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Review

Green analytical methodologies for the discovery of bioactive compounds from marine sources

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ABSTRACT

The application of green analytical chemistry in search of bioactive compounds is of paramount importance, leading to the development of environmentally friendly methodologies for their isolation. This work provides an up-to-date overview of the analytical methodologies based on a green perspective for the discovery of bioactive compounds from marine sources, namely to their extraction and structural characterization. Both the characteristics of marine bioactive compounds and the sustainable evaluation of their bioactivity are also addressed.

Contents

1. Introduction

According to the Europe 2020 strategy [1], three priorities have been considered in order to offer a vision of Europe's social market economy for the 21st century, that is, smart, sustainable, and inclusive growth. The smart growth consists in the development of an economy based on knowledge and innovation, the sustainable growth is based on the promoting of a more resource efficient, greener and more competitive economy, and the inclusive growth purposes a high-employment economy. Thus, the "green" products and technologies become one of the sectors of the future [1]. In this field, the green chemistry can be considered as one of the areas with great interest at industry and educational levels for the practical implementation of the concept of "environmentally friendly". In the final of the 20th century, the term green chemistry was defined by Anastas [2] as the use of chemistry techniques and methodologies that reduce or eliminate the use and generation of feedstocks, products, by-products, solvents, and reagents, that are

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hazardous to human health or the environment. On the other hand, Anastas and Warner [3] have suggested 12 principles of Green Chemistry, being thereafter adapted by Namiesnik [4] as four priorities, that is, (a) elimination or significant reduction of consumption of reagents and organic solvents for analytical procedures; (b) reduction in the emission of vapours and gases, as well as in solid wastes produced in analytical laboratories; (c) elimination of reagents displaying high toxicity and/or ecotoxicity from the analytical procedures; and (d) reduction of labour and energy consumption in analytical procedures.

-According to Anastas [2], one of the most active areas of green chemistry research is in the development of green analytical methodologies and they should be able to provide an environmental performance from pollution preventing through: (a) reduction and elimination of the use and generation of hazardous substances; (b) elimination of solvents from the sample collection, preparation and analysis steps; and (c) reduction of sample handling, transport, and waste minimization. In this way, experimental advances on sampling, preparation, extraction, and characterization of samples have been the centre of the green analytical chemistry in order to reduce any potential adverse environmental impact [5,6]. Although the perfect "green" solvent does not exist, the alternative solvents should be low toxic, easy to recycle, inert, and they do not contaminate the product [7]. Thus, several organic solvents such as volatile organic compounds (*e.g.*, benzene) are inadequate to be used routinely in green analytical chemistry. In particular, the majority of organic solvents belong to the class of volatile organic compounds, which are highly flammable and carcinogen and they cause eye irritation, headaches, and allergic skin reaction [7]. For example, the benzene is genotoxic and carcinogen and other compounds such as dichloromethane and hexane are carcinogen and hazardous air pollutants, respectively. On the other hand, the water, heptane, ethyl acetate, *tert*-butyl methyl ester, methanol, and ethanol are suitable solvents, as well as ethyl lactate, which is less toxic, renewable, and biodegradable solvent [7]. A study defines the "green" solvents as those which minimize the environmental impact resulting from their use, and after the analysis of 26 pure organic solvents, it was reported that simple alcohols such as methanol and ethanol, or alkanes such as heptane and hexane are more environmentally preferable solvents compared to dioxane, acetonitrile, acids, formaldehyde, and tetrahydrofuran [8]. López et al. [9] have studied the effect of solvents (water, water/methanol (1/1), methanol, and ethanol) on the total phenolic content and antioxidant activity of brown alga *Stypocaulon scoparium* extracts. López et al. [9] verified that the aqueous extract showed both the highest antioxidant activity and the highest phenolic content including also higher yield of extractable substances such as gallic acid than the polar solvents. The order of magnitude of the yields achieved from high to low was: water/methanol > water > methanol > ethanol. The authors also stated that the total phenolic content was strongly affected by the extracting solvent with the following order from high to low: water > water/methanol > methanol > ethanol. Also in the study of Capello et al. [8], it was shown that the solvent mixtures such as methanol–water or ethanol–water are environmentally favourable compared to pure alcohols or propanol–water mixtures. Thus, a wide variety of green solvents can be used in analytical processes following the purposes of the green analytical chemistry and preserving the natural environment and its resources.

