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In vitro antioxidant, antibacterial, cytotoxic, and epigenetic screening of crude extract and fractions of the marine sponge *Neopetrosia exigua* from Mauritius waters

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ABSTRACT

The marine sponge *Neopetrosia exigua* is known as a goldmine of novel compounds, yet its pharmacological activities remain poorly characterised. Herein, this study investigates the bioactivities of *N. exigua* collected from Mauritius waters. The crude extract (dichloromethane: methanol), hexane, ethyl acetate and aqueous fractions obtained from *N. exigua* were subjected to *in vitro* antioxidant assays. Their antibacterial activities were evaluated using the broth microdilution method to determine the minimum inhibitory concentration (MIC). The cytotoxic and epigenetic activities were further screened using the MTT assay and a cell-based image system that measures de-repression of a silenced Green Fluorescent Protein (GFP) reporter gene, respectively. Higher antioxidant activity was recorded for the ethyl acetate fraction as demonstrated by its significant ferric reducing antioxidant power, radical scavenging, and metal chelating activities relative to control ($p < 0.05$). The best antibacterial profile was presented by the ethyl acetate fraction against *Cutibacterium acnes* (MIC: 0.039 mg/ml), *Streptococcus mutans* (MIC: 0.078 mg/ml) and *Mycobacterium smegmatis* (MIC: 0.313 mg/ml). Similarly, the fraction displayed significant cytotoxicity against the human liposarcoma SW872 cells with IC_{50} value of 44.34 ± 2.64 µg/ml and GFP re-activation capacity of 43.79 ± 3.19% ($p < 0.05$). This work conveys interesting data on the antioxidant, antimicrobial, and anticancer properties of *N. exigua.* In particular, this study indicates the promising potential of *N. exigua* as a reservoir of epigenetically active agents that can modulate transcription of silenced genes involved in carcinogenesis. Hence, further investigations to isolate the active constituents is actively warranted.

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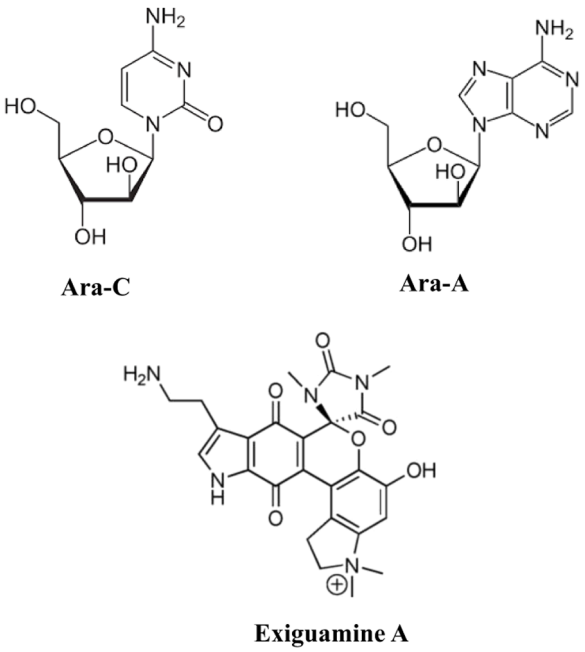


Fig. 1. Bioactive compounds derived from marine sponges.

Introduction

The ocean, the largest biome on earth, hosts an immense biodiversity surpassing that of its terrestrial counterpart [1]. Living in an environment characterised by high competition, marine organisms have evolved to produce toxic compounds as chemical defenses to protect themselves against predators, or as weapons to capture prey [2]. Interestingly, a great number of these compounds have also shown pharmacological activities such as antimicrobial, antifouling, antiviral, cytotoxic, antitumor, antioxidant, antiprotozoal, anti-inflammatory among other bioactivities [3] thus, making them excellent templates for the development of new drug leads. Worldwide bioprospecting efforts have nevertheless not ceased, and a multitude of bioactive molecules are constantly feeding the marine drug armamentarium every year [4].

Among the different marine taxonomical groups, invertebrates primarily marine sponges have widely been acclaimed as an interesting chemical factory, due their immense production of structurally novel bioactive compounds [5]. While the use of marine sponges dates back to Roman times, where they were used against wounds, sunstroke, fractures, infectious diseases and tumours [6], it is only in the early 1950′s that the first bioactive agents, namely the nucleosides spongothymidine and spongouridine were isolated from the Carribean sponge *Cryptotethya crypta* [7]. These compounds possessed remarkable bioactivities and their synthetic analogues eventually led to the development of the anticancer agent cytosine arabinoside (Ara-C) together with adenine arabinoside (Ara-A) as an anti-viral agent (Fig. 1) [8]. Since then, the systematic investigation of marine sponges as a source of novel bioactive metabolites has greatly increased. Recently, marine sponges belonging to the genus *Neopetrosia* have received particular attention due to their unique natural product chemistry including alkaloids [9–12]. Exiguamine A (Fig. 1), a novel alkaloid isolated from the marine sponge *Neopetrosia exigua*, has been found so far, as the most potent inhibitor of the tumor promoter enzyme indoleamine-2,3-dioxygenase from natural sources [13]. Even today, the genus continues to produce a large array of active compounds of potential relevance in drug discovery as reported by the increasing number of publications (Table 1).

