Research Article

Isolation of secondary metabolites from the mediterranean sponge species; *Hemimycale columella* and its biological properties

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Abstract

Despite the richness and biodiversity of invertebrates and algae in the Mediterranean Sea, these organisms are still poorly studied. The objective of our research is the discovery of bioactive lead compounds from the Mediterranean Sea sponge *Hemimycale Collumella* (HC). HC sponge (189.0 g) was collected from Mdiq costs on the Mediterranean Sea and extracted with methanol to yield (10 g) which was then subjected to fractionation. A bio-guided protocol was applied through evaluation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and Oxygen Radical Absorbance Capacity (ORAC), α-amylase, β-glucosidase, pancreatic lipase inhibition as well as anti-collagenase, anti-elastase, antityrosinase and cytotoxic activity. 2,3-*O*-Hexahydroxydiphenoyl-(α/β)-glucose (1) and gentisic acid 2-*O*-β-glucoside (2) were isolated from the water fraction, quercetin-3-*O*-β-glucopyranoside (3), kaempferol 3-*O*-β-glucopyranoside (4) and isorhamnetin 3-*O*-β-glucopyranoside (5) from n-butanol fraction, gallic acid (6) from ethyl acetate fraction and gallic acid-3-methyl ether (7) from methylene chloride fraction. Compound 5 had the highest DPPH and ORAC activity. Compounds 1–5 had promising lipase inhibition activities which exceeded that of the standard Orlistat, while compounds 1–7 showed anti-tyrosinase activity higher than that of the standard Hydroquinone monomethyl ether. This is the first report for evaluation of the biological activities of 2, 3-*O*-hexahydroxydiphenoyl-(α/β)-glucose (1), gentisic acid 2-*O*-β-glucoside (2) and gallic acid-3-methyl ether (7).

Keywords *Hemimycale collumella* · 2,3-O-hexahydroxydiphenoyl-(α/β)-glucose · Quercetin-3-*O*-β-glucopyranoside · Gallic acid · Gallic acid-3-methyl ether

1 Introduction

The increasing incidence of health problems caused by certain cancers, drug-resistant pathogenic microbes, parasitic protozoans and fungi have evoked the interest for newer and more effective therapeutic agents. It is acknowledged that marine invertebrates produce bioactive natural products that may be useful for developing new drugs. By exploring untapped geographical sources and/or novel groups of organisms, one can maximize the search for new marine drugs to treat human diseases. Secondary metabolites obtained from marine sponges have

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been shown to be biologically active compounds with anticancer and antimicrobial activities [1].

From 1963 to 2005, about 24,662 new compounds were isolated and identified from various marine macro- or micro-organisms [2]. Among the oldest multicellular invertebrate organisms are marine sponges that belong to Porifera phylum, which have various colors and shapes [3]. More than 10,000 species of marine sponge were authenticated and described [4]. Marine sponges are a very attractive source of bioactive natural products as they yielded a diverse variety of bioactive compounds, among which about 10 bioactive compounds had reached late phases in clinical trials [5, 6]. This fact demonstrates our interest in marine sponges for drug discovery and development [6]. Marine sponges of phylum Porifera are rich sources of biologically-active compounds, these compounds are biosynthesized by clusters of functional enzyme in the sponges and their associated microorganisms. Each year about 200 new metabolites are reported from sponges [5, 6]. Among these compounds are the anti-viral drug vidarabine [9-β-D-arabinofuranosyladenine (ara-A)], which was effective against herpes simplex encephalitis virus, the beta-carboline alkaloid, manzamine A was proved effectiveness against malaria, tuberculosis and HIV, in addition to the antifungal macrolide compound, lasonolides and the antibacterial psammaplin A [5].

The Mediterranean Sea is acknowledged for its rich biodiversity and high endemism that provide strong motivation to explore the chemical diversity of new interesting metabolites with pharmaceutical applications. However, despite the richness and biodiversity of the Moroccan sea, invertebrates and algae from the seabed are poorly studied. Accordingly, the objective of this research project aims at the discovery of new anti-tumor and bioactive lead compounds from the poorly investigated Moroccan marine sponges collected from the Mediterranean Sea. Members of the genus *Hemimycale* (Family Hymedesmiidae, Order Poecilosclerida) were reported to have many bioactive secondary metabolites such as guanidine alkaloids [7, 8] and hydantoin derivatives which were isolated from *H. Arabica* obtained from the Red Sea [9, 10]. Our plant of interest is *Hemimycale collumella*, the species of Hemimycale, which is widely distributed across the Atlanto-Mediterranean basin. No reports were found concerning its chemical composition and biological activities, so it was deemed of interest for our team to explore its bioactive constituents through a bio-guided fractionation protocol.

2 Materials and methods

2.1 General experimental procedures

1H-NMR spectra were measured by a Jeol ECA 500 MHz NMR spectrometer, at 500 MHz. 1H chemical shifts (δ) were measured in ppm, relative to TMS and ^{13}C NMR chemical shifts to DMSO-*d6* and converted to TMS scale by adding 39.5. FTESIMS spectra were measured on a Finnigan LTQ-FTMS (Thermo Electron, Bremen, Germany) (Department of Chemistry, Humboldt-Universitat zu Berlin). ORAC experiment was performed on fluorometer, FLUOstar OPTIMA, Franka Ganske, BMG LABTECH, Offenburg, Germany. UV recording were made on a Shimadzu UV–Visible-1601 spectrophotometer. Paper chromatographic analysis was carried out on Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) 6% acetic acid in water (6% AcOH); (3) BAW (n-butanol– acetic acid – H_2O, 4:1:5, upper layer). Sephadex LH 20 (Pharmacia, Stockholm, Sweden). All chemicals and reagents for in vitro assays were purchased from (sigma, USA). Plates were purchased from (Mekkawy, Egypt). Spectrophotometric measurements were done on ELX 808 (Bio Tek Instrumental, Italy).

2.2 Hemimycale columella (HC)

The marine sponges were collected in March 2014 from Mdiq costs on the Mediterranean Sea by "Institut National de Recherche Halieutique" (Casablanca, Morocco) and identified later at the zoology department in "naturalis biodiversity center" (Leiden, Netherlands) as *Hemimycale columella* (RMNH POR 10,012) by Nicole J. de Voogd (National Museum of Natural History).

2.3 Extraction of hemimycale columella (HC)

Sponge material was exhaustively extracted with MeOH (2 L × 3) at room temperature (RT) and the total extracts were concentrated under vacuum at 40 °C in a rotary evaporator. The HC collection (189.0 g wet weight) yielded 10 g of crude viscous oily methanol extract. The extract was suspended in distilled water (200 ml) and subjected to subsequent liquid – liquid partition afforded n-hexane (HCH), methylene chloride (HCL), EtOAc (HCE), n-BuOH (HCB) and residual water fractions (HCW). Each fraction was concentrated under vacuum to yield 72 mg, 321 mg, 467 mg, 1.367 g and 1.450 g of HCH, HCL, HCE, HCB and HCW, respectively.