In the second half of the 20th century, a huge biodiversity has been found and characterized in ocean ecosystem [10]. The marine environment provides specific physical, chemical, and biological conditions with potential to be exploited as source of new natural biologically active molecules. Such natural products can be used in pharmaceutical and therapeutical research besides drug development. The bioactive compounds isolated from marine organisms, such as bacteria and fungi [11], algae [12], and microorganisms-host associations [13] are produced as bioactive secondary metabolites due to the lack of natural defences in the majority of the invertebrates of the marine environment, such as innate immune system [14]. Effectively, 63% of new drugs were classified as naturally derived (unmodified natural product, modified natural product, or synthetic compound with a natural product as pharmacophore) [15]. For example, between January 1981 to the middle of October 2008, 68% of anti-infectives (antibacterial, antifungal, antiparasitic, and antiviral) and 63% of drugs used in cancer treatment, respectively, were naturally derived [16].

Therefore, and considering the Europe 2020 strategy [1] on the development of smart, sustainable and inclusive growth, it is urgent to implement the application of green analytical methodologies for the discovery of bioactive compounds. Thus, this review paper discusses the main green analytical methodologies for the discovery of bioactive compounds from marine sources such as algae, bacteria, fishes, as well as rest raw materials from marine organisms. The extraction and the structural characterization of bioactive compounds from marine organisms are discussed following the principles of green chemistry and both the characteristics of marine bioactive compounds and their bioactivity are also addressed with a view to a sustainable development.

2. Characteristics of bioactive compounds isolated from marine sources and bioassays for their discovery

According to Mayer et al. [17], the marine natural products can be classified in six main chemical classes taking into account their structure, that is, polyketides, terpenes, peptides, alkaloids, shikimates, and sugars. Table 1 shows the structures of some marine bioactive compounds belonging to the six main chemical classes identified by Mayer et al. [17].

The final objective of the isolation of marine bioactive compounds is the drug discovery and their potential clinical use. The first marine product to be used in clinical trials was the Didemnin B, which is a depsipeptide isolated from the tunicate *Tridemnum solidum* in the Caribbean Sea [30]. After that, an increasingly number of drugs has been approved for clinical applications, and they are already commercially available. For example, commercial products such as acyclovir have been commonly used as an antiviral agent, specifically, a synthetic derivative of arabinosyl nucleosides, isolated from a marine source (sponge *Tethya cripta*) by Elion et al. [31].

2.1. Characteristics of bioactive compounds isolated from marine sources

Bioactive compounds isolated from marine sources have several biological activities such as antitumor, antimalarial, antibacterial, anticoagulant, antifungal, antiprotozoal, antituberculosis, and antiviral as well as anti-inflammatory and cytotoxic effects, as recently reviewed by Mayer [17] and Rocha-Santos and Duarte [32]. These properties are of particular interest and establish the potential application of natural products. Table 2 shows some examples of the classes of compounds, their marine sources, and corresponding bioactivity.

The latest review paper on the marine natural products [1] refers that there was a pronounced increase in the number of new metabolites reported from molluscs, tunicates, and echinoderms when compared even with recent years. The number of new compounds reported from marine sponges in 2012 (355) has increased by approximately 20% compared with 2011, being the sponges the dominant source of novel natural products in the marine environment. Blunt et al. [43] also concluded that the marine microorganisms such as bacteria, fungi, cyanobacteria,