternes 1998; Petrovic et al. 2005; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014
	Phencyclidine	$C_{17}H_{25}N$	243.394	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	Phenylbutazone	$C_{19}H_{20}N_2O_2$	308.374	-	-	Petrovic et al. 2005; Gros et al. 2008
	Propoxiphene	$C_{22}H_{29}NO_2$	339.471	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Propyphenazone	$C_{14}H_{18}N_2O$	230.305	Water for consumption / Sea	Germany/ Spain	Gros et al. 2008; Gaffney et al. 2014; Moreno-González et al. 2015
	Tramadol	$C_{16}H_{25}NO_2$	263.381	Wastewaters/ Surface waters/ Sea/ Groundwater	Australia/ France/ Greece/ UK	Baker and Kasprzyk-Hordern 2011; Birch et al. 2015; Lopez et al. 2015; Petrie et al. 2015; Alygizakis et al. 2016
	Xylazine	$C_{12}H_{16}N_2S$	220.334	Sea	Spain	Moreno-González et al. 2015
PSYCHOTROPIC	2N-glucuronide	$C_{15}H_{15}Cl_2N_5O_6$	432.214	-	-	Ferrer and Thurman 2012; Gaffney et al. 2014
	Acridone	$C_{13}H_9NO$	195.221	Sea	Spain	Moreno-González et al. 2015
	Alprazolam	$C_{17}H_{13}ClN_4$	308.769	Sea	Spain	Moreno-González et al. 2015
	Amisulpride	$C_{17}H_{27}N_3O_4S$	369.480	Sea	Greece	Alygizakis et al. 2016
	Amitriptyline	$C_{20}H_{23}N$	277.403	Water for consumption /	France/ UK	Baker and Kasprzyk-Hordern 2011; Gaffney

			Wastewaters/ Surface waters		et al. 2014; Petrie et al. 2015
Bupropion	$C_{13}H_{18}ClNO$	239.741	-	-	Ferrer and Thurman 2012; Loos et al. 2013
Carbamazepine	$C_{15}H_{12}N_2O$	236.269	Water for consumption / Wastewater and surface water/ Wastewaters/ Surface waters/ Sea	Australia/ Belgium/ Canada/ France/ Germany/ Greece/ Israel/ Italy/ Portugal/ Spain/ Sweden/ Turkey/ UK/ USA	Ternes 1998; Petrovic et al. 2005; Gros et al. 2008; Ferrer et al. 2010; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014; Birch et al. 2015; Petrie et al. 2015
Chlordiazepoxide	$C_{16}H_{14}ClN_3O$	299.758	Sea/ Wastewaters/ Surface waters	Greece/ UK	Baker and Kasprzyk-Hordern 2011; Alygizakis et al. 2016
Chlorpromazine	$C_{17}H_{19}ClN_2S$	318.863	Sea	Greece	Alygizakis et al. 2016
Citalopram	$C_{20}H_{21}FN_2O$	324.392	Sea	Greece/ Israel/ Spain/ USA	Ferrer and Thurman 2012; Loos et al. 2013; Moreno-González et al. 2014; Nödler et al. 2014; Alygizakis et al. 2016
Clindamycine	$C_{18}H_{33}ClN_2O_5S$	424.983	-	-	Loos et al. 2013
Desvenlafaxine	$C_{16}H_{25}NO_2$	263.375	Water for consumption	Portugal	Ferrer and Thurman 2012; Gaffney et al. 2014
Diazepam	$C_{16}H_{13}ClN_2O$	284.740	Water for consumption/ Rivers/ Wastewaters/ Surface waters/Sea	Germany/ Greece/ Italy/ Spain/ UK	Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Gaffney et al. 2014; Moreno-González et al. 2014; Petrie et al. 2015; Alygizakis et al. 2016
Dosulepin	$C_{19}H_{21}NS$	295.442	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
Duloxetine	$C_{18}H_{19}NOS$	297.416	Sea	Greece	Alygizakis et al. 2016
Erithrohydrobupropion	$C_{13}H_{20}ClNO$	241.757	Water for consumption/ Wastewaters/ Surface waters	Portugal	Ferrer and Thurman 2012; Petrie et al. 2015
Fluoxetine	$C_{17}H_{18}F_3NO$	309.326	Water for consumption/ Rivers/ Wastewaters/ Surface waters/ Sea	Australia/ Netherlands/ Spain/ UK/ USA	Petrovic et al. 2005; Gros et al. 2008; Ferrer and Thurman 2012; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014; Birch et al. 2015; Petrie et al. 2015
Licarbazepine	$C_{15}H_{14}N_2O_2$	254.284	-	-	Ferrer and Thurman 2012; Gaffney et al. 2014
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	321.157	Sea	Spain	Moreno-González et al. 2015

	Nitrazepam	$C_{15}H_{11}N_3O_3$	281.271	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	Norcitalopram	$C_{18}H_{18}ClFN_2O$	332.803	Wastewaters/ Surface waters	UK	Ferrer and Thurman 2012; Petrie et al. 2015
	Nordiazepam	$C_{15}H_{11}ClN_2O$	270.714	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Norfluoxetine	$C_{16}H_{16}F_3NO$	295.299	Rivers/ Wastewaters/ Surface waters	Spain/ UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Nortriptyline	$C_{19}H_{21}N$	263.377	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Norvenlafaxine	$C_{16}H_{25}NO_2$	263.381	Sea	Greece	Alygizakis et al. 2016
	Olanzapine	$C_{17}H_{20}N_4S$	312.435	Sea	Spain	Moreno-González et al. 2015
	Oxcarbazepina	$C_{15}H_{12}N_2O_2$	252.268	Water for consumption	Portugal	Gaffney et al. 2014
	Phenytoin	$C_{15}H_{12}N_2O_2$	252.268	Water for consumption	UK/ USA	Petrovic et al. 2005; Gaffney et al. 2014
	Rivastigmine	$C_{14}H_{22}N_2O_2$	250.342	Sea	Greece	Alygizakis et al. 2016
	Sertraline	$C_{17}H_{17}Cl_2N$	306.230	Sea	Spain	Moreno-González et al. 2015
	Sulpiride	$C_{15}H_{23}N_3O_4S$	341.426	Sea	Greece	Alygizakis et al. 2016
	Trazodone	$C_{19}H_{22}ClN_5O$	371.869	Sea	Spain	Moreno-González et al. 2015
	Venlafaxine	$C_{17}H_{27}NO_2$	277.408	Rivers/ Wastewaters/ Surface waters	Australia/ France/ UK	Baker and Kasprzyk-Hordern 2011; Ferrer and Thurman 2012; Birch et al. 2015; Petrie et al. 2015
LIPID REGULATORS	Atorvastatin	$C_{33}H_{35}FN_2O_5$	558.640	Sea	Spain	Petrovic et al. 2005; Loos et al. 2013; Moreno-González et al. 2014;
	Bezafibrate	$C_{19}H_{20}ClNO_4$	361.819	Water for consumption/ Rivers/ Wastewaters/ Surface waters/ Sea	Belgium/ Brazil/ Canada/ France/ Finland/ Germany/ Greece/ Italy/ Israel/ Spain/ Turkey/ UK	Ternes 1998; Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014; Petrie et al. 2015
	Clofibric acid	$C_{10}H_{11}ClO_3$	214.645	Water for consumption/ River/ Wastewaters/ Surface waters	Germany/Italy	Ternes 1998; Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Gaffney et al. 2014; Petrie et al. 2015

	Fenofibrate	$C_{17}H_{15}ClO_4$	318.752	River/ Water treatment station	France/ Germany/ Greece/ Italy/ Sweden	Ternes 1998; Barceló 2003; Petrovic et al. 2005
	Fluvastatin	$C_{24}H_{26}FNO_4$	411.473	Sea	Spain	Moreno-González et al. 2015
	Gabapentin	$C_9H_{17}NO_2$	171.237	Wastewaters/ Surface waters	UK	Ferrer and Thurman 2012; Petrie et al. 2015
	Gemfibrozil	$C_{15}H_{22}O_3$	250.333	Water for consumption Rivers/ Water treatment station/ Sea	Brazil/ Canada/ France/ Germany/ Greece/ Italy/ Spain/ Sweden/ Turkey/ USA	Ternes 1998; Petrovic et al. 2005; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Gros et al. 2008; Gaffney et al. 2014; Moreno-González et al. 2014; Nödler et al. 2014
	Haloperidol	$C_{21}H_{23}ClFNO_2$	375.864	Sea	Greece/ USA	Loos et al. 2013; Nödler et al. 2014; Alygizakis et al. 2016
	Hydroxybupropion	$C_{13}H_{18}ClNO_2$	255.740	-	-	Ferrer and Thurman 2012
	Lamotrigine	$C_9H_7Cl_2N_5$	256.091	-	-	Ferrer and Thurman 2012
	Lovastatin	$C_{24}H_{36}O_5$	404.547	-	-	Petrovic et al. 2005
	Meprobamate	$C_9H_{18}N_2O_4$	218.250	Water for consumption	USA	Petrovic et al. 2005; Gaffney et al. 2014
	Mevastatin	$C_{23}H_{34}O_5$	390.520	-	-	Petrovic et al. 2005
	Paraxanthine	$C_7H_8N_4O_2$	180.164	Sea/ Wastewaters/ Surface waters/ Groundwater	France/ Germany/ Greece/ Israel/ Italy/ Spain/ Turkey/ UK/ USA	Baker and Kasprzyk-Hordern 2011; Ferrer and Thurman 2012; Nödler et al. 2014; Lopez et al. 2015
	Paroxetine	$C_{19}H_{20}FNO_3$	329.365	Sea	Spain	Gros et al. 2008; Moreno-González et al. 2015
	Pravastatin	$C_{23}H_{36}O_7$	424.534	Sea	Spain	Petrovic et al. 2005; Moreno-González et al. 2014
	Primidone	$C_{12}H_{14}N_2O_2$	218.252	Sea/ Groundwater	Germany /USA	Stuart et al. 2012; Nödler et al. 2014
	Simvastatin	$C_{25}H_{38}O_5$	418.566	Wastewaters/ Surface waters	Spain/UK	Petrovic et al. 2005; Petrie et al. 2015
	Temazepam	$C_{16}H_{13}ClN_2O_2$	300.740	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
ANTI-HYPERTENSIVE, β-BLOCKING AGENTS AND DIURETICS	Acebutolol	$C_{18}H_{28}N_2O_4$	336.426	Sewage treatment plants	France/ Greece/ Italy/ Sweden	Petrovic et al. 2005

Amlodipine	$C_{20}H_{25}ClN_2O_5$	408.879	Sea	Spain	Moreno-González et al. 2014
Atenolol	$C_{14}H_{22}N_2O_3$	266.336	Water for consumption/ River/ Wastewaters/ Surface waters/ Sea	Belgium/ Brazil/ Germany/ Greece/ Italy/ Portugal/ Spain/ South Korea/ Sweden/ Turkey/ UK USA	Petrovic et al. 2005; Gros et al. 2008; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Nödler et al. 2014; Petrie et al. 2015; Pereira et al. 2016
Betaxolol	$C_{18}H_{29}NO_3$	307.434	Rivers	Germany	Ternes 1998; Petrovic et al. 2005
Bisoprolol	$C_{18}H_{31}NO_4$	325.443	Rivers	Germany	Ternes 1998; Petrovic et al. 2005; Loos et al. 2013
Carazolol	$C_{18}H_{22}N_2O_2$	298.386	Sea	Spain	Petrovic et al. 2005; Moreno-González et al. 2014
Clopidogrel	$C_{16}H_{16}ClNO_2S$	321.819	Sea	Spain	Moreno-González et al. 2014
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.518	Wastewaters/ Surface waters/ Sea	Spain/ UK/ USA	Ferrer et al. 2010; Ferrer and Thurman 2012; Loos et al. 2013; Moreno-González et al. 2014; Petrie et al. 2015
Eprosartana	$C_{23}H_{24}N_2O_4S$	424.513	-	-	Loos et al. 2013
Glibenclamide	$C_{23}H_{28}ClN_3O_5S$	404.003	Sea	Spain	Moreno-González et al. 2015
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	297.728	Sea	Greece/ Spain	Moreno-González et al. 2014; Alygizakis et al. 2016
Irbesartan	$C_{25}H_{28}N_6O$	428.529	Sea	Spain	Loos et al. 2013; Moreno-González et al. 2014
Losartan	$C_{22}H_{23}ClN_6O$	422.917	Sea	Brazil/ Spain	Moreno-González et al. 2015; Pereira et al. 2016
Metformin	$C_4H_{11}N_5$	129.167	Groundwater	France	Stuart et al. 2012; Lopez et al. 2015
Metoprolol	$C_{15}H_{25}NO_3$	267.364	Water for consumption Rivers/ Wastewaters/ Surface waters/ Sea	France/ Germany/ Greece/ Israel/ Italy/ Netherlands/ Spain/ Sweden/ Turkey/ UK/ USA	Ternes 1998; Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Ferrer and Thurman 2012; Gaffney et al. 2014; Nödler et al. 2014; Petrie et al. 2015
Metoprolol acid	$C_{14}H_{21}NO_4$	267.321	-	-	Ferrer and Thurman 2012
Nadolol	$C_{17}H_{27}NO_4$	309.406	Sea	Spain	Petrovic et al. 2005
Norverapamil	$C_{26}H_{36}N_2O_4$	440.584	Sea	Spain	Moreno-González et al. 2015

	Oxprenolol	$C_{15}H_{23}NO_3$	265.348	Sewage treatment plants	France/ Greece/ Italy/ Sweden	Petrovic et al. 2005
	Pindolol	$C_{14}H_{20}N_2O_2$	248.326	Sea	Spain	Petrovic et al. 2005
	Propranolol	$C_{16}H_{21}NO_2$	259.343	Water for consumption/ Rivers/ Wastewaters/ Surface waters/ Sea	Australia/ Belgium/ France/ Germany/ Greece / Italy/ Portugal/ Spain/ Sweden/ UK/ USA	Ternes 1998; Barceló 2003; Petrovic et al. 2005; Gros et al. 2008; Ferrer and Thurman 2012; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Birch et al. 2015; Petrie et al. 2015
	Salbutamol	$C_{13}H_{21}NO_3$	239.315	Wastewaters/ Surface waters/ Sea	Spain/ UK	Gros et al. 2008; Moreno-González et al. 2014; Petrie et al. 2015
	Sildenafil	$C_{22}H_{30}N_6O_4S$	474.576	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Sotalol	$C_{12}H_{20}N_2O_3S$	272.363	Groundwater	France	Lopez et al. 2015
	Tamsulosin	$C_{20}H_{28}N_2O_5S$	408.513	Sea	Spain	Moreno-González et al. 2015
	Telmisartana	$C_{33}H_{30}N_4O_2$	514.617	-	-	Loos et al. 2013
	Timolol	$C_{13}H_{24}N_4O_3S$	316.420	Rivers	Germany	Ternes 1998; Barceló 2003; Petrovic et al. 2005
	Trimetazidine	$C_{14}H_{22}N_2O_3$	266.341	Groundwater	France	Lopez et al. 2015
	Torasemide	$C_{16}H_{20}N_4O_3S$	348.421	Sea	Spain	Moreno-González et al. 2014
	Valsartan	$C_{24}H_{29}N_5O_3$	435.528	Wastewaters/ Surface waters/ Sea	Brazil/ Greece/ Spain/ UK	Moreno-González et al. 2014; Petrie et al. 2015; Alygizakis et al. 2016; Pereira et al. 2016
	Verapamil	$C_{27}H_{38}N_2O_4$	454.611	Sea	Spain	Moreno-González et al. 2014
	Warfarin	$C_{19}H_{16}O_4$	308.333	Sea	Spain	Moreno-González et al. 2015
X-RAY CONTRAST	Iopromide	$C_{18}H_{24}I_3N_3O_8$	791.112	Water for consumption/ Sea	Australia/ Germany/ Greece/ Italy/ Turkey	Barceló 2003; Rivera-Utrilla et al. 2013; Gaffney et al. 2014; Birch et al. 2015
	Iopamidol	$C_{17}H_{22}I_3N_3O_8$	777.085	Sea	Germany/ Greece/ Italy/ Turkey/ USA	Barceló 2003; Nödler et al. 2014
	Iohexol	$C_{19}H_{26}I_3N_3O_9$	821.142	Sea	Germany/ Greece/ Israel/ Turkey/ USA	Nödler et al. 2014
	Diatrizoate	$C_{11}H_9I_3N_2O_4$	613.914	Water for consumption	Germany	Barceló 2003; Gaffney et al. 2014

	Gadolinium	Gd	157.924			Loos et al. 2013
	Imeprol	$C_{17}H_{22}I_3N_3O_8$	777.089	Sea	Germany/ Greece/ Italy/ Turkey	Nödler et al. 2014
REGULATORS OF GASTRIC AND ANTIALLERGICS ACIDS	Bleomycin	$C_{55}H_{84}N_{17}O_{21}S_3$	1415.551	Water for consumption	UK	Gaffney et al. 2014
	Cetirizine	$C_{21}H_{25}ClN_2O_3$	388.888	Sea	Germany/ Italy/ USA	Ferrer and Thurman 2012; Nödler et al. 2014
	Cyclophosphamide	$C_7H_{15}Cl_2N_2O_2P$	261.086	Water for consumption	Portugal	Gaffney et al. 2014
	Cimetidine	$C_{10}H_{16}N_6S$	252.339	Wastewaters/ Surface waters/ Sea	Spain/ UK	Moreno-González et al. 2014; Petrie et al. 2015
	Desloratadine	$C_{19}H_{19}ClN_2$	310.825	Sea	Spain	Moreno-González et al. 2014
	Diphenhydramine	$C_{17}H_{21}NO$	255.355	Wastewater/ Surface water	USA	Ferrer et al. 2010; Ferrer and Thurman 2012; Loos et al. 2013
	Famotidine	$C_8H_{15}N_7O_2S_3$	377.435	Sea	Spain	Moreno-González et al. 2014
	Furosemide	$C_{12}H_{11}ClN_2O_5S$	330.744	Wastewaters/ Surface waters/ Sea	Spain/ UK	Petrovic et al. 2005; Moreno-González et al. 2014; Petrie et al. 2015
	Ifosfamide	$C_7H_{15}Cl_2N_2O_2P$	261.086	River	Germany	Ternes 1998
	Loratadine	$C_{22}H_{23}ClN_2O_2$	382.883	Sea	Germany/ Greece/ Spain/ Turkey/ USA	Nödler et al. 2014; Moreno-González et al. 2014
	Omeprazole	$C_{17}H_{19}N_3O_3S$	345.416	-	-	Petrovic et al. 2005; Gros et al. 2008
	Pantoprazole	$C_{16}H_{15}F_2N_3O_4S$	383.370	-	-	Petrovic et al. 2005; Gros et al. 2008
	Ranitidine	$C_{13}H_{22}N_4O_3S$	314.404	Wastewaters/ Surface waters/ Sea	Spain/ UK	Petrovic et al. 2005; Gros et al. 2008; Rivera-Utrilla et al. 2013; Moreno-González et al. 2014; Petrie et al. 2015
	Tamoxiphen	$C_{26}H_{29}NO$	371.515	Wastewaters/ Surface waters/ Sea	UK/ USA	Nödler et al. 2014; Petrie et al. 2015
CONTRACEPTIVES	Diethylestilbestrol	$C_{18}H_{20}O_2$	268.350	-	-	Barceló 2003; Santana 2013
	Estradiol	$C_{18}H_{24}O_2$	272.382	Wastewaters/ Surface waters/ Sewage treatment plants	Italy/ UK	Baronti et al. 2000; Barceló 2003; Gros et al. 2008; Rivera-Utrilla et al. 2013; Petrie et al. 2015
	Estriol	$C_{18}H_{24}O_3$	288.381	Sewage treatment plants	Italy	Baronti et al. 2000; Barceló 2003; Gros et al.

