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ORIGINAL RESEARCH

Biochemical composition and antioxidant properties of the sponge *Suberites domuncula* from two regions of Moroccan Mediterranean waters

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Abstract Although the sponge is known to harbor secondary metabolites with biomedical potential, the chemical composition or primary metabolites of most sponge species remain understudied. Studies of their intraspecific variation are scarce. *Suberites domuncula* is one of the most studied sponges across the East Atlantic and Mediterranean soft bottoms, particularly in the trawlable bottoms of its continental shelves. Nevertheless, very little information if any is currently available regarding the biochemical composition of *S. domuncula*. This study aims to determine the antioxidant activity and assess the influence of geographical variability on the content of primary metabolites and the mineral composition of this species. The results show that the specimens from the regions of Oued Laou and Al Hoceima (Northern Morocco) contain significant amounts of polyphenols (TPC) and flavonoids (TFC) with interesting antioxidant activity revealed by DPPH test and the IC_{50} method. Additionally, the preliminary screening of the samples show a significant presence of sterol compounds and coumarins, free quinones and tannins in *S. domuncula* in the two areas. Furthermore, while geographical distance has no apparent effect the sponge's primary metabolites, ICP-OES findings show that the origin of the specimens has a significant effect on mineral elements. In summary, we find that *S. domuncula* shows a significant mineral concentration, especially Ca, Na, K, S, Mg, P and Fe. Overall, this first characterization shows that *S. domuncula* could have potential medicinal values and could also be a useful tool to monitor water quality in the Mediterranean coast of Morocco.

Keywords Antioxidant activity . Coastal water . Marine sponge . Mediterranean littoral . Mineral composition . Primary metabolites . Secondary metabolites . *Suberites domuncula*

Introduction

Marine sponges, classified in the phylum Porifera, represent some of the earliest multicellular animals on Earth (Li et al. 2023). They inhabit polar, tropical, and temperate seas, as well as various marine environments (Laport et al. 2009). As they do not have a physical defense system, sponges have developed a highly effective chemical defense mechanism, enabling them to synthesize defensive and toxic compounds.

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The compounds of the sponges exhibit chemical structures including lipids, sterols, alkaloids, quinones and others molecules. They are responsible for antimicrobial, anticancer, antibacterial, antifungal, antiviral and antimalarial activity (El-Shitany et al. 2015; Mabhiza et al. 2016). Sponge species have the highest concentration of natural antioxidants compared to all marine species. The antioxidants, which slow down oxidation by preventing the production of free radicals, are widely used in medicine, cosmetics, and the food industry (Tangman et al. 2015). The free radicals are unstable substances formed during oxidation reaction, they contribute to the development of a number of health problems such as cancer, cardiovascular diseases, Alzheimer's disease and nervous system abnormalities (Balakrishnan et al. 2014). Polyphenols and flavonoids are among the most natural antioxidants. They limit the effects of free radicals, aging, and diseases, and provide a wide range of benefits for human health (Yi et al. 2017).

Marine sponges exhibit a distinct skeletal framework and chemical composition, including inorganic and organic components. Functioning as filter feeders, they consume microorganisms and plankton present in the filtered water (Riisgård et al. 2016). The organic content of marine sponges is influenced by factors such as food availability, geographic location, and environmental stress. During autumn and winter, the sponge exhibits reduced food consumption (Koopmans et al. 2015).

Furthermore, demosponges are regarded as exceptional marine biomonitoring organisms due to their distinctive characteristics and adaptability to diverse ecological niches (Mayzel et al. 2014). Additionally, it is widely acknowledged that the geochemistry of the ocean significantly influences marine life and all associated biological processes. In the realm of biological processes, only approximately a dozen elements from the marine environment are categorized as macro-elements. Phosphorus, sodium, potassium, calcium, magnesium, and sulfur constitute the predominant components of living biomass (Mayzel et al. 2014). The growth and development of organisms rely on these macro-elements as well as on first-row transition metals like manganese, iron, and zinc (Morel et al. 2003). Specifically, sponges exhibit selectivity in accumulating these dissolved metals in seawater (Kubiak et al. 2022) . However, the contribution of this study lies in the lack of research on the marine demosponge *S. domuncula* in Moroccan coastal waters.

The objective of our work is to examine the variability in oxidative activity, primary and secondary metabolites, and elemental composition of *S. domuncula* specimens gathered from the northern Moroccan regions of Oued Laou and Al Hoceima.

Materials and methods

Sponge collection

The sponge *Suberites domuncula* was collected at Oued Laou and Al Hoceima on the Mediterranean Sea (35.57833 N, -5.07 W and 35,2959488N, -3,7909930W, respectively) (Fig.1). After the collection, the samples were washed, frozen at -30°C and then placed in a freeze-drying chamber equipped with a heating tray. Freeze-drying was done at -70°C and at low pressure.

