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Chemical characterization of *Cinachyrella tarentina*: Sponge of Atlantic Moroccan Coast

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ABSTRACT : *Currently, marine organisms are a very important source of new molecules in pharmacology and thus in the development of new bioactive products. Sponges, in particular, given their very primitive origin and persistence during evolution, have developed a chemical defense system.The chemical study of Cinachyrella tarentina, marine sponge recognized by its antibacterial and antifungal activity was investigated for the first time in Morocco. The screening of Cinachyrella tarentina revels that it contains different levels of primary and secondary metabolites. The dosage of polyphenols was carried out using the reagent Foulin-Ciocalteu. The antioxidant activity was evaluated by the DPPH test. The fatty acid composition determined by Gas chromatography-mass spectrometry (GC/MS) showed a predominance of palmitic and stearic acids. Furthermore, we found the presence of several sterols which cholesterol and sitosterol are the most abundants.*
Keywords: *Antioxydant activity, Chemical composition, Cinachyrella tarentina, Marine sponge, Polyphenols.*

INTRODUCTION

The marine environment represents unquestionably a treasure of organisms generating plethora of primary and secondary metabolites that would benefit from further exploration [1]. This environment is very diverse and marine organisms, particularly the most primitive of them, produce a variety of bioactive molecules [2]. Marine sponges are the most primitive multicellular animals and contain many primary and secondary metabolites, including lipids, alkaloids, and polyphenols [3]. Sponges are sessile marine filter feeders that have developed efficient defense mechanisms against foreign attackers such as viruses, bacteria, or eukaryotic organisms. Marine sponges are among the richest sources of pharmacologically-active chemicals from marine organisms. It is suggested that (at least) some of the bioactive secondary metabolites isolated from sponges are produced by functional enzyme clusters, which originated from the sponges and their associated microorganisms. More than 5,300 different products are known from sponges and their associated microorganisms, and more than 200 new metabolites from sponges are reported each year [4]. Most of the bioactive compounds from sponges consist of anti-inflammatory, antitumor, immunosuppressive (or) neurosuppressive, antiviral, antibiotics, antifouling and antimalarial properties [5]. The chemical composition of Moroccan sponges has not been extensively investigated. The aim of the present work was to analyse the chemical composition of the marine sponge *Cinachyrella tarentina* and to investigate the antioxidant activity by evaluating the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. Also we studied the chemical composition of fatty acid and sterols of iso-octanic extract of the marine sponge *Cinachyrella tarentina*.

MATERIALS AND METHODS

2.1. Biological material and sampling site

The studied sponge (*Cinachyrella Tarentina*) was collected during summer season, in June 2011 to the beach Deauville of El Jadida (N 33 15 140 ; W8° 29 930) at low tide to a depth of 3 to 5 meters (Figure 1, harvesting site). After harvest, marine sponge was washed, frozen and lyophilized.

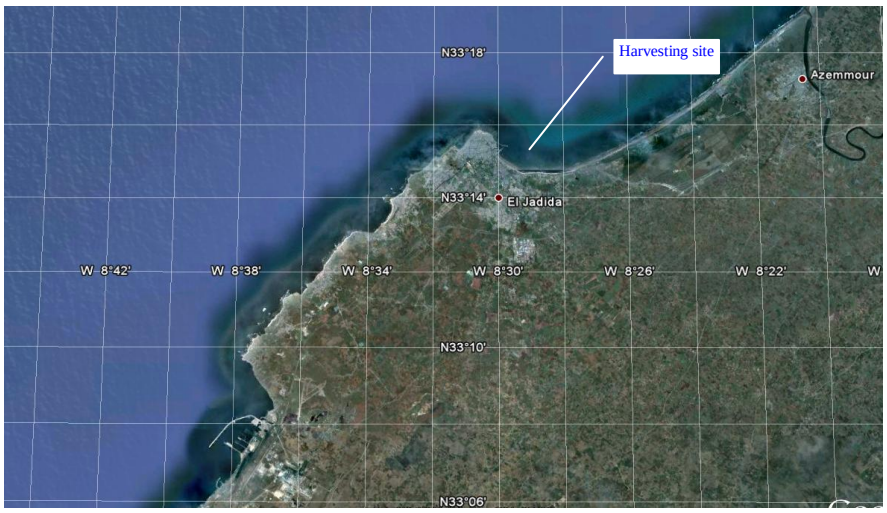

Fig. 1. Map showing the harvesting site of marine sponge *Cinachyrella tarentina*