						2008; Petrie et al. 2015
	Estrone	$C_{18}H_{22}O_2$	270.366	Wastewaters/ Surface waters/ Springs/ Water for consumption/ Sewage treatment plants/ Groundwater	Italy/ Spain/ UK	Baronti et al. 2000; Barceló 2003; Gros et al. 2008; Raimundo 2011; Jurado et al. 2012; Petrie et al. 2015
	Etynylestradiol	$C_{20}H_{24}O_2$	296.403	Wastewaters/ Surface waters/ Sewage treatment plants	Brazil/ Canada/ Germany/ Italy/ UK/ USA	Baronti et al. 2000; Gros et al. 2008; Melo et al. 2009; Rivera-Utrilla et al. 2013; Petrie et al. 2015
	Levonorgestrel	$C_{21}H_{28}O_2$	312.446	Groundwater	France	Stuart et al. 2012; Santana 2013
	Mestranol	$C_{21}H_{26}O_2$	310.430	Wastewaters/ Surface waters	Brazil	Santana 2013; Silva et al. 2016
	Progesterone	$C_{21}H_{30}O_2$	314.462	Groundwater/ Springs/ Water for consumption	Brazil/ France	Raimundo 2011; Stuart et al. 2012; Santana 2013
	Testosterone	$C_{19}H_{28}O_2$	288.431	Groundwater/ Springs/ Water for consumption	Brazil/ France	Raimundo 2011; Stuart et al. 2012; Santana 2013
STIMULANTS AND THEIR METABOLITES	1-methylxanthine	$C_6H_6N_4O_2$	166.137	Sea	Greece/ Italy/ Turkey	Nödler et al. 2014
	2-ethyl-5-methyl-3-3-diphenylpyrroline	$C_{19}H_{23}N$	265.393	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	2-ethylidene-1,5-dimethyl-3,3-Diphenylpyrrolidine (EDDP)	$C_{20}H_{24}ClNO_4$	377.865	Sea/ Wastewaters/ Surface waters	Greece/ UK	Baker and Kasprzyk-Hordern 2011; Alygizakis et al. 2016
	3-methylxanthine	$C_6H_6N_4O_2$	166.140	Sea	Greece/ Italy/ Turkey	Nödler et al. 2014
	6-acetylmorphine	$C_{19}H_{21}NO_4$	327.374	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Amphetamine	$C_9H_{13}N$	135.206	River/ Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
	Anhydroecgonine methyl ester	$C_{10}H_{15}NO_2$	181.235	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	Benzoylecgonine	$C_{16}H_{19}NO_4$	289.326	Wastewaters/ Surface waters/ Sea	Brazil/ Greece/ Italy/ Turkey/ UK	Baker and Kasprzyk-Hordern 2011; Nödler et al. 2014; Petrie et al. 2015; Pereira et al. 2016
	Benzylpiperazine	$C_{11}H_{16}N_2$	176.258	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015

Caffeine	$C_8H_{10}N_4O_2$	194.191	Water for consumption/ Wastewaters/ Surface waters/ Sea	Brazil/ Canada/ France/ Gernnany/ Greece/ Israel/ Italy/ Portugal/ Spain/ Turkey/ UK/ USA	Ferrer et al. 2010; Ferrer and Thurman 2012; Loos et al. 2013; Gaffney et al. 2014; Petrie et al. 2015; Nödler et al. 2014; Pereira et al. 2016
Cocaethylene	$C_{18}H_{23}NO_4$	317.379	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Cocaine	$C_{17}H_{21}NO_4$	303.353	Wastewaters/ Surface waters/ River/ Sea/ Groundwater	Brazil/ France/ Italy/ UK	Zuccato et al. 2005; Lopez et al. 2015; Petrie et al. 2015; Pereira et al. 2016
Cotinine	$C_{10}H_{12}N_2O$	176.215	Sea/ Groundwater	France/ Greece/ Italy/ Turkey	Ferrer and Thurman 2012; Nödler et al. 2014; Lopez et al. 2015
Desmethyl-dextrorphan	C16H21NO	243.340	-	-	Ferrer and Thurman 2012
Dextrorphan	$C_{17}H_{23}NO$	257.371	-	-	Ferrer and Thurman 2012
Ecgonidine	$C_9H_{13}NO_2$	167.208	River/ Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
Ephedrine	$C_{10}H_{15}NO$	165.232	River/ Wastewaters/ Surface waters/ Sea	Greece/ UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015; Alygizakis et al. 2016
Fexofenadine	$C_{32}H_{39}NO_4$	501.656	-	-	Loos et al. 2013
Methamphetamine	$C_{10}H_{15}N$	149.233	River/ Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Methcathinone	$C_{10}H_{13}NO$	163.220	River/ Wastewaters and surface	UK	Baker and Kasprzyk-Hordern 2011
Nicotine	$C_{10}H_{14}N_2$	162.232	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Stuart et al. 2012; Petrie et al. 2015
Norbenzoylecgonine	$C_{15}H_{17}NO_4$	275.304	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Norcocaine	$C_{16}H_{19}NO_4$	289.326	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Norephedrine	$C_9H_{13}NO$	151.206	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
Theobromine	$C_7H_8N_4O_2$	180.164	Sea	Germany/ Greece/ Israel/ Italy/ Spain/ Turkey/ USA	Nödler et al. 2014
Theophylline	$C_7H_8N_4O_2$	180.164	Wastewaters/ Surface waters/ Sea	Germany/ Greece/ Italy/ Turkey/ UK/	Nödler et al. 2014; Petrie et al. 2015; Alygizakis et al. 2016

					USA	
	Trifluoromethyl-phenylpiperazine	$C_{13}H_{14}F_3NO_2$	273.251	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011; Petrie et al. 2015
HALLUCINOGENS AND THEIR METABOLITES	2-oxo-3-hydroxy-Lysergic acid diethylamide (O-H-LSD)	$C_{20}H_{25}N_3O_3$	355.438	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	3,4-(methylenedioxyphenyl)-2-butanamine (BDB)	$C_{17}H_{19}NO_2$	269.344	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	3,4-methylenedioxyamphetamine (MDA)	$C_{10}H_{13}NO_2$	179.219	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	3,4-methylenedioxymethamphetamine (MDMA) – (Ecstasy)	$C_{11}H_{15}NO_2$	193.246	Wastewaters/ Surface waters /Sea	Greece/ UK	Baker and Kasprzyk-Hordern 2011; Alygizakis et al. 2016
	3,4-methylenedioxy-N-ethylamphetamine (MDEA)	$C_{12}H_{17}NO_2$	207.273	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	3,4-methylenedioxyphenyl-N-methylbutanamine (MBDB)	$C_{12}H_{17}NO_2$	207.273	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	Lysergic acid diethylamide (LSD)	$C_{20}H_{25}N_3O$	323.440	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
	Mescaline	$C_{11}H_{17}NO_3$	211.261	Wastewaters/ Surface waters	UK	Baker and Kasprzyk-Hordern 2011
HYGIENE AND PERSONAL CARE PRODUCTS						
FRAGRANCES	Ambrettolide	$C_{16}H_{28}O_2$	252.398	Wastewater treatment plants	Spain	Vallecillos 2014
	Cashmeran	$C_{14}H_{22}O$	206.329	Wastewater treatment plants	Spain	Regueiro et al. 2008; Matamoros et al. 2012; Vallecillos 2014
	Celestolide	$C_{17}H_{24}O$	244.378	Wastewater treatment plants	Spain	Regueiro et al. 2008; Vallecillos 2014
	Civetone	$C_{17}H_{30}O$	250.426	Wastewater treatment plants	Spain	Vallecillos 2014

	Exaltolide	$C_{15}H_{28}O_2$	240.387	Wastewater treatment plants	Spain	Barceló 2003; Vallecillos 2014
	Exaltone	$C_{15}H_{28}O$	224.388	Wastewater treatment plants	Spain	Vallecillos 2014
	Galaxolide	$C_{18}H_{26}O$	258.398	Wastewater treatment plants/ Groundwater	France/ Germany/ Spain	Regueiro et al. 2008; Stuart et al. 2012; Santana 2013; Vallecillos 2014; Lopez et al. 2015
	Galaxolidone	$C_{18}H_{24}O_2$	272.388	Wastewater treatment plants	Spain	Vallecillos 2014
	Isoborneol	$C_{10}H_{18}O$	154.249	-	-	Santana 2013
	Methyl dihydrojasmonate	$C_{13}H_{22}O_3$	226.316	Water treatment plants	Spain	Matamoros et al. 2012
	Muscone	$C_{16}H_{30}O$	238.415	Wastewater treatment plants	Spain	Barceló 2003; Vallecillos 2014
	Musk ambrette	$C_{12}H_{16}N_2O_5$	268.269	Groundwater	France	Lopez et al. 2015
	Musk ketone	$C_{14}H_{18}N_2O_5$	294.307	Wastewater treatment plants/ Groundwater	France/ Spain	Regueiro et al. 2008; Barceló 2003; Vallecillos 2014; Lopez et al. 2015
	Musk MC4	$C_{14}H_{24}O_4$	256.340	Wastewater treatment plants	Spain	Barceló 2003; Vallecillos 2014
	Musk moskene	$C_{14}H_{18}N_2O_4$	278.308	Wastewater treatment plants	Spain	Regueiro et al. 2008; Barceló 2003; Vallecillos 2014
	Musk NN	$C_{15}H_{26}O_4$	270.369	Wastewater treatment plants	Spain	Barceló 2003; Vallecillos 2014
	Musk xylene	$C_{12}H_{15}N_3O_6$	297.267	Wastewater treatment plants/ Groundwater	France/ Spain	Barceló 2003; Regueiro et al. 2008; Vallecillos 2014; Lopez et al. 2015
	Phantolide	$C_{17}H_{24}O$	244.378	Wastewater treatment plants	Spain	Regueiro et al. 2008; Vallecillos 2014
	Tonalide	$C_{18}H_{26}O$	258.405	Wastewater treatment plants	Germany/ Spain	Regueiro et al. 2008; Matamoros et al. 2012; Stuart et al. 2012; Vallecillos 2014
	Traseolide	$C_{18}H_{26}O$	258.405	Wastewater treatment plants	Spain	Regueiro et al. 2008; Vallecillos 2014
COSMETIC PRESERVATIVES	Butylparaben	$C_{11}H_{14}O_3$	194.227	Wastewaters/ Surface waters	UK	Petrie et al. 2015
	Ethylparaben	$C_9H_{10}O_3$	166.174	Wastewaters/ Surface waters	UK	Petrie et al. 2015
	Methylparaben	$C_8H_8O_3$	152.147	Wastewaters/ Surface	UK	Stuart et al. 2012; Petrie et al. 2015

			waters		
	Propylparaben	$C_{10}H_{12}O_3$	180.201	Wastewaters/ Surface waters/ Groundwater	France/ UK	Stuart et al. 2012; Lopez et al. 2015; Petrie et al. 2015
SUN PROTECTION AGENT	Benzofenone	$C_{13}H_{10}O$	182.218	Wastewaters/ Surface waters	UK	Barceló 2003; Petrie et al. 2015
	Enzacamene	$C_{18}H_{22}O$	254.370	-	-	Barceló 2003
	Octocrylene	$C_{24}H_{27}NO_2$	361.485	Groundwater	France	Lopez et al. 2015
	Octyl methoxycinnamate	$C_{18}H_{26}O_3$	290.397	-	-	Barceló 2003
	Oxybenzone	$C_{14}H_{12}O_3$	228.247	Wastewater treatment plants	Spain	Matamoros et al. 2012
INSECTS REPELLENT	N,N-diethyltoluamide (DEET)	$C_{12}H_{17}NO$	191.270	River	Brazil/ South Korea/ UK	Barceló 2003; Stuart et al. 2012; Santana 2013
ANTISÉPTICS	Methyltriclosan	$C_{13}H_9Cl_3O_2$	303.563	Lake/ River/ Wastewater treatment plants	USA	Coogan and Point et al. 2008
	Triclocarban	$C_{13}H_9Cl_3N_2O$	315.582	Lake/ River/ Wastewater treatment plants	USA	Barceló 2003; Coogan and Point et al. 2008
	Triclosan	$C_{12}H_7Cl_3O_2$	289.536	Lake/ River/ Wastewaters/ Surface waters	Denmark/ Spain/ UK/ USA	Barceló 2003; Coogan and Point et al. 2008; Matamoros et al. 2012; Stuart et al. 2012; Santana 2013; Petrie et al. 2015
			SUBSTANCES OF VARIOUS USES			
SURFACTANTS AND SURFACE METABOLISM	Alkylbenzyl dimethylammonium chloride	$C_{32}H_{70}Cl_2^{+4}$	525.812	River	Brazil	Penteado et al. 2006
	Ethoxylated oleic alcohol of phosphate	$C_{20}H_{43}O_6P$	410.532	River	Brazil	Penteado et al. 2006
	Nonylphenol	$C_{15}H_{24}O$	220.350	Groundwater	Autria/ Denmark/ Spain	Barceló 2003; Jurado et al. 2012; Lapworth et al. 2012; Santana 2013
	Octylphenol	$C_{14}H_{22}O$	206.329	Groundwater	Spain	Barceló 2003; Jurado et al. 2012; Santana 2013

	Sodium dodecylbenzenesulfate	$C_{18}H_{29}NaO_3S$	348.480	River	Brazil	Penteado et al. 2006
	Sodium dodecylsulfate	$NaC_{12}H_{25}SO_4$	288.372	River	Brazil	Penteado et al. 2006
	Sodium lauryl ether sulfate	$C_{12}H_{25}O4S.Na$	288.378	-	-	Barceló 2003
FLAME RETARDANTS	Decabromodiphenyl ether (BDE 209)	$C_{12}\ Br_{10}O$	959.171	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Hexabromocyclododecan e	$C_{12}H_{18}Br_6$	641.700	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Hexabromodiphenyl ether (BDE 154)	$C_{12}H_4Br_6O$	643.587	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Octabromodiphenyl ether (BDE 196)	$C_{12}H_2Br_8O$	801.379	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Pentabromodiphenyl ether (BDE 100)	$C_{12}H_5Br_5O$	564.691	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Tetrabromobisphenol A	$C_{15}H_{12}Br_4O_2$	543.875	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Tetrabromodiphenyl ether (BDE 47)	$C_{12}H_6Br_4O$	485.795	Sewage treatment plants	USA	Barceló 2003; Stevens 2010; Pazin et al. 2015
	Tris (2-chloroethyl) phosphate	$C_6H_{12}Cl_3O_4P$	285.489	Sewage treatment plants/ Water treatment plants	Spain/ USA	Barceló 2003; Stevens 2010; Matamoros et al. 2012; Pazin et al. 2015
ADDITIVES AND INDUSTRIAL AGENTS	1H-benzotriazole	$C_6H_5N_3$	119.127	Sea/ Water treatment plants	Germany/ Greece/ Israel/ Italy/ Spain/ Turkey/ USA	Matamoros et al. 2012; Nödler et al. 2014
	2-Anthraquinone Sulfonate	$C_{14}H_7O$	287.268	River	Germany	Loos and Niessner 1998
	4-Toluene Sulfonate	$C_7H_7O_3S^-$	171.190	River	Germany	Loos and Niessner 1998
	Benzene sulphonate	$C_6H_5O_3S^-$	157.163	River	Germany	Loos and Niessner 1998
	Dibutyltin	$C_8H_{18}Sn$	232.942	Groundwater	France	Lopez et al. 2015
	Dimercaptosuccinic acid (DMSA)	$C_4H_6O_4S_2$	182.220	-	-	Barceló 2003
	Ethylenediamine tetra acetic acid (EDTA)	$C_{10}H_{16}N_2O_8$	292.244	-	-	Barceló 2003

	Methylglycine di-acetic acid (MGDA)	$C_7H_{11}NO_6$	205.166	-	-	Barceló 2003
	Monobutyltin	$C_4H_{10}O_2Sn$	208.830	Groundwater	France	Lopez et al. 2015
	Tolyltriazole	$C_9H_9N_3$	159.190	Sea/ Groundwater	France/ Germany/ Greece/ Israel/ Italy/ Spain/ Turkey/ USA	Nödler et al. 2014; Lopez et al. 2015
GASOLINE ADDITIVES	Tert-amyl ethyl ether (TAEE)	$C_6H_{14}O$	102.177	-	-	Barceló 2003
	Tert -Butyl methyl ether (TBME)	$C_5H_{12}O$	88.150	-	-	Barceló 2003
DISINFECTION PRODUCTS	Bromate	BrO_3^-	127.901	Groundwater	France	Barceló 2003; Lopez et al. 2015
	Bromoacetonitrile	C_2H_2BrN	119.949	-	-	Barceló 2003
	Cyanoformaldehyde	C_2HNO	55.036	-	-	Barceló 2003
	Iodine-(THM)	CHI_3	393.732	-	-	Barceló 2003
	N-nitrosodimethylamine (NDMA)	$C_2H_6N_2O$	74.083	-	-	Barceló 2003
PLASTICIZERS	Bis(2-ethylhexyl) phthalate (DEHP)	$C_{24}H_{38}O_4$	390.564	Freshwater/ River/ Groundwater/ Sea	Canada/ China/ France/ India/ Iraq/ Japan/ Netherlands/ South Africa/ Spain/ Taiwan/ UK	Stuart et al. 2012; Santana 2013; Lopez et al. 2015; Gao and Wen 2016
	Bisphenol A (BPA)	$C_{15}H_{16}O_2$	228.291	Wastewaters and surface/ Springs/ Water for consumption/ River/ Groundwater	Austria/ Brazil/ France/ Germany/ Spain/ UK/ USA	Raimundo 2011; Jurado et al. 2012; Lapworth et al. 2012; Stuart et al. 2012; Santana 2013; Lopez et al. 2015; Petrie et al. 2015
	Butylbenzyl phthalate (BBP)	$C_{19}H_{20}O_4$	312.365	Freshwater/ River/ Sea	Canada/ China/ France/ India/ Korea/ Spain/	Regueiro et al. 2008; Souza et al. 2014; Gao and Wen 2016
	Diethyl phthalate (DEP)	$C_{12}H_{14}O_4$	222.240	Freshwater / River/ Sea	Brazil/ Canada/ China/ France/ India/ / Iraq/ Japan/ South Africa/ Spain/ Taiwan	Regueiro et al. 2008; Santana 2013; Gao and Wen 2016; Silva et al. 2016