It was confirmed the sponge identity by spicule preparation for light microscopy, according to Cristobo et al. (1993). In brief, a small representative piece of the specimen was dissolved with an industrial bleaching agent and mounted on a microscopic slide. Then, observations of the spicules were made using a Leica DM IRB inverted microscope in the Instituto de Ciencias Del Mar (ICM-CSIC) and were used to identify the species using the relevant literature for the area.

Reagents

All the preparations were carried out using analytical-grade reagents supplied by Sigma Aldrich, with the exception of the standard solutions, which were used for calibration. They were prepared by diluting a stock solution (1000 µg/mL) for each element studied (K, Ca, Na, Mg, Mn, Fe, S, Zn and B). The stock solutions were supplied by Merck Millipore (Certiur®, Darmstadt, Germany). Nitric acid (HNO3, 65%) and argon has a purity of 99.99% and has been used as a plasma and nebulizing gas.

Determination of polyphenol

To prevent polyphenol oxidases from degrading phenolic compounds, the extraction of polyphenols was carried out by macerating 50mg of powdered sponge in 2ml of 80% acetone for an hour at 4°C. The centrifugation process was then started to recover the supernatant containing polyphenol. For a full extraction of the extra polyphenol and to have a more complete dose, the extraction was done on the pellet a second time over the course of one night. In the end, the two supernatants were added to the dosage (Boizot and Charpentier 2006).

Determination of polyphenols using the folin-ciocalteu reagent

To evaluate the presence of polyphenols in the samples, we put an extract of 100 µl in tubes together with 400 µl of carbonate sodium (75 g/l), and 500 µl of folin reagent, which had been diluted ten times. We then shook the tubes and incubated them at 40°C for five minutes. After 60 minutes, the absorbance test was carried out using a (Model Jenway 6705 UV/Vis) spectrophotometer at 735. We used gallic acid to elaborate the standard range, with concentrations ranging from 0 to 500 ppm (Boizot and Charpentier 2006).

Determination of flavonoids

The flavonoid levels in the extracts were quantified using the colorimetric method based on the procedure outlined by Bijla et al. (2022). With minor modifications. In short, a 10 ml volumetric flask was filled with 1 ml of diluted sample to which 0.3 ml of 5% sodium nitrite ($NaNO_2$) was then added. After five minutes, 0.3 ml of aluminum chloride (AlCl3, 10%) was added, the mixture was allowed to stand for another six minutes, followed by the addition of 2 ml of 1 M sodium hydroxide (NaOH) and finally 10 ml of distilled water to obtain the final solution. After mixing the solution completely, it was allowed to stand for 30 minutes. At 415 nm, absorbance was finally measured using a (Model Jenway 6705 UV/Vis) spectrophotometer. The rutin equivalent of TFC was calculated using the calibration curve of rutin standard solutions, expressed as mg rutin/g extract.