The systematic identification of the marine sponge was carried out by Dr. Maria-Jesús Uriz, Professor at the Centro de Estudios Avanzados de Blanes (CEAB) and Consejo Superior de Investigaciones Científicas (CSIC), Spain. The in situ photo of Cinachyrella tarentina is shown in figure 2.

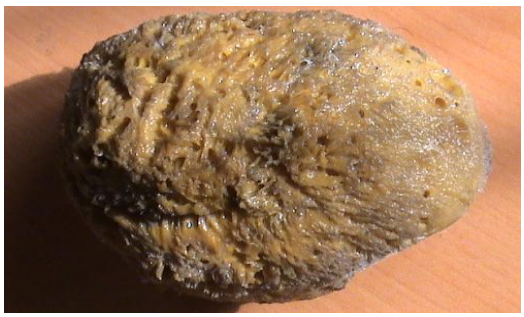

Fig. 2. Collected sponge *Cinachyrella tarentina*

2.2. Chemical composition of the marine sponge

The determination of the chemical composition has consisted in dosage of the primary metabolites and to search some secondary metabolites known for their biological activity.

2.2.1. Compounds belonging to the primary metabolism

Dosage of lipids

The dosage of lipids was realized by using a soxhlet. The sample was continuously extracted with hexane which gradually dissolves the fat. The solvent containing the fat returns into the balloon by successive spills. Once the extraction was complete, the solvent was evaporated, and the fat was weighed and the percentage was calculated using following formula:

$$\%lipides = \frac{M(lipides) \times 100}{M(échantillon)} \qquad (1)$$

Dosage of total sugars

The dosage of total sugars was performed using phenol /sulfuric acid method as described previously [6]. Briefly, in the presence of concentrated sulfuric acid, the oses were dehydrated and the products condense with phenol to give yellow-orange complex. Then, we measured the optical density at 490nm. A standard was prepared from a solution of glucose with different dilutions ranging from 0 to 0.4 mg /ml.

2.2.2. Study of the chemical composition of the marine sponge (preliminary tests)

The tests were conducted according to the standard techniques described by Paris and Moyse [7], Bouquet [8], and Debray et al [9]. These tests consist to highlight the presence of a certain number of chemical groups known to their biological activities [10].

Research of alkaloids

The test based on the ability of alkaloids to combine with the heavy metal or with iodine was performed.
Mayer Test: 0.5 g of powder material was added to 15 ml of 70% ethanol. After a long agitation, the extract was allowed to stand until complete decantation, followed by filtration and evaporation. The residue was taken up in a few ml of 50% HCl. After adding a few drops of Mayer's reagent (mercuritetraiodure potassium), the formation of a yellow precipitate, indicated the presence of alkaloids.

Research of sterol

The research was based on the Lieberman-Burchard reaction [9]. Three grams of dry ground material were macerated in 15 ml of chloroform for 20 minutes. Then, the mixture is filtered and concentrated to 2 ml. One milliliter of acetic anhydride and 1 ml of concentrated sulfuric acid were successively added. The presence of sterol compounds gives a red-brown color veering to purplish-brown.