The Exclusive Economic Zone of Mauritius Island has a diverse assemblage of *Neopetrosia* species. However, their population density has significantly declined over the last few years (personal communication, 2019). As part of our ongoing screening program to search for marine natural products that can spawn avenues in the discovery of lead compounds, we report the *in vitro* antioxidant and antibacterial (anti-tuberculosis, anti-acne and anti-oral infections) activities of the crude extract and fractions derived from the Mauritian sponge *Neopetrosia exigua*. In addition, we also reveal the potential of *N. exigua* as a source of cytotoxic compounds that can reverse epigenetic alterations linked with oncogenes activation and inactivation of tumor suppressor genes particularly involved in carcinogenesis. This preliminary screening will subsequently set the stage for more in-depth mechanistic studies and chemical characterization of potential novel drug leads from the genus *Neopetrosia*. Furthermore, it will also back-up existing framework oriented towards the conservation of some threatened *Neopetrosia* species in Mauritius waters.

Materials and methods

Sampling and identification

The marine sponge *Neopetrosia exigua* (Fig. 2) was collected at Amber Island (20°03′52.5″S and 57°41′19.7″E) on the northeast coast of the Republic of Mauritius (Fig. 3). The sponge was sampled by hand at depths of 2–3 metres by snorkeling at low tide. It was transferred to the laboratory under seawater, cleaned of debris and frozen at − 80 °C until use. Taxonomic identification of *N. exigua* was confirmed via the World Porifera Database [22].

Preparation of crude extract and fractions

The marine sponge *N. exigua* was cut into small pieces, freeze dried and powdered. The freeze-dried powder (250 g) was macerated exhaustively in a mixture of dichloromethane and methanol (1:1) for 48 h. The macerate was filtered, and flash evaporated under vacuum at 37 °C (LABOROTA 4003, Heidolph, Germany). The crude extract was mixed with 200 ml of distilled water and defatted with n-hexane (200 ml x 3). The hexane fractions were eluted, and the aqueous fractions were successively partitioned with ethyl acetate (200 ml x 3) to furnish n-hexane, ethyl acetate and aqueous fractions. The total crude extract and fractions were stored at −20 °C.

In vitro antioxidant activity screening

A multi-method approach was used to assess the antioxidant potency of the total crude extract and fractions of *N. exigua*. Seven independent antioxidant methods were used to provide a mechanistic insight of the antioxidant actions of the extracts under study. Gallic acid were used as positive control in all the antioxidant assays.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay measures the reduction of ferric ion (Fe^{3+})-ligand complex to the intensely blue-colored ferrous (Fe^{2+}) complex by antioxidants in an acidic medium. The reducing power of *N. exigua* crude extract and fractions were assessed as previously described by Benzie and Strain [23]. The FRAP reagent was freshly prepared by mixing together 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride in 0.25 M acetate buffer, pH 3.6. The crude extract or fractions (20 µl) at 1 mg/ml were mixed with 180 µl FRAP reagent in wells of a 96-well plate, left for 6 min at room temperature and the absorbance was measured at 593 nm. Results were expressed as millimoles of ferrous sulfate per gram freeze-dry weight (mMol Fe (II)/g FDW).

$$Fe^{3+}\text{ - TPTZ} \xrightarrow{\textbf{Antioxidant}} Fe^{2+}\text{ - TPTZ}$$

(Intense blue colour at 593 nm)

Ferric reducing antioxidant power (FRAP) mechanism

Trolox equivalent antioxidant capacity (TEAC) assay

The ability of *N. exigua* extracts to scavenge the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was assessed according to a modified protocol of Campos and Lissi [24]. To 180 µl of the $ABTS^{\bullet+}$ solution, generated by a reaction between 0.5 mM ABTS and activated manganese dioxide (MnO_2) (1 mM) in phosphate buffer (0.1 M, pH 7), 20 µl of the crude extract or fractions at 1 mg/ml were added and the absorbance was recorded at 734 nm after 30 min incubation at room temperature. The ABTS

Table 1
Chemical active constituents of the *Neopetrosia* genus.

Metabolites	Species	Class	Bioactivity	References
Neopetrocyclamines A and B	*N. exigua*	Alkaloid	Cytotoxic	[10]
Araguspongines M, B, D	*N. exigua*	Alkaloid	Cytotoxic	[12]
Papuamine	*N. exigua*	Alkaloid	Cytotoxic	[12]
Araguspongine C	*N. exigua*	Alkaloid	Anti-tuberculosis activity	[14]
Petrosin A	*N. exigua*	Alkaloid	Anti-HIV activity	[15]
Renieramycin J	*Neopetrosia* sp.	Alkaloid	RNA and/or protein synthesis inhibition	[16]
Renieramycin A	*Neopetrosia* sp.	Alkaloid	Cytotoxic & Anti-protozoal activity	[16,17]
Demethylxestopongin B	*N. exigua*	Alkaloid	Cytotoxic	[18]
Neopetrosamine A	*N. exigua*	Alkaloid	Anti-plasmodial activity	[19]
Halenaquinone	*N. exigua*	Quinone	Inhibit phosphatidylinositol 3-kinase activity	[20]
Neopetrosiquinones A and B	*N. exigua*	Quinone	Cytotoxic	[21]