	Diisobutyl phthalate (DBP)	$C_{16}H_{22}O_4$	278.348	Freshwater / river/ Sea	Brazil/ Canada/ China/ France/ India/ Iraq/ Japan/ Netherlands/ Spain/ South Africa/ Taiwan	Regueiro et al. 2008; Souza et al. 2014; Gao and Wen 2016; Silva et al. 2016
	Diisoctyl phthalate (DOP)	$C_{28}H_{46}O_4$	446.672	Freshwater / River/ Sea	Canada/ China/ Grance/ India/ Iraq/ Korea Spain	Regueiro et al. 2008; Souza et al. 2014; Gao and Wen 2016
	Dimethyl phthalate (DMP)	$C_{10}H_{10}O_4$	194.186	Freshwater/ River	Canada/ China/ France/ India/ Japan/ Korea/ South Africa/ Spain	Regueiro et al. 2008; Gao and Wen 2016
	Tributyl phosphate	$C_{12}H_{27}O_4P$	266.318	Water treatment plants	Spain	Matamoros et al. 2012
HERBICIDES, INSETICIDES AND PESTICIDES	2,4-Dichlorophenoxyacetic acid	$C_8H_6Cl_2O_3$	221.033	River/ Groundwater	Australia/ Denmark/ Mediterranean marine/ Swiss /UK	Stuart et al. 2012; Birch et al. 2015
	2-methyl-4-chlorophenoxyacetic acid (MCPA)	$C_9H_9ClO_3$	200.618	River/ Groundwater	Australia/ Denmark/ Dutch/ Italy/ Mediterranean marine/ Swiss	Stuart et al. 2012; Birch et al. 2015
	3,4-dichloroaniline	$C_6H_5Cl_2$	162.017	River	Australia	Birch et al. 2015
	Alachlor	$C_{14}H_{20}ClNO_2$	269.767	Groundwater	Spain	Jurado et al. 2012
	Aquacide	$C_{12}H_{12}Br_2N_2$	344.050	-	-	Santana 2013
	Atrazine	$C_8H_{14}ClN_5$	215.685	Sea/ Springs/ Water for consumption/ Groundwater	Brazil/ Denmark/ France/ Greece/ Israel/ Italy/ Spain/ Turkey/ UK/ USA	Raimundo 2011; Jurado et al. 2012; Stuart et al. 2012; Loos et al. 2013; Santana 2013; Nödler et al. 2014; Lopez et al. 2015
	Bentazone	$C_{10}H_{12}N_2O_3S$	240.277	Groundwater	France/ Denmark/ Italy/ Norway	Stuart et al. 2012; Lopez et al. 2015
	Carbaril	$C_{12}H_{11}NO_2$	201.221	River	Australia	Birch et al. 2015
	Carbendazim	$C_9H_9N_3O_2$	191.187	-	-	Loos et al. 2013; Santana 2013
	Carbofuran	$C_{12}H_{15}NO_3$	221.256	-	-	Santana 2013

Chloridazon	$C_{10}H_8ClN_3O$	221.644	Groundwater	Germany	Stuart et al. 2012
Chlortoluron	$C_{10}H_{13}ClN_2O$	212.677	Groundwater	France/ Spain	Jurado et al. 2012; Lopez et al. 2015
Clopyralid	$C_6H_3Cl_2NO_2$	191.995	Groundwater	Norway	Stuart et al. 2012
Cyanazine	$C_9H_{13}ClNO_6$	240.692	Groundwater	Spain	Jurado et al. 2012
Deisopropylatrazine	$C_5H_8ClN_5$	173.604	Groundwater	France/ Spain	Jurado et al. 2012; Lopez et al. 2015
Deisopropyldeethylatrazine	$C_3H_4ClN_5$	145.550	Groundwater	France	Lopez et al. 2015
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	505.206	-	-	Santana 2013
Desethylatrazine (DEA)	$C_6H_{10}ClN_5$	187.631	Sea/ Groundwater	France/ Germany/ Greece/ Spain/ Turkey	Jurado et al. 2012; Nödler et al. 2014; Lopez et al. 2015
Diazinon	$C_{12}H_{21}N_2O_3PS$	304.345	Water treatment plants/ Groundwater	Spain	Jurado et al. 2012; Matamoros et al. 2012
Dichlorobenzamide	$C_7H_5Cl_2NO$	190.023	Groundwater	France	Lopez et al. 2015
Dichlorodiphenyldichlorethylene (DDE)	$C_{14}H_8Cl_4$	318.018	-	-	Loos et al. 2013
Dichlorodiphenyltrichloroethane (DDT)	$C_{14}H_9Cl_5$	354.476	-	-	Loos et al. 2013
Dichlorprop	$C_9H_8Cl_2O_3$	235.060	Groundwater	Denmark/ Norway	Stuart et al. 2012
Dimethoate	$C_5H_{12}NO_3PS_2$	229.249	Groundwater	Norway/ Spain	Jurado et al. 2012; Stuart et al. 2012
Diuron	$C_9H_{10}Cl_2N_2O$	233.092	River/ Sea/ Groundwater	Australia/ Germany/ Greece/ Israel/ Italy/ Japan/ Malasian/ San Francisco/ Spain/ Southern Adriatic/ Turkey/ UK US	Jurado et al. 2012; Stuart et al. 2012; Loos et al. 2013; Nödler et al. 2014; Birch et al. 2015
Isoproturon	$C_{12}H_{18}N_2O$	206.289	Sea/ Groundwater	France/ Germany/ Israel/ Norway/ Spain	Jurado et al. 2012; Stuart et al. 2012; Nödler et al. 2014; Lopez et al. 2015
Lindane	$C_6H_6Cl_6$	290.814	River/ Effluent	Spain/ UK	Regueiro et al. 2008; Stuart et al. 2012
Linuron	$C_9H_{10}Cl_2N_2O_2$	249.091	Groundwater	Norway/ Spain	Jurado et al. 2012; Stuart et al. 2012

Malation	$C_{10}H_{19}O_6PS_2$	330.350	Groundwater	Spain	Jurado et al. 2012
Manab	$CH_2NS_2^-$	92.154	Groundwater	Spain	Jurado et al. 2012
Mecoprop	$C_{10}H_{11}ClO_3$	214.645	River/ Sea	Australia/ Germany/ Greece/ Italy/ Norway/ Portugal/ San Francisco/ Swiss/ Turkey/ USA	Stuart et al. 2012; Nödler et al. 2014; Birch et al. 2015
Metalaxyl	$C_{15}H_{21}NO_4$	279.336	Groundwater	Norway	Stuart et al. 2012
Metazachlor	$C_{14}H_{16}ClN_3O$	277.752	Sea/ Groundwater	France/ Israel	Stuart et al. 2012; Nödler et al. 2014
Metolachlor	$C_{15}H_{22}ClNO_2$	283.796	Groundwater	France/ Spain	Jurado et al. 2012; Lopez et al. 2015
Metribuzin	$C_8H_{14}N_4OS$	381.287	Groundwater	Denmark/ Norway	Stuart et al. 2012
Metsulfuron-methyl	$C_{14}H_{15}N_5O_6S$	381.363	Groundwater	France	Lopez et al. 2015
Oxadixyl	$C_{14}H_{18}N_2O_4$	278.308	Groundwater	France	Lopez et al. 2015
Propachlor	$C_{11}H_{14}ClNO$	211.689	Groundwater	Norway	Stuart et al. 2012
Propazine	$C_9H_{16}ClN_5$	229.710	Groundwater	Spain	Jurado et al. 2012
Pyrethroids and pyrethrins	$C_{21}H_{28}O_3$	328.452	River/ Surface waters	Canada/ South Africa/ USA	Oros and Werner 2005; Sereda and Meinhardt 2003
Simazine	$C_7H_{12}ClN_5$	201.658	River/ Groundwater	Australia/ Denmark/ France/ Greece/ Spain Swiss/ Mediterranean marine/ Portugal/ UK	Jurado et al. 2012; Stuart et al. 2012; Loos et al. 2013; Birch et al. 2015; Lopez et al. 2015
Tebuthiuron	$C_9H_{16}N_4OS$	228.314	-	-	Loos et al. 2013
Terbutryn	$C_{10}H_{19}N_5S$	241.357	Water treatment plants/ Groundwater	Spain	Jurado et al. 2012; Matamoros et al. 2012
Terbuthylazine (TBA)	$C_9H_{16}ClN_5$	229.712	Sea/ Groundwater	Greece/ Italy/ Norway/ Spain/ Turkey	Jurado et al. 2012; Stuart et al. 2012; Nödler et al. 2014
Tributyltin	$C_{12}H_{27}Sn$	290.058	-	-	Loos et al. 2013; Santana 2013
Trfluralin	$C_{13}H_{16}F_3N_3O_4$	335.283	Groundwater	Spain	Jurado et al. 2012
Triphenyl phosphate	$C_{18}H_{15}O_4P$	326.288	-	-	Souza et al. 2014

However, all these pollutants are still not included in the guidelines for bathing, surface, and drinking water, and effluent control in any country of the world; therefore, the current data about their respective toxicities are not enough to establish their daily intake and reference doses. These unregulated pollutants are being considered challenges for water quality regulators. (Barceló 2003; Farré et al. 2008; Sousa et al. 2018).

Table 1 provides, as well, molecular formulas and masses of these emerging concern contaminants, useful information for analyzing human and animal tissues and fluids, and water samples by chromatographic techniques coupled to mass spectrometry.

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Capítulo 4. *Sesquiterpenos de* Dictyopteris delicatula *J.V. Lamouroux (Dictyotales, Ochrophyta)*

Sesquiterpenos de *Dictyopteris delicatula* J.V. Lamouroux (Dictyotales, Ochrophyta)

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Resumo: A análise do extrato hexânico da macroalga marinha *Dictyopteris delicatula* J.V. Lamouroux (Dictyotaceae-Ochrophyta), em cromatográfo a gás acoplado a espectrômetro de massas, levou à caracterização de treze sesquiterpenos, dois dos quais já haviam sido dela isolados, em estudo anterior. A identificação desses sesquiterpenos, que pertencem à família dos cadinanos, foi realizada com assistência de bibliotecas virtuais e de material existente na literatura mundial. Dois dos sesquiterpenos identificados são biologicamente ativos e dez outros estão presentes em óleos essenciais que apresentam atividades terapêuticas.

Palavras-chave: algas pardas, cadinanos, terpenoma.

Abstract: An analysis of the components of *Dictyopteris delicatula* Lamouroux (Dictyotaceae-Phaeophyta) hexane extract, by gas chromatography/mass spectrometry, led to the characterization of thirteen sesquiterpenes, two of which had already been isolated from this marine macroalga, in previous studies. The identification of these sesquiterpenes, which belong to the family of the cadinanes, was carried out with the assistance of virtual libraries and of existing material in the world literature. Two of the herein identified sesquiterpenes are biologically active and ten others are present in essential oils which exhibit therapeutic activities.

Key-words: brown algae, cadinanes, terpenome.

Introdução

Os organismos que habitam o ambiente marinho estão sujeitos a uma série de agentes agressores entre os quais encontram-se os organismos herbívoros e incrustantes, os patógenos, as substâncias alelopáticas (Solan & Whiteley, 2016) e os fatores ambientais tais como radiação solar e temperaturas extremas (Bernardi et al., 2016). As ações deletérias desses agentes determinam sobre seus antagonistas, as mais diversas reações: nas macroalgas, uma notável reação a herbívoros e incrustantes é a síntese de numerosos metabolitos especiais (Molis et al., 2008; Hay et al., 1988).

Essas substâncias, que têm a função de contribuir para a sobrevivência de seus produtores (Molis et al., 2008) e pertencem principalmente às classes dos terpenos, acetogeninas, alcaloides e polifenóis, possuem estruturas bastante diversificadas e apresentam um leque de atividades extremamente abrangente (Blunt et al. 2018; Alves et al., 2018). Porém, os terpenos destacam-se pela diversidade e abundância, especialmente os produzidos por *Laurencia* (Ceramiales, Rhodophyta): são sesqui, di e triterpenos, com estruturas diversas e importantes atividades biológicas (Fujii et al., 2011; Irianto & Devi, 2013).

As macroalgas pardas também produzem notável quantidade de terpenos. Por exemplo, na família Dictyotaceae, sintetizam numerosos diterpenos, membros dos gêneros *Dictyota* e *Canistrocarpos* (Faulkner, 2002; De-Paula et al., 2018; 2012; 2011; Oliveira et al., 2008) e sintetizam sesquiterpenos algas do gênero *Dictyopteris* (Faulkner, 2002; Blunt et al., 2018).

De *Dictyopteris divaricata* foi isolada a maior parte dos 59 sesquiterpenos produzidos por este último gênero de algas, do qual também foram extraídos mono e diterpenos (Zatelli et al., 2018; Ji et al., 2009; Song et al., 2004, 2005a, 2005b, 2006; Suzuki et al.,1981). Dos extratos de *D. delicatula*, que foi objeto de nosso estudo, foram isolados os sesquiterpenos α-cadinol, torreiol, cubenol, 3-cubenolona, 5α-diidroxicubenol (König & Wright, 1995). A maior parte desses sesquiterpenos possui esqueletos pertencentes a classe dos cadinanos, principalmente cadalanos e selinanos, mas também, entre eles, são encontrados copaanos, cubebanos e elemanos. Também de *D. delicatula* foram extraídos um diterpeno (Wright & Coll, 1990) e oito esteróis (Fleury et al., 1994a, 1994b e 1996).

Parte dos terpenos produzidos por algas são portadores de atividades biológicas; as ações biológicas exercidas por diversos extratos de *D. delicatula* são enumerados por Zatelli et al. (2018) e por Soares et al. (2012). Entretanto, quanto aos sesquiterpenos contidos nos óleos essenciais das macroalgas, poucos foram analisados, individualmente, quanto à ação biológica exercida (Scherer et al, 2009). Dos identificados em *D. delicatula*, o α-cadinol tem propriedades antifúngicas (Ho et al., 2011) e o cubenol, ação citotóxica frente às células tumorais NCI-H187 (Jiangseubchatveera et al., 2015).

Neste estudo, nosso intuito foi colaborar com o conhecimento dos metabolitos de *D. delicatula* empregando cromatografia gasosa/espectrometria de massas, que é um método capaz de identificar inequívoca e rapidamente substâncias voláteis, termicamente estáveis e de baixa polaridade (Stranska-Zachariasova et al., 2017; Shobier et al., 2016; Gressler et al., 2012; Mackintosh et al., 2004); Nosso objetivo foi determinar a existência de sesquiterpenos no extrato hexânico de *D. delicatula* submetendo-o à análise por cromatografia gasosa/espectrometria de massas.

Material e Métodos

O organismo

Os espécimes de *Dictyopteris delicatula* J.V. Lamouroux foram coletados manualmente na Praia de Ubu, em Anchieta, no litoral do estado de Espírito Santo (20° 48' 07'' S, 40° 35' 34'' O), em março de 2016; a biomassa coletada foi limpa, identificada e seca à sombra. Parte da amostra foi utilizada para a confecção de exsicata que foi depositada (SP428.538) no Herbário Científico "Maria Eneyda Pacheco Kauffman Fidalgo" do Instituto de Botânica do Estado de São Paulo.

Obtenção do extrato hexânico (HE)

A biomassa algácea foi moída em moinho Wiley e submetida à extração, assistida por ultrassom (5 x, 30 s, 100 W), com hexano PA. O extrato obtido foi submetido à filtração em papel de filtro GF/C, concentrado em rotoevaporador e analisado por cromatografia gasosa/espectrometria de massas.

Análise qualitativa, por cromatografia gasosa/espectrometria de massas

Essa análise foi realizada em cromatógrafo gasoso, conectado a espectrômetro de massas (GC/MS-QP2010 Plus, Shimadzu, Kyoto, Japan), dotado de coluna HP-5MS (5%-fenilmetilpolisiloxano, 30 m x 0,25 mm ϕ, 0.25 μm espessura do filme); o gás de arraste foi o hélio e o fluxo, de 1.0 ml.min^{-1}. A temperatura de injeção foi de 250 °C e a temperatura inicial do forno, que foi de 60 °C teve incrementos de 3 °C por minuto, até atingir 260 °C, temperatura que foi mantida por 40 min.

A temperatura da interface do espectrômetro de massas, que operou em modo full scan, de 40 a 1.000 m/z, foi de 260 °C; a amostra foi ionizada por corrente eletrônica de 70 eV.

A identificação dos constituintes químicos foi feita com a assistência das bibliotecas NIST08, NIST08s, Wiley9 and Nist Mass Spectral Search Program from Nist/ Epa/ Nih Mass Spectral Library Version 2.0 e do material disponível na literatura mundial. Somente foram consideradas substâncias com índices de similaridade (S) maiores ou iguais a 95% e também as com espectros de massas idênticos aos dos encontrados na literatura.

Resultados e Discussão

Foram identificados 13 sesquiterpenos, os quais estão compilados na tabela 1. Deles, apenas o cubenol e a 3-cubenolona haviam sido isolados de *D. delicatula.* Não foram observados, neste estudo, os outros sesquiterpenos descritos por König & Wright (1995).

Esses sesquiterpenos pertencem à família dos cadinanos cujo esqueleto é resultado de ciclização 1→10 do cátion farnesila E,Z, que forma um intermediário tipo-germacrano e por uma sequência de etapas de transciclização descrita minuciosamente por Phyllips (1971).

Neste grupo, são observados quatro tipos de configuração isomérica com relação à orientação dos átomos de hidrogênio ligados aos anéis A e B (Figura 1). Nos cadinanos e bulgaranos essas ligações têm orientação trans e nos muurolanos e os amorfanos, configuração cis; os copaanos derivam dos muurolanos (Sukh, 1989).

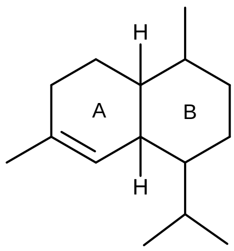


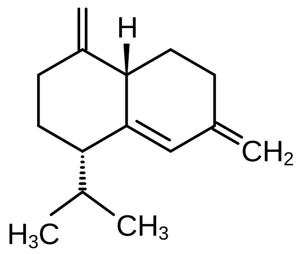
Figura 1. Esqueleto dos cadinanos.

Os sesquiterpenos de *D. delicatula* identificados no presente estudo estão listadas na Tabela 1, em que os picos moleculares estão em destaque e os demais picos relativos às massas dos íons das quebras moleculares estão colocados em ordem decrescente.

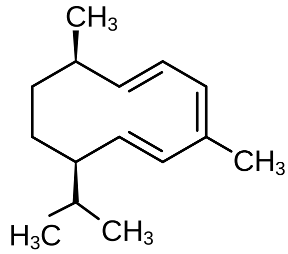
Tabela 1. Sesquiterpenos identificados no extrato hexânico de *D. delicatula*.

Sesquiterpeno	TR (min)	Massa molecular (D)	Massa dos fragmentos iônicos (m/z)						
			Pico base (100%)	2º. maior pico	3º. maior pico	4º. maior pico	5º. maior pico	Demais picos (em ordem decrescente de tamanho)	Referência
Cis- muurola-4(14),5-dieno	24,025	204	161	105	119	91	204	133; 91; 93; 81; 79; 41; 55	Adams, 2017, p. 461
Cis- calameneno	27,100	202	157	202	131	105	144	117; 91; 57; 41	Adams, 2017, p. 526
Cubenol	30,692	222	161	105	119	43	121	204; 179; 81; 93;95; 91; 55; 41; 189	Adams, 2017, p. 605
1-10-di-epi-cubenol	35,908	222	161	119	105	179	204	55; 79; 81; 95; 43; 91; 93; 41	Adams, 2017, p. 586
1-epi-cubenol	36,233	222	119	105; 161	204	95; 179	55; 93	179; 82; 41; 81; 43; 69; 79; 67; 91; 189	Adams, 2017, p.592
Muurola- 4, 10 (14)-dien-1-β-ol	39,442	220	159	97	107	93	91	177; 105; 202; 79; 187; 131; 119; 117;41; 67; 53	Adams, 2017, p.594
δ-cadineno	40,358	222	161	105	204	81; 119	95	43; 189; 134; 93; 121; 41	Adams, 2017, p. 218
Tau-muurolol	40,950	222	95	121	43	161; 204; 109		105; 164; 94; 93; 71; 79; 81; 58; 41; 189; 179; 222	Borg-Karlson, 1981; Biblioteca NIST
α-copaeno	41,950	204	161	105; 119	95	121	204	81;79; 120; 55; 71; 69; 41; 189; 175	Adams, 2017, p. 387
Cadaleno	41,775	198	183	198	168	153; 165	83	91; 99;115; 128;;57;51;41	Adams, 2017, p. 629
α- cadinol	42,525	222	95	121	43	109	105	204; 161; 164; 93; 81;71; 79; 41; 137; 189; 148; 179	Borg-Karlson, 1981
Calamenen-10-ona	47,033	202	159	131	202	115	145	91; 57; 82; 96; 41; 43	Adams, 2017, p. 648
Cubenolona	64,642	236	175	109	193	43	85	236; 135; 147; 165; 69; 79; 218; 127; 41	König & Wright, 1995

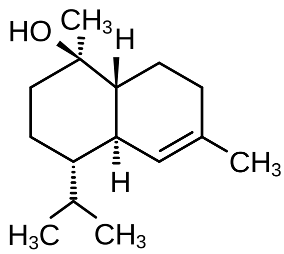
As estruturas desses sesquiterpenos identificados estão dispostas na figura 2.