Research of saponosides

It is based on the determination of the index of moss. In a 500 ml conical flask, 100 ml of boiling water and 2 g of ground material were introduced. Boiling was maintained for 30 minutes. The mixture was filtered, cooled and adjusted to 100 ml.
In a series of ten test tubes were successively introduced 1, 2, 3, 4, ... 10 ml of decocted, all tubes were filled to 10 ml with distilled water and agitated vigorously for 15 seconds. After standing for 15 minutes, the height of moss was measured. If the latter was equal to 1 cm, the dilution of the substance in the tube corresponds to the index of moss searched. An index greater than 100 was considered as a positive reaction indicating a wealth of material in saponosides.

Research of tannins

1.5 g of dry ground material was placed in 10 ml of 80% methanol. After 15 minutes of agitation, the extracts were filtered and placed in tubes. The addition of 1% $FeCl_3$ can detect the presence or not of tannins. The color turns to blue-black in the presence of gallic tannins and to greenish brown in the presence of catechin tannins.

Research of free quinines

One gram of dry ground material was placed in a tube with 15-30 ml of petroleum ether. After agitation and standing for 24 h, the extract was filtered and concentrated on a rotary evaporator. The presence of free quinones was confirmed by adding a few drops of NaOH 1/10, when the aqueous phase turns yellow, red or purple.

Research of anthraquinones

At the chloroform extract was added aqueous KOH 10% (v/v). After agitation, the presence of anthraquinones is confirmed by a turn of the aqueous phase to red.

Research of coumarins

An infused at 10% or an alcoholic extract was examined under UV light. A bluish fluorescence indicates a positive reaction.
Note: Other compounds also show a marked fluorescence to UV. This revelation is therefore only an indication.

Research of flavonoids (Reaction to cyanidin)

At 2 ml of infused, 2 ml of hydrochloric alcohol and some magnesium turnings were added. An orange to red color appears in the presence of flavonoids.

2.2.3. Dosage of the polyphenols

Extraction of phenolic compounds

The polyphenols were extracted by maceration of 1g of powder in 20 ml of absolute ethanol, for 48 hours. After centrifugation, the supernatant containing the polyphenols was recovered. Therefore, we proceed to a second extraction overnight on the base to extract supplementary polyphenols to obtain a dosage more comprehensive. The two supernatants were added to the dosage.

Dosage of the polyphenols using the Folin-Ciocalteu reagent

To 0.5 mL of extract (diluted 100 times), 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times) and 2 mL of sodium carbonate (75g / l) were added to the tubes. After agitation, the tubes were incubated 5 min at 50 ° C at the dark, and the absorbance was read at 760 nm by a spectrophotometer. A standard range was prepared by gallic acid in concentrations ranging from 0 to 500 ppm [11].

2.2.4. Evaluation of the antioxidant activity DPPH test

The antioxidant activity was measured by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. In this test the antioxidants reduce the DPPH from a violet color to a yellow compound, the diphenyl picryl hydrazine, the intensity of the color is inversely proportional to the ability of the antioxidants present in the medium to give protons. The solution of DPPH was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol. 0.5 ml of the ethanolic extract at different concentrations [0-10 mg/ml] was added to 1.5 ml of the ethanolic DPPH solution. After agitation by a vortex, the mixture was left in the dark for 30 min at room temperature and discoloration relative to the negative control containing only DPPH solution was measured at 517 nm by a spectrophotometer. The positive control was represented by a standard solution of an antioxidant, ascorbic acid (vitamin C) and the absorbance was measured in the same conditions as the test sample. The antioxidant activity, that expresses the capacity of trapping the free radical, was estimated by the percentage of discoloration of the DPPH dissolved in ethanol [12]. Inhibition of DPPH free radical in percent (I%) was calculated as follows: [13]

$$I\% = \frac{A_C - A_t}{A_C} \times 100 \quad (2)$$

A_c is the absorbance of the negative control, and A_t is the absorbance of the test compound.
Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.2.5. Study of the composition of fatty acids and sterols

Extraction and isolation of sterols and fatty acids

In the laboratory, the lyophilized biological material, ground and weighed is emerged in a mixture of chloroform-ethanol solvent (CHCl3 / EtOH; v / v) for 48 h. Then the solution obtained is evaporated under reduced pressure at 40°C. The extraction is repeated several times until complete exhaustion of the biological material. The crude extract obtained after concentration is weighed to calculate the yield. It is then taken up in a mixture equivolume of isooctane and methanol to give the methanolic and isooctanic phases. The two phases are then subjected to evaporation of the solvent to achieve the methanolic and isooctanic extracts. The isotonic extract of the studied sponge was subsequently subjected to two specific protocols in order to get fatty acids and sterols.