Fig. 2. *Neopetrosia exigua.*

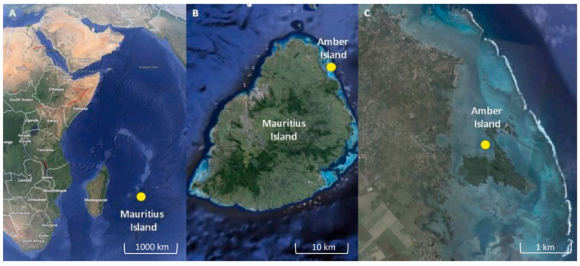


Fig. 3. Satellite images showing Mauritius island (A) located at 20.2000° S, 57.5000° E in the Indian Ocean (B), Research site map area of Amber Island located at 20°03′52.5″S and 57°41′19.7″E (C) (Source: Google Earth 2022).

radical scavenging activity was expressed as millimoles of Trolox equivalents per gram of freeze-dry weight (mMol TE/g FDW).

$$\mathrm{ABTS} + \mathrm{MnO_2} \longrightarrow \mathrm{ABTS^{\bullet+}} \xrightarrow{\textbf{Antioxidant}} \mathrm{ABTS}$$

(Bluish-green colour at 734 nm) Colourless

ABTS radical scavenging mechanism

DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging capacity was evaluated according to the method described by Brand-Williams [25]. The crude extract or fractions of *N. exigua* (20 µl) at concentrations ranging from 10 mg/ml to 0.039 mg/ml were added to DPPH solution (180 µl of 0.1 mM). The mixture was mixed and left to stand at room temperature in the dark for 30 min. The reduction of DPPH radicals was evaluated by measuring the absorbance at 517 nm. The inhibition of the DPPH radicals was calculated according to equation 1 and the percentage scavenging activity was plotted against different concentrations of the extracts to obtain the fifty percent inhibitory concentration (IC_{50}) value (mg/ml).

$$\mathrm{DPPH} \xrightarrow{\textbf{Antioxidant}} \mathrm{DPPH\text{-}H}$$

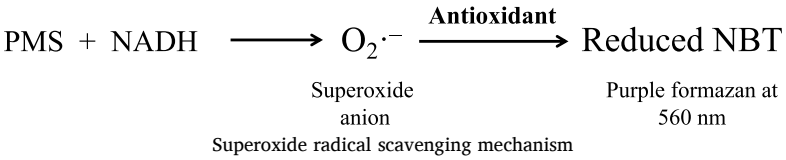
Purple at 517 nm Colourless

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging mechanism

Equation 1: $\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sponge\ samples}{Absorbance\ of\ control} \times 100$

Superoxide anion radical scavenging activity

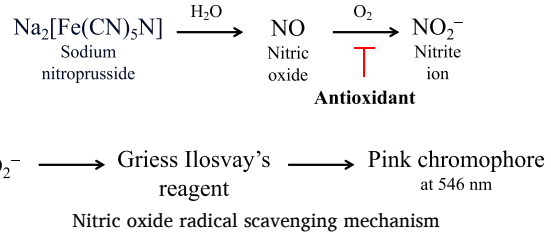
The superoxide anion scavenging activity was determined according to a modified protocol of Nishikimi et al. [26]. The phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) non-enzymatic system produces superoxide radicals that reduce nitroblue tetrazolium (NBT) into an insoluble purple formazan. Briefly, 500 µl NBT solution (156 µM in 100 mM phosphate buffer, pH 8.0), 500 µl of beta-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH) solution (468 µM in 100 mM phosphate buffer, pH 8.0) and 50 µl of the crude extract or fractions at different concentrations (10 mg/ml - 0.039 mg/ml) were mixed. The reaction was started by adding 50 µl of PMS solution (60 µM in 100 mM phosphate buffer, pH 8.0) and was incubated for 20 min at 25 °C. The absorbance was recorded at 560 nm. The scavenging capacity was calculated according to equation 1 and results were expressed as mean IC_{50} (mg/ml).



Superoxide radical scavenging mechanism

Nitric oxide radical scavenging activity

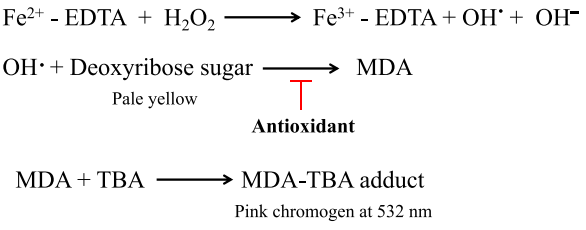
The nitric oxide radical inhibition potential was estimated according to the method described by Leone et al. [27]. N-(1-naphthyl) ethylendiamine dihydrochloride (0.1%) was used to prepare the Griess Ilosvay's reagent. The reaction mixture consisted of 250 µl of different concentrations of the crude extract or fractions (10 mg/ml - 0.039 mg/ml), 1 ml of aqueous sodium nitroprusside (10 mM), and 250 µl 1X PBS. The mixture was incubated for 180 min at room temperature. 1 ml of Griess Ilosvay's reagent (0.33% sulphanilic acid in 20% glacial acetic acid and 0.1% naphthylethylenediamine dichloride) was mixed with 250 µl of the reaction mixture and allowed to stand at room temperature for 30 min. The absorbance of the pink chromophore formed was recorded at 546 nm and the scavenging capacity was calculated according to equation 1. The results were expressed as mean IC_{50} (mg/ml).



