Determination of polyphenols, tannins, flavonoids and evaluate the antioxidative properties of extracts of five marine sponges of Atlantic Morrocan coast by DPPH.

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Abstract— Marine sponges have been regarded as a treasure trove of drugs compared to a great potential regarding their secondary metabolites. Most studies have been conducted on compounds derived from the sponge to examine its pharmacological properties. Such compounds have been found to have antibacterial, antiviral, antifungal, antitumor, immunosuppressive and cardiovascular activities.
Marine sponges produce a variety of unusual chemical compounds, but the ecological functions of these metabolites remain largely unknown. The objective of this study was to evaluate the content of polyphenols, flavonoids, and condensed tannins, as well as the antioxidant activity by evaluating the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent in the various extracts.
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Index Terms— Sponges, polyphenols, tannins, flavonoids, antioxedant activity.

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1 Introduction

Sponges are simple, multicellular, sessile animals with no true tissue layers or organs. They inhabit every type of marine environment, from polar seas to temperate and tropical waters, and are often more abundant and diverse in the tropics than stony and soft corals [1]. The marine organisms, such as sponges represent one of the richest sources of natural primary and secondary metabolites. Recently, the research focus for natural products has been shifted from terrestrial to marine sources because of the superior chemical and biological novelties of marine compounds. In general, natural molecules isolated from marine environment show higher and more significant bioactivity than those from terrestrial environment [2]. Most of the studies have been conducted on sponge's derived compounds to examine its pharmacological properties. Such compounds proved to have antibacterial, antiviral, antifungal, antimalarial, antitumor, immunosuppressive, and antioxydant activity [3]. The marine environment has proved to be a promising source of antioxidant compounds derived from sponges, sea weed and marine microbes exhibited good antioxidant properties [4].

This study is focused on the quantitative identification of organic substances such as: polyphenols, tannins, and flavonoids found in by various methods. The aim of this work is to evluate the antioxidative properties of extracts of five marine sponges of Atlantic Morrocan coast by the DPPH.

2 Materials and Methods

2.1 Sponge Materials

The marine sponges were collected in winter 2015 at the littoral Atlantic of El-Jadida (Morocco). All the sponges were identified by Dr. Maria-Jesús Uriz, Research Professor at the Centro de Estudios Avanzados de Blanes (CEAB) and Consejo superior de investigaciones científicas (CSIC) Spain. The collected materials were immediately frozen for one night prior to extraction [5].

2.2 Preparation of the extracts

Each sponge (100 g wet weight) was thawed, homogenized with ethanol-chloroform (50:50), allowed to stand in a dark chamber for 24 h and the solid-liquid separation is performed with a centrifuge (5000trs/min). The residue was again extracted with ethanol-chloroform (50:50). The extracts were combined, evaporated at reduced pressure until total evapora-

tion of solvents. The suspension is completed with distilled and sterile water to 100 ml as final volume and extracted with hexane. The hexane extracts were combined, dried on anhydrous sodium sulphate (Na_2SO_4), filtered and concentrated at reduced pressure to give a crude lipid extract (C). The aqueous phase is lyophilised and is twice dissolved in absolute ethanol then filtered and concentrated at reduced pressure to give a crude ethanol extract (B).

2.3 Determination of polyphenols contents

The determination of phenolic compounds was performed according to the method of reagent Folinciocalteu, 2.5ml of folin (diluted 10 times) was added to 0.5ml of the liquid extract (diluted 100 times). 2mL of Sodium carbonate Na_2CO_3 (75g/L) are added. The mixture was placed in a water bath maintained at a temperature of 50°C for 5 minutes protected from light. Absorbance was measured at 760 nm by a spectrophotometer UV-3100PC VWR. The total polyphenols content is calculated from the calibration curve established with gallic acid (calibration range 0-80µg/ml).The results obtained are equivalent in micrograms/mLgallic acid per gram of the raw material (mg GAE/g)[6].