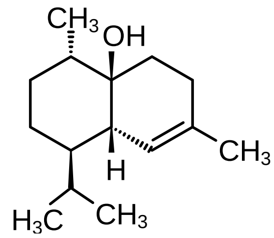
Cis-muurola-4(14),5-dieno



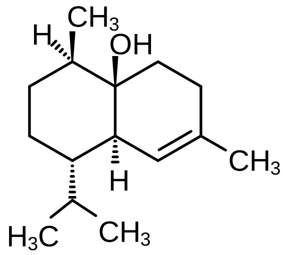
Cis-calameneno



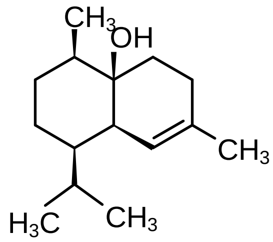
α-Cadinol



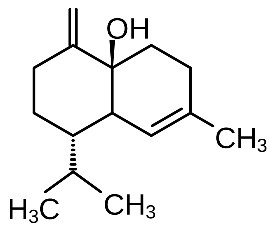
Epi-cubenol



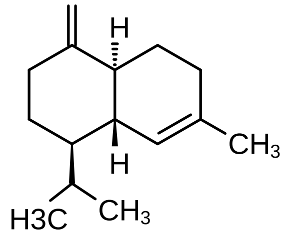
-(-)-Cubenol



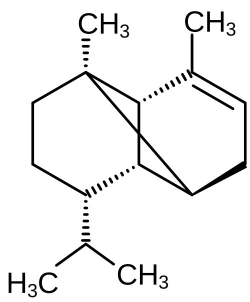
1,10-Di-epicubenol



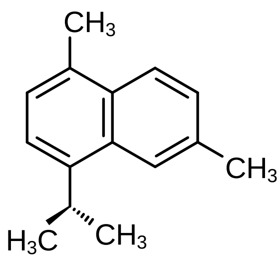
Muurola-4,10(14)-dien-1-β-ol



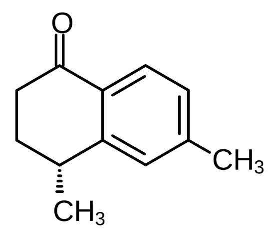
γ-Cadineno



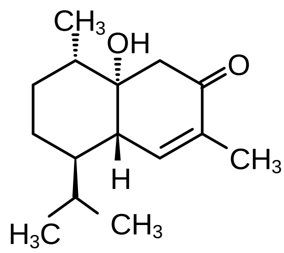
α-Copaeno



Cadaleno



Calamenen-10-ona



Cubenolona

Figura 2. Sesquiterpenos identificados *Dictyopteris delicatula* J.V. Lamouroux.

Na tabela 2 estão dispostos os 10 sesquiterpenos, identificados em *D. delicatula*, que são componentes majoritários de óleos essenciais biologicamente ativos; entre eles estão inclusos o α-cadinol e o cubenol, supracitados como ativos per se (Ho et al., 2011; Jiangseubchatveera et al., 2015).

Tabela 2. Sesquiterpenos apontados como substâncias majoritárias de óleos essenciais biologicamente ativos, que também são componentes do extrato hexânico de *D. delicatula*.

Substância	**Atividade**	**Referência**
Cis- muurola-4(14),5-dieno	Anticolinesterasica, antibacteriana	Chéraif et al, 2007; Silva et al., 2009; Lima et al., 2011
Cis- calameneno	Antibacteriana, antioxidante, antitripanosômica	Jaramillo-Colorado et al., 2016; Fratini et al., 2017; Oliveira de Souza et al., 2017
Cubenol	Antibacteriana, antioxidante, citotóxica, repelente de insetos	Rad et al., 2013; Suleiman et al., 2014; Jiangseubchatveera et al., 2015; Espinoza et al., 2016; Juárez et al., 2016; Aouam et al., 2018; Kumar et al., 2018
1-10-di-epi-cubenol	Acaricida, antileishmaniose, antibacteriana, citotóxica	Kabouss et al., 2002; Del-Vechio-Vieira et al., 2009; Simionatto et al., 2011; De Oliveira et al., 2017; Koffi, et al., 2018; Oliveira et al., 2019
1-epi-cubenol	Antifúngica, antileishmaniose, antibacteriana, citotóxica	Stefanello et al., 2008; González et al., 2012; Rad et al., 2013; Saroj et al., 2015; Oliveira et al., 2019
d-cadineno	Acaricida, antifúngica, anti-inflamatória, antilitiase, antibacteriana, antioxidante, antitripanosômica, citotóxica, larvicida, repelente de insetos	Santos et al., 2006; Gazim et al., 2008; Martins et al., 2008; Karapandzova et al., 2011; González et al., 2012; Usta et al., 2012; Ornano et al., 2015; Espinoza et al., 2016; Govindarajan et al., 2016; Guerrini et al., 2016; Rungqu et al., 2016; De Sena Filho et al., 2017; Monggoot e Pripdeevech, 2017; Santos et al., 2017; Olieveira de Souza et al., 2017; Kumar et al., 2018; Karapandzova et al., 2019; Mahboubi et al., 2019
Tau-muurolol	Antifúngica, anti-inflamatória,	Cheng et al., 2004; Stefanello et al., 2008; Karapandzova et al., 2011; Ho et al.,

	antibacteriana,antioxidante, antitermiticida	2012; Usta et al., 2012; Chien et al., 2014; Guerrini et al., 2016; Kumar et al., 2018
α-copaeno	Antifúngica, anti-inflamatória, antileishmaniose, antibacteriana, antioxidante, antiproliferativa, citotóxica	Mishra et al., 2010; Kobayashi et al., 2011; Ashraf et al., 2014; Pereira et al., 2017; Velandia et al., 2017; Kumar et al., 2018; Oliveira et al., 2019
Cadaleno	Acaricida, antifúngica, anti-inflamatória, antibacteriana, larvicida	Guerreiro et al., 2005; Tung et al., 2008; Zhu et al., 2015; Conceição et al., 2017
α- cadinol	Antifúngica, anti-inflamatória, antilitíase, antibacteriana, antioxidante, antitermiticida, citotóxica, larvicida	Chang et al., 2001; Cheng et al., 2004; El-Shazly e Hussein 2004; Rojas et al., 2004; Gazim et al., 2008; Stefanello et al., 2008; Karapandzova et al., 2011; González et al., 2012; Ho et al., 2012; Usta et al., 2012; Carvalho et al., 2014; Chien et al., 2014; Zhu et al., 2015; Guerrini et al., 2016; Su e Ho, 2016; Santos et al., 2017; Karapandzova et al., 2019; Mahboubi et al., 2019

Houve maior prevalência das atividades bactericida, (10 ocorrências) e fungitóxica (6 ocorrências), nesses óleos essenciais (Tabela 2); esses resultados estão em concordância com as observações existentes na literatura sobre as ações exercidas pelos terpenos, frente à patógenos de plantas e de algas (Freeman & Beattie, 2008). Em alguns desses óleos, foram observadas, também, atividades interessantes como antitripanosômica, antileishmaniose e anticolinesterásica.

Extratos de *D. delicatula* apresentaram atividade frente ao vírus *herpes simplex* (Soares et al., 2012). Não foram encontradas informações sobre ações biológicas de óleos essênciais contendo o muurola-4, 10 (14)-dien-1-β-ol, calamenen-10-ona e a cubenolona.

Conclusão

Foram identificados 13 sesquiterpenos no extrato hexânico de *D. delicatula*, 11 dos quais ainda não haviam sido identificados nesta macroalga. Também foram indicadas ações biológicas exercidas por dois desses sesquiterpenos e a presença de 10 deles, como substâncias majoritárias, em óleos essenciais biologicamente ativos.

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Capítulo 5. *As macroalgas como indicadoras da poluição do ambiente marinho*

As macroalgas como indicadoras da poluição do ambiente marinho

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Resumo: Os poluentes descartados no meio marinho afetam consideravelmente os organismos que nele habitam, quer pelas ações tóxicas que exerçam, quer pelo fato de poderem se acumular em determinados elos da cadeia alimentar aquática e assim, tornarem-se um risco à saude humana. A procura por novas substâncias terapêuticas entre os metabolitos de algas, levou à identificação de compostos exógenos, cuja retenção, pelas macroalgas, é atribuída aos polímeros que constituem seus talos. Os extratos hexânicos e em diclorometano de três macroalgas, uma pertencente ao grupo das Chlorophytas (*Cladophora prolifera*), outra, ao das Rhodophytas (*Acanthophora spicifera*) e o terceiro, ao das Ochrophytas (*Lobophora variegata*), foram submetidos a estudos para a identificação de seus compostos naturais e dos possíveis poluentes nelas retidos. Em *Lobophora variegata* foram identificados dois sesquiterpenos. Nos extratos de todas as algas foram encontrados, além dos componentes naturais, diversos contaminantes alguns dos quais presentes em quantidade majoritária, como por exemplo o mono (2-etilhexil) ftalato. Esses resultados indicam que as macroalgas podem ser consideradas indicadoras de poluição.

Palavra-chave: bioindicadoras, sesquiterpeno, polissacarídeos, poluentes.

Introdução

A poluição do ambiente marinho afeta todos os organismos que nele habitam. Os poluentes, que são, em sua quase totalidade, resíduos de produtos fabricados pelo homem, e têm como destino final o oceano, incluem pesticidas, herbicidas, fertilizantes químicos, farmácos, detergentes, óleo, esgoto, plásticos e outros sólidos. Além do mais, alguns desses poluentes podem sofrer biodegradação ou ainda degradação química ou fotoquímica, o que os torna mais persistentes no meio marinho (Farré et al., 2008; Beiras, 2018).

Um dos mais frequentes e abundantes contaminantes aquáticos é o resíduo de plásticos (macro, micro e nanoplásticos), que além de poluir o ambiente, é um risco para a saúde humana, pois pode se acumular nos tecidos de plantas e de animais ingeridos pelo homem (Pellini et al., 2018; Zhu et al., 2018). Um dos componentes do plástico, o que confere flexibilidade e suavidade a ele, é o ftalato (Christia et al., 2018; Luo et al., 2018). Esta substância é comumente encontrada nas águas do mar e em extratos de organismos marinhos desde o século passado tanto que tem sido erroneamente considerado um componente de alga (Ali et al., 2010; Garcia et al., 2018, no prelo).

O que determinou a descoberta dos contaminantes, no ambiente marinho, foi a procura, entre os metabolitos de algas e de outros organismos, por compostos ativos a serem empregados como fármacos ou modelos de novas substâncias terapêuticas (Newman e Cragg, 2012; Alygizakis et al., 2016). As algas marinhas são um dos organismos mais estudados por serem produtores de numerosos metabolitos com interessantes atividades. Nos processos de isolamento dessas substâncias, têm sido encontrados compostos exógenos, cuja retenção pela alga é atribuida aos polímeros que constituem seus talos (Dhargalkar e Verlecar, 2009; Beiras 2018).

Esses polímeros são característicos de cada um dos três grupos em que estão divididas, quimiotaxonomicamente, as macroalgas: o primeiro grupo é formado por algas vermelhas (Rhodophyceae), o segundo, por algas verdes (Chlorophyceae) e o terceiro, por pardas (Phaeophyceae) (Coppejans et al. 2009; Kharkwal et al. 2012; Deniaud-Bouët et al., 2017).

Os talos das algas vermelhas são formados por matrizes de celulose associadas a polímeros de xilose (β-1,3 e β-1,4-xilanos) ou de manose (β-1,4-mananos) e principalmente por matrizes mucilaginosas formadas por polímeros de galactose (galactanas sulfatadas). Algumas espécies sintetizem outros tipos de polissacarídeos como, por exemplo, xilanas neutras e xilomananas sulfatadas (Tiwari e Troy, 2015; Paula et al., 2018). As algas vermelhas também podem possuir depósitos de carbonato de cálcio em sua parede celular, sendo então denominadas algas coralináceas (Barsanti e Gualtieri, 2015; Tiwari e Troy, 2015; Paula et al., 2018).

Em algas verdes, a parede celular é composta por uma camada de celulose e outra de pectina; em algumas espécies também são identificadas hemiceluloses. Seus polissacarídeos apresentam uma grande diversidade estrutural em termos de composição monossacarídica e de padrão de sulfatação, não havendo um único tipo de polissacarídeo amplamente distribuído neste grupo (Domozych et al., 2012; Paula et al., 2018). Um importante polissacarídeo é o ulvano, um polissacarídeo sulfatado composto principalmente de L-ramnose, D-xilose, D-glicose e ácido D-glucurônico. Já foram encontrados polissacarídeos sulfatados constituídos por unidades de β-D-galactopiranose, e também já foram isolados polímeros constituídos principalmente por ramnose e ácidos urônicos, de elevada massa molar, altamente ramificados e sulfatados (Pankiewicz et al., 2016; Tiwari e Troy, 2015; Paula et al., 2018).

A parede celular de algas pardas, em geral, é formada por três componentes: celulose, polissacáridos aniônicos, chamados de alginatos (cadeias lineares de ácido α-1,4-L-glucurônico e β-1,4-D-manurônico, associados a íons) e, polissacarídeos sulfatados, os fucoidanos (polímeros de α-L-fucose e açúcares adicionais que são sulfatados) (Kim e Chojnacka, 2015; Michel, 2000; Tiwari e Troy, 2015). As algas pardas também possuem componentes adicionais que contribuem para a formação da estrutura da parede celular: são proteínas, glicoproteínas halogenadas, compostos fenólicos sulfatados conhecidos como florotaninos, além e diversos compostos halogenados (Deniaud-Bouët et al., 2017).

Observa-se que as paredes celulares de todos os três grupos de algas têm em comum um grande número de hidroxilas, pois todas são formadas por polímeros de açúcares. As hidroxilas, por formarem dipolos, ligam-se facilmente a outras moléculas que tenham um de par de elétrons disponível (Smith e March, 2007), o que explicaria a retenção das substâncias que podem estar diluídas ou em suspensão no ambiente marinho.

A detecção dessas substâncias não produzidas pela alga ficou facilitada com o desenvolvimeto de técnicas cromatográficas como cromatógrafo a gas acoplado a espectrômetro de massas. Essas técnicas são extremamente sensíveis pois captam e identificam nanogramas de material (Cole et al., 2016; Arulkumar et al., 2018).

Nesse estudo, selecionamos três macroalgas para serem estudadas quanto aos seus componentes naturais e à possível presença de poluentes entre eles.

Materiais e métodos

O organismo

Os espécimes de *Acanthophora spicifera* (M.Vahl) Børgesen (Rhodophyta, Ceramiales) foram coletados na Praia da Fortaleza, em Ubatuba, litoral de São Paulo (23° 31' 49'' S, 45° 10' 05'' O), no dia 05 de fevereiro de 2015 (SP428.537). Os de *Cladophora prolifera* (Roth) Kützing (Chlorophyta, Cladophorales) foram coletados na Praia de Ubu, em Anchieta, no litoral do estado de Espírito Santo (20° 48' 07'' S, 40° 35' 34'' O), no dia 06 de outubro de 2010 (SP428.539). E os de *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira (Ochrophyta, Dictyotales) foram coletados na Praia do Coqueirinho, no município do Conde, no litoral da Paraíba (07° 19' 44'' S, 34° 47' 42'' O), no dia 15 de julho de 2015 (SP428.536).

De parte dos exemplares coletados foram confeccionadas exsicatas que se encontram depositados no Herbário Científico "Maria Eneyda Pacheco Kauffman Fidalgo" do Instituto de Botânica do Estado de São Paulo.

Obtenção dos extratos algáceos

Após a coleta, as macroalgas foram lavadas com água do mar esterilizadas, secas à temperatura ambiente (+/- 25 °C), trituradas em moinho Willey e submetidas à extração a frio, com os solventes hexano 100% e diclometano 100%, até esgotamento. Os extratos foram concentrados a vácuo e pesados, para a determinação do rendimento (%) do processo de extração (Carvalho et al., 2003; Bhagavathy et al., 2011; Conserva et al., 2011).

Estudo químico dos extratos hexânicos e em diclorometano, por CG/EM

Os extratos em hexano e em diclorometano foram submetidos à cromatografia gasosa/espectrometria de massas (CG/EM) nas seguintes condições: os extratos diluídos foram injetados em equipamento Shimadzu (GCMS-QP2010 Plus, Kyoto), acoplado a espectrômetro de massas quadrupolo (5973), dotado de coluna capilar HP 5-MS (5%-fenilmetilpolisiloxano, 30 m x 0,25 mm de diâmetro interno, com 0,25 μm de espessura), operando a 70 eV. As temperaturas do injetor e do detector foram fixadas em 280 °C. A temperatura inicial do forno, em 70 °C, com acréscimos de 5 °C por minuto, até atingir 280 °C, temperatura que foi mantida por 8 minutos. O tempo total de análise foi de 95 minutos; o gás de arraste foi o hélio (1 mL min^{-1}), em velocidade de 37 cm/seg.

Para a obtenção do índice de retenção linear (IK), calculado segundo o método de Kovats, foi empregada a série homóloga de alcanos C_8 a C_{40} (padrões Sigma-Aldrich), submetida às mesmas condições de análise cromatográfica. A identificação das substâncias foi feita por comparação de seus espectros com os registrados na base de dados das bibliotecas NIST08, NIST08s, Wiley9 e Nist Mass Spectral Search Program from Nist/ Epa/ Nih Mass Spectral Library Version 2.0.Willey e NIST. Somente substâncias com identificação inequívoca foram incluídos na tabela, ou seja, apenas aquelas cujos índices de similaridade (IS) eram iguais ou superiores a 80%.

Resultados e discussão

Rendimento dos extratos

Os rendimentos dos processos de extração das três espécies em estudo estão reunidos na tabela 1.

Tabela 1. Rendimentos dos processos de extração aos quais foram submetidas as espécies estudadas.

Espécie	Biomassa após moagem (g)	Extrato Hexano (%)	Extrato Diclorometano (%)
Acanthophora spicifera (M.Vahl) Børgesen	22,4559	0,57	0,53
Cladophora prolifera (Roth) Kützing	110,1800	3,34	1,43
Lobophora variegata (J.V.Lamouroux) Womersley ex E.C.Oliveira	31,6900	0,73	0,64

Tanto os rendimentos do extrato hexânico quanto o do em diclorometano foram maiores para a alga *Cladophora prolifera.*

Identificação das substâncias componentes dos extratos hexânicos e em diclorometano, por CG/EM, das três macroalgas

Em cada um dos extratos (hexânico e em diclorometano) de cada uma das algas estudadas, o conjunto de substâncias detectadas por CG-EM é constituído por um grupo de compostos sintetizados pela alga (aqui denominado de grupo **A**), outro, cujos componentes podem ser produzidos pelas algas e industrialmente (grupo **B**) e por um terceiro grupo, formado por poluentes (grupo **C**) (Anexos A ao F). Os integrantes do grupo **B** são utilizados em produtos alimentícios e como componentes de cosméticos, de fármacos e de insumos industriais. Entre os membros do grupo **C** foram encontrados hidrocarbonetos de cadeias muito longas, componentes de petróleo, que não são produzidos por algas (Ji et al., 2018, Carvalho et al., 1999). Estes, apesar de terem sido caracterizados quimicamente, não puderam ser identificados.

1. **Estudo dos extratos de *A. spicifera*.**

No extrato hexânico foram identificadas 45 substâncias, que estão compiladas no Anexo A. A figura **1a** mostra a distribuição percentual de cada um dos componentes dos grupos **A**, **B** e **C,** em relação ao total dos constituintes do extrato. A substância majoritária foi o ácido palmítico (grupo **B**) (7,84%), seguido pelo heptadecano (grupo **A**) (5,22%) e pela 2-metil-2-ciclopentanona (grupo **B**) (2,81%) (Ali et al., 2018).