Nitric oxide radical scavenging mechanism

Inhibition of deoxyribose degradation activity

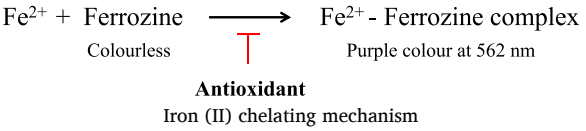
The hydroxyl radical scavenging potential was assessed using the deoxyribose assay [28]. The reacting mixture contained the following reagents: 200 µl of 100 mM KH_2PO_4- KOH, 200 µl of 0.5 mM $FeCl_3$, 100 µl of 1 mM EDTA, 100 µl varied concentrations of the crude extract or fractions (10 mg/ml - 0.039 mg/ml), 200 µl of 15 mM deoxyribose, 100 µl of 10 mM H_2O_2, and 100 µl of 1 mM ascorbic acid. The reaction mix was incubated at 37 °C for 1 h. After incubation, 1 ml 1% (w/v) thiobarbituric acid (TBA) was added to the mixture followed by 1 ml 2.8% (w/v) trichloroacetic acid (TCA). The solution was further heated in a water bath at 80 °C for 20 min. The malondialdehyde and thiobarbituric acid (MDA-TBA) pink chromogen formed was extracted into 3 ml butan-1-ol and its absorbance measured at 532 nm. The scavenging capacity was calculated according to equation 1 and results were expressed as mean IC_{50} (µg/ml).



Inhibition of deoxyribose degradation reaction mechanism

Iron (II) chelating activity

The ability of the extracts to inhibit the formation of iron (II)-ferrozine complex was evaluated according to the method developed by Neergheen et al. [29]. The reaction mixture contained 200 µl of variable concentrations of the crude extract or fractions (10 mg/ml - 0.039 mg/ml) and 50 µl of $FeCl_2 \cdot 4H_2O$ (0.5 mM). The total reaction volume was made up to 1 ml with DMSO and incubated for 5 min at room temperature. After incubation, the reaction was initiated by the addition of 50 µl of 2.5 mM ferrozine solution. The mixture was mixed and left to stand at room temperature for 10 min. The purple colouration observed was read at 562 nm. Chelating activity was calculated according to equation 1 and results were expressed as mean IC_{50} (mg/ml).



Iron (II) chelating mechanism

Antibacterial screening

Microorganisms

The antibacterial potential of *N. exigua* was assessed using the microbroth dilution method adapted from Lall et al. [30]. The panel of bacterial strains investigated in this study including *Cutibacterium acnes* (ATCC 11827), *Streptococcus mutans* (ATCC 25175) and *Prevotella intermedia* (ATCC 25611), were obtained from the American Type Culture Collection (ATCC). The inhibitory activity of *Mycobacterium smegmatis* (MC^2 155) was also assessed. The strains were maintained on an agar slant at 4 °C. In particular *S. mutans* and *P. intermedia* were cultured under anaerobic conditions, in the Department of Plant and Soil Sciences at the University of Pretoria where the tests were conducted.

Antibacterial activity against C. acnes

C. acnes cultures were grown for 72 h at 37 °C on Brain Heart Infusion (BHI) agar supplemented with 1% glucose and then inoculated in BHI broth at a concentration of 1.5×10^6 CFU/ml for the microdilution assay. The crude extract and fractions of *N. exigua* (10 mg/ml) and the positive control tetracycline (0.2 mg/ml) were diluted with BHI broth to obtain concentrations ranging from 2.5 to 0.039 mg/ml and 0.05 - 0.00039 mg/ml respectively. 100 µl of the bacterial suspension were added into a 96-well microplate followed by 100 µl of the sponge crude extract or fractions. The microplate was incubated in an Anaerocult jar with Anaerocult A for 72 h at 37 °C (Merck, Darmstadt, Germany). Negative controls consisted of untreated *C. acnes* culture, the extract solvent DMSO (2.5% v/v) and media. After 72 h, 20 µl of the PrestoBlue viability reagent (LTC Tech (Pty) Ltd., Johannesburg, South Africa) was added to all the wells. The plates were incubated for 1 h, and the Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of extract that inhibits bacterial growth.