5g of the isotonic extract is saponified in alcoholic potassium hydroxide, the saponifiable fraction contains fatty acids in salt form. An acidification followed by extraction with diétylic ether leads to obtaining a mixture of fatty acids in free form (0.97g). The obtained fatty acids are methyled by esterification with methanol in the presence of boron trifluoride (20%) in excess and reflux at 100 ° C for 15 min. The obtained methyl esters of fatty acids are analyzed by gas chromatography coupled to mass spectrometry.

The analysis of the unsaponifiable fraction of the isooctanic extract of the studied sponge, by thin layer chromatography (TLC) in an eluent system 2,2,4- trimethyl - pentane (TMP) / ethyl acetate (EA) 7/3 shows that it has the same frontal report as cholesterol. Spraying with sulfuric acid (50%) followed by oven drying at110 °C for 10 minutes reveals purple spots, characteristic of sterols. The fractionation of the unsaponifiable fraction (4g) is followed on an open column of silica gel using the same couple of eluent (TMP / EA) in order to get the sterol fraction. The sterol fraction (yield (1.47g); frontal report (0.3); white color) obtained by eluent system TMP / EA (8/2) was recrystallized in methanol, silylated with 50µL of pyridine and 40µL of MSTFA at 50 °C for 2h, and injected in gas chromatography coupled to mass spectrometry (CG/MS) After adding 910µL of isooctane.

Chromatographic conditions of analysis by GC / MS

DB-5 column ((30 m × 0.32 mm)
Injector: Initial temperature 80 °C and then increase to 100 °C / min up to 300 °C.
Column: initial temperature 80 °C (maintained 2min) and then increase to 6 °C / min up to 320 °C (maintained 25min).

Identification of compounds by GC/MS analyses

The identification of a part of compounds was accomplished using computer searches on a NIST MS Data library and another from the mass spectra of reference compounds analyzed.

RESULTS AND DISCUSSION

3.1. Compounds belonging to the primary metabolism

The averages levels in lipids and carbohydrates of *Cinachyrella tarentina* were shown in Table 1:

Table 1. Averages levels of lipids and carbohydrates expressed in percentage relative to the dry weight.

Lipids	Carbohydrates
4% (+/- 0.05)	17.85% (+/- 0.2)

From the Table 1, *Cinachyrella tarentina* represents a source of primary metabolites with a significant proportion of carbohydrates compared to lipids. The carbohydrates are recognized by their role in the mechanism of recognition and cellular interactions of the sponge. Some sugars may be related to lipids, thus forming glycolipids: molecules implicated in many physiological processes and showed a proven pharmacological activity [14-15].

3.2. Compounds belonging to the secondary metabolism:

These substances are known to be responsible for the pharmacological activity. The results of tests of the chemical composition are presented in the table 2.

Table 2. Determination of chemical composition of the studied sponge

Chemical substances	**Results of the tests**
Sterol substances	+++
Saponins	+++
Tannins	+
free quinones	+
Anthraquinones	+
Flavonoids	+
Alkaloids	+++
Coumarins	+

(+ + + : presence in abundance, +: Presence in low quantity)

Acording to table 2, we observed the presence of different chemical compounds recognized by their biological activity with high concentration of alkaloids, sterol and saponins substances.
Therefore according to our preliminary tests, we found that *Cinachyrella tarentina* produced molecules with potential biological interest. In the case of particular species of sponges, it seems that the production of these active metabolites does either not directly related to the organisms themselves but rather to heterotrophic endosymbionts (bacteria) or autotrophic (microalgae and cyanobacteria). The ability to produce such metabolites is of obvious interest in the context of the search for new molecules presenting interesting therapeutic potential [16].