2.4 Radical scavenging activity using DPPH assay

The estimation was done according to the method of Brand-Williams et al. (1995) [11]. All experiments were carried out in triplicate. Ascorbic acid was used as positive control.

2.5 Oxygen radical absorbance capacity (ORAC assay)

The antioxidant capacity in phosphate-buffered saline (10 mM, pH 7.4) were assayed by measuring the time of fluorescein fluorescence decay (Sigma), produced by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) in comparison with the positive control trolox control [12, 13].

2.6 Alpha amylase inhibition assay

The α-amylase bioassay method was adopted [14] using acarbose as a standard. The starch solution (0.5% w/v) was obtained by stirring potato starch in 20 mmol/L sodium phosphate buffer (pH 7.0) and 6.7 mmol/L sodium chloride. The tested sample was dissolved in the buffer (31.25–1000 µgm/L). The colorimetric reagent was prepared by mixing sodium potassium tartrate solution (12.0 g /8.0 ml of 2 M Na OH) and 96 mmol/L of 3,5-dinitro salicylic acid solution. Both control and extracts were mixed with starch solution and left to react with α-amylase solution in alkaline conditions at room temperature. The generated maltose by measuring the absorbance of 3-amino5-nitrosalicylic acid resulted from the reduction of 3,5-dinitrosalicylic acid at 540 nm.

2.7 β-Glucosidase inhibition assay

1.0 ml of 0.1 M acetate buffer (pH 5.0) was mixed with 0.5 ml of 20 mM (603 mg p-nitrophenyl-β-D-glucopyranoside/100 ml of H_2O) (PNPG) and incubated at 37 °C for 5 min then mixed with 0.5 ml of the β-glucosidase enzyme (50 mM in Tris–HCl buffer pH 7.8 and diluted in 10 mM phosphate buffer pH 7.0) and incubated again for 15 min at 37 °C. 2.0 ml Na_2CO_3 solution was used to stop the reaction. The absorbance was measured at 400 nm [15–17].

2.8 Pancreatic lipase inhibition assay

The inhibition of pancreatic lipase was determined as described by [18] using orlistat as a positive control.

100 µL of the substrate 4-nitrophenyl octanoate (NPC) (5 mmol.L^{-1} in DMSO) was mixed with 4 mL of Tris–HCl buffer (pH 8.5), 100 µL of each tested sample and 100 µL of aqueous solution of porcine pancreatic lipase (1 mg/mL). The mixture was incubated at 37 °C for 25 min. The absorbance was measured at 412 nm.

2.9 Anti-collagenase assay

25 µl of collagenase type 1 from Clostridium histolyticum (1 mg/ml), 25 ul tris(hydroxymethyl)-methyl-2-aminoethane sulfonate (TES) buffer (50 mM) with 0.36 mM calcium chloride (pH 7.4) and test sample or epigallocatechin gallate which was used as a positive standard (EGCG) (1.4 mg/ml in DMSO) were incubated at 37 °C for 20 min. 100 µl N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) was added to the mixtures and kept again for 1 h at 37 °C. 200 µl of a mixture of equal volumes of 200 mM citrate buffer (pH 5) and ninhydrin were then added. The mixtures were incubated at 100 °C for 5 min then 200 µl of 50% isopropanol were added. The absorbance was measured at 540 nm [19].

2.10 Anti-elastase assay

The anti-elastase activity was assessed in accordance with the method of Kraunsoe et al. [20], with minor modifications. The test samples (25 µl) or standard (1.4 mg/ml in DMSO) were incubated at 25 °C for 20 min with 25 µl of human leukocyte elastase (1 µg/ml) and 25 µl 4-(2-hydroxyethyl)- 1-piperazineethane sulfonic acid (HEPES) buffer (pH 7.5). The substrate N-Methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide (1 mM) (100 µl) was then added and the mixtures were incubated for 40 min at room temperature. Absorbance was read at 405 nm using Elafin as a positive control.

2.11 Anti-tyrosinase assay

Inhibition of tyrosinase was determined using hydroquinone monomethyl ether as positive standard [21]. Mushroom tyrosinase enzyme (5600 units/ml) (80 µl) was incubated with 80 µl of 1 mg/ ml test sample or standard at 37 °C for 15 min. Then 40 µl of L-3, 4-dihydroxyphenylalanine (L-DOPA) was added and incubated at 37 °C for 30 min. Absorbance of the formed Dopachrome is measured at 475 nm.

2.12 Neutral red uptake assay (NRU)

Non-tumorigenic HaCaT cells, bladder carcinoma cell line (5637 cells), breast carcinoma (MCF7), hepatocellular

carcinoma (Huh7) were obtained from the Vaccera (Giza, Egypt). Cytotoxicity of the fractions and isolates against the four cell lines was carried out using the neutral red uptake (NRU) assay [22]. Measurement was done at 450 nm in a plate reader and IC_{50} values were determined and expressed in mean ± SD. All samples were tested in triplicate using Etoposide as positive control.

2.13 Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard deviations (SDs). The IC_{50} (concentration necessary for 50% inhibition of enzyme activity) was calculated by constructing a linear regression curve showing extracts concentrations percentage inhibition on the y-axis[23]. All analysis were done using the SPSS v. 22.0 (IBM, Chicago, USA). Microsoft Excel 2010 was used for graph construction.

2.14 Isolation and identification of the major phenolic compounds from the bioactive fractions

HCW (1 g) was fractionated using Sephadex LH-20 column (50 gm) and elution was done by H_2O/ H_2O–MeOH mixtures where fractions (I and II) were obtained and investigated using 2D-PC (two dimensional paper chromatography). Fraction I (eluted with 10% MeOH in water, 320 mg) was then purified over Sephadex LH-20 (20 g) using H_2O for elution to yield compound **1** (20 mg) in a pure from. Compound **2** (15 mg) was separated by purification of fraction II (eluted with 20%, MeOH in water, 730 mg) over Sephadex LH-20 (25 × 1 cm, 20 g) using H_2O as eluent.

HCB (1 g) was fractionated as for HCW to yield three fractions (III-V). Fraction IV yield Compound **3** (32 mg) (elution with 30% MeOH in water, 210 mg) using preparative PC and 6% AcOH (solvent). Fraction V yields compound **4** (25 mg) and **5** (30 mg) (eluted with 60% MeOH in water, 435 mg) by preparative PC using 6% AcOH (solvent). HCE fraction (300 mg) was fractionated over Sephadex LH-20, using MeOH–H_2O mixtures of different polarities. The fraction eluted with 80% MeOH in water (200 mg) from which pure samples of compound **6** (40 mg) was isolated using preparative PC and BAW as solvent system.

HCL (100 mg) was purified over several Sephadex LH-20 (20 g) using MeOH for elution to yield compound **7** (18 mg).