Antibacterial activity against P. intermedia and S. mutans

The bacterial strains *P. intermedia* and *S. mutans* were inoculated in Tryptone Soy Broth and BHI broth respectively and 100 µl of each inoculum (3×10^8 CFU/ml) were added in a 96-well plate. To that, 100 µl of different concentrations of sponge crude extract or fractions (2.5 - 0.039 mg/ml) was added. Chlorhexidine (0.00488 – 0.625 mg/ml) was used as positive control while, the untreated bacterial cultures, DMSO (2.5% v/v) and their respective were used as negative control. *S. mutans* and *P. interdia* inocula were put in an anaerobic jar containing Anaerocult A and incubated at 37 °C under anaerobic conditions. After 24 h, 20 µl of PrestoBlue were added to the test wells and incubated for an additional 30 min to determine the MIC.

Anti-mycobacterial activity

Cultures of *M. smegmatis*, previously maintained on 7H11 agar plates were transferred into Middlebrook 7H9 broth medium, containing 2% glycerol and 0.5% Tween-80 and allowed to grow for 24 h at 37 °C. To 100 µl of *M. smegmatis* inocula (1.5×10^6 CFU/ml), 100 µl of the sponge crude extract or fractions (2.5 - 0.039 mg/ml) were added and incubated at 37 °C for 24 h, followed by the addition of 20 µl of PrestoBlue. The plates were left to incubate for an additional 3 h from where the MIC was determined. Ciprofloxacin (0.005 - 0.0004 mg/ml) was used as the positive control whereas the negative controls included the untreated *M. smegmatis* culture, DMSO (2.5% v/v) and media.

Cell culture and in vitro cytotoxic screening

The human liposarcoma SW872 cell line (ATCC® HTB-92) was used for the cytotoxic assay. The cells were grown in high glucose Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/l streptomycin-penicillin and 0.5 µg/ml amphotericin B and maintained at 37 °C in an atmosphere of 5% CO_2 and 95% humidity.

The cell viability was assessed by the ability of SW872 cells to cleave the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyl-2H-tetrazolium bromide), by the mitochondrial succinate dehydrogenase enzyme. SW872 cells were seeded at 1×10^4 cells/well in a 96-well plate and allowed to incubate for 24 h at 37 °C. Subsequently, the cells were treated with the crude extract or fractions at concentrations ranging from 2 to 0.156 mg/ml for 24 h. DMSO (0.5% v/v) was used as negative control and etoposide (0.02−0.00016 mg/ml) was used as positive control. After treatment, the old medium was replaced with 100 µl of fresh medium, containing MTT (5 mg/ml) and the plate was further incubated for 4 h at 37 °C. The formazan crystals were dissolved in 100 µl DMSO and the absorbance was read at 595 nm and 690 nm (Biotek Synergy HT, USA). The cytotoxicity potential was expressed as IC_{50} and presented as mean ± SD from three independent experiments.

Locus de-repressing (LDR) screening assay

The *N. exigua* extracts were screened for epigenetically active agents using a cell-based assay in which a locus containing a quantifiable marker, green fluorescent protein (GFP) was chemically de-repressed by known epigenetic modulators. The experiment was carried out in HeLa cells which contained multiple copies of the GFP reporter genes, epigenetically silenced by DNA hypermethylation. The HeLaGFP cells were obtained from Prof. Richard Katz lab (Fox Chase Cancer Center, PA).

The rationale of this assay was if the extracts could overcome the hyper-methylation based repression of the GFP reporters, they may have a similar effect on tumor suppressors which are also repressed via hypermethylation, including, the well-known tumor repressor, p53. Briefly, HeLaGFP cells were seeded into 96-well Nunc glass-bottom black plates at 1×10^4 cells/well and cultured at 37 °C and 5% CO_2 overnight. The cells were exposed to the active extract of *N. exigua* at different concentrations (1 - 0.0078 mg/ml) for 24 h and the IC_{50} value was calculated (Supplementary).

HeLaGFP cells were treated with the extract at its IC_{50} concentration. Following 24 h incubation, the medium was removed, the cells were washed twice with ice-cold PBS and fixed with 4% paraformaldehyde solution for 30 min. The cells were washed again with ice-cold PBS and stained with 30 µl/well 1 µg/ml DAPI (4′,6-diamidino-2-phenylindole) [31]. DMSO (0.5%) and HeLa cells transfected with empty vectors served as negative controls while the epigenetic drug trichostatin (TSA 10 µMol) was used as the positive control. After 30 min exposure in the dark, the cells were washed and visualised by fluorescent microscopy using a Zeiss Observer Z1 microscope (Zeiss FLUAR 10x/0.5) with MetaMorph v7.8.8.0 software from Molecular Devices (Sunnyvale, CA). The CellProfiler v2.1.1 software was used to calculate the proportion of GFP fluorescent cells as a percentage of total cells. Only when a cell was tested positive for DAPI staining, deemed of appropriate size, and had a significantly high level of GFP staining compared to control, did this count as a cell with re-activated GFP reporters.

Statistical analysis

The results were expressed as means ± standard deviation ($n = 3$) from three independent assays. Statistical analysis was performed using Prism, version 5.01, from GraphPad Software (USA). Tests were carried out to determine the homogeneity of variance and to evaluate normality. The results were analysed using one-way ANOVA followed by Least Significant Difference (LSD) test for parametric data. For the analysis of non-parametric data, the Kruskal-Wallis test followed by Dunn's test were used. Statistical comparison between the mean values and solvent control was performed using Dunnett's post hoc test of significance wherein $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ were considered to be statistically significant. The heatmap was generated from the heatmap package using R software (R Core Team, 2015).