3.3. Determination of polyphenols

The polyphenols content was determinate from ethanolic extract, expressed in milligram of gallic acid equivalent per gram of dry weight. The polyphenol content was 3.61 % (+/- 0.02) mg GAE/g DW.

3.4. Evaluation of the antioxidant activity by DPPH Test

The results of antioxidant power show that the percentage of inhibition of ethanolic extract of Cinachyrella tarentina is equal to 77.2% (+/- 0.8) at a concentration of 10 mg/ml.
The IC50 value determined in mg / ml expressing the inhibitory concentration of extract required to inhibit 50% of the initial DPPH free radical (Table 3).

Table 3. Result of the antioxidant test expressing the inhibitory concentration (IC50) in mg / ml

Extracts / Standard	IC50
Ethanolic extract	1.8 mg/ml (+/- 0.5)
Ascorbic acid	0,13 mg/ml (+/- 0.03)

The IC50 of the ethanolic extract of the sponge is 1.8 mg/ml, this value remains low compared to ascorbic acid; and used as a reference is 0.13 mg/ml and thus indicates that this sponge contains the modest antioxidants, which is consistent with the result obtained for the low content of polyphenols in the studied sponge.

3.5. Study of the composition of sterols and fatty acids

3.5.1. Sterols composition

The silylated sterols of *Cinachyrella tarentina* were investigated by GC/MS (qualitative analysis). The data are reported in Table 4. The studied sponge contains the usual complex mixtures of sterols for marine invertebrates, all of them identified earlier in different sponges. Cinachyrella tarentina contains therefore principally the unsaturated sterols, with a predominance of cholesterol and sitosterol.

Table 4: Chemical composition of silylated sterols of the studied sponge

Peak	Ret Time (min)	Area%	Component Name	Formula
1	40.141	236648	-	-
2	40.632	1.311e+6	Cholesterol TMS	$C_{30}H_{54}OSi$
3	41.089	556246	Brassicasterol TMS	$C_{31}H_{54}OSi$
4	41.814	328392	Campesterol TMS	$C_{31}H_{56}OSi$
5	42.112	212427	Stigmasterol TMS	$C_{32}H_{56}OSi$
6	42.897	2.363e+6	Sitosterol TMS	$C_{32}H_{58}OSi$

(TMS= Trimethylsilyl)

3.5.2. Fatty acids composition

The methyl esters of fatty acids of *Cinachyrella tarentina* were investigated by GC/MS (qualitative analysis). The data are reported in Table 5. No study has been performed in Morocco on the fatty acid composition of *Cinachyrella tarentina.* This study allowed us to identify the variety of fatty acids contained in *Cinachyrella tarentina* with predominance of palmitic and stearic acids. The mixture of sodium salts of these two acids led to the preparation of ordinary hard soap.

Table 5: Chemical composition of methyl esters of fatty acids of the studied sponge

Peak	Ret Time (min)	Area%	Component Name	Formula
1	4.9	8.42E+07	Tridecylic acid, methyl ester	$C_{14}H_{28}O_2$
2	5.6	1.21E+08	Stearic acid, methyl ester	$C_{19}H_{38}O_2$
3	7.8	7.37E+07	Palmitoleic acid, methyl ester	$C_{17}H_{32}O_2$
4	8.2	1.90E+08	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$

5	9.5	4.90E+06	Heinicosylic acid , methyl ester	$C_{22}H_{44}O_2$
6	11.9	4.40E+07	Oleic acid , methyl ester	$C_{19}H_{36}O_2$
7	12.5	5.43E+07	Margaric acid, methyl ester	$C_{18}H_{36}O_2$