No extrato em diclorometano, foram identificadas 46 substâncias (Anexo B); a figura **1b** mostra as participações percentuais dos grupos **A** (tabela 2), **B** (tabela 3) e **C** (tabela 4) no extrato, tendo-se como referência a totalidade de seus componentes. Também neste extrato, o ácido palmítico foi a substância majoritária (grupo **B**) (12,56%), seguido pelo mono (2-etilhexil) ftalato (5,79 %) e pelo cicloteno (2,10%) ambos pertencentes ao grupo **C** (Rowdhwal e Chen, 2018, Kocadagli e Gokmen, 2019).

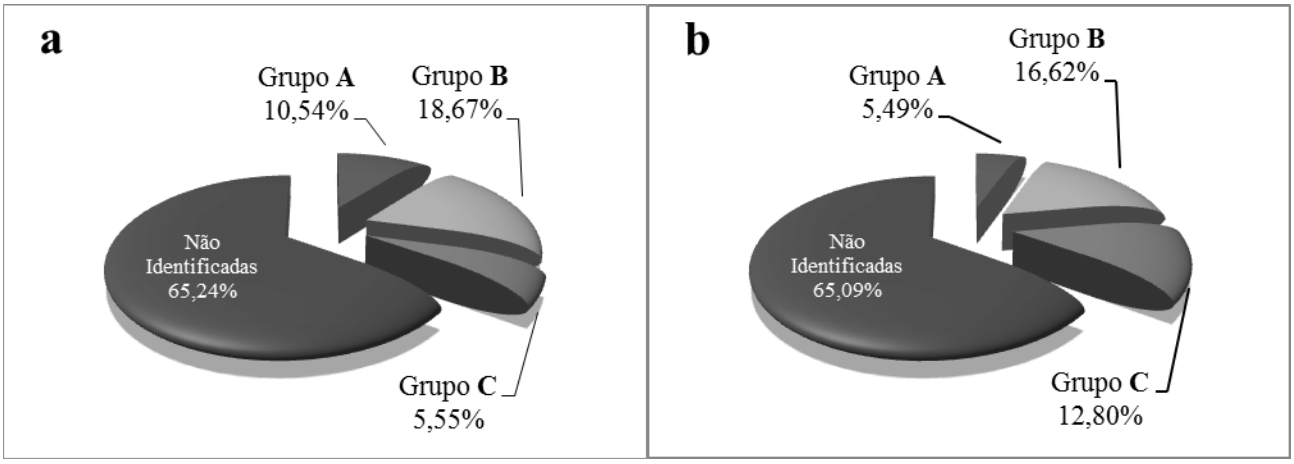

Figura 1. Distribuição percentual dos componentes dos extratos em hexano (1a) e em diclorometano (1b) de *A. spicifera*, tendo-se como base o número total de seus componentes.

2. **Estudo dos extratos de *C. prolifera*.**

No extrato hexânico foram caracterizados 48 compostos (Anexo C) e a estimativa percentual dos grupos a que pertencem, em relação aos componentes totais deste extrato, está disposta na figura **2a**. A substância majoritária foi o clionasterol (11,72%) seguida pelo fitol (9,50%) e pelo ácido palmítico (5,41%), sendo os dois primeiros pertencentes ao grupo **A** e o último, ao grupo **B**. (Dzeha et al., 2003, Yasmeen et al., 2018, Ali et al., 2018). Do total de componentes do extrato em diclorometano de *C. prolifera*, foram identificadas 44 substâncias (Anexo D), cuja distribuição percentual, em relação às suas origens, está mostrada na figura **2b**. O ácido palmítico (grupo **B**) (10,07%) foi a substância predominante seguindo-se a ele o acído cis-vaccênico (grupo **A**) (7,76%) e o ácido oleico (grupo **B**) (7,53%) (Ali et al., 2018, Zielinka et al., 2014).

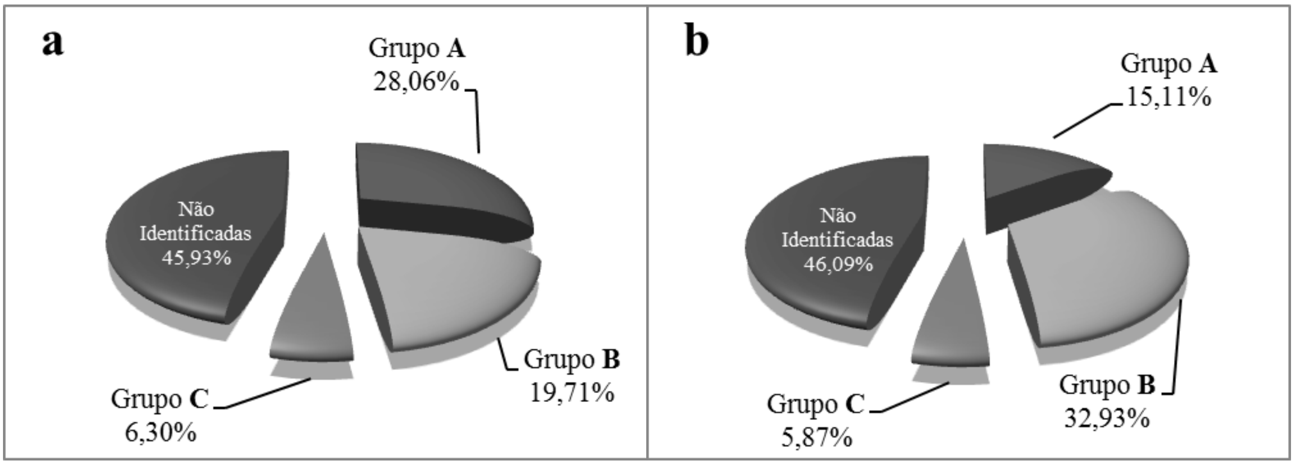

Figura 2. Distribuição percentual dos componentes dos extratos em hexano (2a) e em diclorometano (2b) de *C. prolifera*, tendo-se como base o número total de seus componentes.

3. **Estudo dos extratos de *L. variegata*.**

No extrato hexânico, foram caracterizadas 52 substâncias (22,70% do total) (Anexo E), cuja catalogação percentual, segundo suas origens, encontra-se na figura **3a.** Novamente o ácido palmítico foi a substância majoritária (grupo **B**) (2,43%) seguido pelo mono (2-etilhexil) ftalato (grupo **C**) (1,86%) e pelo pentadecano (grupo **B**) (1,35%).

No extrato em diclorometano foram caracterizadas 43 substâncias (Anexo F) que, agrupadas segundo a classificação aqui adotada, em grupos **A**, **B** e **C**, originaram a figura **3b**. A substância majoritária foi o mono (2-etilhexil) ftalato (6,31%), (grupo **C**), seguida pelo ácido palmítico (4,39%) (grupo **B**) e pelo 3-metil-1,2-ciclopentanediona (2,31%) (grupo **B**) (Ali et al., 2018, Saeidnia, 2014).

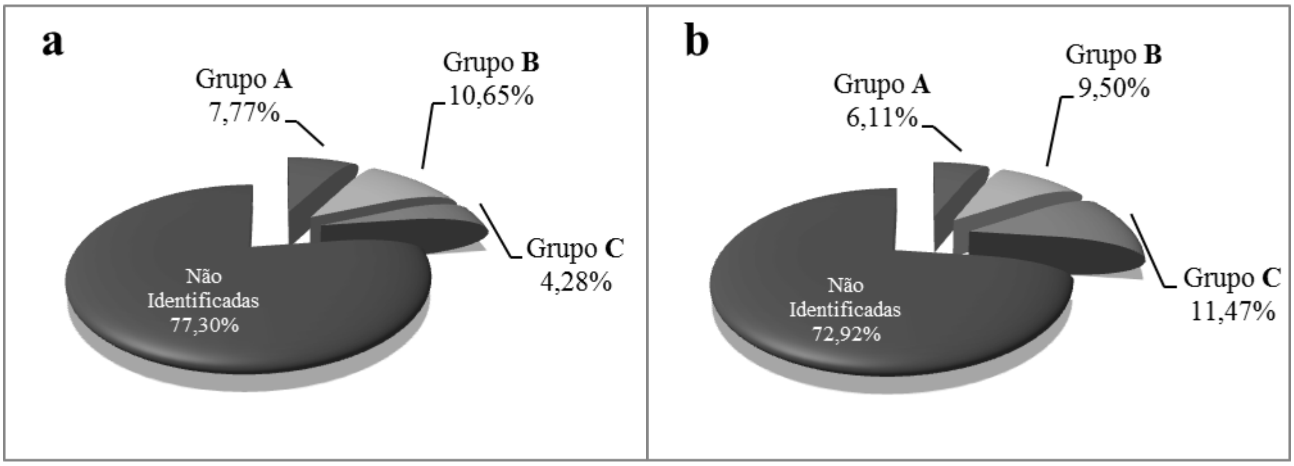

Figura 3. Distribuição percentual dos componentes dos extratos em hexano (3a) e em diclorometano (3b) de *L. variegata*, tendo-se como base o número total de seus componentes.

Foram encontrados poluentes nos extratos das três macroalgas estudadas, ou seja, em *A. spicifera* (Rhodophyta), em *C. prolifera* (Chlorophyta) e em *L. variegata* (Ochrophyta), sugerindo que podem ser encontradas algas capazes de adsorverem substâncias dissolvidas ou em suspensão, em cada um dos grandes grupos de algas. O número total de poluentes encontrados nestas três macroalgas foi de 51 substâncias, sendo alguns deles encontrados em extratos das três algas, caso do mono (2-etilhexil) ftalato e do diisobutil ftalato, substâncias amplamente empregadas como plastificante e que têm efeitos tóxicos tanto sobre humanos quanto sobre animais (Rowdhwal e Chen 2018, Saeidnia, 2014) (tabelas 2, 3 e 4). Os plastificantes têm sido recorrentemente encontrados em extratos de representantes de diversas outras algas (Osman et al. 2013, Shobier et al. 2016, Stranska-Zachariasova et al. 2017) e, por vezes, citados como componentes sintetizados por esses organismos (Ali et al., 2010). Esses resultados embasam o conceito de que algas são importantes indicadores da poluição marinha (Garcia et al., 2019, no prelo).

Tabela 2. Substâncias sintetizadas por algas identificadas nos extratos hexânicos e em diclorometano das macroalgas estudadas.

Nº	Substância	*Acanthophora spicifera*		*Cladophora prolifera*		*Lobophora variegata*	
		HEX	DCM	HEX	DCM	HEX	DCM
		Área do pico %		Área do pico %		Área do pico %	
1	1-heptacosanol	0,17					
2	1 hexacosanol	0,03					
3	2-metilpropanoato de 2-etil-3-hidroxi-hexil					0,56	
4	3,4-dimetil-2-ciclopenten-1-ona	1,15		0,07		0,06	
5	3,4-dimetilciclohexanol				0,04		
6	3,7,11-trimetil-1-dodecanol		0,13				
7	3-etil-1,4-hexadieno	1,15					
8	3-etil-4-metil-1h-pirrole-2,5-diona			0,24		0,12	
9	4- (4-hidroxi-2,2,6-trimetil-7-oxabiciclo [4.1.0] hept-1- il) -3-buten-2-ona				0,75		
10	4,8,12,16-tetrametil-heptadecano-4-olídeo			0,03		0,10	
11	4-hidroxi-3,5,5-trimetil-4- (3-oxo-1-butenil) -2-ciclo-hexen-1-ona		0,12		0,08		
12	5,6-dimetil-decano	0,05				0,04	
13	Ácido araquidônico						0,24
14	Calameneno					0,17	
15	Campesterol			0,28			
16	Colest-5-en-3-ol	1,01	1,94	0,24		1,18	0,17
17	Ácido cis-vaccênico			2,51	7,76		
18	Clionasterol			11,72			
19	Doconexent (triglicerídeos marinhos omega 3)			0,64	2,24		
20	Docosahexaenoato de metila					0,73	0,29
21	9-hexadecenoato de etila					0,30	
22	Ácido gamolênico				0,61		
23	Germacreno-D					0,39	0,10
24	Heptadecano	5,22	1,00	1,95	0,25	0,47	0,17
25	Icosapent (triglicerídeos marinhos omega 3)		0,13		0,19	0,06	0,19
26	Loliolida						0,36
27	(Z) -5,11,14,17-eicosatetraenoato de metila					0,25	
28	Metiletilmaleimida	0,14	0,22		0,36		0,11
29	Metilvinilmaleimida		0,07		0,22		0,03

30	Neofitadieno	0,10				1,22	1,74
31	Nonacosano						0,08
32	Nonadecano				0,02	0,26	0,10
33	Pentacosano		0,17				
34	Pentadecanal	1,41	1,58			0,07	
35	Fitol	0,12	0,12	9,50	2,58	0,23	0,44
36	Fitona			0,30		0,44	
37	Esqualeno					0,67	1,87
38	Estigmasterol					0,37	0,21
39	Tricosano			0,03		0,06	
40	Z-5-nonadeceno			0,55			

HEX -extrato hexânico; DCM - extrato em diclorometano

Tabela 3. Substâncias sintetizadas por algas e também produzidas industrialmente para usos diversos, identificadas no extrato hexânico e em diclorometano das macroalgas estudadas.

N°	**Substância**	***Acanthophora spicifera***		***Cladophora prolifera***		***Lobophora variegata***	
		HEX	**DCM**	**HEX**	**DCM**	**HEX**	**DCM**
		Área do pico %		**Área do pico %**		**Área do pico %**	
1	1-hidroxi-4-metyl-2,6-di-tert-butilbenzeno		0,04				
2	2,6,10,14-tetrametilpentadecano	0,42		0,16		0,36	
3	2,6,10-trimetildodecano						0,03
4	2-etilbutanal		0,07		0,06		0,07
5	2-metil-1-penten-3-ol	0,30	0,16	0,41	0,50	0,17	
6	2-metil-2-ciclopentenona	2,81	0,45		2,47		0,30
7	2-pentanona	0,91					
8	3-metil-1,2-ciclopentanediona				0,85		2,31
9	4-metil-3-penten-2-ona			0,73	1,49		
10	Ácido 9,12-octadecadienoico				7,41		
11	Ácido benzoico			0,06	0,32		
12	Dihidroactinidiolida	0,18	0,11	0,22	0,15		
13	Docosano	0,04				0,04	0,06
14	Dodecanal	0,34	0,37				
15	Eicosano			0,05		0,16	0,08
16	Araquidonato de Etila					0,08	
17	Miristato de Etila					0,41	
18	Oleato de Etila					0,84	
19	Palmitato de Etila					1,01	

20	Heneicosano					0,13	
21	Hexadecano	0,78		0,32	0,05	0,86	0,07
22	Hexahidrofarnesil acetona	1,31					
23	Ácido isovalérico			0,25	0,08		
24	Ácido láurico		0,19	0,19	0,17		
25	Ácido linoleico			5,19			
26	Ácido mirístico	0,89		2,24		0,98	
27	Octacosano			0,02		0,05	
28	Octadecano					0,27	0,10
29	Ácido oleico	0,69	0,92	3,39	7,53		1,55
30	Ácido palmítico	7,84	12,56	5,41	10,07	2,43	4,39
31	Ácido palmitoleico	0,44		0,15	1,25	0,25	
32	Pentadecano		0,11	0,45	0,07	1,35	0,07
33	Ácido pentadecanoico	0,34	0,61				
34	Ácido fenilacético		0,20		0,43		0,27
35	Esqualano	0,31		0,12		0,26	
36	Ácido esteárico	0,18	0,39				0,19
37	Tetradecanal	0,09	0,10				
38	Tetradecano	0,58	0,23	0,34	0,04	0,88	
39	Tridecano	0,23				0,11	
40	Ácido Tridecanoico		0,11				

HEX -extrato hexânico; DCM - extrato em diclorometano

Tabela 4. Poluentes identificados nos extratos hexânicos e em diclorometano das macroalgas estudadas.

N°	Substância	***Acanthophora spicifera***		***Cladophora prolifera***		***Lobophora variegata***	
		HEX	**DCM**	**HEX**	**DCM**	**HEX**	**DCM**
		Área do pico %		**Área do pico %**		**Área do pico %**	
1	1-(1,2,2,3-tetrametilciclopentil)-etanona		0,45				
2	1,1,2,2-tetracloroetano		0,14		0,13		0,07
3	1,1,2,2-tetrametil-ciclopropano	0,47		0,27		0,05	
4	1,3-diazepano-2,4-diono		0,09				
5	1,8-dimetil-naftaleno	0,10		0,03			
6	Oxido1-metil-1,2-ciclohexeno					0,15	
7	1-metilnaftaleno			0,11			
8	2,3-hexanediol		0,29		0,14		
9	2,4-dimetil-2,4-pentanediol		0,09				0,07
10	2,4-hexadien-2-ol	0,09				0,08	

11	2,5-dihidro-3,5-dimetil 2-furanona	0,15		0,09	0,27		
12	2,5-heptanediol		0,74		0,32		
13	2,5-hexanediona					0,04	
14	2,6,10-trimetiltridecano	0,40		0,27		0,74	
15	2,6-dimetil-4-hepten-3-ona	0,26				0,10	
16	2-cyclohexenol		0,12				0,09
17	2-etil-2-metil-succinimida						0,09
18	2-hexen-4-ona			0,19			
19	2-metil-2-(1-metilpropil)-oxirano			0,32			
20	2-metil-2,4-pentanediol		0,36				0,33
21	Ácido 2-metil-2-pentenóico				0,22		
22	3,6-dimetiloctano			0,17			
23	3,7-dimetil-3-octanol					0,08	
24	3-etil-3-metil-2-pentanol	1,75					
25	3-hepteno-2-ona			0,11			
26	3-hexeno-2,5-diol		0,27				0,96
27	3-metil-1,2-ciclopentanediona			0,06			
28	3-metil-2-ciclopenteno-1-ona			2,77			0,05
29	3-metil-4-undeceno	0,59					
30	3-metilpent-2-eno-1,5-diol	0,24				0,25	0,10
31	4,6-dimetil dodecano	0,09		0,03			
32	Ácido 4-acetilbutírico		0,22				
33	Ácido 4-metoxi-benzeneacético		0,06				
34	4-metil-1-heptanol	0,06					
35	4-metil-2,3-dihidropirano		0,37	0,89	1,34		
36	Ácido adípico, butil 2-metoxietil ester						0,05
37	Bis(2-etilhexil) adipato	0,34	0,46	0,17		0,41	1,13
38	Butil metacrilato				0,01		
39	Butil palmitato	0,08					
40	Ciclohexeno-2-ona				0,09		
41	Ciclopentacicloheptenο			0,04			
42	Cicloteno		2,10		0,23		0,12
43	Diisobutil ftalato	0,20	0,23	0,09	0,11	0,34	1,31
44	Etil isopropenil eter				0,03		
45	Metil isobutil cetona					0,15	
46	Mono(2-etilhexil) ftalato	0,73	5,79	0,67	2,34	1,86	6,31
47	Metilpiperidina				0,24		
48	Succinimida		0,14				
49	Tetradecilciclohexano		0,00				

50	Tetrahidropirano-2-carbinol		0,31		0,15		0,11
51	Vinil 2,2-dimetilpentanoato		0,59		0,25		0,68

HEX -extrato hexânico; DCM - extrato em diclorometano

Foram identificados, nos extratos de *L. variegata*, dois sesquiterpenos conhecidos: o calameneno (0,17%), no extrato hexânico e o germacreno D, tanto no extrato hexânico (0,39%) quanto no em diclorometano (0,10%) (Elias et al., 1997; Zatelli et al., 2018 (figura 4).

