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Chemical investigations on the spiny sea-star Marthasterias glacialis collected in Portugal as a source of bioactive molecules

Investigação química da Marthasterias glacialis da costa portuguesa, como fonte de compostos com actividade farmacêutica

D.M. Pereira[1,2], F. Ferreres[3], P. Valentão[1], N. Teixeira[2,4], P.B. Andrade[1]

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ABSTRACT

Marine organisms are increasingly regarded as a promising source of bioactive molecules for human pharmacotherapy, being used in conditions such as cancer, inflammation, infection and pain. *Marthasterias glacialis*, also known as the spiny sea-star, is an echinoderm common in the Portuguese coast, however little information is available regarding its chemical constituents. In this work, the chemistry of this organism is reviewed taking into account the latest reports on several classes of natural products, namely carotenoids, sterols, fatty acids and amino acids.

Keywords: bioactive molecules, *Marthasterias glacialis*, carotenoids, fatty acids, sterols

RESUMO

Os organismos marinhos tem sido considerados como uma fonte promissora de moléculas com actividade farmacoterapêutica para os humanos, sendo utilizadas em distintas patologias como neoplásicas, inflamatórias, infecciosas ou álgicas. A *Marthasterias glacialis*, também conhecida como estrela do mar espinhosa, é um equinoderme muito comum na costa portuguesa, existindo, contudo, pouca informação sobre a sua composição quimica. Pretende-se rever a química deste organismo tendo em conta os ultimos dados sobre as diferentes classes de produtos, nomeadamente carotenoides, esteróis, ácidos gordos e aminoácidos.

Palavras-chave: actividade biofarmacêutica, *Marthasterias glacialis*, carotenóides, ácidos gordos, esteróis

[1]REQUIMTE/Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal.

[2]Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal.

[3]Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain

[4]IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal

INTRODUCTION

From a historical point of view, Nature has been a very important source of new molecules with application in pharmacotherapy. Even today, a significant number of medicines are either obtained directly or derived from natural products[1]. Although plants have been the main source of molecules, in the past few years marine organisms have received considerable attention. In particular, the marine environment has been increasingly shown to be a remarkable source of chemical diversity, which frequently results in interesting biological properties[2-5]. For this reason, several pharmaceutical industries are now using non-conventional sources of natural products in their pipelines for drug discovery, marine products included.

Portugal has a rich cost, which harbours a diversified ecosystem. In the next few pages we will address some of the recent findings regarding the chemistry of the spiny sea-star *M. glacialis* L. collected along Peniche in 2009, namely its composition in carotenoids, fatty acids and sterols.

CAROTENOIDS

Carotenoids constitute a class of natural products widely distributed in Nature, mainly in lower trophic levels. They are found both in terrestrial and aquatic environments and are present across several taxonomical groups. In natural sources, the most common carotenoids are fucoxanthin, neoxanthin, violaxanthin and lutein[6].

Carotenoids comprise two chemically distinct groups: the hydrocarbons carotenes and their oxygenated derivatives, xanthophyls. Due to the long system of conjugated double bonds, which constitutes the chromophore, nearly all carotenoids absorb light in the 400-500 nm range, however this conjugation system turn these molecules unstable in the presence of light, heat or oxidative atmosphere and susceptible to isomerization from all-*trans* (all-E) to *cis* (Z) isomers.

In humans, carotenoids can be found in plasma and also in the eye, were they are part of the macular antioxidant system.

Biological properties of carotenoids include pro-vitamin A activity, antioxidant activity and modulation of immune responses, among others[7,8]. Several works address the cancer prevention effect of carotenoid-rich. Although there seems to be enough evidence to support these claims, it should be highlighted that in some cases, such as smokers or individuals exposed to asbestos, high intake of carotenoids may be associated to a higher risk of developing cancer. As reviewed recently, high intakes of carotenoids in individuals with lung damage may result in an increase of the odds of developing cancer up to 30%[9].

Carotenoids in *M. glacialis*

HPLC-PAD is an important tool for the preliminary analysis of carotenoids due to the fact that the chemical characteristics responsible for UV spectra shape and maxima are fairly understood. The position of long-wave absorbance bands is a function of the number of conjugated double bonds, with an increase in this number resulting in increased wavelength of maximal absorption[10]. The introduction of a carbonyl group in a cyclic end group in conjugation with the polyene chain has 2 consequences: for one, there is a marked loss of fine structure, resulting in rounded, sometimes symmetrical, shape. Secondly, a bathochromic shift takes place.