2.14.1 Compound (1)

R*f* values: 0.66 (H_2O), 0.78 (6% AcOH in water), 0.22 (BAW), UV λ_{max} (nm) in MeOH: 259 (inflection).
^{1}H- NMR spectral data (DMSO-*d6*) δ (ppm):

Glucose moiety: 5.32 (d, *J* = 3.5 Hz, *α*-H-1″), 4.90 (*J* = 8 Hz, *β*- H-1″),4.64 (dd, *J* = 8 &7.5, *β*-H-2″), 4.83 (dd, *J* = 3.5 & 8 Hz, *α*-H-2″), 4.93 (t, *J* = 7.5 Hz, *β*-H-3″), 5.25 (t, *J* = 8 Hz, *α*-H-3″), 3.3–3.8 (m, H-4″'-H-6″).Hexahydroxydiphenoyl (HHDP) in *α*-& *β*-anomers: 6.53, 6.54, 6.61, 6.62 (s, H-3 & H-3′).

^{13}C-NMR Spectral Data (DMSO-*d6*)δ (ppm): *α*-glucose moiety: 90.50 (C-1″), 72.06 (C-2″), 77.43 (C-3″), 67.24 (C-4″), 77.01 (C-5″), 61.05 (C-6″).*β*-glucose moiety:93.77 (C-1″), 74.72 (C-2″), 79.71 (C-3″), 67.53 (C-4″), 77.01 (C-5″), 61.05(C-6″). (HHDP) in *α*-& *β*-anomers: 113.93, 113.77 (C-1 & C-1′), 125.67, 125.93, 126.17 (C-2 & C-2′), 106.60, 106.84 (C-3 & C-3′), 143.64, 144.57 (C-4, C-4′and C-6 & C-6′),135.71, 135.72 (C-5 & C-5′), 168.88, 169.02, 169.55 (C = O).

2.14.2 Compound (2)

R*f* values: 0.35 (H_2O), 0.48 (6% AcOH in water), 0.19 (BAW), UV λ_{max} (nm) in MeOH: 282,393.
^{1}H- NMR spectral data (DMSO-*d6*) δ (ppm):
Sugar moiety:4.71(1H, d, *J* = 7 Hz, H-1). Aglycone:7.20 (1H, d, *J* = 2.5 Hz, H-6′), 6.71 (1H, dd, *J* = 2.5, 8.4 Hz, H-4′), 6.52 (1H, d, *J* = 8.4 Hz, H-3′).

^{13}C-NMR Spectral Data (DMSO-*d6*) δ (ppm): *β*-glucose moiety: 96.8 (C-1), 72.9 (C-2), 76.6 (C-3), 69.6(C-4), 76.4 (C-5), 61.0 (C-6).
Aglycone: 116.5(C-1′), 148.2 (C-2′), 115.0 (C-3′), 121 (C-4′), 154.3 (C-5′), 115.0 (C-6′), 171.9 (C = O).

2.14.3 Compound (3)

R*f* values: 0.8 (H_2O), 0.44 (6% AcOH in water), 0.32 (BAW).), UV λ_{max} (nm) in MeOH:256, 265 sh., 358; + NaOMe 268, 327, 403 + NaOAc 273,323,387; + NaOAc-H_3BO_3 262, 377; + $AlCl_3$ 273, 430.
^{1}H- NMR spectral data (DMSO-*d6*) δ (ppm):
Quercetin moeity: 7.68(2H, m, H-2′ and H-6′), 6.87 (1H, d, *J* = 8.5 Hz, H-5′), 6.39 (IH, d, *J* = 2 Hz, H-8), 6.17 (IH, d, *J* = 2 Hz, H-6).Glucoside moiety: 5.53 (1H, d, *J* = 8 Hz, H-1"), 3.2–3.9 (glucoside protons overlapped with hydroxyl and water protons).

^{13}C-NMR Spectral Data (DMSO-*d6*) δ (ppm): Quercetin moeity: 156.6 (C-2), 133.80 (C-3),177.9 (C-4), 161.71 (C-5), 99.20(C-6), 164.8 (C-7), 95.62(C-8), 156.91 (C-9), 104.42 (C-10), 122.10 (C- 1′), 115.70 (C-2′), 145.30 (C-3′), 149.0 (C-4′), 116.7 (C-5′), 122.10 (C-6′); *β*-glucose moiety: 101.32(C-1″), 74.60(C-2″), 76.05(C-3″), 70.40(C-4″), 77.70(C-5″), 61.50 (C-6″).

2.14.4 Compound (4)

R*f* values: 0.24 (H_2O), 0.47 (6% AcOH in water), 0.76 (BAW). UV λ_{max} (nm) in MeOH: 266, 345 + NaOMe: 271,

376, + NaOAC: 270, 346, + NaOAC + H_3BO_3: 270, 346 sh., 405, + Al Cl_3: 268, 340 sh., 385.

^{1}H- NMR spectral data (DMSO-$d6$)δ (ppm):

kaempferol moiety: 6.186 (d, J = 2.5 Hz, H-6), 6.38 (d, J = 2.5 Hz, H-8), 7.7 (d, J = 2.5, H-2', H-6'), 6.89 (d, J = 8 Hz, H-3', H-5'). β-glucose moiety: 5.26 (d, J = 8 Hz, H-1''), 3.4–4.0 (m, H-2''- H-6'').

^{13}C-NMR Spectral Data (DMSO-$d6$)δ (ppm):

Kaempferol moiety: 156.93 (C-2), 134.20 (C-3), 177.93 (C-4), 160.28 (C-5), 99.33 (C-6), 165.20 (C-7), 94.24 (C-8), 157.86 (C-9), 104.16 (C-10), 121.20 (C-1'), 131.28 (C-2', C-6'), 115.36 (C-3', C-5'), 161.33 (C-4'). β-glucose moiety: 103.23 (C-1''), 74.33 (C-2''), 76.49 (C-3''), 69.71 (C-4''), 76.69 (C-5''), 61.18 (C-6'').

2.14.5 Compound (5)

Rf values: 0.11 (H_2O), 0.12 (6% AcOH in water), 0.52 (BAW). UV λ_{max} (nm) in MeOH: 256, 300 sh., 354, + NaOMe: 275, 325 sh., 408, + NaOAC: 275, 323, 371, + NaOAC + H_3BO_3: 276, 295 sh., 355 + Al Cl_3: 269, 365, 403.

^{1}H- NMR spectral data (DMSO-$d6$)δ (ppm):

Isorhamnetin moiety: 6.21 (d, J = 1.6 Hz, H-6), 6.42 (d, J = 1.6 Hz, H-8), 3.83(s, OCH_3), 7.93 (d, J = 1.6 Hz, H-2'), 6.92 (d, J = 8.4 Hz, H-5'), 7.55 (dd, J = 1.6 and 8.4 Hz, H-6'). β-glucose moiety: 5.57 (d, J = 8 Hz, H- 1''), 3.0–3.8 (m, H-2''- H-6'').