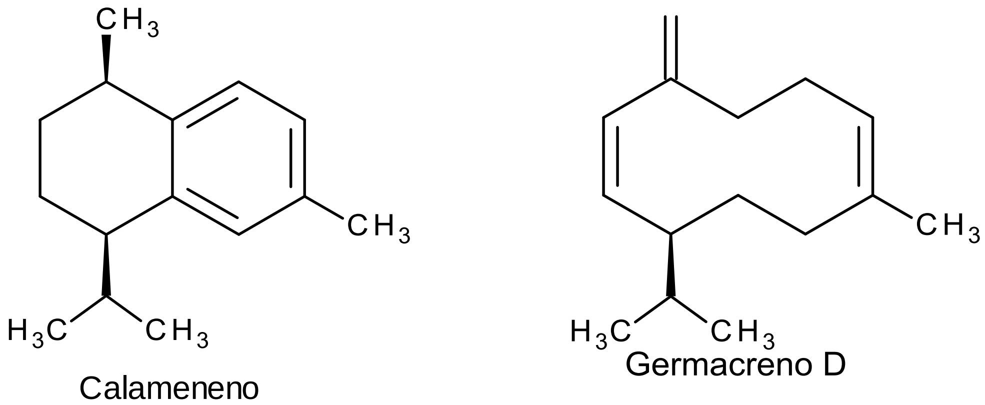

Figura 4. Sesquiterpenos identificados em *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira.

Na tabela 5 estão dispostos as massas e os dados de fragmentação de ambos os sesquiterpenos, dados esses obtidos também pela análise dos extratos por CG-EM.

Tabela 5. Sesquiterpenos identificados nos extratos hexânico e em diclorometano de *Lobophora variegata*.

Sesquiterpeno	**TR (min)**	**Massa molecular (D)**	**Massa dos fragmentos iônicos (m/z)***					
			Pico base (100%)	**2º. maior pico**	**3º. maior pico**	**4º. maior pico**	**5º. maior pico**	**Demais picos (em ordem decrescente de tamanho)**
Calameneno	28,583	202	157	202	131	105	144	117; 91; 57; 41
Germacreno	27,350	204	161	105	91	41	119	79; 81; 93; 77; 27; 39; 120; 133; 55; 67; 43; 204; 53; 65

* Referência utilizada: Adams, 2017.

Não há relatos de que essas substâncias tenham sido identificadas anteriormente, em *L. variegata*.

Conclusão

Nessas macroalgas, além de substâncias de interesse, como os sesquiterpenos calameneno e germacreno D, foram identificados, a par de metabolitos comuns de algas, uma considerável quantidade de resíduos. Os contaminantes mais comuns, presentes nas três espécies de algas são Mono (2-etilhexil) ftalato, Diisobutil ftalato e Bis(2-etilhexil) adipato que são substâncias amplamente empregadas como plastificante e que têm efeitos tóxicos tanto sobre humanos quanto sobre animais (Rowdhwal e Chen 2018, Saeidnia, 2014). Cada uma das algas aqui analisadas pertence a um grupo distinto (Chlorophyta, Ochrophyta e Rhodophyta) e sem exceção, adsorveu substâncias com as quais esteve em contato. Esta observação sugere que, de um modo geral, macroalgas sejam bioindicadoras da presença de poluentes no ambiente marinho e ressalta a importância da existência de um controle rígido dos descartes desses compostos nos meios aquáticos, em geral.

Anexo A. Substâncias identificadas por CG/EM no extrato hexânico da alga vermelha *Acanthophora spicifera*.

Nº	Tr. (min.)	Área do pico (%)	IS	IK Exp.	IK Lit.	Substância	Fórmula Molecular	Peso Molecular g/mol	Referências/ Patentes
1	4,300	0,09	85	-	-	2,4-hexadieno-2-ol	$C_6H_{10}O$	98,145	JECFA, 2018
2	4,400	0,30	91	-	-	2-metil-1-penten-3-ol	$C_6H_{12}O$	100,161	Carpino et al., 2010
3	4,708	0,24	88	-	-	3-metlpent-2-eno-1,5-diol	$C_6H_{12}O_2$	116, 160	PubChem, 2018 - US20070197696A1
4	5,600	0,06	85	926	-	4-metil-1-heptanol	$C_8H_{18}O$	130,231	PubChem, 2018
5	6,200	1,75	86	949	-	3-etil-3-metil-2-pentanol	$C_8H_{18}O$	130,231	PubChem, 2018 - EP0324610A2
6	6,508	2,81	96	921	925[b]	2-metil-2-ciclopentenona	C_6H_8O	96,129	Yannai, 2004; JECF, 2018
7	6,708	0,91	93	968	969[r]	2-pentanona	$C_5H_{10}O$	86,134	López-Pérez et al., 2017; HMDB, 2018
8	6,908	1,15	89	916	903[p]	3-etil-1,4-hexadieno	C_8H_{14}	110,200	Quirós et al., 2000; Izzreen e Ratnam, 2011
9	7,342	0,47	89	992	-	1,1,2,2-tetrametil-ciclopropano	C_7H_{14}	98,189	PubChem, 2018
10	7,417	0,59	88	995	-	3-metil-4-undecene	$C_{12}H_{24}$	168,324	PubChem, 2018
11	8,283	1,15	84	1421	1439[s]	3,4-dimetil-2-ciclopenten-1-ona	$C_7H_{10}O$	110,156	Choi et al., 2017
12	10,325	0,15	91	1078	-	2,5-dihidro-3,5-dimetil 2-furanono	$C_6H_8O_2$	112,128	Yannai, 2004
13	12,542	0,05	86	1135	1135[t]	5,6-dimetil-decano	$C_{12}H_{26}$	170,340	Kailas e Nair, 2015
14	16,517	0,14	87	1242	1265[u]	Metiletilmaleimido	$C_7H_9NO_2$	139,154	Pinho et al, 2009
15	18,592	0,26	86	1281	-	2,6-dimetil-4-hepten-3-ona	$C_9H_{16}O$	140,226	Yannai, 2004
16	19,442	0,23	92	1301	1300[a]	Tridecano	$C_{13}H_{28}$	184,367	Yamamoto et al., 2014; HMDB, 2018
17	23,633	0,58	92	1400	1400[a]	Tetradecano	$C_{14}H_{30}$	198,394	Fujimura et al., 1990; Kalhor et al., 2017; EPA/TSCA, 2018; JECFA, 2018

18	24,000	0,34	94	1410	1408[a]	Dodecanal	$C_{12}H_{24}O$	184,323	Blunt e Munro, 2008; HMDB, 2018; JECFA, 2018
19	24,225	0,10	92	1425	1441[d]	1,8-dimetil-naftaleno	$C_{12}H_{12}$	156,228	Alexander et al., 1984
20	26,167	0,40	90	1463	1463[m]	2,6,10-trimetiltridecano	$C_{16}H_{34}$	226,448	PubChem, 2018 - US8080581A1
21	27,533	0,09	85	1497	1325[g]	4,6-dimetil dodecano	$C_{14}H_{30}$	198,394	Kostecki et al., 1992
22	28,750	0,18	88	1528	1525[i]	Dihidroactinidiolida	$C_{11}H_{16}O_2$	180,247	Yamamoto et al., 2014; JECFA, 2018
23	31,558	0,78	96	1600	1600[a]	Hexadecano	$C_{16}H_{34}$	226,448	El-Din e El-Ahwany, 2016
24	32,017	0,09	91	1613	1612[a]	Tetradecanal	$C_{14}H_{28}O$	212,377	Demirel et al., 2009; JECFA, 2018
25	33,392	0,31	92	1650	-	Squalano	$C_{30}H_{62}$	422,826	Blunt e Munro, 2008; EPA/TSCA, 2018, Nakaji et al., 2018
26	35,267	5,22	98	1701	1700[a]	Heptadecano	$C_{17}H_{36}$	240,475	Sugisawa et al., 1990; Oliveira et al., 2012
27	35,458	0,42	94	1706	1707[e]	2,6,10,14-tetrametilpentadecano	$C_{19}H_{40}$	268,529	Michalak et al., 2015, HMDB, 2018
28	35,767	1,41	95	1715	1715[c]	Pentadecanal	$C_{15}H_{30}O$	226,404	Kajiwara et al., 1990; HMDB, 2018; Maria e Narendra, 2018
29	37,458	0,89	94	1763	1763[k]	Ácido mirístico	$C_{14}H_{28}O_2$	228,376	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
30	40,117	0,10	90	1840	1840[c]	Neofitadieno	$C_{20}H_{38}$	278,524	Oliveira et al., 2012; Rajkumar e Bhavan, 2017
31	40,308	1,31	94	1846	1846[q]	Hexahidrofarnesil acetona	$C_{18}H_{36}O$	268,485	Blunt e Munro, 2008; Abou-El-Wafa et al., 2009
32	40,817	0,34	93	1861	1861[h]	Ácido pentadecanóico	$C_{15}H_{30}O_2$	242,403	El-Din e El-Ahwany, 2016
33	41,067	0,20	92	1868	1868[l]	Diisobutil ftalato	$C_{16}H_{22}O_4$	278,348	HMDB, 2018; Rowdhwal e Chen, 2018
34	43,467	0,44	93	1942	1953[j]	Ácido palmitoleico	$C_{16}H_{30}O_2$	254,414	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
35	44,275	7,84	94	1967	1960[a]	Ácido palmítico	$C_{16}H_{32}O_2$	256,430	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018

36	48,800	0,12	89	2113	2113[c]	Fitol	$C_{20}H_{40}O$	296,539	Souza e Nes, 1969; Jassbi et al., 2016; Yasmeen et al., 2018
37	49,675	0,69	90	2143	2142[a]	Ácido oleico	$C_{18}H_{34}O_2$	282,468	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
38	50,242	0,18	87	2162	2161[n]	Àcido esteárico	$C_{18}H_{36}O_2$	284,484	Blunt e Munro, 2008; Chen et al., 2016; HMDB, 2018
39	50,975	0,08	85	2187	2188[i]	Butil palmitato	$C_{20}H_{40}O_2$	312,538	HMDB, 2018
40	51,350	0,04	92	2200	2200[a]	Docosano	$C_{22}H_{46}$	310,610	Karabay-Yavasoglu et al., 2007; HMDB, 2018
41	56,883	0,34	92	2399	2398[c]	Bis(2-etilhexil) adipato	$C_{22}H_{42}O_4$	370,574	EwG's Skin Deep, 2018; HMDB, 2018
42	60,717	0,73	97	2548	2152[f]	Mono(2-etilhexil) ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018
43	63,658	0,03	82	2867	2848[v]	1-hexacosanol	$C_{26}H_{54}O$	382,717	Okunowo et al., 2016
44	68,433	0,17	90	3013	3016[w]	1-heptacosanol	$C_{27}H_{56}O$	396,744	Murugan e Lyer, 2014; Unnikrishnan et al., 2014
45	76,250	1,01	90	3096	3098[o]	Colest-5-en-3-ol	$C_{27}H_{46}O$	386,664	Plouguerné et al., 2006

* Tr. (min.) - tempo de retenção (minutos) / IS - índice de similaridade/ IK Exp. – índice de Kovats experimental. / IK Lit. – índice de Kovats da literatura [a]Adams, 2017/ [b]Ames et al., 2001/ [c]Andriamaharavo, 2014/ [d]Gerasimenko e Nabivach, 1997/ [e]Kenig et al., 2005/ [f]Liang, 2010/ [g]Liu et al., 2007/ [h]Okumura, 1991/ [i]Pino et al., 2005/ [j]Quijano et al., 2007/ [k]Raina et al., 2006/ [l]Saroglou et al., 2006/ [m]Shlyakhov, 1984/ [n]Srivastava et al. 2006/ [o]Steiger et al., 2011/ [p]Stern et al., 1985/ [q]Stojanovic et al., 2000/ [r]Vinogradov, 2004/ [s]Welke et al., 2012/ [t]Widmer, 1967/ [u]Xian et al., 2006/ [v]Yusuf e Bewaji, 2011/ [w]Zheng e White, 2008.

Anexo B. Substâncias identificadas por CG/EM no extrato em diclorometano da alga vermelha *Acanthophora spicifera*.

Nº	Tr. (min.)	Área do pico (%)	IS	IK Exp.	IK Lit.	Substância	Fórmula Molecular	Peso Molecular g/mol	Referências/ Patentes
1	4,008	0,37	86	-	-	4-metil-2,3-dihidropirano	$C_6H_{10}O$	98,145	PubChem, 2018 - US2015111873A1
2	4,433	0,16	90	-	-	2-metil-1-penten-3-ol	$C_6H_{12}O$	100,161	Carpino et al., 2010
3	4,525	0,07	86	-	-	2-etilbutanal	$C_6H_{12}O$	100,161	HMDB, 2018; JECFA, 2018
4	4,667	0,12	87	-	-	2-ciclohexenol	$C_6H_{10}O$	98,145	PubChem 2018 - US6303640A1
5	5,075	0,45	94	907	925[c]	2-metil-2-ciclopentenono	C6H8O	96,129	JECFA, 2018
6	5,200	0,14	93	911	920[p]	1,1,2,2-tetracloroetano	$C_2H_2Cl_4$	167,838	ATSDR, 2008; HMDB, 2018
7	5,325	0,36	93	916	913[t]	2-metil-2,4-pentanediol	$C_6H_{14}O_2$	118,176	EwG's Skin Deep, 2018, PubChem, 2018 - US2014349969A1
8	6,292	0,09	90	952	-	2,4-dimetil-2,4-pentanediol	$C_7H_{16}O_2$	132,203	PubChem, 2018 - US5304550A1
9	6,842	0,29	91	973	-	2,3-hexanediol	$C_6H_{14}O_2$	118,176	Ray et al., 2012
10	7,033	0,27	88	980	-	3-hexeno-2,5-diol	$C_6H_{12}O_2$	116,160	Kumar e Gupta, 2008
11	8,500	0,31	96	1027	-	Tetrahidropirano-2-carbinol	$C_6H_{12}O_2$	116,160	PubChem 2018 - US9708274A1
12	8,642	0,74	88	1031	-	2,5-heptanediol	$C_7H_{16}O_2$	132,203	PubChem, 2018 - US2012020906A1
13	10,383	0,45	84	1080	-	1-(1,2,2,3-tetrametilciclopentil)etanona	$C_{11}H_{20}O$	168,280	Sarker e Rashid, 2014
14	10,842	2,10	85	1093	1042[j]	Cicloteno	$C_6H_8O_2$	112,128	JECFA, 2018
15	11,292	0,59	88	1105	-	Vinil 2,2-dimetilpentanoato	$C_9H_{16}O_2$	156,225	PubChem, 2018 - US4367220A
16	12,075	0,14	97	1124	2458[m]	Succinimida	$C_4H_5NO_2$	99,082	Schumacher, 1984; Kibet, 2013
17	13,250	0,22	92	1153	-	Ácido 4-acetilbutirico	$C_6H_{10}O_3$	130,143	Nakahashi et al., 2003
18	16,533	0,22	89	1232	1265[u]	Metiletilmaleimido	$C_7H_9NO_2$	139,154	Pinho et al, 2009

19	17,242	0,20	94	1249	1248[o]	Ácido Fenilacético	$C_8H_8O_2$	136,150	Blunt e Munro, 2008; Korasick et al., 2013; HMDB, 2018
20	17,592	0,07	90	1257	1261[d]	Metilvinilmaleimido	$C_7H_7NO_2$	137,138	Köst e Benedikt, 1982; Kurtin, 1977; Suzuki e Shioi, 1999
21	19,433	0,09	88	1300	-	1,3-diazepane-2,4-diona	$C_5H_8N_2O_2$	128,131	PubChem, 2018
22	23,300	0,23	94	1393	1400[a]	Tetradecano	$C_{14}H_{30}$	198,394	Rajkumar e Bhavan, 2017; JECFA, 2018
23	24,000	0,37	95	1410	1408[a]	Dodecanal	$C_{12}H_{24}O$	184,323	Blunt e Munro, 2008; HMDB, 2018; JECFA, 2018
24	27,258	0,06	89	1490	1496[g]	Ácido 4-metoxi-benzeneacético	$C_9H_{10}O_3$	166,176	HMDB, 2018
25	27,533	0,11	88	1497	1500[a]	Pentadecano	$C_{15}H_{32}$	212,421	El-Din e El-Ahwany, 2016; JECFA, 2018
26	28,217	0,04	80	1514	1514[b]	1-hidroxi-4-metil-2,6-di-tert-butilbenzeno	$C_{15}H_{24}O$	220,356	HMDB, 2018
27	28,758	0,11	90	1528	1525[l]	Dihidroactinidiolida	$C_{11}H_{16}O_2$	180,247	Yamamoto et al., 2014; JECFA, 2018
28	30,133	0,19	93	1564	1566[a]	Ácido Láurico	$C_{12}H_{24}O_2$	200,322	El-Shoubaky et al., 2008; HMDB, 2018
29	32,025	0,10	94	1613	1612[a]	Tetradecanal	$C_{14}H_{28}O$	212,377	Demirel et al., 2009; JECFA, 2018
30	33,825	0,11	88	1662	1662[e]	Àcido Tridecanóico	$C_{13}H_{26}O_2$	214,349	El-Shoubaky et al., 2008; EPA/TSCA, 2018
31	35,258	1,00	97	1701	1700[a]	Heptadecano	$C_{17}H_{36}$	240,475	El-Din e El-Ahwany, 2016
32	35,767	1,58	95	1715	1715[d]	Pentadecanal	$C_{15}H_{30}O$	226,404	Kajiwara et al., 1990; HMDB, 2018; Maria e Narendra, 2018
33	36,358	0,13	89	1732	1576[h]	3,7,11-trimetil-1-dodecanol	$C_{15}H_{32}O$	228,420	El-Din e El-Ahwany, 2016; HMDB, 2018
34	37,283	0,00	87	1758	-	Tetradecilciclohexano	$C_{20}H_{40}$	280,540	Koma et al., 2003; Carroll et al., 2006
35	38,350	0,12	91	1788	1760[f]	4-hidroxi-3,5,5-trimetil-4-(3-oxo-1-butenil)-2-ciclohexen-1-ona	$C_{13}H_{18}O_3$	222,284	HMDB, 2018; Zatelli et al., 2018
36	40,833	0,61	95	1861	1861[k]	Ácido Pentadecanóico	$C_{15}H_{30}O_2$	242,403	Blunt e Munro, 2008; Plaza et al., 2010

37	41,075	0,23	94	1869	1868[n]	Diisobutil ftalato	$C_{16}H_{22}O_4$	278,348	HMDB, 2018, Rowdhwal e Chen, 2018
38	44,317	12,56	94	1968	1960[a]	Ácido Palmítico	$C_{16}H_{32}O_2$	256,430	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
39	48,800	0,12	95	2113	2113[d]	Fitol	$C_{20}H_{40}O$	296,539	Souza e Nes, 1969; Jassbi et al., 2016; Yasmeen et al., 2018
40	49,675	0,92	92	2143	2142[a]	Ácido oleico	$C_{18}H_{34}O_2$	282,468	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
41	50,242	0,39	91	2162	2161[q]	Ácido esteárico	$C_{18}H_{36}O_2$	284,484	Blunt e Munro, 2008; Chen et al., 2016; HMDB, 2018
42	54,325	0,13	87	2305	2334[s]	Icosapent (triglicérides marinho ômega 3)	$C_{20}H_{30}O_2$	302,458	HMDB, 2018
43	56,883	0,46	91	2399	2398[d]	Bis(2-etilhexil) adipato	$C_{22}H_{42}O_4$	370,574	EwG's Skin Deep, 2018; HMDB, 2018
44	59,500	0,17	88	2500	2500[a]	Pentacosano	$C_{25}H_{52}$	352,691	El-Shafay et al., 2016; HMDB, 2018
45	60,742	5,79	98	2549	2152[i]	Mono(2-etilhexil) ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018
46	76,275	1,94	89	3096	3098[r]	Colest-5-en-3-ol	$C_{27}H_{46}O$	386,664	Plouguerné et al., 2006

* Tr. (min.) - tempo de retenção (minutos) / IS - índice de similaridade/ IK Exp. – índice de Kovats experimental. / IK Lit. – índice de Kovats da literatura
[a]Adams, 2017/ [b]Adams et al., 2005/ [c]Ames et al., 2001/ [d]Andriamaharavo, 2014/ [e]Benkaci-Ali et al., 2007/ [f]Guyot-Declerck et al., 2000/ [g]Jerkovic et al., 2010/ [h]Kurashov et al., 2013/ [i]Liang, 2010/ [j]Mateo et al., 1997/ [k]Okumura, 1991/ [l]Pino et al., 2005/ [m]Prososki et al., 2007/ [n]Saroglou et al., 2006/ [o]Schwambach e Peterson, 2006/ [p]Sorimachi et al., 1995/ [q]Srivastava et al., 2006/ [r]Steiger et al., 2011/ [s]Tret'yakov, 2007 / [t]Vijayanand et al., 2001/ [u]Xian et al., 2006.