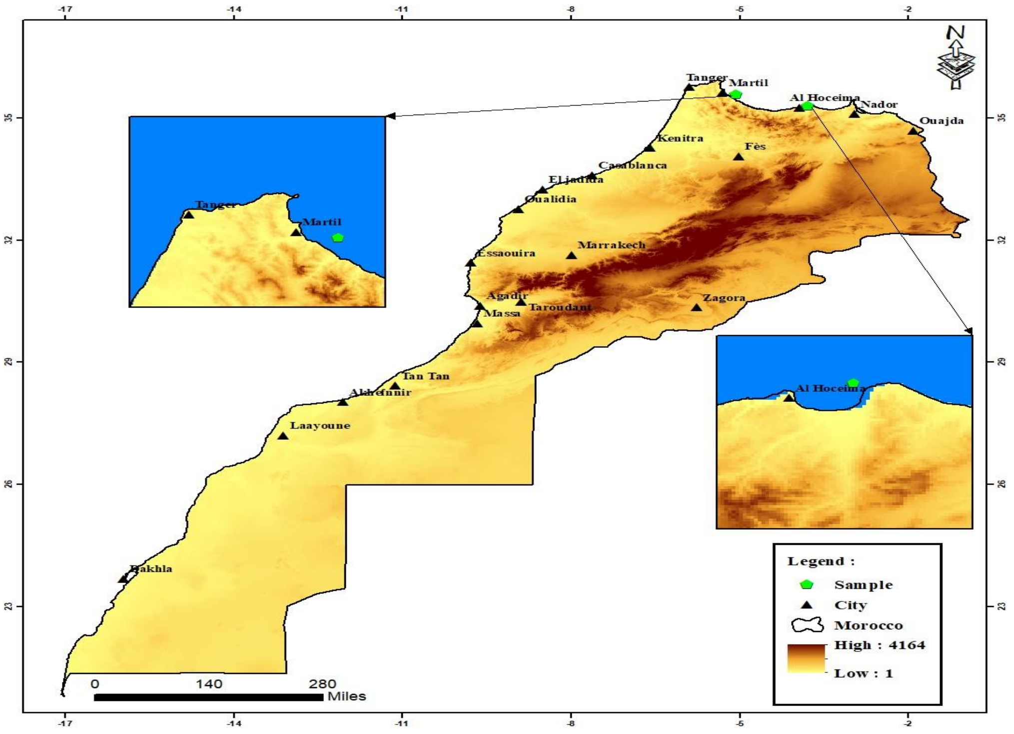

Fig. 1 The collect sites of *S. domuncula* in Northern Morocco

Antioxidant activity

Using the Krimat et al. (2017) approach, we used DPPH (1,1-diphenyl-2-picrylhydrazyl) to measure antioxidant activity. In the presence of antioxidants, DPPH changed from its initial purple color to a yellow hue. The capacity of antioxidants to provide protons to the media affects how intense the color is. By saturating 4 mg of DPPH in 100 ml of ethanol, the DPPH solution was made. The obtained extract was then mixed with 1.5 ml of the DPPH solution at a variety of ethanol strengths. The next step was stirring and a 30-minute dark incubation period. The spectrophotometer (Model Jenway 6705 UV/Vis) was used to assess the mixture discoloration at 517 nm in comparison to the negative control, which contained only the DPPH solution. The extract under test was used as the positive control and the absorbance measurement was carried out using ascorbic acid solution as the reference antioxidant. By running a linear regression on the computed inhibition percentages in accordance with various quantities of produced samples, the IC_{50} value, the concentration of the extract needed to neutralize 50% of free radicals was determined.

Secondary metabolites

To screen secondary metabolites, preliminary tests were performed using methodologies of (Rhandour et al. 2016). The tests revealed the presence of several chemical groups known for biological activities (Dohou et al. 2003).

Coumarins

Coumarins were detected using the Békro method (Bekro et al. 2007). In a nutshell, it was made by adding 0.5 ml of 10% NaOH to 2 ml of ethanoic solution. After heating, cooling and adding 4 ml of distilled water to the solution, the mixture would make it transparent in comparison to the control. A few drops of concentrated HCl were used to confirm the presence of coumarins. When the solution lost its yellow color, it formed a precipitate or became cloudy.

Free quinones

One gram of powdered sponge was mixed with 15 to 30 ml of petroleum ether in a tube. After 24 hours of stirring and resting, the extract was filtered and placed in the evaporator. When a few drops of 1/10 NaOH were added, the aqueous phase turned yellow, red, or purple, indicating the presence of free quinones.

Tannins

Tannin screening began with the addition of 1.5 g of powdered sponge sample in 10 ml of 80% methanol (MeOH), followed by 15 minutes of shaking, filtration, and pouring the filtrate into tubes. The presence of tannins was detected by adding FeCl3. If the color changed to blue-black, it indicated the presence of gallic tannins, and if it changed to greenish-brownish, it indicated the presence of catechic tannins.

Saponosides

The identification of saponosides started with the preparation of 10 ml of fully aqueous sponge extracts to be poured into test tubes. The tubes were shaken for 15 seconds after standing for 15 minutes. Saponosides were detected by the presence of persistent foam with a height exceeding 1 cm.

Sterol substances

This study used the Liebermann-Burchard reaction as a reference for identifying sterol compounds. The experiment was carried out as follows: three grams of powdered sample were macerated in 15 ml of chloroform for 20 minutes, then filtered and the mixture concentrated to 2 ml, followed by adding 1 ml of acetic anhydride and 1 ml of concentrated sulfuric acid. If sterol compounds were present, the color would change from red-brown to brown-violet.

Macromolecules

Lipids

We used the Soxhlet extraction to determine the presence of lipids. The sample was extracted with hexane, which progressively solubilized the fat. Through a series of pours, the solvent containing the fat was pulled into the flask. Once the extraction finished, the solvent was evaporated according to the technique of (Rhandour et al. 2016). Using the following formula, we measured and calculated the amount of fatty matter and its percentage:

$$\%\text{lipids} = \frac{\text{P2} - \text{P1}}{\text{PE}} \times 100$$

P1: Weight of the empty flask, P2: Weight of the flask filled with grease, and PE: Weight of the test sample.

Proteins

Using the Dumas technique in accordance with AOAC 992.15, PC was measured based on nitrogen measurement using combustion by an LECO FP628 Protein Analyzer (LECO Corp., MI, USA) (AOAC 2005). The measured nitrogen was then converted to crude protein (CP) reported as a percentage using a 6.25 factor.

Carbohydrates

We used the Dubois technique, thoroughly documented Dubois et al. (1956) , to determine the total amount of sugar. Its basis is the dehydration of the simple carbohydrates with strong sulfuric acid, condensation of the resulting orange-yellow complex with phenol, and determination of the optical density at 490 nm. The standard range was created using a glucose solution with a series of dilutions between 0 and 0.4 mg/ml.

Moisture content

The method used to calculate moisture content, represented as a percentage of mass, was somewhat different from that used by Gharby et al. (2017). A vented oven (Precision Scientific Co., USA) was used to bake 5g of sponge powder for 3 hours at 103 °C to get a consistent weight. The findings of the final two analyses differed by 0.01g of moisture per 100g of material.