^{13}C-NMR Spectral Data (DMSO-$d6$)δ (ppm):

Isorhamnetin moiety: 156.30 (C-2), 133.20 (C-3), 177.30 (C-4), 161.1 (C-5), 98.70 (C-6), 164.31 (C-7), 93.61 (C-8), 156.21 (C-9), 103.90 (C-10), 55.60 (OCH_3), 120.90 (C-1'), 113.40 (C-2'), 148.41 (C-3'), 149.31 (C-4'), 115.10 (C-5'), 122.0 (C-6'). β-glucose moiety: 100.8 (C-1''), 74.21 (C-2''), 76.30 (C-3''), 70.40 (C-4''), 77.30 (C-5''), 60.51 (C-6'').

2.14.6 Compound (6)

R_f values: 44 (H_2O),0.55 (6% AcOH in water), 0.72 (BAW); UV Spectral Data λ_{max} (nm): 272.

^{1}H- NMR Spectral Data (DMSO-$d6$) δ (ppm): 6.98 (S, H-2 and H-6).

2.14.7 Compound (7)

Rf values: 0.0.50 (H_2O), 0.53 (6% AcOH in water), 0.84 (BAW), UV λ_{max} (nm) in MeOH; 273.

^{1}H- NMR spectral data (DMSO-$d6$)δ (ppm):7.24 (d, J = 2.5 Hz, H-2),7.15 (d, J = 2.5 Hz, H-6),3.8 (s, 3H, 3-OMe).

^{13}C-NMR Spectral Data (DMSO-$d6$) δ (ppm):120.90 (C-1), 105.21 (C-2), 148.61 (C-3), 139.50 (C-4),145.90 (C-5), 111.26 (C-6), 167.32 (C=O), 56.32 (3-OMe).

3 Results and discussion

The ocean covers nearly 75% of the Earth's surface, which offers a huge biodiversity and interesting source of natural products. Marine sponges play an indispensable ecological role in preserving a stable marine ecosystems by factor of their symbiosis with other organisms [24]. Currently more than 8,500 sponge species are confirmed valid from more than 11,000 species, which could be classified in four different classes including Calcarea (calcareous sponges), Hexactinellida (glass sponges), Sclerospongiae (coralline sponges) and Demospongiae (horny sponges, siliceous sponges or common sponges) [25], 25 orders, 680 genera and 128 families [26]. Diversity of marine sponges and especially their secondary metabolites are considered as a gold mine for discovering bioactive components with great therapeutic, cosmetic and nutritional importance. In fact, the sponge biomass culture may conduct alternative compounds for new drug development in pharmaceutical aspects [25]. However, the lack of appropriate ethno-medical history, technical problems in collection or culturing and the variety of marine species organisms may directly affect the development of marine-derived products as beneficial agents.

Marine sponges are aquatic invertebrates, which are a rich source of bioactive metabolites (i.e. amino acids, alkaloids, aliphatics, sterols, macrolides, glycosides, ketones, lipids, macrolides, peptides, nucleosides, porphyrins, fatty acids, esters, steroids, terpenes, terpenoids, terpenoids) and enzymes [27]. Marine organisms contain secondary bioactive metabolites, which were proved to have medicinal, biological and pharmacological effects including antioxidant, antiviral, anti-malarial, anti-tuberculosis, antibacterial, anti-inflammatory, immunosuppressive, antifungal, antiviral, anticancer, antitumor, anticoagulant, neurosuppressor and enzyme inhibitory agents [28]. However, the search for those secondary metabolites includes three steps: chemical structure identification, kind of pharmacological potential and biological mechanism of action.

Determining the antioxidant potential by measuring antiradical scavenging has been widely applied to indicate the antioxidant properties in plants, food, beverages and marine products. However, antioxidant potential of marine sponges and its metabolites are poorly investigated [29–31].

Antioxidant refers to any substance that inhibits a free radical reaction. These substances possess importance as health promoters in the treatment of cardiovascular diseases, atherosclerosis and anti-cancer ageing [32]. Few studies concerning antioxidant activity of marine sponges were reported, such as antioxidant and cytotoxic activities of some Philippian marine sponges [33]; antioxidant

potential of *Petrosia* sp., *Oceanapia ramsayi*, *Clathria* sp., *Ancorina cerebrum* and *Haliclona fascigera* sponges [34]. In the current study, the antioxidant activity of the Mediterranean sponge *Hemimycale columella* different fractions (n-hexane (HCH), methylene chloride (HCL), ethyl acetate (HCE), n-butanol (HCB) and water (HCW) fractions) were evaluated using two methods 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and oxygen radical absorbance capacity (ORAC). The antioxidant capacities issued by DPPH showed a highly inhibitory potential of HCB, HCE and HCW (Table 1). HCB, followed by HCE and HCW fractions exhibited the highest ORAC antioxidant capacity which exceeded that of the standard Trolox. The activity of HCL extract is comparable to the activity of Trolox. The obtained results were promising and indicated that *H. columella* sponge exhibited an antioxidant ability.

The antidiabetic potential of marine sponge has been poorly investigated, where evaluation of the α-amylase inhibition activity of 24 Antarctic marine sponge was reported [35]; α-amylase inhibitory of Antarctic marine sponges (Porifera) and *α*-glucosidase inhibition of marine sponges collected in Mauritius waters [36]; *α*-glucosidase Inhibitors from the *Penares schulzei* marine Sponge [37]; *α*-glucosidase inhibition of 1,4-dideoxy-1,4-imino-d-arabinitol isolated from two marine sponges collected from Western Australia [38]; *β*-glucosidase inhibition of fungal endophyte of the marine sponge *Latrunculia corticata* [39].

Table 1 the antioxidant activity of the extracts and isolated compounds

The tested sample	ED$_{50}$ (µg/ml)	
	DPPH	ORAC
HCH	36.15 ± 1.77	35.91 ± 2.15
HCL	27.18 ± 2.91	26.89 ± 2.37
HCE	10 ± 0.88	10 ± 0.13
HCB	8 ± 2.15	7.81 ± 1.18
Trolox	–	27.0 ± 13.41
Vit. C	1.83 ± 1.41	–
The tested compound	ED$_{50}$ (µM)	DPPH
Compound 1	40.456 ± 1.35	39.419 ± 1.34
Compound 2	71.46 ± 3.67	69.25 ± 3.12
Compound 3	81.83 ± 2.13	79.676 ± 2.59
Compound 4	73.599 ± 2.58	27.261 ± 2.55
Compound 5	25.54 ± 1.22	26.65 ± 1.32
Compound 6	455.267 ± 1.22	452.62 ± 2.13
Compound 7	233.505 ± 1.45	217.214 ± 2.66
Trolox	–	107.87 ± 13.41
Vit. C	10.391 ± 1.41	–