Anexo C. Substâncias identificadas por CG/EM no extrato hexânico da alga verde *Cladophora prolifera*.

Nº	Tr. (min.)	Área do pico (%)	IS	IK Exp.	IK Lit.	substância	Fórmula Molecular	Peso Molecular g/mol	Referências/ Patentes
1	3,608	0,25	91	-	-	Ácido isovalérico	$C_5H_{10}O_2$	102,133	Maia et al., 2016; HMDB, 2018
2	3,675	0,19	94	-	-	2-hexen-4-ona	$C_6H_{10}O$	98,145	Sandoval-Montemayor et al., 2012; HMDB, 2018
3	3,767	0,73	87	-	-	4-metil-3-penten-2-ona	$C_6H_{10}O$	98,145	Abdel-Aal et al, 2015; HMDB, 2018
4	3,975	0,89	87	-	-	4-metil-2,3-dihidropirano	$C_6H_{10}O$	98,145	PubChem, 2018 - US2015111873A1
5	4,392	0,41	92	-	-	2-metil-1-penten-3-ol	$C_6H_{12}O$	100,161	Carpino et al., 2010
6	6,5	2,77	95	960	976[l]	3-metil-2-ciclopenten-1-ona	C_6H_8O	96,129	JECFA, 2018
7	7,067	0,32	82	982	-	2-metil-2-(1-metilpropil)-oxirano	$C_7H_{14}O$	114,188	PubChem, 2018 - US2016053102A1
8	7,325	0,27	90	991	-	1,1,2,2-tetrametil-ciclopropano	C7H14	98,189	PubChem, 2028
9	8,25	0,07	87	1420	1439[u]	3,4-dimetil-2-ciclopenten-1-ona	$C_7H_{10}O$	110,156	Choi et al., 2017
10	8,542	0,11	84	1028	933[a]	3-hepten-2-ona	$C_7H_{12}O$	112,172	JECFA, 2018
11	10,325	0,09	88	1078	-	2,5-dihidro-3,5-dimetil 2-furanona	$C_6H_8O_2$	112,128	Yannai, 2004
12	10,783	0,06	81	1091	1043[e]	3-metil-1,2-ciclopentanediona	$C_6H_8O_2$	112,128	Yang et al., 2016; HMDB, 2018
13	13,492	0,06	84	1159	1159[h]	Ácido benzóico	$C_7H_6O_2$	122,123	,Chojnacka et al 2012; HMDB, 2018; JECFA, 2018
14	14,425	0,04	82	1182	1298[a]	Ciclopentacicloheptenо	$C_{10}H_8$	128,174	EwG's Skin Deep, 2018
15	16,508	0,24	89	1231	1231[p]	3-etil-4-metil-1h-pirrole-2,5-diono	$C_7H_9NO_2$	139,154	Abdel-Aal et al, 2015
16	18,275	0,17	92	1273	-	3,6-dimetiloctano	$C_{10}H_{22}$	142,286	PubChem, 2018, US6087322A1
17	19,033	0,11	90	1291	1299[x]	1-metilnaftaleno	$C_{11}H_{10}$	142,201	PubChem, 2018

18	23,617	0,34	93	1400	1400[a]	Tetradecano	$C_{14}H_{30}$	198,394	Rajkumar e Bhavan, 2017; JECFA, 2018
19	24,208	0,03	90	1415	1441[c]	1,8-dimetil-naftaleno	$C_{12}H_{12}$	156,228	Alexander et al., 1984
20	26,15	0,27	90	1463	1463[r]	2,6,10-trimetiltridecano	$C_{16}H_{34}$	226,448	PubChem, 2018 - US8080581A1
21	27,525	0,03	86	1496	1325[j]	4,6-dimetil dodecano	$C_{14}H_{30}$	198,394	Kostecki et al., 1992
22	27,667	0,45	96	1500	1500[a]	Pentadecano	$C_{15}H_{32}$	212,421	El-Din e El-Ahwany, 2016; JECFA, 2018
23	28,733	0,22	88	1527	1525[m]	Dihidroactinidiolida	$C_{11}H_{16}O_2$	180,247	Yamamoto et al., 2014; JECFA, 2018
24	30,108	0,19	90	1563	1566[a]	Ácido láurico	$C_{12}H_{24}O_2$	200,322	El-Shoubaki et al., 2008; HMDB, 2018
25	31,542	0,32	96	1600	1600[a]	Hexadecano	$C_{16}H_{34}$	226,448	El-Din e El-Ahwany, 2016
26	33,367	0,12	90	1649	-	Esqualano	$C_{30}H_{62}$	422,826	Blunt e Munro, 2008; Nakaji et al., 2018; EPA/TSCA, 2018
27	33,892	0,03	87	1664	-	Tricosano	$C_{23}H_{48}$	324,637	Moustafa et al., 2008
28	34,45	0,55	95	1679	-	Z-5-nonadecene	$C_{19}H_{38}$	266,153	Alarif et al., 2010
29	35,242	1,95	97	1700	1700[a]	Heptadecan0	$C_{17}H_{36}$	240,475	El-Din e El-Ahwany, 2016
30	35,458	0,16	93	1706	1707[g]	2,6,10,14-tetrametilpentadecano	$C_{19}H_{40}$	268,529	Michalak et al., 2015; HMDB, 2018
31	37,5	2,24	95	1764	1763[o]	Ácido mirístico	$C_{14}H_{28}O_2$	228,376	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
32	40,292	0,30	93	1845	1845[f]	Fitono	$C_{18}H_{36}O$	268,485	Blunt e Munro, 2008; Maheswari et al. 2017
33	41,05	0,09	93	1868	1868[q]	Diisobutil ftalato	$C_{16}H_{22}O_4$	278,348	Gao e Wen, 2016; HMDB, 2018, JECFA, 2018
34	43,058	0,64	86	1929	1806[d]	Doconexent (triglicérides marinho ômega 3)	$C_{22}H_{32}O_2$	328,496	Ginneken et al., 2011; Martinsson, 2016; HMDB, 2018; JECFA, 2018
35	43,733	0,15	89	1950	1953[n]	Ácido palmitoleico	$C_{16}H_{30}O_2$	254,414	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018

36	44,233	5,41	94	1966	1960[a]	Ácido palmítico	$C_{16}H_{32}O_2$	256,430	Blunt e Munro, 2008; Sahu et al., 2013; Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
37	45,325	0,05	91	2000	2000[a]	Eicosano	$C_{20}H_{42}$	282,556	Karabay-Yavasoglu et al., 2007
38	48,808	9,50	97	2113	2113[w]	Fitol	$C_{20}H_{40}O$	296,539	Souza e Nes, 1969, Jassbi et al., 2016, Yasmeen et al., 2018
39	49,475	5,19	95	2136	2133[a]	Ácido linoleico	$C_{18}H_{32}O_2$	280,452	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
40	49,642	3,39	89	2142	2142[a]	Ácido oleico	$C_{18}H_{34}O_2$	282,468	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
41	49,758	2,51	94	2145	2161[t]	Ácido cis-vaccênico	$C_{18}H_{34}O_2$	282,468	Khotimchenko et al., 2002; Zielinka et al., 2014
42	55,575	0,03	83	2351	2353[k]	4,8,12,16-tetrametilheptadecan-4-olida	$C_{21}H_{40}O_2$	324,549	Sujatha e Vijayalakshmi, 2013; Kalita et al., 2018
43	56,867	0,17	92	2399	2398[b]	Bis(2-etilhexil) adipato	$C_{22}H_{42}O_4$	370,574	EwG's Skin Deep, 2018; HMDB, 2018
44	60,683	0,67	85	2547	2152[i]	Mono(2-etilhexil) ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018
45	66,775	0,02	83	2799	2800[a]	Octacosano	C28H58	394,772	El-shafay et al., 2016; HMDB, 2018
46	76,2	0,24	85	3094	3098[s]	Colest-5-en-3-ol	$C_{27}H_{46}O$	386,664	Plouguerné et al., 2006
47	81,142	0,28	87	3197	3131[v]	Campesterol	$C_{28}H_{48}O$	400,691	Lopes et al., 2013; Kendel et al., 2015; HMDB, 2018
48	86,408	11,72	91	-	-	Clionasterol	$C_{29}H_{50}O$	414, 718	Dzeha et al., 2003; HMDB, 2018

* Tr. (min.) - tempo de retenção (minutos) / IS - índice de similaridade/ IK Exp. – índice de Kovats experimental. / IK Lit. – índice de Kovats da literatura

[a]Adams, 2017/ [b]Andriamaharavo, 2014/ [c]Gerasimenko e Nabivach, 1997/ [d]Herath et al., 2018/ [e]Hill et al., 1999/ [f]Karioti et al., 2003/ [g]Kenig et al., 2005/ [h]Lalel, et al., 2003/ [i]Liang, 2010/ [j]Liu et al., 2007/ [k]Mann et al, 2017/ [l]Mateo et al., 1997/ [m]Pino et al., 2005/ [n]Quijano et al., 2007/ [o]Raina et al., 2006/ [p]Sabik et al., 2016/ [q]Saroglou et al., 2006/ [r]Shlyakhov, 1984/ [s]Steiger et al., 2011/ [t]Tret'yakov, 2007/ [u]Welke et al., 2012/[v]Xu et al., 2012/ [w]Yasmeen et al., 2018/ [x]Zeng et al., 2007.

Anexo D. Substâncias identificadas por CG/EM no extrato em diclorometano da alga verde *Cladophora prolifera*.

Nº	Tr. (min.)	Área do pico (%)	IS	IK Exp.	IK Lit.	Substância	Fórmula Molecular	Peso Molecular g/mol	Referências/ Patentes
1	3,567	0,08	96	-	-	Ácido isovalérico	$C_5H_{10}O_2$	102,133	Maia et al., 2016; HMDB, 2018,; JECFA, 2018
2	3,792	1,49	84	-	-	4-metil-3-penten-2-ona	$C_6H_{10}O$	98,145	Abdel-Aal et al, 2015; HMDB, 2018
3	4,000	1,34	87	-	-	4-metil-2,3-dihidropirano	$C_6H_{10}O$	98,145	PubChem, 2018 - US2015111873A1
4	4,417	0,50	92	-	-	2-metil-1-penten-3-ol	$C_6H_{12}O$	100,161	Carpino et al., 2010
5	4,525	0,06	87	-	-	2-etilbutanal	$C_6H_{12}O$	100,161	HMDB, 2018; JECFA, 2018
6	5,058	2,47	93	906	925[b]	2-metil-2-ciclopentenona	C_6H_8O	96,129	JECFA, 2018
7	5,183	0,13	92	911	920[t]	1,1,2,2-tetracloroetano	$C_2H_2Cl_4$	167,838	ATSDR, 2008; HMDB, 2018
8	5,700	0,09	91	930	927[j]	Ciclohexen-2-ona	C_6H_8O	96,129	JECFA, 2018
9	6,408	0,22	81	957	974[g]	Ácido 2-metil-2-pentenóico	$C_6H_{10}O_2$	114,144	JECFA, 2018
10	6,817	0,14	87	972	-	2,3-hexanediol	$C_6H_{14}O_2$	118,176	Ray et al., 2012
11	8,475	0,15	95	1026	-	Tetrahidropirano-2-carbinol	$C_6H_{12}O_2$	116,160	PubChem, 2018 - US9708274A1
12	8,608	0,32	84	1030	-	2,5-heptanediol	$C_7H_{16}O_2$	132,203	PubChem, 2018 - US2012020906A1
13	8,958	0,24	82	1040	1020[o]	Metilpiperidina	$C_6H_{13}N$	99,177	CAMEO Chemicals, 2018
14	9,742	0,01	85	1062	1190[m]	Butil metacrilato	$C_8H_{14}O_2$	142,198	CAMEO Chemicals, 2018; FDA/CFSAN, 2018
15	10,333	0,27	84	1078	-	2,5-dihidro-3,5-dimetil 2-furanona	$C_6H_8O_2$	112,128	Yannai, 2004
16	11,258	0,25	90	1104	-	Vinil 2,2-dimetilpentanoato	$C_9H_{16}O_2$	156,225	PubChem, 2018 - US4367220A1

17	11,458	0,04	84	1109	1126[f]	3,4-dimetilciclohexanol	$C_8H_{16}O$	128,215	Oliveira et al., 2009
18	11,892	0,03	83	1119	-	Etil isopropenil éter	$C_5H_{10}O$	88,150	PubChem, 2018 - US6878703A1
19	12,467	0,23	87	1134	1042[n]	Cicloteno	$C_6H_8O_2$	112,128	JECFA, 2018
20	12,725	0,85	87	1140	1043[i]	3-metil-1,2-ciclopentanediona	$C_6H_8O_2$	112,128	Yang et al., 2016, HMDB, 2018
21	13,558	0,32	87	1160	1159[k]	Ácido benzóico	$C_7H_6O_2$	126,122	Chojnacka et al 2012; HMDB, 2018; EU Pesticides, 2018
22	16,500	0,36	88	1231	1265[v]	Metiletilmaleimida	$C_7H_9NO_2$	139,154	Pinho et al, 2009
23	17,217	0,43	95	1248	1248[s]	Ácido fenilacético	$C_8H_8O_2$	136,150	Blunt e Munro, 2008; Korasick et al., 2013; HMDB, 2018
24	17,558	0,22	90	1256	1261[c]	Metilvinilmaleimida	$C_7H_7NO_2$	137,138	Köst e Benedikt, 1982; Kurtin, 1977; Suzuki e Shioi, 1999
25	23,608	0,04	90	1400	1400[a]	Tetradecano	$C_{14}H_{30}$	198,394	Rajkumar e Bhavan, 2017; JECFA, 2018
26	27,667	0,07	92	1500	1500[a]	Pentadecano	$C_{15}H_{32}$	212,421	El-Din e El-Ahwany, 2016; JECFA, 2018
27	28,733	0,15	91	1527	1525[p]	Dihidroactinidiolida	$C_{11}H_{16}O_2$	180,247	Yamamoto et al., 2014; JECFA, 2018
28	30,083	0,17	91	1562	1566[a]	Ácido láurico	$C_{12}H_{24}O_2$	200,322	El-Shoubaky et al., 2008 HMDB, 2018
29	31,533	0,05	91	1600	1600[a]	Hexadecano	$C_{16}H_{34}$	226,448	El-Din e El-Ahwany, 2016
30	34,742	0,75	90	1687	1690[c]	4-(4-hidroxi-2,2,6-trimetil-7-oxabiciclo[4.1.0]hept-1-il)-3-buten-2-ona	$C_{13}H_{20}O_3$	224,300	Abdel-Aal et al, 2015
31	35,233	0,25	95	1700	1700[a]	Heptadecano	$C_{17}H_{36}$	240,475	El-Din e El-Ahwany, 2016
32	38,333	0,08	82	1788	1796[e]	4-hidroxi-3,5,6-trimetil-4-(3-oxo-1-butenil)-2-ciclohexen-1-ona	$C_{13}H_{18}O_3$	222,284	Zatelli et al., 2018; HMDB, 2018
33	41,050	0,11	90	1868	1868[r]	Diisobutil ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018; HMDB, 2018
34	42,108	0,02	87	1899	1900[a]	Nonadecano	$C_{19}H_{40}$	268,529	Moustafa et al., 2008

35	43,083	2,24	90	1930	1806[h]	Doconexent (triglicérides marinho ômega 3)	$C_{22}H_{32}O_2$	328,496	Ginneken et al., 2011; Martinsson, 2016; HMDB, 2018; JECFA, 2018
36	43,475	1,25	95	1942	1953[q]	Ácido palmitoleico	$C_{16}H_{30}O_2$	254,414	Alencar et al., 2016; Zielinka et al., 2014; HMDB, 2018
37	44,225	10,07	94	1965	1960[a]	Ácido palmítico	$C_{16}H_{32}O_2$	256,430	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
38	48,775	2,58	97	2112	2113[c]	Fitol	$C_{20}H_{40}O$	296,539	JECFA, 2018; Souza e Nes, 1969, Jassbi et al., 2016; Yasmeen et al., 2018
39	48,933	0,61	86	2117	2143[u]	Ácido gamolênico	$C_{18}H_{30}O_2$	278,436	Park et al, 2013
40	49,433	7,41	94	2134	2134[d]	Ácido 9,12-octadecadienóico	$C_{18}H_{32}O_2$	280,452	Sugisawa et al., 1990; Patra et al., 2015
41	49,642	7,53	93	2142	2142[a]	Ácido oleico	$C_{18}H_{34}O_2$	282,468	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
42	49,783	7,76	94	2146	2161[u]	Ácido cis-vaccênico	$C_{18}H_{34}O_2$	282,468	Khotimchenko et al., 2002; Zielinka et al., 2014
43	54,308	0,19	91	2304	2334[u]	Icosapent (triglicérides marinho omega 3)	$C_{20}H_{30}O_2$	302,458	El-Din e El.Ahwany, 2016; HMDB, 2018
44	60,708	2,34	98	2548	2152[l]	Mono(2-etilhexil) ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018

* Tr. (min.) - tempo de retenção (minutos) / IS - índice de similaridade/ IK Exp. – índice de Kovats experimental. / IK Lit. – índice de Kovats da literatura
[a]Adams, 2017/ [b]Ames et al., 2001/ [c]Andriamaharavo, 2014/ [d]Asuming et al., 2005/ [e]Boulanger e Crouzet, 2000/ [f]Cardeal et al., 2006/ [g]Collin et al., 2012/ [h]Herath et al., 2018/ [i]Hill et al., 1999/ [j]Kim e Chung, 2009/ [k]Lalel et al., 2003/ [l]Liang, 2010/ [m]MacLeod e Pieris, 1981/ [n]Mateo et al., 1997/ [o]Peng et al., 1991/ [p]Pino et al., 2005/ [q]Quijano et al., 2007/ [r]Saroglou et al., 2006/ [s]Schwambach e Peterson, 2006/ [t]Sorimachi et al., 1995/ [u]Tret'yakov, 2007/ [v]Xian et al., 2006.

Anexo E. Substâncias identificadas por CG/EM no extrato hexânico da alga parda *Lobophora variegata*.