Ash content

In a freeze-dryer, the sponge samples were frozen and dried. The samples were all then finely ground. Afterwards, one gram of dry powder was calcined for four hours at 500°C in the oven. To treat the resultant ash, 4ml of 65% HNO_3 and 10ml of HCL were added (AOAC 1990).

Determination of mineral elements

Mineral elements (K, Ca, Mg, B, Fe, Zn, Mn, P, and Na) were identified according to Faez and colleagues (Mohammed et al. 2013). One gram of each powdered sample was weighed into crucibles and then placed in a muffle furnace at 525°C for 4 hours to complete calcination. The resultant ash was subsequently broken down by nitric acid (65%) in the hood. Each crucible received around 2ml of concentrated acid while the remaining 100ml were made up of ultra-pure water. The resultant liquid was put into polypropylene flasks that had been smoothed and sealed so that the suspended particles could settle for 24 hours. Following decantation, the measurement of mineral elements was carried out with the use of an ICP-OES spectrometer

(Jobin Yvon, Optima).

Statistical analysis

We conducted the ANOVA tests using the SPSS 21 software. The Pearson correlation test was completed using OriginPro software.

Results

Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity with DPPH methods

In this study, we examined the polyphenol content (TPC) of acetone extract, flavonoid content (TFC) and 50% DPPH free radical inhibition capacity of dichloromethane extract and ethanolic extract of *S. domuncula* collected from two different regions (Table 1). The results of spectrometric methods showed that the amount of polyphenol in *S. domuncula* collected in Al Hoceima (29.51 mg GAE /g DW ±0.05) was higher than the amount of TPC of *S. domuncula* collected in Oued Laou (13.072 mg GAE /g DW ±0.01). Similarly, the ethanolic extract of *S. domuncula* of Al Hoceima had a higher amount of TFC (39.66mg RE /g DW±0.003) compared to that of Oued other Laou (28.15 mg RE /g DW±0.03). The dichloromethane extract of the specimen of Oued Laou had the highest amount of TFC (73.92 mg RE /g DW± 0.04) compared to that of the other area (73.92 mg RE /g DW± 0.04).

In addition, the capacity to scavenge 50% of DPPH free radicals by *S. domuncula* from Al Hoceima is higher than that of *S. domuncula* from Oued Laou. Moreover, we observed that the ethanolic extract of *S. domuncula* from Al Hoceima had a higher antioxidant activity (IC_{50} equal to 173 µg/ml ±0.003) than the dichloromethane extract (IC_{50} equal to 240 µg/ml ±0. 004). The dichloromethane extract of *S. domuncula* from Oued Laou (IC_{50} equals 182.04 µg/ml ±0.04) was higher than that of the ethanol extract (IC_{50} equals 307 µg/ml ±0.03). Furthermore, the two-way ANOVA analysis showed highly significant differences between extract types, origin of sponge and their interaction.

Secondary metabolites

In this preliminary research, the presence of secondary metabolites in *S. domuncula* extracts from different regions was examined. The results of this study showed the presence of sterol substances, coumarins, tannins, free quinones and saponins in specimens from Oued Laou and Al Hoceima. The presence of saponins was only detected in specimens from Al Hoceima (Table 2).

Macromolecules

The results of this study are summarized in Table 3. They show variation between the macromolecular content of different *S. domuncula* samples. In the study, we found that protein content was higher in *S. domuncula*. The specimens from Oued Laou presented 27.57% ± 0.001 of protein while the one collected from Al Hoceima presented 21.87% ± 0.03 of protein, followed by carbohydrates that were moderately abundant macromolecules. We found that *S. domuncula* collected from Al Hoceima had a carbohydrate content of 4.89% while the specimens collected from Oued Laou had 4.21%. However, we report low lipid content

Table 1 Mean values of antioxidant activity, flavonoids and polyphenols of *S. domuncula* from Al Hoceima and Oued Laou

Components or activity		*S. domuncula*	
		Oued Laou	Al Hoceima
Polyphenols	Acetone extract (mg GAE /g DW)	13.072 ±0.0^a	29.51±0.05^b
Flavonoids	Ethanolic extracts (mg RE /g DW)	28.15±0.03^a	39.66±0.003^b
	Dichloromethane extracts (mg RE/g DW)	73.92 ± 0.04^d	67.522±0.004^c
Antioxidant activity (IC_{50})	Ethanolic extracts (µg/ml)	307±0.03^a	173±0.003^c
	Dichloromethane extracts (µg/ml)	182.04±0.04^c	240±0.004^b

The results are presented as mean ± SD of triplicates measurements. Different superscript letters for each extract of each parameter indicate significant differences at P ≤0.05.

in *S. domuncula*. Samples collected from Oued Laou had 2.15% ±0.14 lipids while those collected from Al Hoceima had 2.02%± 0.11 lipids. The ANOVA test results presented in Table 3 reveal that the origin of primary sponge metabolites does not have a significant effect, including proteins (p=0.618), carbohydrates (p=0.537) and lipids (p=0.366).