In the preliminary screening of carotenoids from *M. glacialis*, HPLC-DAD-APCI--MS/MS with a C18 column was used. Several peaks compatible with carotenoids were found (Figure 1), with two types of UV spectra being noticed: spectra with rounded shape with just one maximum (compound 2) and spectra with a fine structure with 3 UV absorption maxima (such as compound 5) (Figure 2). Compound 2, with no fine structure and a maximum at 474 nm, had to be a xanthophyll with a

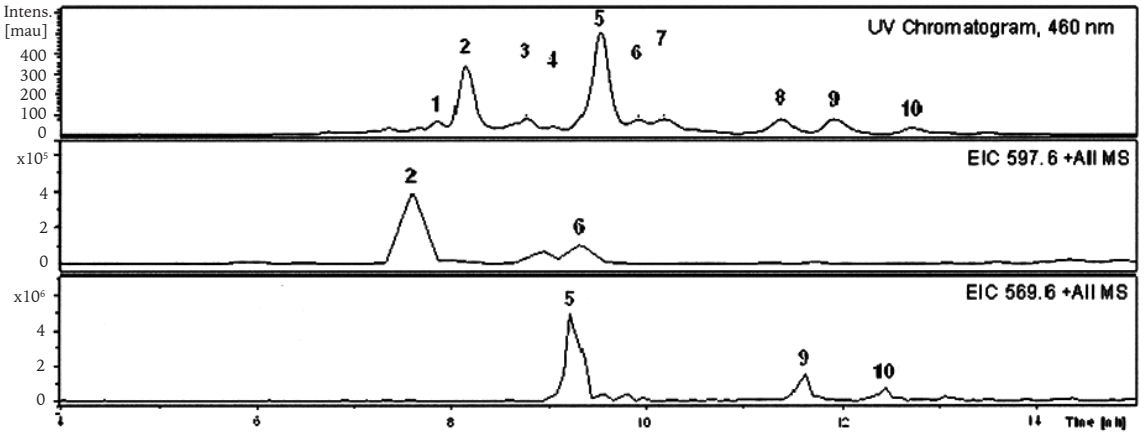

Figure 1. UV Chromatogram (460 nm) of the extract from *M. glacialis* and Extraction Ion Chromatogram (EIC) of the ions $[597,6]^+$ (2 and 6), $[569,6]^+$ (5, 9 and 10) and $[567,6]^+$ (8).

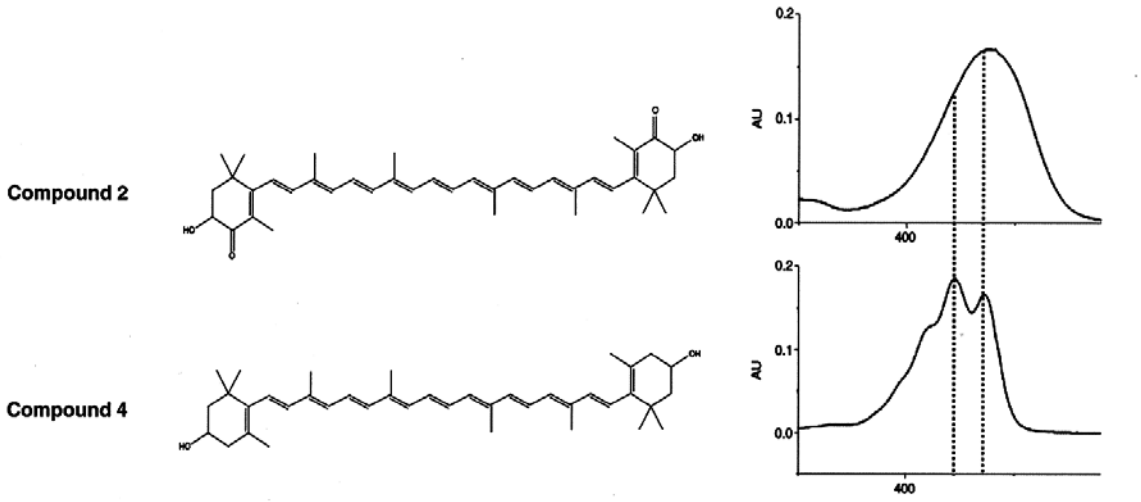

Figure 2. Influence of chemistry in the UV-spectra of carotenoids.

carbonyl group in conjugation with the chromophore. Due to the presence of a fine structure and multiple UV maxima for the remaining compounds, these were thought to be either carotenes or xanthophylls with no conjugated carbonyl groups.