Results are given as mean values ± SD of n = 3 independent experiments

α-Amylase, *α*- and *β*-glucosidase inhibition is an effective approach for diabetic care [40]. Thus, isolation of glucosidase and amylase inhibitors from natural sources such as marine organisms can represent a promising strategy in drug discovery [40]. Few sponges were subjected to evaluation of their *α*-amylase, *α*- and *β*-glucosidase inhibition activity such as some Antarctic marine sponges (Porifera) from Mauritius waters [36], *Penares schulzei* marine Sponge [37],1,4-dideoxy-1,4-imino-d-arabinitol isolated from two marine sponges collected from Western Australia [38]; endophyte of the marine sponge *Latrunculia corticata* [39]. Our findings showed that HCL (85%) exhibited the highest α-amylase inhibitory activity, followed by HCB (68%), HCW (68%), HCE (65%) and HCH (21%) extract. HCB only exhibited *β*-glucosidase inhibitory effect (64%), while the other fractions were inactive (Table 2).Our results revealed a good anti-diabetic potential of *H. columella* sponge fractions regarding the α-amylase inhibition. Moreover, the pancreatic lipase formed by pancreatic acinar-cells is responsible for 50%–70% hydrolysis of dietary triacylglycerols into monoacylglycerol and fatty acids; its inhibition contribute in treatment of both diabetes and obesity [41]. Our results highlighted a potent pancreatic lipase of *H. columella* sponge five fractions (Table 2) where HCW, HCH, HCL and HCE had about 91% inhibitory effect. They showed IC$_{50}$ values that were even lower than that of the standard Orlistat.

Collagenase and elastase are two enzymes responsible for dehydration and wrinkle formation on the skin surface. Secondary metabolites isolated from natural products including flavonoids, tocopherols, phenolics and tannins have demonstrated anti-collagenase and anti-elastase activities [42]. The anti-aging properties of plant extracts are generally attributed to their antioxidants, which reduce free radical scavenging and provide skin protection [43]. Some metabolites such as mycosporine-like amino-acids isolated from marine organisms were reported to possess in vitro anti-aging and wound-healing properties including inhibition of collagenase [44]. Three tiglic acid-containing cyclodepsipeptides (largamides) metabolites isolated from marine cyanobacterium *Lyngbya confervoides* showed an elastase inhibitory activity [45]. Secondary metabolites such as mycosporine-like amino acids isolated from marine red algae *Porphyra* sp. and *Palmaria palmata* exhibited collagenase inhibitory potential [46].

Tyrosinase is an important enzyme which catalyzes melanin synthesis in melanocytes [47, 48]. Currently, tyrosinase inhibition is a promising approach for the development of skin whitening and cosmetic products. In our study, the collagenase, elastase inhibitory effects of *H. columella* sponge fractions were also examined. The results (Table 3), showed that HCE and HCH had about a 75% inhibition effect on the collagenase enzyme. HCE

Table 2 α-Amylase, β-glucosidase and pancreatic lipase inhibition effect of the extracts and compounds (Mean $\pm$ S.D.) and their IC_{50} ($IC_{50} \pm$ S.D.)

The tested sample	Inhibition of α-amylase		Inhibition of β-glucosidase		Inhibition of pancreatic lipase	
	% Inhibition of at 600 µg/ml	IC_{50} µg/ml	% Inhibition of at 600 µg/ml	IC_{50} µg/ml	% Inhibition at 100 µg/ml	IC_{50} µg/ml
HCH	21.4 ± 6.4	1061.1 ± 11.8	–	–	92.9 ± 5.9	12.6 ± 6.4
HCL	85.1 ± 7.1	181.67 ± 9.8	–	–	92.2 ± 5.1	13.79 ± 4.6
HCE	64.7 ± 5.7	321.83 ± 7.9	–	–	91.01 ± 5.6	11.69 ± 6.9
HCB	67.7 ± 5.6	312.16 ± 12.4	64 ± 5.9	343.17 ± 8.8	85.4 ± 5.9	15.64 ± 5.1
HCW	67.5 ± 5.3	294.7 ± 8.5	–	–	94.5 ± 6.4	9.926 ± 5.8
Acarbose	94.1 ± 7.2	230.85 ± 2.45	–	–	–	–
1-Deoxynojrimycin	–	–	98.3 ± 0.4	27.72 ± 0.02	–	–
Orlistat	–	–	–	–	92 ± 6.4	28.96 ± 6.4
The tested sample	Inhibition of α-amylase		Inhibition of β-glucosidase		Inhibition of pancreatic lipase	
	% Inhibition of at 600 µg/ml	IC_{50} (µM)	% Inhibition of at 600 µg/ml	IC_{50} (µM)	% Inhibition at 100 µg/ml	IC_{50} (µM)
Compound 1	80 ± 5.1	571.16 ± 10.1	–	–	93.4 ± 5.4	16.57 ± 2.43
Compound 2	72.4 ± 6.2	1113.64 ± 8.6	–	–	94 ± 5.1	35.79 ± 3.1
Compound 3	32.4 ± 6.5	2111.18 ± 14.3	–	–	92.4 ± 4.1	25.41 ± 3.9
Compound 4	59.2 ± 5.37	1137.88 ± 9.6	–	–	84.4 ± 5.2	27.72 ± 3.95
Compound 5	25.3 ± 5.8	2086.12 ± 11.5	–	–	85.9 ± 4.7	25.15 ± 4.2
Compound 6	76.2 ± 5.9	2314.24 ± 12.3	–	–	93.8 ± 4.8	66.54 ± 4.2
Compound 7	52 ± 8.7	3157.15 ± 10.8	–	–	83 ± 4.5	91.12 ± 4.7
Acarbose	94.1 ± 7.2	357.57 ± 2.45	–	–	–	–
1-Deoxynojrimycin	–	–	98.3 ± 0.4	169.884 ± 0.02	–	–
Orlistat	–	–	–	–	92 ± 6.4	58.41 ± 6.4

Results are given as mean values $\pm$ SD of n = 3 independent experiments

exhibited the highest collagenase inhibition activity. The elastase inhibitory capacity of the five fractions (Table 3) was higher than on collagenase, which might contribute to their antiwrinkle properties. The tyrosinase inhibition effects of *H. columella* sponge fractions were also summarized in Table 3. Both HCL and HCE showed a higher tyrosinase inhibitory potential (81%) than the three other fractions with IC_{50} values lower than that of the standard Hydroquinone monomethyl ether (149.9 ± 5.8 and 155.6 ± 7.3 µg/ml, respectively). This study revealed that the different fractions of *H. columella* sponge exhibited high or satisfactory anti-collagenase, anti- elastase and tyrosinase inhibitory effects.

Marine sponges derived metabolites including terpenes, alkaloids, peptides, aromatics, lactones, steroids and miscellaneous compounds are evidenced cytotoxic and anticancer agents [49]. However, the anti-cancer drugs from marine products as sources of natural metabolites is still in research development [50]. The cytotoxicity potential of the five fractions of *H. columella* sponge was assessed against four tumorous human cell lines: bladder carcinoma cell line (5637 cells), hepatocellular carcinoma (Huh7), breast carcinoma (MCF7) and immortalized

human keratinocytes (HaCaT cells) while etoposide was used as a positive control. The highest cytotoxic potential against bladder carcinoma cell line were obtained for HCE (IC_{50} = 8.52 µg/ml) and HCB (IC_{50} = 11.52 µg/ml). On the other hand, HCB (IC_{50} = 2.98 µg/ml) revealed a good inhibitory activity against hepatocellular carcinoma. Moreover, the five fractions showed high safety on the immortalized human keratinocytes normal cells. Based upon the obtained results, HCL, HCE, HCB and HCW were investigated for the isolation of their major compounds.