Moisture content and ash content

As shown in Table 3, we found a very significant difference between the moisture content in the sponges in the two regions, p=0.002. Specimens from the Oued Laou region had a higher moisture value (6.9% ±0.14) than those from the Al Hoceima region (4.97% ± 0.02). Moreover, the ANOVA test showed a very significant difference between the inorganic matter content of *S. domuncula* in the two regions, p=0.000. The specimens of *S. domuncula* collected in Al Hoceima had a high inorganic matter content (66.16% ± 0.02) compared to those collected in Oued Laou (58.77% ± 0.17).

Composition of mineral elements

We analyzed four microelements in *S. domuncula*: zinc, manganese, iron and boron in addition to six macroelements: sodium, phosphorus, potassium, calcium, magnesium and sulfur. As Table 4 shows, calcium is the most abundant element in Oued Laou specimens while the most abundant element in Al Hoceima specimens is sodium. The other mineral compositions of this sponge from the two areas are listed in the following order: S > K > Mg > P > Fe > Zn > Mn > B. Moreover, the specimens of *S. domuncula* collected from Oued Laou presented the highest concentrations of macroelements Ca, Na, K, S, P and microelements Zn and B. The specimens from Oued Laou presented higher concentration of Mg, Fe, B and Mn. The ANOVA test shows that the origin of the sponge has a highly significant effect on its mineral elements. Specifically, the mineral elements, including Ca, K, Fe, Mn, Zn, B and P, all have p=0.00 values, compared to Na (p=0.005), Mg (p=0.002) and S (p=0.041).

Table 2 Determination of the chemical composition belonging to the secondary metabolites of the sponge *S. domuncula* from Al Hoceima and Oued Laou areas

Substances	Al Hoceima	Oued Laou
Coumarins	+++	+
Free quinones	++	++
Tanins	+++	++
Saponins	+	-
Sterol substances	+++	+++

(-): none detected, (+): trace amounts, (++): medium amounts (+++): high amounts

Table 3 Mean values of primary metabolites, moisture and ash in *S. domuncula* from Al Hoceima and Oued Laou areas

Chemical composition	Site	Mean ± SD	P-value
Lipids (g/100g)	Al Hoceima	2.02± 0.11	0.366
	Oued Laou	2.15± 0.14	
Protein (g/100g)	Al Hoceima	21.87± 0.03	0.618
	Oued Laou	27.57± 0.001	
Carbohydrates (g/100g)	Al Hoceima	4.89 ± 0.007	0.537
	Oued Laou	4.19 ± 0.017	
Moisture (g/100g)	Al Hoceima	4.97± 0.02	0.002
	Oued Laou	6.9± 0.14	
Ash (g/100g)	Al Hoceima	66.16± 0.02	0.000
	Oued Laou	58.77± 0.17	

Correlation study

The correlation matrix between the variables is presented in Fig. 2. Significant positive and negative correlations were found between the dependent variables. We found that Ca was positively correlated with Na, K, Zn, P and S. Na also showed a positive correlation with K, Zn, P and S. Thus, K was positively correlated with Zn, P and S. Mg was positively correlated with Fe, Mn, B and ash. Fe showed a positive correlation with Mn, B and ash. Mn had a strong positive correlation with B and ash. Zn was positively correlated with P and S. Finally, lipids were positively correlated with carbohydrates.

Discussion

Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity with DPPH methods

Regarding the set objectives related to the antioxidant properties of marine sponges, several studies such as El-Shitany et al. (2015) show that marine sponges represent a natural and rich source of new substances that have antioxidant properties. Seradj et al. (2012) showed that dichloromethane extract *Fascaplysinopsis* reticulate sponge had a significant antioxidant activity. Furthermore, dichloromethane extracts from the sponge in our study have very high antioxidant activity compared to dichloromethane extracts from the sponges reported in Seradj et al. (2012). Additionally, previous studies indicate that sterols from marine

Table 4 Mean values of mineral elements (μg/ g) in *S. domuncula* from Al Hoceima and Oued Laou

Mineral elements	Site	Mean ± SD	P-value
Ca	Al Hoceima	19642.71±65.67	0.000
	Oued Laou	34274.04±55.65	
Na	Al Hoceima	27300.76±179.69	0.005
	Oued Laou	29144.24±52.81	
K	Al Hoceima	3875.81±7.75	0.000
	Oued Laou	6485.038±19.55	
Mg	Al Hoceima	2782.60±4.95	0.002
	Oued Laou	2699.62±3.83	
Fe	Al Hoceima	1165.72±7.08	0.000
	Oued Laou	319.57±0.47	
Mn	Al Hoceima	40.21±0.11	0.000
	Oued Laou	30.28±0.06	
Zn	Al Hoceima	126.84±1.04	0.000
	Oued Laou	176.64±0.19	
B	Al Hoceima	23.38±0.04	0.000
	Oued Laou	19.02±0.04	
P	Al Hoceima	1361.80±7.39	0.000
	Oued Laou	1571.45±3.51	
S	Al Hoceima	9705.49±80.66	0.041
	Oued Laou	10023.69±50.26	

sponges have antioxidant properties (e.g., Aknin et al. 2010). In this study, *S. domuncula* specimens showed the presence of sterol compounds, which could indicate a role in the antioxidant activity observed. However, the antioxidant activity may be due to the high content of TPC (Sulaiman et al. 2011). The higher amount of polyphenol in *S. domuncula* from Al Hoceima may explain the high free radical scavenging capacity (50%) of this sponge compared to the others.