By combining data from MS fragmentation, UV spectra and co-chromatography, 3 compounds could be identified: astaxanthin (peak 2), lutein (peak 4) and zeaxanthin (peak 5). With the MS conditions used herein, it was noticed that in the process of ion transfer a loss of water occurred in compounds 4 and 5, thus originating the ion $[M+H-18]^+$, being very abundant in the case of lutein (*m/z* 551,6), with the protonated molecule being frequently absent.

As several peaks whose UV-spectra were compatible with carotenoids could not be identified at the time, a method in which HPLC-DAD-APCI-MS/MS, equipped with a C30 column, instead of a C18, was used. This approach rendered the identification of twenty compounds, eight of them reported for the first time in this marine organism. Differentiation of carotenoid isomers was also achieved[10].

FATTY ACIDS

Fatty acids are lipophilic molecules present in all living organisms, where they can occur in their free form or integrate more complex

lipids, namely triglycerides, phospholipids or glycolipids. Fatty acids exhibit a very diversified chemistry, which can result in structures comprising saturated/unsaturated, branched and cyclic molecules.

Classical nomenclature for fatty acids indicates chain length, number and position of double bonds when present, with the carbon associated with the methyl end group being designated ω carbon. For this reason, unsaturated FA in which the position of the first double bond is located 3, 6 and 9 carbons away from the ω carbon are named ω-3 (omega-3), ω-6 (omega-6) and ω-9 (omega-9), respectively.

Fatty acids, as it happens with other lipids, play three major roles in organisms: structural components of biological membranes, source and reserves of energy and biochemical mediators in a wide range of biological processes. They are endogenous ligands of several receptors, which in part play important roles in controlling a number of metabolic pathways. Unsaturated fatty acids with 18-20 carbon atoms are precursors of prostaglandins, thromboxanes and leucotrienes, which display several autocrine and paracrine effects and have a number of regulatory properties. Molecules with 20 and 22 carbons can be precursors of other biologically important molecules, such as non-classic eicosanoids, which include resolvins, lipoxins and neuroprotectins [11].

Fatty acids in *M. glacialis*

A GC-MS method was used for the identification and quantification of fatty acids in *M. glacialis*[13]. Several samples, comprising individuals collected in different months from distinct geographical origins were used.

Overall, 16 compounds could be studied, comprising both saturated and unsaturated fatty acids. Stearic and palmitic acids were major saturated fatty acids, with arachidonic and *cis* 11-eicosaenoic acids being their most abundant unsaturated counterparts. Samples from July and September presented palmitic acid as major compound, while samples from February had arachidonic acid was the major compound, far exceeding palmitic acid. In all samples studied, unsaturated fatty acids were present in higher amounts than saturated ones. Subsequent studies, not covered in this review, showed the role of some of these fatty acids in the pro-apoptotic and anti-inflammatory activities of a purified extract from this organism[14, 15].

STEROLS

Sterols are, in a general way, C27 steroid alcohols synthesized by higher plants, algae, nearly all fungi and also vertebrates, although through different biosynthetic pathways. The presence of the C3 hydroxyl group turns esterification possible and thus sterols can be esterified with molecules such as fatty acids, sugars and hydroxycinnamic acids.

Sterols play a pivotal role in most organisms, mainly due to their function as membrane constituents, where they interfere with membrane fluidity and permeability. In the context of human health, the most explored biological property of sterols is related to their well-known ability to lower total cholesterol and low density lipoproteins, an effect that has been known for the last 5 decades[16,17]. This effect is dose-dependent, with a dose of 2,0-2,5 g/day eliciting a reduction of about 10% - 15%[18]. When higher reduction values are required, supplementation with sterols has been shown to act synergically with pharmacotherapy involving cholesterol-lowering agents, such as statins[19]. This cholesterol-lowering effect of sterols can result either from interference with intestinal absorption of dietary cholesterol (exogenous cholesterol) or from inhibition of one of the key enzymes in cholesterol biosynthesis, 3-hydroxy-3-methyl-glutaryl-CoA reductase (endogenous cholesterol).