The isolated compounds (1–7) showed chromatographic, UV absorption and hydrolytic data identical with those reported for 2,3-*O*-hexahydroxydiphenoyl-(*α/β*)-glucose (1) [51], gentisic acid 2-*O*-β-glucoside (2) [52] which were isolated from HCW, quercetin-3-*O*-β-glucopyranoside (3) [53], kaempferol 3-*O*-β-glucopyranoside (4) [54], isorhamnetin 3-*O*- β-glucopyranoside (5) [55] from HCB; gallic acid (6) [56] from HCE and gallic acid-3-methyl ether (7) [57, 58] from HCL. Structures of the isolated compounds are shown in Fig. 1.

Compound (1), (20 mg) off-white amorphous powder, appeared as a blue spot under short UV light on PC and gives deep blue color upon spraying with $FeCl_3$. It gives red

Table 3 Collagenase, elastase and tyrosinase inhibition of different extracts and isolated compounds (Mean ± S.D.) and their IC_{50} (IC_{50} ± S.D.)

The tested Sample	Inhibition of collagenase		Inhibition of elastase		Inhibition of tyrosinase	
	% Inhibition at 500 μg/ml	IC_{50} μg/ml	% Inhibition at 300 μg/ml		% Inhibition at 500 μg/ml	IC_{50} μg/ml
HCH	75.1 ± 7.65	200.4 ± 6.6	87.7 ± 4.8	106.09 ± 5.4	75.8 ± 6.1	188.9 ± 6.9
HCL	71.2 ± 6.6	217.19 ± 7.5	90.2 ± 5.9	100.6 ± 4.7	80.6 ± 7.6	149.9 ± 5.8
HCE	75.2 ± 4.9	196.5 ± 5.1	88.2 ± 4.7	102.2 ± 7.5	81.2 ± 5.4	155.6 ± 7.3
HCB	71 ± 5.7	224.5 ± 5.6	87.8 ± 7.6	105.7 ± 7.1	72 ± 5.8	207.8 ± 5.3
HCW	72 ± 7.4	224.11 ± 7.4	88.1 ± 7.2	111.94 ± 6.9	64 ± 6.3	323.75 ± 7.8
EGCG	89.10 ± 2.7	112.12 ± 9.4	–	–	–	–
Elafin	–	–	59.5 ± 7.1	250.0 ± 12.6	–	–
Hydroquinone mono-methyl ether	–	–	–	–	55.2 ± 2.2	330.0 ± 4.1
The tested Sample	Inhibition of collagenase		Inhibition of elastase		Inhibition of tyrosinase	
	% Inhibition at 500 μg/ml	IC_{50} (μM)	% Inhibition at 300 μg/ml	IC_{50} (μM)	% Inhibition at 500 μg/ml	IC_{50} (μM)
Compound 1	56 ± 4.6	1237.34 ± 6.5	79.8 ± 7.5	369.295 ± 4.3	74.2 ± 6.9	382.99 ± 7.2
Compound 2	69 ± 6.7	1129.261 ± 6.3	77.1 ± 6.9	564.41 ± 5.5	75 ± 5.8	574.84 ± 4.6
Compound 3	71.5 ± 7.3	563.116 ± 4.9	93 ± 6.1	222.232 ± 7.6	76 ± 5.3	459.959 ± 4.6
Compound 4	65.1 ± 6.3	857.75 ± 6.8	87.9 ± 5.2	228.61 ± 6.4	72 ± 7.3	421.8995 ± 7.1
Compound 5	70.2 ± 5.8	710.97 ± 6.4	81.2 ± 4.1	320.65 ± 4.7	72.8 ± 6.2	385.66 ± 4.3
Compound 6	58.5 ± 6.1	2671.64 ± 8.6	90.4 ± 4.6	626.62 ± 5.9	81.1 ± 7.3	889.078 ± 5.7
Compound 7	74 ± 6.7	1152.1 ± 6.75	92.5 ± 5.5	566.929 ± 4.6	62 ± 5.6	1510.72 ± 6.7
EGCG	89.10 ± 2.7	244.605 ± 9.4	–	–	–	–
Elafin	–	–	59.5 ± 7.1	41.67 ± 12.6	–	–
Hydroquinone mono-methyl ether	–	–	–	–	55.2 ± 2.2	2658.35 ± 4.1

Results are given as mean values ± SD of n = 3 independent experiments

with nitrous acid specific for ellagitannins. In the 1H- NMR spectrum of (DMSO-d_6, room temperature), it showed two doublets appearing at δ 5.32 (J = 3.5 Hz) and 4.90 (J = 8 Hz) of the α- and β-anomeric glucose protons, respectively, confirming the free anomeric hydroxyl group. The H-2″ glucose proton appears at δ ppm 4.64 (dd, J = 8 and 7.5 Hz) and at 4.83 (dd, J = 3.5 and 8 Hz), in both β- and α- anomers, respectively. Also, the H-3″ glucose protons in the β- and α-anomers appeared at δ ppm 4.93 (t, J = 7.5 Hz) and at 5.25 (t, J = 8 Hz), respectively. The downfield shifts of H-2″ and H-3″, confirmed the attachment of HHDP moiety at C-2″ and C-3″ of glucose. The HHDP moiety appeared as two pairs of singlets in the aromatic region at δ 6.62, 6.61, 6.54 and 6.53, referring to the H-3 and H-3′ in both anomers. The remaining glucose proton appeared as a multiplet from δ 3.3 to 3.8 ppm. The ^{13}C-NMR spectrum (DMSO-d_6, room temperature) finally confirmed the structure of (1) and the measured chemical shift values of the glucose carbon resonances proved that the sugar core is in the pyranose form.

Compound (2), (20 mg), amorphous off- white powder. It appeared as a blue spot under short UV light on PC and gives dirty blue color upon spraying with FeCl₃. Normal

acid hydrolysis (2 N aqueous HCL, 3 h, 100 °C) yields gentisic acid and glucose (CoPC). In ^{1}H-NMR (DMSO-d_6, room temperature) the β-glucose anomeric proton appeared at δ 4.71 (d, J = 7 Hz) whose germinal OH is involved in the acetal linkage and the attachment of gentisic acid and glucose moiety confirmed by ^{13}C -NMR (DMSO-d_6, room temperature) as seven chemical shift values appeared for gentisic acid in which the C-2 hydroxyl group is substituted by the anomeric glucose carbon at δ 96.80 (C-1). This was concluded from the upfield shift of the aromatic C-2 carbon all in comparison with the corresponding chemical shifts of free gentisic acid [52]. Also, β-glucose configuration was confirmed from the C-l chemical shift.