Polyphenols and flavonoids are two natural antioxidants that provide defense against oxidative damage such as cardiovascular disease (Yi et al. 2017). Yet, the quantity of TPC and TFC can vary between species due to the external variables as light, depth, salinity, nutritional content, seasonality and environmental stresses. Thus, intrinsic factors like tissue type and age influence the amount of TPC in species (Connan et al. 2004). The variation in TPC and TFC between the two specimens in this study may be due to one or both of the factors above. We found that our TFC results are higher than those of *Hyrtios aff. Erectus* sponge from the Red Sea coast in Egypt (Nabil-Adam et al. 2020). In addition, our TPC results are superior to those of TPC of different extracts from 46 sponge specimens collected in Mauritius (Oogarah et al. 2020). However, they are lower than the results of extracts of sponges in the coast of Turkey (Orhan et al. 2021).

Secondary metabolites

Marine sponges proved to be the richest source of bioactive compounds such as saponins, coumarins, tannins, free quinones and sterol substances. Some of them have demonstrated a wide range of biological activities that are very useful in various disciplines like medicine (Krishnan and Keerthi 2016). The production of active metabolites is often attributed to the endosymbiotic microorganisms present in the sponges and not directly to the sponges themselves, as observed in Sibero et al. (2019) and Zhang et al. (2022). One study showed the presence of certain secondary metabolites such as sterols and tannins in the ethanolic extract of *Aaptos suberitoides* (Cuong et al. 2019). Furthermore, this preliminary study shows that *S. domuncula* is relatively rich in various secondary metabolites, which indicates it could have a potential use in the pharmacological field. This requires further research using more precise analytical methods.

Macromolecules

Macromolecules are the main constituents of various life forms. They help determine the biological structures of species. In terms of composition, the most common macromolecules are proteins, and *S. domuncu-*

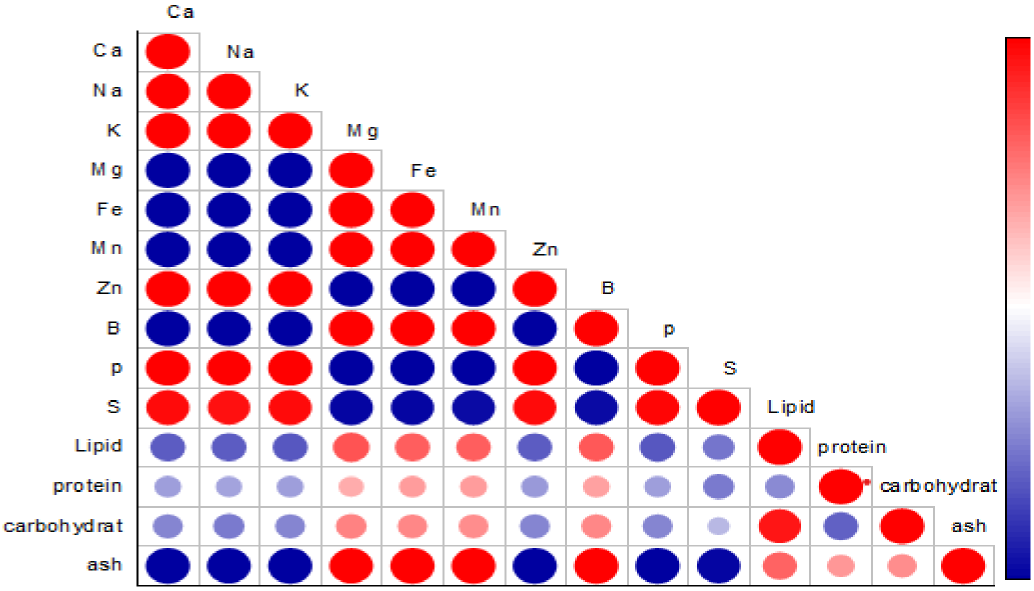

Fig. 2 Coefficients of correlation among parameters
Large dark blue circles indicate a strong negative correlation between parameters, while small light blue circles indicate a negative but lower correlation. On the other hand, dark red circles represent a strong positive correlation, while smaller red circles indicate a weaker positive correlation between parameters.

la has higher protein content (Hadas et al. 2005). The protein content found in *S. domuncula* in this study is higher than those reported in Pallela and Janapala (2013). According to several studies, the physical stress which sponges are subjected to can result in an increase or decrease in protein concentration. Sponges exposed to wave force and water movement, which varies by location, may require more structural protein while those in relatively calm waters may require less (Criddle 2017).