Sterols in *M. glacialis*

The GC-MS sterol profile of *M. glacialis* revealed the presence of 8 compounds, with

cholesten-7-en-3-ol, ergosta-7.22-dien-3-ol (Figure 3) and an unidentified ergosterol derivative being the compounds present in higher amounts[12,13].

As it had been done with fatty acids, for the analysis of sterols several samples, comprising individuals collected in different months from distinct geographical origins were used. There was a clear difference between samples from February and July/September, with the former displaying higher amounts and diversity of sterols and also greater diversity. Ergosterol was the only compound present in all analyzed samples under study. Other compounds, such as β-sitosterol, fucosterol, betulin, lupeol and lupeol acetate were searched by using their MS data, however none could be found.

CONCLUSION

Sea-derived molecules will have an undeniable role in human pharmacotherapy in the next few years, a trend that is well represented if we consider the high number of molecules currently undergoing clinical trials. In this regard, the process of drug discovery from marine organisms requires detailed metabolomics studies on several species as a first step.

We have taken part in this process by focusing on the chemical investigation of several species from Portuguese ecosystems, of which *M. glacialis*, discussed here, is an example. The qualitative and quantitive composition described can be the basis of future works aiming to address the biological properties of these compounds and their potential use in pathological conditions.

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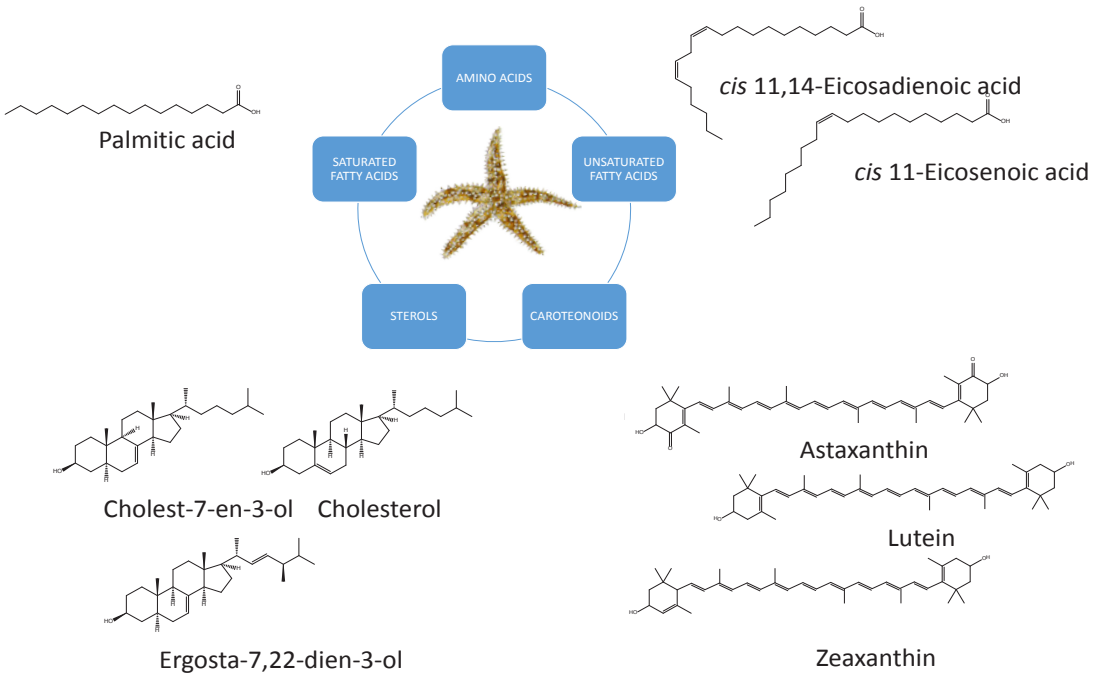

Figure 3. Overview of chemical classes and representative compounds present in *M. glacialis*.

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