Compound (3), (32 mg) a yellowish brown amorphous powder. It appeared as a dark purple spot under short UV light on PC and turned orange when fumed with ammonia. Normal acid hydrolysis (2 N HCl, 3 h, 100 °C) yields quercetin and glucose (CoPC). Also, upon enzymatic hydrolysis with β-glucosidase, quercetin was released (CoPC). In ^{1}H-NMR (DMSO-d_6, room temperature) the β-glucose anomeric proton appeared δ 5.53 ppm (d, J = 8 Hz) and other resonance chemical shifts were agreed with the suggested structure of both sugar and flavonoid
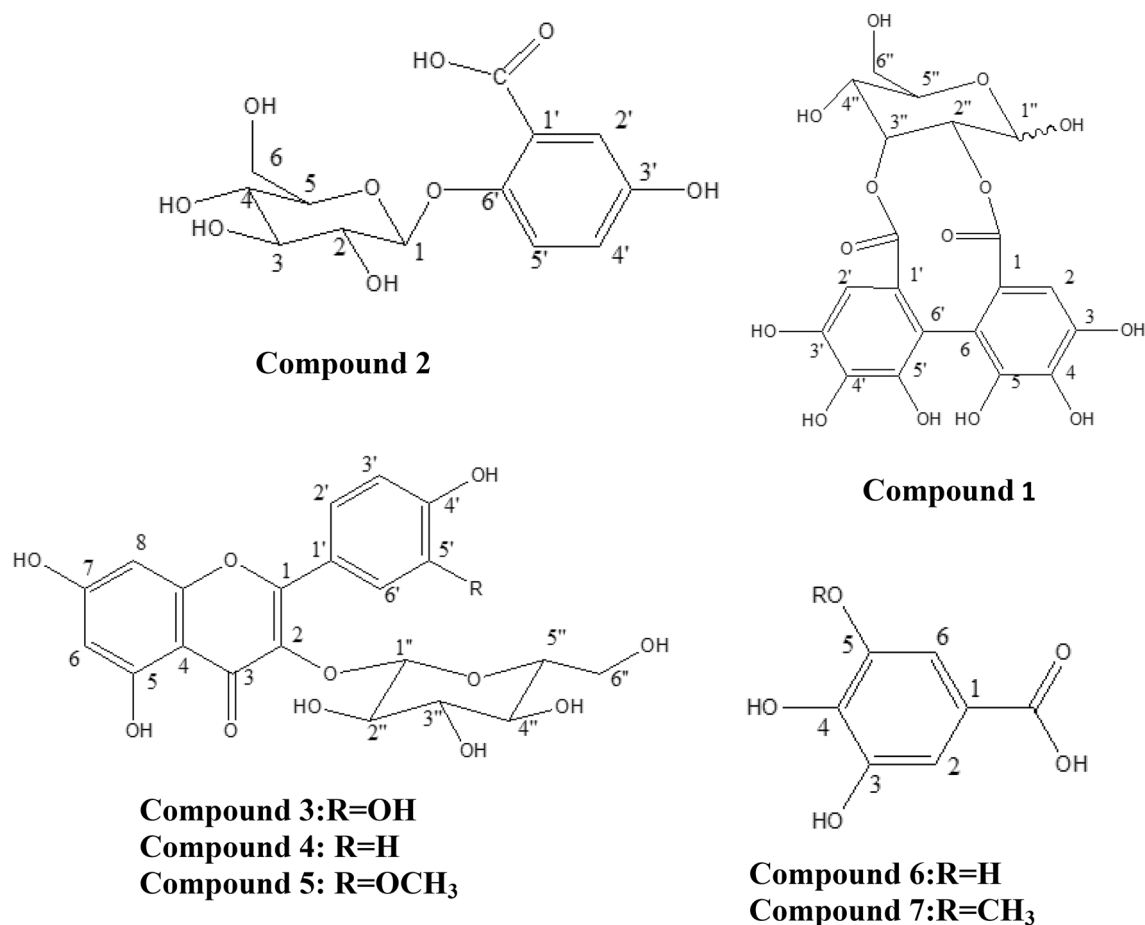
Compound 2

Compound 1

Compound 3:R=OH
Compound 4: R=H
Compound 5: R=OCH$_3$

Compound 6:R=H
Compound 7:R=CH$_3$

Fig. 1 Structures of the isolated compounds

moieties [53]. In ^{13}C-NMR (*DMSO-d6*, room temperature) the presence of one *β*-glucose moiety confirmed from the anomeric carbon resonance at δ101.32 ppm and from the chemical shift values of the remaining sugar resonances in the region from δ 61.50 to 76.05 ppm. The flavonoid moiety resonances were in agreement with the corresponding published signals [53].

Compound (4), (25 mg) a brown amorphous powder. It appeared as a dark purple spot under short UV light on PC and turned yellow when fumed with ammonia. Normal acid hydrolysis (2 N HCl, 3 h, 100 °C) yields kaempferol and glucose (CoPC). Also, upon enzymatic hydrolysis with *β*-glucosidase, kaempferol was released (CoPC). In ^{1}H-NMR (*DMSO-d$_6$*, room temperature) the *β*-glucose anomeric proton appeared δ 5.26 ppm (d, *J* = 8 Hz) and other resonance chemical shifts were agreed with the suggested structure of both sugar and flavonoid moiety [54]. In ^{13}C-NMR (*DMSO-d$_6$*, room temperature) the presence of one *β*-glucose moiety confirmed from the anomeric carbon resonance at δ103.23 ppm and from the chemical shift values of the remaining sugar resonances in the region from δ 61.18 to 76.69 ppm. The flavonoid moiety

resonances were in agreement with the corresponding published signals [53].

Compound (5), (30 mg) a yellow amorphous powder. It appeared as a dark purple spot under short UV light on PC and turned lemon yellow when fumed with ammonia. Normal acid hydrolysis (2 N HCl, 3 h, 100 °C) yields isorhamnetin and glucose (CoPc and UV). In ^{1}H-NMR (*DMSO-d$_6$*, room temperature) the *β*-glucose anomeric proton appeared δ 5.57 ppm (d, *J* = 8 Hz). The flavonol moiety H-6 and H-8 appeared at δ 6.21 ppm (d, *J* = 1.6 Hz) and δ 6.42 ppm (d, *J* = 1.6 Hz), respectively. Also, H-5' and H-6' appears at δ ppm 6.92 (d, *J* = 8.4 Hz, H-5'), 7.55 (dd, *J* = 8.4 & 1.6 Hz, H-6') while the methoxy group appears at δ 3.83 ppm. Furthermore, ^{13}C-NMR analysis (*DMSO-d$_6$*, room temperature) confirmed the structure of (5) in comparison with the corresponding published data [55].