Nº	Tr. (min.)	Área do pico (%)	IS	IK Exp.	IK Lit.	Substância	Fórmula Molecular	Peso Molecular g/mol	Referências/ Patentes
1	4,292	0,08	87	-	-	2,4-hexadieno-2-ol	$C_6H_{10}O$	98,145	JECFA, 2018,
2	4,400	0,17	91	-	-	2-metil-1-penten-3-ol	$C_6H_{12}O$	100,161	Carpino et al., 2010
3	4,700	0,25	87	-	-	3-metilpent-2-eno-1,5-diol	$C_6H_{12}O_2$	116, 160	PubChem, 2018 - US20070197696A1
4	5,542	0,04	83	924	931[h]	2,5-hexanediona	$C_6H_{10}O_2$	114,144	Antunes et al., 2011
5	6,050	0,15	86	943	-	1-metil-1,2-ciclohexeno oxido	$C_7H_{12}O$	112,172	PubChem, 2018
6	7,325	0,05	88	991	-	1,1,2,2-tetrametil-ciclopropano	C_7H_{14}	98,189	PubChem, 2018
7	8,250	0,06	84	1440	1439[w]	3,4-dimetil-2-ciclopenten-1-ona	$C_7H_{10}O$	110,156	Choi et al., 2017
8	8,492	0,15	87	1026	1025[f]	Metil isobutil cetona	$C_6H_{12}O$	100,161	HMDB, 2018,
9	11,033	0,08	94	1098	1098[g]	3,7-dimetil-3-octanol	$C_{10}H_{22}O$	158,285	Lapczynski et al., 2008; HMDB, 2018; JECFA, 2018
10	12,525	0,04	89	1135	1135[x]	5,6-dimetil-decano	$C_{12}H_{26}$	170,340	Kailas e Nair, 2015
11	16,500	0,12	88	1231	1231[q]	3-etil-4-metil-1h-pirrole-2,5-diono	$C_7H_9NO_2$	139,154	Abdel-Aal et al, 2015
12	18,575	0,10	83	1280	-	2,6-dimetil-4-hepten-3-ona	$C_9H_{16}O$	140,226	Yannai, 2004
13	19,425	0,11	90	1300	1300[a]	Tridecano	$C_{13}H_{28}$	184,367	Yamamoto et al., 2014; HMDB, 2018
14	22,500	0,56	92	1373	1373[e]	2-etil-3-hidroxihexil 2-metilpropanoato	$C_{12}H_{24}O_3$	216,321	Zhao et al, 2008; Monte et al., 2016; Hosoglu, 2017
15	23,625	0,88	95	1400	1400[a]	Tetradecano	$C_{14}H_{30}$	198,394	Rajkumar e Bhavan, 2017; JECFA, 2018
16	26,158	0,74	91	1463	1463[s]	2,6,10-trimetiltridecano	$C_{16}H_{34}$	226,448	PubChem, 2018 - US8080581A1
17	27,342	0,39	88	1492	1481[a]	Germacreno-D	$C_{15}H_{24}$	204,357	Zatelli et al., 2018

18	27,675	1,35	97	1500	1500[a]	Pentadecano	$C_{15}H_{32}$	212,421	El-Din e El-Ahwani, 2016; JECFA, 2018
19	28,583	0,17	86	1524	1524[c]	Calameneno	$C_{15}H_{22}$	202,341	Elias et al., 1997
20	31,542	0,86	97	1600	1600[a]	Hexadecano	$C_{16}H_{34}$	226,448	El-Din e El-Ahwany, 2016
21	33,383	0,26	92	1650	-	Esqualano	$C_{30}H_{62}$	422,826	Blunt e Munro, 2008; EPA/TSCA, 2018; Nakaji et al., 2018
22	33,900	0,06	89	1664	-	Tricosano	$C_{23}H_{48}$	324,637	Moustafa et al., 2008
23	35,242	0,47	97	1700	1700[a]	Heptadecano	$C_{17}H_{36}$	240,475	Sugisawa et al., 1990, Oliveira et al., 2012
24	35,450	0,36	95	1706	1707[j]	2,6,10,14-tetrametilpentadecano	$C_{19}H_{40}$	268,529	Michalak et al., 2015; HMDB, 2018
25	35,750	0,07	91	1714	1715[b]	Pentadecanal	$C_{15}H_{30}O$	226,404	Kajiwara et al., 1990; HMDB, 2018; Maria e Narendra, 2018
26	37,442	0,98	94	1762	1763[p]	Ácido Mirístico	$C_{14}H_{28}O_2$	228,376	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
27	38,592	0,41	96	1795	1795[a]	Etil miristato	$C_{16}H_{32}O_2$	256,430	HMDB, 2018
28	38,758	0,27	96	1800	1800[a]	Octadecano	$C_{18}H_{38}$	254,502	El-Din e El-Ahwany, 2016; HMDB, 2018
29	40,108	1,22	94	1840	1840[b]	Neofitadieno	$C_{20}H_{38}$	278,524	Oliveira et al., 2012; Rajkumar e Bhavan, 2017
30	40,292	0,44	93	1845	1845[i]	Fitono	$C_{18}H_{36}O$	268,485	Blunt e Munro, 2008; Maheswari et al. 2017; JECFA, 2018
31	41,058	0,34	95	1868	1868[r]	Diisobutil ftalato	$C_{16}H_{22}O_4$	278,348	HMDB, 2018, Rowdhwal e Chen, 2018
32	42,117	0,26	96	1900	1900[a]	Nonadecano	$C_{19}H_{40}$	268,529	Moustafa et al., 2008
33	43,433	0,25	94	1941	1953[o]	Ácido palmitoleico	$C_{16}H_{30}O_2$	254,414	Zielinka et al., 2014; Alencar et al., 2016; HMDB, 2018
34	44,150	2,43	93	1963	1960[a]	Ácido palmítico	$C_{16}H_{32}O_2$	256,430	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
35	44,475	0,30	91	1973	1975[v]	Etil 9-hexadecenoato	$C_{18}H_{34}O_2$	282,468	Sudha e Balasundaram, 2018

36	45,175	1,01	97	1995	1993[a]	Etil palmitato	$C_{18}H_{36}O_2$	284,484	Peres et al., 2012; HMDB, 2018
37	45,325	0,16	94	2000	2000[a]	Eicosano	$C_{20}H_{42}$	282,556	Karabay-Yavasoglu et al., 2007
38	46,625	0,73	91	2042	2413[m]	Ácido docosahexaenóico metil ester	$C_{23}H_{34}O_2$	342,523	Ginneken et al., 2011; Martinsson, 2016
39	48,408	0,13	90	2100	2100[a]	Heneicosano	$C_{21}H_{44}$	296,583	Abou-El-Wafa et al., 2011; El-shafay et al., 2016; EPA/TSCA, 2018
40	48,775	0,23	94	2112	2113[b]	Fitol	$C_{20}H_{40}O$	296,539	Souza e Nes, 1969; Jassbi et al., 2016; Yasmeen et al., 2018
41	50,425	0,84	92	2168	2168[b]	Etil oleato	$C_{20}H_{38}O_2$	310,522	Shobier et al., 2016; HMDB, 2018; JECFA, 2018
42	51,350	0,04	89	2200	2200[a]	Docosano	$C_{22}H_{46}$	310,610	Karabay-Yavasoglu et al., 2007
43	52,183	0,25	89	2229	2231[d]	Metil (Z)-5,11,14,17-eicosatetraenoato	$C_{21}H_{34}O_2$	318;501	Yagi et al., 2016; Salama et al., 2017
44	54,758	0,08	87	2321	2302[n]	Etil araquidonato	$C_{22}H_{36}O_2$	332,528	Refaai et al., 2002; Refaai et al., 2003; Hattab et al., 2011; HMDB, 2018
45	54,950	0,06	87	2328	2334[u]	Icosapent (triglicérides marinho ômega 3)	$C_{20}H_{30}O_2$	302,458	El-Din e El-Ahwany, 2016; HMDB, 2018
46	55,567	0,10	81	2351	2353[l]	4,8,12,16-tetrametilheptadecan-4-olida	$C_{21}H_{40}O_2$	324,549	Sujatha e Vijayalakshmi, 2013; Kalita et al., 2018
47	56,875	0,41	92	2399	2398[b]	Bis(2-etilhexil) adipato	$C_{22}H_{42}O_4$	370,574	EwG's Skin Deep, 2018; HMDB, 2018
48	60,717	1,86	98	2548	2152[k]	Mono(2-etilhexil) ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018
49	66,775	0,05	85	2799	2800[a]	Octacosano	$C_{28}H_{58}$	394,772	El-shafay et al., 2016
50	67,483	0,67	95	2826	2835[b]	Esqualeno	$C_{30}H_{50}$	410,730	Kendel et al., 2015; HMDB, 2018
51	76,225	1,18	89	3095	3098[t]	Colest-5-en-3-ol	$C_{27}H_{46}O$	386,664	Plouguerné et al., 2006
52	85,450	0,37	81	-	-	Estigmasterol	$C_{29}H_{48}O$	412,702	Kendel et al., 2015; HMDB, 2018

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[a]Adams, 2017/ [b]Andriamaharavo, 2014/ [c]Elias et al., 1997/ [d]Golovnya e Kuzmenko, 1977/ [e]Gomez e Ledbetter, 1994/ [f]Héberger et al., 2002/ [g]Hemmateenejad et al., 2007/ [h]Jerkovic et al., 2010/ [i]Karioti et al., 2003/ [j]Kenig et al., 2005/ [k]Liang, 2010/ [l]Mann et al, 2017/ [m]Mjos et al., 2006/ [n]Nowack et al., 2017/ [o]Quijano et al., 2007/ [p]Raina et al., 2006/ [q]Sabik et al., 2016/ [r]Saroglou et al., 2006/ [s]Shlyakhov, 1984/ [t]Steiger et al., 2011/ [u]Tret'yakov, 2007/ [v]Watanabe et al., 2008/ [w]Welke et al., 2012/ [x]Widmer, 1967.

Anexo F. Substâncias identificadas por CG/EM no extrato em diclorometano da alga parda *Lobophora variegata*.

Nº	Tr. (min.)	Área do pico (%)	IS	IK Exp.	IK Lit.	Substância	Fórmula Molecular	Peso Molecular g/mol	Referências/ Patentes
1	4,425	0,07	87	-	-	2-etilbutanal	$C_6H_{12}O$	100,161	JECFA, 2018; HMDB, 2018
2	4,475	0,05	83	-	-	3-metil-2-ciclopenten-1-ona	C_6H_8O	96,129	JECFA, 2018
3	4,667	0,09	81	-	-	2-ciclohexenol	$C_6H_{10}O$	98,145	PubChem, 2018- US6303640A1
4	4,733	0,10	82	-	-	3-metilpent-2-eno-1,5-diol	$C_6H_{12}O_2$	116, 160	PubChem, 2018 - US20070197696A1
5	5,075	0,30	95	907	925[b]	2-metil-2-ciclopentenona	C_6H_8O	96,129	JECFA, 2018
6	5,200	0,07	90	911	920[l]	1,1,2,2-tetrachloroetano	$C_2H_2Cl_4$	167,838	ATSDR, 2008; HMDB, 2018
7	5,317	0,33	86	916	913[q]	2-metil-2,4-pentanediol	$C_6H_{14}O_2$	118,176	EwG's Skin Deep, 2018, PubChem, 2018 - US2014349969A1
8	5,592	0,11	89	926	-	Tetrahidropirano-2-carbinol	$C_6H_{12}O_2$	116,160	PubChem, 2018 - US9708274A1
9	6,283	0,07	91	952	-	2,4-dimetil-2,4-pentanediol	$C_7H_{16}O_2$	132,203	PubChem, 2018 - US5304550A1
10	7,400	0,96	93	994	-	3-hexene-2,5-diol	$C_6H_{12}O_2$	116,160	Kumar e Gupta, 2008
11	8,875	0,12	80	1037	1042[g]	Cicloteno	$C_6H_8O_2$	112,128	JECFA, 2018
12	11,275	0,68	89	1104	-	Vinil 2,2-dimetilpentanoato	$C_9H_{16}O_2$	156,225	PubChem, 2018 - US4367220A
13	12,783	2,31	87	1141	1043[d]	3-metil-1,2-ciclopentanediono	$C_6H_8O_2$	112,128	Yang et al., 2016; HMDB, 2018

14	16,508	0,11	86	1231	1265[r]	Metiletilmaleimida	$C_7H_9NO_2$	139,154	Pinho et al, 2009
15	17,225	0,27	95	1248	1248[k]	Ácido fenilacético	$C_8H_8O_2$	136,150	Blunt e Munro, 2008; Korasick et al., 2013; HMDB, 2018
16	17,392	0,09	84	1252	1225[h]	2-etil-2-metil-succinimida	$C_7H_{11}NO_2$	141,170	Yende et al., 2018; HMDB, 2018
17	17,583	0,03	87	1257	1261[c]	Metilvinilmaleimida	$C_7H_7NO_2$	137,138	Köst e Benedikt, 1982; Kurtin, 1977; Suzuki e Shioi, 1999
18	27,350	0,10	83	1492	1481[a]	Germacreno-D	$C_{15}H_{24}$	204,357	Zatelli et al., 2018
19	27,675	0,07	90	1500	1500[a]	Pentadecano	$C_{15}H_{32}$	212,421	El-Din e El-Ahwany, 2016; JECFA, 2018
20	31,550	0,07	91	1600	1600[a]	Hexadecano	$C_{16}H_{34}$	226,448	El-Din e El-Ahwany, 2016
21	33,408	0,03	83	1650	1392[f]	2,6,10-trimetildodecano	$C_{15}H_{32}$	212,421	Sun et al., 2012; EPA/TSCA, 2018
22	35,242	0,17	93	1700	1700[a]	Heptadecano	$C_{17}H_{36}$	240,475	El-Din e El-Ahwany, 2016
23	36,450	0,36	84	1734	1784[c]	Loliolida	$C_{11}H_{16}O_3$	196,246	Grabarczyk et al., 2015; HMDB, 2018
24	38,758	0,10	90	1800	1800[a]	Octadecano	$C_{18}H_{38}$	254,502	El-Din e El-Ahwany, 2016; HMDB, 2018
25	40,108	1,74	94	1840	1840[c]	Neofitadieno	$C_{20}H_{38}$	278,524	Oliveira et al., 2012, Rajkumar e Bhavan, 2017
26	41,058	1,31	97	1868	1868[j]	Diisobutil ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018; HMDB, 2018
27	42,125	0,10	90	1900	1900[a]	Nonadecano	$C_{19}H_{40}$	268,529	Moustafa et al., 2008
28	42,192	0,05	81	1902	-	Ácido adipico acid, butil 2-metoxietil ester	$C_{13}H_{24}O_5$	260,330	PubChem, 2018
29	44,158	4,39	93	1963	1960[a]	Ácido palmítico	$C_{16}H_{32}O_2$	256,430	Blunt e Munro, 2008; Sahu et al., 2013 HMDB, 2018
30	45,333	0,08	92	2000	2000[a]	Eicosano	$C_{20}H_{42}$	282,556	Karabay-Yavasoglu et al., 2007
31	46,450	0,24	91	2036	2324[o]	Ácido araquidonico	$C_{20}H_{32}O_2$	304,474	Shanab et al., 2018; HMDB, 2018
32	46,625	0,29	92	2042	2413[i]	Ácido docosahexaenóico metil ester	$C_{23}H_{34}O_2$	342,523	Ginneken et al., 2011; Martinsson, 2016

33	48,775	0,44	94	2112	2113[c]	Fitol	$C_{20}H_{40}O$	296,539	Souza e Nes, 1969; Jassbi et al., 2016; Yasmeen et al., 2018
34	49,517	1,55	93	2137	2142[a]	Ácido oleico	$C_{18}H_{34}O_2$	282,468	Blunt e Munro, 2008; Sahu et al., 2013; HMDB, 2018
35	50,200	0,19	88	2161	2161[m]	Ácido esteárico	$C_{18}H_{36}O_2$	284,484	Blunt e Munro, 2008; Chen et al., 2016; HMDB, 2018
36	51,342	0,06	90	2199	2200[a]	Docosano	$C_{22}H_{46}$	310,610	Karabay-Yavasoglu et al., 2007; HMDB, 2018
37	52,050	0,19	86	2224	2334[p]	Icosapent (Triglicérides marinho ômega 3)	$C_{20}H_{30}O_2$	302,458	El-Din e El-Ahwany, 2016; HMDB, 2018
38	56,875	1,13	94	2399	2398[c]	Bis(2-etilhexil) adipato	$C_{22}H_{42}O_4$	370,574	EwG's Skin Deep, 2018; HMDB, 2018
39	60,725	6,31	97	2548	2152[e]	Mono(2-etilhexil) ftalato	$C_{16}H_{22}O_4$	278,348	Rowdhwal e Chen, 2018
40	67,483	1,87	96	2826	2835[c]	Esqualeno	$C_{30}H_{50}$	410,730	Kendel et al., 2015; HMDB, 2018
41	69,350	0,08	86	2899	2900[a]	Nonacosano	$C_{29}H_{60}$	408,799	Moustafa et al., 2008; HMDB, 2018
42	76,200	0,17	82	3094	3098[n]	Colest-5-en-3-ol	$C_{27}H_{46}O$	386,664	Plouguerné et al., 2006
43	85,425	0,21	81	-	-	Estigmasterol	$C_{29}H_{48}O$	412,702	Kendel et al., 2015; HMDB, 2018

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[a]Adams, 2017/ [b]Ames et al., 2001/ [c]Andriamaharavo, 2014/ [d]Hill et al., 1999/ [e]Liang, 2010/ [f]Maarse, 1975/ [g]Mateo et al., 1997/ [h]Maurer, 1990/ [i]Mjos et al., 2006/ [j]Saroglou et al., 2006/ [k]Schwambach e Peterson, 2006/ [l]Sorimachi et al., 1995/ [m]Srivastava et al., 2006/ [n]Steiger et al., 2011/ [o]Tret'yakov, 2007/ [p]Tret'yakov, 2007/ [q]Vijayanand et al., 2001/ [r]Xian et al., 2006.

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Capítulo 6. ***Considerações finais***

Considerações finais

No presente estudo, foram submetidas a análises, quanto à presença de contaminantes aquáticos, *Dichotomaria marginata*, *Acanthophora spicifera*, *Cladophora prolifera* e *Lobophora variegata*, pertencentes respectivamente ao grupo das Rhodophyceae, Chlorophyceae e Phaeophyceae. De todas foram dessorvidos contaminantes; estas observações e mais os dados da literatura que registram a presença de poluentes em extratos algáceos (Aboutbl et al., 2010) ampliam e embasam o conceito de macroalgas como indicadoras da poluição marinha

Porém, em virtude dos inúmeros poluentes aquáticos identificados nos extratos dessas algas, parte do nosso objetivo, relacionado à identificação de compostos com atividades biológicas, foi descontinuado, pois alguns desses contaminantes eram fungitóxicos (ftalatos) e outros, antioxidantes (como oxibenzona e octinoxate ambos encontrados em protetor solar).

O compulsório redirecionamento desse estudo levou à execução de um imprescindível levantamento de contaminantes aquáticos; este poderá auxiliar no reconhecimento de resíduos encontrados em extratos de algas e de plantas, evitando que substâncias como os ftalatos (Aboutbl et al., 2010) continuem a ser citadas como metabolitos desses organismos.

Na identificação dos poluentes de *D. marginata* foram utilizadas, com êxito, as técnicas de CG/EM e CLAE/EM, esta última com deconvolução (Goulitquer et al. 2012). Visto que essas técnicas analíticas apresentam alta sensibilidade, foram tomadas todas as precauções (ou cuidados especiais) para evitar qualquer contaminação externa, em quaisquer das amostras, em todas as etapas de suas análises. A técnica de CG/EM mostrou-se também valiosa no estudo do extrato de *D. delicatula*, permitindo que fossem nele identificados 13 sesquiterpenos, sete além dos já conhecidos como produzidos por esta macroalga. Possibilitou, também, que fossem caracterizados dois sesquiterpenos em *L. variegata*.

Houve, pois, também, concreta contribuição ao conhecimento dos metabolitos naturais das macroalgas *Dichotomaria marginata*, *Acanthophora spicifera*, *Cladophora prolifera*, *Lobophora variegata* e de *Dictyopteris delicatula*.

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