2.4 Determination of condensed tannins (Cyanidin equivalent content)

Proanthocyanidin content was determined with a BuOH/HCl test as described by Rhandour et al [7] : 0.5 mL of aqueous extract was the tubes were covered and placed in a water bath at 95°C for 15 min. The absorbance was read at 530 nm and results were expressed as follows: milligram of cyaniding equivalent (Cya) per gram of dry (mgCya/g).The condensed tannins content was calculated using the formula given below:

$$\mathbf{ECya/g} = \frac{\mathbf{A \times V1 \times D \times M \times V2}}{\mathbf{l \times £ \times v \times m}}$$

Where A is the sample absorbance at 530 nm; V is the total reaction volume (ml); D is the dilution factor; M is the cyanidin molar mass (g mol^{-1}); V2 is the aqueous volume extract, recovered after extraction (ml); l is the path length (cm−1); £ is the molar extinction coefficient (34.700 l $mol^{-1}cm^{-1}$); **v** is 0.5 mL; and **m** is the dry weight mass of sponge (g).

2.5 Determination of flavonoids (Quercetin equivalent content)

The flavonoids are quantified by a colorimetric method with aluminum trichloride ($AlCl_3$) and sodium hydroxide (NaOH). Aluminum trichloride forms a yellow complex with flavonoids and soda form a pink complex which absorbs in the visible at 510nm [8]. 0.5 ml of each extract (or quercetin) is mixed with 2ml of distilled water, 0.15 ml of 150g/l solution of sodium nitrite ($NaNO_2$), 0.15mL of a 100g/l solution of aluminum trichloride ($AlCl_3$ $6H_2O$). After 6 min incubation at ambient temperature and addition of 2mL NaOH (1mol/l), the volume was brought to 5 mL distilled water, and after a further incubation of 15 min, the absorbance measured at 510 nm.

A standard range was performed under the same operating conditions using quercetin at different final concentrations. The results obtained expressed in µg.

Eqquercetin/mg of extract is calculated using the following formula:

$$[\text{Flavonoid}] = \frac{a \times f}{c}$$

Where a: flavonoid concentration (µg/ml) determined from the standard, f: dilution factor c: concentration of the extract.

2.6 Determination of DPPH, The activity of trapping of the radicals

The extracts (C & B) were dried after elimination of the solvent by using a rotary evaporator in 60°C under vacuum, then frozen and freeze-dried with a lyophilizator (ALPHA-2 LD) shielded from the light, and obtained a powder dry product. The antioxidant power of extracts was estimated with the method described by Yuanting Zhang [9], Based on the reduction of the free radical 1,1- diphenyl-2-picrylhydrazyl DPPH·, who is relatively stable. The extracts which have an antioxi-

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dant power changes their tint (coloring) from violet to yellow during the reduction of diphenylpicrylhydrazine DPPH. This essay requires the preparation of a range of concentrations of the samples from 0 to 10000µg/ml. In the assay, 0.5mL of diluted extract was mixed with 1.5mL of 0.1mmol/L solution of DPPH in ethanol. The mixture was incubated in the dark at room temperature for 30 min, and the absorbance at 517 nm was measured. All tests were performed in triplicate. The scavenging capacity was calculated as:

$$\% (AA) = [(Ac - As)/Ac] \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the tested sample after 30 min.

Butylatedhydroxytoluene (BHT) was used as standard. The free radical scavenging capacities of samples were expressed as IC_{50} values (concentration of samples required to scavenge 50% of DPPH. radicals).

2 RESULTS AND DISCUSSION

2.1 Dosage of polyphenols, tannins and total flavonoids

The determination of the total content of polyphenols, tanninsand flavonoids in different sponges is not homogeneous. The highest levels of all classes of polyphenols studied were EM3, EM9, and EM6 followed by EM13 (Table 1).

Table 1: The yields in polyphenols, Tannins, and Flavonoids of the extracts.