In this study, lipids were the least abundant macromolecules in *S. domuncula*. Under climate change, they are likely to play an important role in the sponge's stress response (Bennett et al. 2018).Our results are lower than those reported in Watson et al. (2014). In the same context, Bell et al. (2017) show that lipids are primarily a source of metabolic energy. The high lipid content in sponges may indicate that there is an accumulation of energy storage products in response to increased food availability and, therefore, a variable dependence on food abundance (Watson et al. 2014). In addition, lipid concentration in sponges varies with season; it is high in spring and very low in winter. The variation is consistent with the variation in lipid concentration in suspended particles (Watson et al. 2017). In this regard, it seems that the high protein and low lipid content in *S. domuncula* sponges in this study is due to food shortage and probably the collection season. Also, the regions are known for their harsh environmental conditions during the collection time. However, carbohydrates are moderately abundant macromolecules. Carbohydrates of marine origin, especially sponges, have received much attention because of their health benefits including antioxidant, anti-infectious, anticoagulant, anti-inflammatory and antidiabetic effects (Kang et al. 2015). The data obtained from the sponge in Al Hoceima and Oued Laou are superior to that of *Amphimedon queenslandica*, collected from Heron Island Reef, Australia (Watson et al. 2017). The variation in carbohydrate content is due to different factors.

Furthermore, according to Bayona et al. (2021), the metabolic composition of sponges undergoes significant changes with age and depth.

Moisture content

According to numerous studies, sponges are known for their ability to filter seawater. The high water retention that occurs in their skeletons allows them to retain moisture (Gaino et al. 2010). The results of this research are lower than those previously observed (e.g., Cuong and Hien 2020). Previous research suggested that the season and temperature in the collection area may affect the change in moisture content of marine sponges (Batista et al. 2014). In our study, we collected the samples from Oued Laou in November and those from Al Hoceima in February. The result of the variation in moisture content is due to the conditions associated with the collection season and the temperature where the specimens live. Furthermore, variations in humidity have an effect on microbial populations such that the bacterial community grows in proportion to moisture content.

Ash content

Spicules of inorganic material are the major skeletal components of sponges. Silicon is also the first enzyme involved in the formation of the inorganic siliceous skeleton of *S. domuncula* (Krasko et al. 2000)are spicules formed from inorganic material. The data obtained in this study was superior to those found in *Geodia barretti* , *Geodia atlantica* , *Stelletta normani* , *Phakellia ventilabrum*, and *Axinella infundibuliformis* collected in Korsfjord, Norway (Martins et al. 2021). However, physiological and morphological adaptations related to their environment are common in sponge species. Thus, an increase in the inorganic skeleton of the sponge makes it stiffer and more resistant to physical pressures (Dahihande and Thakur 2021). In this regard, it seems that these characteristics are probably responsible for the difference found among the specimens under study.

Composition of mineral elements

This study indicates that *S. domuncula* had a very high amount of calcium especially the samples collected from Oued Laou. The amount is necessary for the adhesion of sponge cells and essential in the composition of the skeletons of organisms. This allows them to cope with climatic stress as shown in several studies (Vilanova et al. 2016; Ribeiro et al. 2021). The values we find are higher than those reported in Aguilar-Camacho et al. (2019). Since the industrial revolution, the world has suffered from increasing global warming global which threatens marine environment (WMO 2020). According to Ribeiro et al. (2021), temperature increase and pH decrease influence Ca content in the ocean. According to the literature, both collection areas have a higher amount of Ca (Achahbar et al. 2020; Benaissa et al. 2022). Therefore, the variation in Ca concentration between the two locations is related to the environment of each specimen. Furthermore, according to Wang et al. (2013), juvenile *S. domuncula* have more sodium content than adults.

One of the most abundant chemical elements in nature is sulfur. There are over 3,000 distinct sulfur compounds found in a variety of biological environments, including the oceans (Sandford 2003). According to scientific research, sulfur was found in the skeletons of *verongiid demosponges* (Klinger et al. 2019). Based on our study, *S. domuncula* from the two regions have a high amount of sulfur. However, these amounts are lower than those reported in Nabil-Adam et al. (2020). Small sulfur-containing compounds are widely recognized as antioxidant agents that reduce oxidative damage (Battin and Brumaghim 2009). Thus, they play a crucial role in highly oxidative environments and they are necessary for normal cell function and health. Potassium content is essential for nutrient transport in the cell. Our results show that the potassium content is higher than that found in *Geodia barretti, Geodia atlantica, Stelletta normani*, and *Axinella infundibuliformis* and lower than the content found in *Phakellia ventilabrum* (Martins et al. 2021) Previous studies show that newly synthesized spicules of *S. domuncula* have a concentration of five to six times higher than spicules in adult specimens. Thus, high potassium concentration in specimens give a reflection on specimen age (Wang et al. 2013). Specifically, *S. domuncula* from Oued Laou appears to be younger because it has twice the potassium content of the Al Hoceima specimen.