Compound (6), (40 mg) a white powder. It appeared as a blue spot under short UV light on PC which gave a blue colour with FeCl$_3$. Structure confirmation was carried out by CoPc and further by ^{1}H-NMR analysis (*DMSO-d$_6$*, room temperature). The spectrum showed one singlet signal in

Table 4 The cytotoxic activity of the extracts and isolated compounds

The tested samples	Cytotoxicity [IC$_{50}$ (µg/mL)]			
	Bladder carcinoma cell line (5637 cells)	Hepatocellualr carcinoma (Huh 7)	Breast cancer cell line (MCF7)	Immortalized human keratinocytes (HaCaT cells)
HCH	32.10±0.16	9.10±2.11	10.8±0.69	410±6.17
HCL	28.33±1.32	7.11±0.12	9.10±0.17	379±11.12
HCE	8.52±0.21	5.10±1.11	5.33±1.27	520±3.83
HCB	11.52±1.11	2.98±0.12	3.15±1.22	569±9.11
HCW	22.51±0.18	5.32±1.21	8.81±3.51	460±4.57
Etoposide	9.40±3.34	1.52±0.15	10.90±0.24	388±7.87
The tested samples	Cytotoxicity [IC$_{50}$ (µg/mL)]			
	Bladder carcinoma cell line (5637 cells)	Hepatocellualr carcinoma (Huh 7)	Breast carcinoma cell line (MCF7)	Immortalized human keratinocytes (HaCaT cells)
Compound 1	12.45±1.85	10.37±2.41	6.66±1.73	220.98±10.22
Compound 2	22.134±0.89	34.05±1.87	54.765±2.22	948.587±4.56
Compound 3	27.801±	12.081±1.05	13.35±1.11	925.966±7.76
Compound 4	38.026±2.31	18.69±3.60	10.73±2.88	838.56±10.41
Compound 5	63.148±1.78	23.45±1.06	17.18±2.6	1168.77±12.10
Compound 6	29.39±4.77	111.686±2.45	65.31±1.33	587.82±3.55
Compound 7	239.18±0.61	88.623±2.13	82.54±3.12	2172.14±6.33
Etoposide	15.97±3.34	2.58±0.15	18.52±0.24	659.19±7.87

Results are given as mean values±SD of n=3 independent experiments

the aromatic region at δ 6.98 ppm for the two equivalent H-2 and H-6 protons [56].

Compound (7), (18 mg) an off-white powder. It appeared as a blue spot under short UV light on PC which gave a blue colour with FeCl$_3$. Structure confirmation was carried out by CoPC and further by ^{1}H-NMR analysis (*DMSO-d$_6$*, room temperature). The spectrum showed two doublet signals in the aromatic region at δ 7.15 ppm and δ 7.24 ppm for the two H-2 and H-6 protons and a singlet at δ 3.80 ppm for the methoxy group [56]. The ^{13}C-NMR spectrum (*DMSO-d$_6$*, room temperature) confirmed the achieved structure [56].

The isolated compounds were also subjected to evaluation of their in vitro activities, compound 5, followed by 1 and 2, showed good radical scavenging activity among the tested compounds using DPPH (Table 1). The radical scavenging potential of compound 5 was comparable with that of HCW, while compounds 4 and 5 indicated the highest ORAC antioxidant potential which were stronger than that of the standard Trolox. The isolated compounds had no inhibitory effect on the β-glucosidase. Meanwhile, the α-amylase inhibitory capacity was highest for compound 1 (80%) and 2 (72.4%). Compounds (1–5) showed significant lipase inhibition activity, where they had IC$_{50}$ values lower

than that of Orlistat (Table 2). Moreover, at 100 µg/ml, compound 1, 2, 3 and 6 exhibited pancreatic lipase inhibition potential above 92%, while compound 3, 5 and 6 had inhibitory activity of more than 83%. Compound 3 (71.5%) and compound 7 (74%) showed the highest collagenase inhibition activity, but with IC$_{50}$ values higher than that of EGCG (Table 3). Concerning the elastase inhibition activity, compound 3,4, 6 and 7 revealed an elastase potential of more than 85%. These results were in accordance with the reported data about quercetin-3-*O*-β-glucopyranoside (3) and kaempferol 3-*O*-β-glucopyranoside (4) which can be used as valuable agents in cosmetics due to their important chemical characteristics and their ability to possess skin protective effect against UV radiation [59]. Meanwhile, compounds 1–7 showed strong tyrosinase inhibitory activity with IC50 values lower than the standard Hydroquinone monomethyl ether. Gallic acid was reported to significantly inhibit both melanin synthesis and tyrosinase activity in a dose- and time-dependent manner [60]. Compound 1 exhibited the highest cytotoxic potentials against bladder carcinoma cell line (5637 cells) and hepatocellular carcinoma (Huh7) (Table 4). Moreover, compounds 1,3,4,5 had promising anticancer activity against the tested breast cancer cell line (MCF7). On the other hand, the isolated

compounds except compounds 1 and 6 showed a good safety margin as they had high IC_{50} against immortalized human keratinocytes, respectively (Table 4). These findings demonstrated a good cytotoxic potential and selectivity of the isolated compounds against different types of cancer cell lines.

Our work presented the initial evaluation of the biological activities of 2, 3-*O*-hexahydroxydiphenoyl-(α/β)-glucose (1), gentisic acid 2-*O*-β-glucoside (2) and gallic acid-3-methyl ether (7) whose ORAC activities were comparable to that of the standard Trolox. It is also the first study to report the effectiveness of the three compounds as pancreatic lipase and tyrosinase inhibitors as they showed IC_{50} lower than the standard Orlistat and Hydroquinone monomethyl ether, respectively. 2, 3-*O*-hexahydroxydiphenoyl-(α/β)-glucose (1) and gentisic acid 2-*O*-β-glucoside (2) were proved to be promising cytotoxic compounds against Bladder carcinoma cell line (5637 cells), hepatocellular carcinoma (Huh7) and Breast cancer cell line (MCF7) where their IC_{50} were somewhat comparable to that of the standard Etoposide. It is worthy also to mention that our study is the first report about the collagenase, elastase, tyrosinase and lipase inhibiting activity as well as the cytotoxic activity of isorhamnetin 3-*O*- β-glucopyranoside (5).

4 Conclusion

The present study is a deep investigation of the biological activities of marine sponges from the Mediterranean. Seven secondary metabolites were isolated, characterized and their biological properties were highlighted. To the best of our knowledge, the present results are the first one to establish antioxidant, antidiabetic, collagenase, elastase, tyrosinase and cytotoxic effects of *H. columella* sponge and its metabolites, especially 2, 3-*O*-hexahydroxydiphenoyl-(α/β)-glucose (1), gentisic acid 2-*O*-β-glucoside (2) and gallic acid-3-methyl ether (7). The data showed that *H. columella* sponge can provide appreciated resources for new pharmaceutical based molecules.

Compliance with ethical standards

Conflict of interest The authors declare no conficts of interest.

Human and animal rights No human and animal experiments were performed in this study.

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