Results

Antioxidant activity

The ethyl acetate fraction of *N. exigua* recorded significantly higher FRAP value of 5.17 ± 0.60 mMol Fe(II)/g FDW followed by the crude extract (3.88 ± 0.73 mMol Fe(II)/g FDW), hexane (2.04 ± 0.46 mMol Fe(II)/g FDW) and aqueous fraction (1.34 ± 0.15 mMol Fe (II)/g FDW) ($p < 0.05$) (Fig. 4). The same trend was observed in the TEAC assay with the highest activity displayed by the ethyl acetate fraction (9.49 ± 0.43 mMol TE/g FDW) followed by the crude extract (7.30 ± 0.51 mMol TE/g FDW), hexane (3.93 ± 0.61 mMol TE/g FDW) and aqueous fraction (2.15 ± 0.40 mMol TE/g FDW) ($p < 0.05$) (Fig. 4). However, these values were significantly lower when compared to the positive control gallic acid (FRAP: 22.25 ± 1.06 mMol Fe(II)/g FDW and TEAC: 94.05 ± 5.03 mMol TE/g FDW) ($p < 0.01$). The ethyl acetate fraction displayed dose-dependent antioxidant activities with five additional antioxidant assays whereby the IC_{50} value was determined. The results are summarised in Table 1. The sponge fraction registered significantly higher DPPH (IC_{50}: 0.294 ± 0.06 mg/ml), superoxide (IC_{50}: 0.556 ± 0.03 mg/ml) and nitric oxide (IC_{50}: 0.613 ± 0.05 mg/ml) radical scavenging activity. It also showed significant metal chelating activity (IC_{50}: 2.00 ± 0.15 mg/ml) and protective effects against oxidative degradation of deoxyribose substrates (IC_{50}: 1.48 ± 0.21 mg/ml). The standard gallic acid, however registered superior antioxidant activity than the sponge crude extract and fractions under these assays ($p < 0.05$). In general, the ethyl acetate fraction displayed peak antioxidant

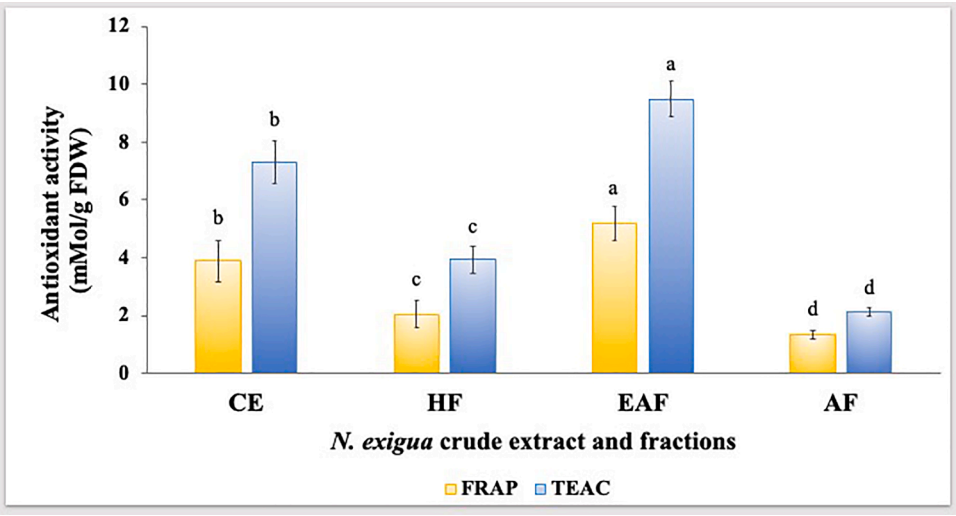


Fig. 4. Antioxidant activity of *N. exigua* crude extract and fractions: Ferric Reducing Antioxidant Potential (FRAP, mMol Fe(II)/g FDW) and Trolox Equivalent Antioxidant Capacity (TEAC, mMol TE/g FDW). Data expressed as mean values ± standard deviations (n = 3). Abbreviations: CE: Crude extract; HF: Hexane fraction; EAF: Ethyl acetate fraction; AF: Aqueous fraction; Different letters represent significant differences between the sponge samples and with a denoting highest antioxidant activity ($p < 0.05$).

Table 1
Antioxidant potential (IC_{50}, mg/ml) of *N. exigua* derived crude extract and fractions.