CONCLUSION

The dosage of primary metabolites revels that *Cinachyrella tarentina* represents a source of carbohydrates and lipids with a significant proportion of carbohydrates compared to lipids. The Screening of Cinachyrella tarentina revels that it contains different levels of secondary metabolites. The dosage of polyphenols was carried out using the reagent Foulin-Ciocalteu. The antioxidant activity was evaluated by the DPPH test, the results of this test indicates that *Cinachyrella tarentina* contains the modest antioxidants, which is consistent with the result obtained for the low content of polyphenols in the studied sponge. The fatty acid composition determined by Gas chromatography-mass spectrometry (GC/MS) showed a predominance of palmitic and stearic acids. Furthermore, we found the presence of several sterols which cholesterol and sitosterol are the most abundants. In the current investigation, the extracts from *Cinachyrella tarentina* gave good results indicating that it possesses significant amount of chemicals compounds and in vitro antioxidant activity. Phenolic compounds and other chemicals compounds appear to be responsible for the in vitro antioxidant activity of the extracts and may contribute to the therapeutic activity observed. On the basis of the results obtained, *Cinachyrella tarentina* extracts are rich sources of natural antioxidants appears to be an alternative to synthetic antioxidants. Further investigation to determine antioxidant activity by in vivo methods could be considered.

REFERENCES

[1] J.M Kornprobst, Encyclopedia of Marine Natural Products, *Wiley-Blackwell, 3 vol*, 2010.

[2] J.P Bergé, and G Barnathan, Recent advances in fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically-active compounds and economical aspects. In: Le Gal Y, Ulber R (eds)Marine Biotechnology. *Adv Biochem Eng Biotechnol, Springer,96*, 2005, 49-125.

[3] Adv Biochem Engin/Biotechnol, 96, 2005, 49-125.

[4] M. S Laport, O.C.S Santos, and G Muricy, Marine Sponges: Potential Sources of New Antimicrobial Drugs, *Bentham Science,10(1)*, 2009, 86-105

[5] E Fattorusso, and O Taglialatela-Scafati, *Mar Drugs, 7(2)*, 2009, 130-152.

[6] M Dubois, K.A Gilles, J.K Hamilton, P.A Rebers, and F Smith. *Analytical Chemistry , 28 (3)*, 1956, 350-356.

[7] R Paris, and H Moses, "Précis of medicinal material", Paris, Masson (1969).

[8] M Bouquet, "Works and documents of Orstom", (1971) (Paris, No. 13).

[9] M Debray, H Jacquemin, and R Razafindrambo, "Works and documents of the Orstom" (1971) (Paris, No. 8).

[10] N Dohou, K Yamni, S Tahrouch, L.M Idrissi Hassani, A Badoc, and N Gmira, Bull. *Soc. Pharm. Bordeaux , 142*, 2003, 61-78.

[11] V.L Singleton, and J.A Rossi, Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am. J. Enol. Vitic. 16*, 1965, 144–158.

[12] Y Zhang, Q Li, H. Xing, X Lu, L Zhao, K Qu, and K Bi, Evaluation of antioxidant activity of ten compounds in different tea samples by means of an on-line HPLC–DPPH assay, 53(2), 2013, 847–856.

[13] N BOUGANDOURA, N BENDIMERAD, Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de Satureja calamintha ssp.Nepeta(L.) Briq, Revue « Nature & Technologie ». *B- Sciences Agronomiques et Biologiques, n° 09,* 2013, 14-19

[14] E Fattorusso, A Mangoni , Marine Glycolipide. *Progress in the Chemistry of Organic Natural Products 72*, 1997, 215-301.

[15] V Costantino, E Fattorusso, A Mangoni , M Di Rosa , A Ianaro , *Bioorg. Med. Chem. Letters 9*, 1999, 2-276.

[16] A Bendaoud, Photosynthetic symbionts of sponges: Isolation, cultivation and production of active metabolites, Thesis (2007).