Magnesium is another element present in seawater. In this study, the values we found are lower than those reported in Batista et al. (2014). This is expected because previous research demonstrated that demosponges were rich in Mg (Batista et al. 2014). In addition, Jones and Jenkins (1969) showed that Mg content increased with the size of the elements that made up the sponge skeleton (and thus perhaps the rate of growth). Through relationship with the Mg content, some research found that there was an increase in Mg in an environment composed of warmer water (Niedermayr et al. 2013). However, other studies such as Smith et al. (2013) showed that temperature had no effect on Mg content in sponges. Studies are needed to show results that confirm whether or not heat has an effect on Mg increase. The slight variation between Mg content in *S. domuncula* specimens is probably related to physiological conditions.

The last macronutrient in our screening is phosphorus. It is essential in several vital biological processes. It is typically supplied to the marine environment in dissolved and particulate form by rivers and runoff (Karl 2014). Sponges can play an important role in providing it to other species such as coral reefs that would otherwise be unable to receive sufficient P from nutrient-poor waters (López-Acosta et al. 2019). The amount we found in our samples are lower than those in Zhang et al. (2015). Moreover, the composition of the microbiome associated with the sponge directly influences the amount of P (López-Acosta et al. 2019). This may explain the difference recorded between the *S. domuncula* specimens in this work. In terms of microelements, the major element is iron. It is also the fourth most prevalent element in the earth crust (Zahra et al. 2021). It is present in seawater in dissolved form and is considered a vital nutrient for marine organisms. Iron is available in seawater from several sources, such as wastewater discharge (Perez et al. 2005) . The results we find show a lower content than that of *S. domuncula* collected in an industrial area in Brittany (Gentric et al. 2016). However, the Al Hoceima area shows rejection, while in the Oued Laou area there is no rejection. In line with Vacelet et al. (1988), the amount of iron varies considerably following the environment and the age of the fiber. In this regard, the significant difference between *S. domuncula* from different areas seems to be due to the environmental and physiological characteristics of the sponge.

The second most abundant element is zinc. It is an essential microelement for the growth of microorganisms, animals and plants. It plays as much a role as micronutrients do for phytoplankton and ensures their growth (Sinoir et al. 2012). It is also essential for enzymes like carbonic anhydrase (Lane and Morel 2000). In this study, the amounts of *S. domuncula* are lower than those of *Suberites* cf. collected in Lake Macquarie (de Mestre et al. 2012). According to Hansen et al. (1995), demosponges have very weak correlation between Zn accumulation and environmental factors. However, the Zn content of the demosponge most likely reflects the nutritional and physiological processes that influence Zn fractionation or utilization efficiency (Hendry and Andersen 2013). Thus, an alternative hypothesis is that the primary source of Zn input to the sponge is particulate organic matter (POM) rather than dissolved Zn in seawater (Mayzel et al. 2014). In this regard, we cannot suggest that the considerable variation between the sponges from two areas is attributed to physiology and the POM on which the organism feeds.

Manganese is one of the most widespread metals in the natural environment. It can get into oceanic environment by domestic and industrial wastewater. In this study, we find that *S. domuncula* samples have a much lower amount of Mn than *Phyllospongia foliascens* collected in Mkomani Beach and Nyali Beach (Ohowa et al. 2021). Yet, the area of Al Hoceima is known for the presence of a treatment plant, which is not the case for the area of Oued Loau. Hence, the difference between specimens from the two areas is probably related to the environment of the sponge samples.

In addition, the last element is boron, found in rocks, soil and water; it stimulates cell growth in animals. These findings are similar to what is reported in Mayzel et al. (2014). According to Furst (1981), sponges from warm regions contain much less boron than those from temperate regions with high productivity. Therefore, the low amounts of bore in our sponge samples can be attributed to the fact that they are from less temperate, low-productivity marine regions.

In this context, a study of nine *Haliclona* species from the Irish and Mediterranean coasts confirmed that the mineral composition of spicules was strongly influenced by the interactions between sponges and their environment (Aguilar-Camacho et al. 2019).

Conclusion

Numerous biochemical compounds with antioxidant properties are present in natural marine species, particularly sponges. The first part of this study considers the variation in energy content between *S. domuncula* from the Al Hoceima and Oued Laou areas. The results show that the origin has no influence on the energy content of the sponge samples. Thus, we find that proteins are the most abundant macromolecules, followed by carbohydrates, and last lipids. On the other hand, the sponge of the two regions presents a significant number of polyphenols (TPC), flavonoids (TFC) and antioxidant activity (DPPH). There are potentially sterol substances, coumarins, free quinones and tannins in *S. domuncula* from both regions. This indicates that the sample sponge might have medical applications.

In the second part of the study, we investigate the geographical influence on the variability of macro and microelement concentrations in *S. domuncula* from the Al Hoceima and Oued Laou regions. The data show that the geographical origin has a significant influence on the mineral composition of the sponge under study. Consequently, sponges can be a useful tool to monitor the quality of the Mediterranean coast in Morocco.

Declaration of interest conflict The authors affirm that they do not have any conflicts of interest.

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