Antioxidant Activity	*N. exigua* crude extract and fractions $IC_{50} \pm SD$ (mg/ml)				Positive control
	CE	HF	EAF	AF	Gallic acid
DPPH radical scavenging	0.893 ± 0.09^{b} **	3.30 ± 0.49^{c} ***	0.294 ± 0.06^{a} *	5.14 ± 0.84^{d} ***	0.002 ± 0.00
Superoxide radical scavenging	1.52 ± 0.30^{b} ***	4.12 ± 0.15^{c} ***	0.556 ± 0.03^{a} *	5.23 ± 1.11^{d} ***	0.030 ± 0.00
Nitric oxide radical scavenging	2.25 ± 0.47^{b} **	4.85 ± 0.81^{c} ***	0.613 ± 0.05^{a} **	7.52 ± 1.73^{d} ***	0.045 ± 0.00
Iron (II) chelating	4.66 ± 1.13^{b} *	5.94 ± 1.54^{b} ***	2.00 ± 0.15^{a} *	7.06 ± 1.29^{c} ***	1.53 ± 0.03
Inhibition of deoxyribose degradation	2.17 ± 0.17^{b} ***	4.61 ± 0.61^{c} ***	1.48 ± 0.21^{a} **	7.27 ± 1.38^{d} ***	0.141 ± 0.01

Abbreviations: CE: Crude extract; HF: Hexane fraction; EAF: Ethyl acetate fraction; AF: Aqueous fraction. Data for IC_{50} are expressed as mean ± SD (n = 3). Different letters between columns represent significant differences between the crude extract and fractions with a denoting highest antioxidant activity ($p < 0.05$). Asterisks represent significant differences between the extracts and gallic acid (positive control), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

activity while the hexane and aqueous fraction displayed lowest activity.

Antibacterial activity

A preliminary screening of the antibacterial activity of the *N. exigua* crude extract and fractions was carried out against the bacterial strains *C. acnes, S. mutans, P. intermedia* and *M. smegmatis*. As shown in Table 2, the crude extract, hexane and ethyl acetate fractions of *N. exigua* exhibited varying degrees of activity against the test bacterial species with MIC values within a concentration range of 0.039 to 2.5 mg/ml. The ethyl acetate fraction had the most potent antibacterial activity particularly against *C. acnes* (MIC: 0.039 mg/ml), *S. mutans* (MIC: 0.078 mg/ml) and *M. smegmatis* (MIC: 0.313 mg/ml). Although the crude extract displayed minimal inhibitory activity in general, it recorded higher activity exceptionally against *P. intermedia* (MIC: 1.25 mg/ml) compared to its fractions. Weak antibacterial activity was observed for the hexane fraction whereas the aqueous fraction had no effect at all. Furthermore, the sponge crude extract and fractions appeared to have a broad spectrum of activity as their activities observed were independent of whether they were Gram-positive or Gram-negative bacteria. Among the four bacterial strains investigated, maximum growth suppression was noted against the Gram-positive bacteria *C. acnes, S. mutans* and *M. smegmatis* while *P. intermedia* was the least sensitive.

In vitro cytotoxic activity

The *in vitro* cytotoxic potential of *N. exigua* was further investigated against the human liposarcoma SW872 cells. The cells were exposed to increasing concentrations of the crude extract and fractions (0.0156−2 mg/ml) for 24 h and the percentage cytotoxicity was determined using the MTT assay. A 24 h incubation with the extracts resulted in a concentration-dependent decrease in the proliferation of SW872 cells (Fig. 5). The ethyl acetate fraction displayed the highest activity by decreasing the cell viability from 87.53% to 13.57% followed by the crude extract (90.42% to 20.76%), hexane fraction (98.53% to 67.87%) and the aqueous fraction (100% to 90.93%). Based on this preliminary cytotoxic screening, the IC_{50} values were also determined (Table 3). The ethyl acetate fraction and crude extract exhibited inhibitory effects with IC_{50} values of 44.34 ± 2.64 μg/ml and 165.87 ± 37.69 μg/ml, respectively which were significantly lower than the positive control, etoposide (IC_{50}: 1.71 ± 0.27 μg/ml) ($p < 0.05$).

Table 2
Antibacterial activity (mg/ml) of *N. exigua* derived crude extract and fractions.

Crude extract and fractions/ Control	Minimum Inhibitory Concentration (mg/ml)			
	Cutibacterium Acnes	*Streptococcus Mutans*	*Prevotella Intermedia*	*Myco-bacterium Smegmatis*
CE	0.16	2.5	1.25	1.25
HF	0.31	2.5	2.5	2.5
EAF	0.04	0.78	2.5	0.31
AF	NI 2.5	NI 2.5	NI 2.5	NI 2.5
Positive control	0.003[a]	0.0005[b]	0.0005[b]	0.0004[c]

Abbreviations: CE: Crude extract; HF: Hexane fraction; EAF: Ethyl acetate fraction; AF: Aqueous fraction; NI 2.5: No inhibition at 2.5 mg/ml; [a]Tetracycline; [b]Chlorhexidine; [c]Ciprofloxacin. Values are the mean of Minimum Inhibitory Concentration (MIC) (mg/ml) ($n = 3$) of three independent determinations.