	Marine sponges				
	EM3	EM9	EM6	EM13	EM15
Polyphenols (mg GAE/g dry matter)	23.25±0.78	21,03±1.38	11.33±1.11	8.82±0.48	2.53±0.02
Flavoloids (µg eq quercetin/ mg of extract)	33952.3±2407.8	109871.7±4556.2	52314.8±2084.8	20477.7±435	3446.1±100.8
Tannins (mg Cya/gdry matter)	0.44± 0.03	0.17±0.00076	0.16±0.007	0.06±0.001	0,22±0,005

The variation in the total polyphenol content in the various sponges is remarkable, give the highest levels (23.25 and 21.03 mg GAE / g dry matter) respectively for EM3 and EM9, as well as moderately high levels in Sponges EM6 and EM13 (11.33 and 8.82 mg GAE / g dry matter).

The results of the flavonoid assay in the sponges (Table 1) show that the different crude extracts of EM6, EM9, EM13, EM15, and EM3 have high Flavonoid contents.

The results of the determination of the condensed tannins showed that the studied spices all contain condensed tannins. The highest levels are observed in EM3 than in EM15. This study of the different phenolic sponges could more accurately characterize biological activity.

2.2 Evaluation of the antioxidant activity by DPPH Test

The DPPH method is usually used to evaluate radical effects. The present invention relates to an antioxidant-based antioxidant. The DPPH test provides information on the potential reactivity of the test extracts with a stable free radical. It gives a strong absorption band at 517 nm of visible Spectrophotometer. As the electron becomes depleted in the presence of a free radical altering agent, the DPPH absorption and solution are decolorized in the oven and as the color changes from deep violet to light yellow. The degree of reduction in the measurement of absorbance is indicative of the antioxidant activity of the crude extracts. Table 2 present the Extract (C) of EM3 sponges and extract (B) of EM13 & EM6 sponges have the highest antioxidant activity; Their IC_{50} table 2 is the lowest among all study sponges. Extracts of EM3 and EM6 sponges possessed the concentrated polyphenols and flavonoids. Their high antioxidant activity (figure1) compared to other extracts, suggests the antiradical potential due to polyphenols and flavonoids.

Samples	Percentage inhibition of DPPH˙	IC_{50} (µg/µl)
EM13 (B)	67.06%	2339.74
EM6 (C)	92.91%	93.18
EM3(C)	83.57%	258.67
EM15(C)	63.06%	6707.48
EM13(B)	56.10%	8407.43
EM6(B)	59.4%	4987.4
EM9(B)	58.69%	4711.65

Table 2: Percentage inhibition and IC_{50} of samples

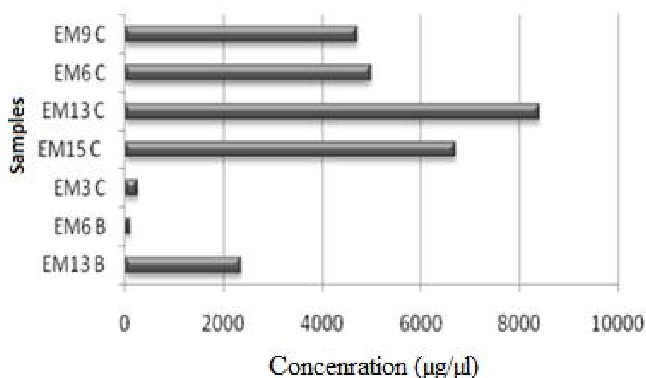

Figure1: Reducing power of the extracts studied.

3 Conclusion

This study shows that the content of phenolic compounds, flavonoids and condensed tannins depends on the type of sponge. The EM3 and EM9 sponges have the largest amounts of polyphenols, EM9 and EM6 are rich in flavonoids, EM3 and EM15 have high concentrations of condensed tannins.

As regards antioxidant activities, it is not found in all the sponge extracts of this study. However, the genera EM13 (C), EM6 (B), and EM3 (B) show the strongest activities with variations.

So it would be interesting to find out if compounds would cause antioxidant activities.

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