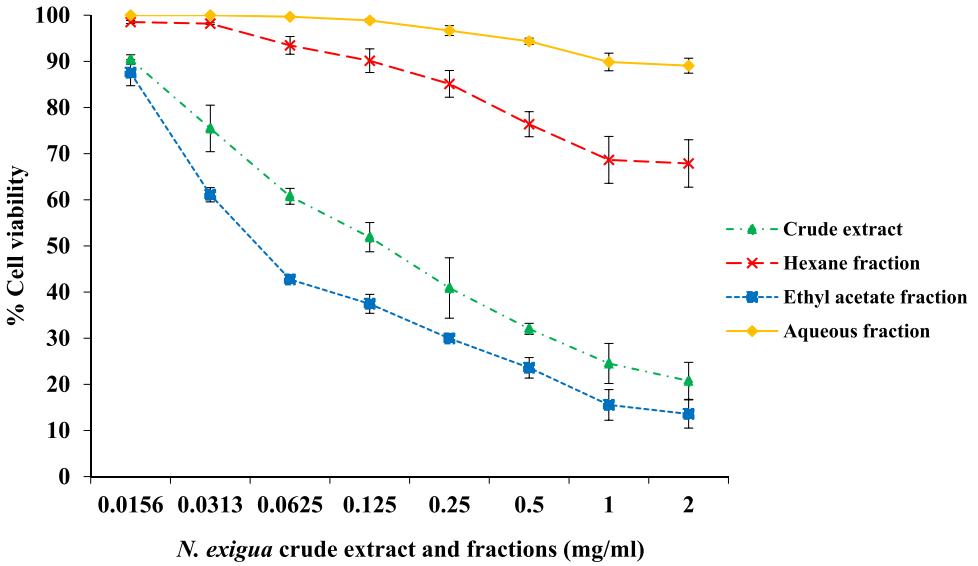


Fig. 5. Percentage cell viability of *N. exigua* crude extract and fractions against SW872 cells at different concentrations (2–0.0156 mg/ml). Data expressed as mean values ± standard deviation ($n = 3$) of three independent determinations.

Table 3
In vitro cytotoxic activity (IC_{50} ± SD, µg/ml) of *N. exigua* derived crude extract and fractions against SW872 liposarcoma cells.

Crude extract and fractions/ Control	CE	HF	EAF	AF	Etoposide
IC_{50} (µg/ml)	165.87 ± 37.69[b] ***	ND	44.34 ± 2.64[a] *	ND	1.71 ± 0.27

Abbreviations: CE: Crude extract; HF: Hexane fraction; EAF: Ethyl acetate fraction; AF: Aqueous fraction; ND: extract failed to induce 50% growth; hence, no IC_{50} value was determined. Data for IC_{50} are expressed as mean ± standard deviation ($n = 3$) in three independent experiments. Different letters between columns represent significant differences between the crude extract and fractions with the letter a denoting highest cytotoxic activity ($p < 0.05$). Asterisks represent significant differences between the extracts and etoposide (positive control), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Epigenetic activity

Given the interesting cytotoxic activity of the ethyl acetate fraction of *N. exigua*, its ability to overcome hypermethylation was further assessed in a HeLaGFP cell model via the LDR assay. HeLaGFP cells were treated with the fraction at its IC_{50} concentration (35.6 ± 4.23 µg/ml) overnight (Supplementary). After staining, the images acquired were analysed and the effect on GFP expression in the cell population was measured, based on the proportion of cells with intensity above the background. An average was taken from three independent experiments and plotted in a bar graph (Fig. 6[a]). The GFP re-activation capacity of the ethyl acetate fraction measured in terms of the total mean fluorescent intensity was 43.79 ± 3.19%, was 1.9-folds lower than the positive control TSA (total mean fluorescent intensity: 84.20 ± 5.02%), 12–13-folds higher than the negative control 0.5% DMSO (3.63 ± 1.07%) and null (3.12 ± 0.78%) ($p < 0.05$). Fluorescent image micrographs showing the effect of the ethyl acetate fraction on GFP expression in the HeLaGFP cells were compared to the controls (Fig. 6[b]).

Discussion

Recently, natural product chemists have intensified their bioprospecting efforts towards unfolding the pharmacological activities *Neopetrosia exigua,* a marine sponge species that has generated enormous attention as revealed by the increasing number of bioactive extracts/compounds reported during the last two decades [9,10,12,13]. Contributing to this endeavor, the present study examined the *in vitro* antioxidant, antibacterial, cytotoxic and epigenetic activities of the Mauritian sponge *N. exigua*-derived crude extract and fractions to ascertain any potential therapeutic effect.

A rising demand requisite on welcoming novel and alternative sources for natural antioxidants prototypes. In this view, the potential of *N. exigua* as a source of antioxidant was investigated using an array of *in vitro* antioxidant systems. It is important to employ different assays when taking into consideration the chemical constituents of extracts that act through various mechanisms such as prevention of chain initiation and continued hydrogen abstraction, decomposition of peroxides, binding of transition metal ion catalysts reductive capacity and radical scavenging [32]. In the present study, we observed that the ethyl acetate fraction exhibited the highest antioxidant activity by virtue of its ferric reducing, free radical scavenging (DPPH, $ABTS^{\cdot+}$, superoxide, nitric oxide and hydroxyl) and Fe^{2+} chelating activities (Table 1). Therefore, this indicates that the ethyl acetate fraction is a potential repertoire of

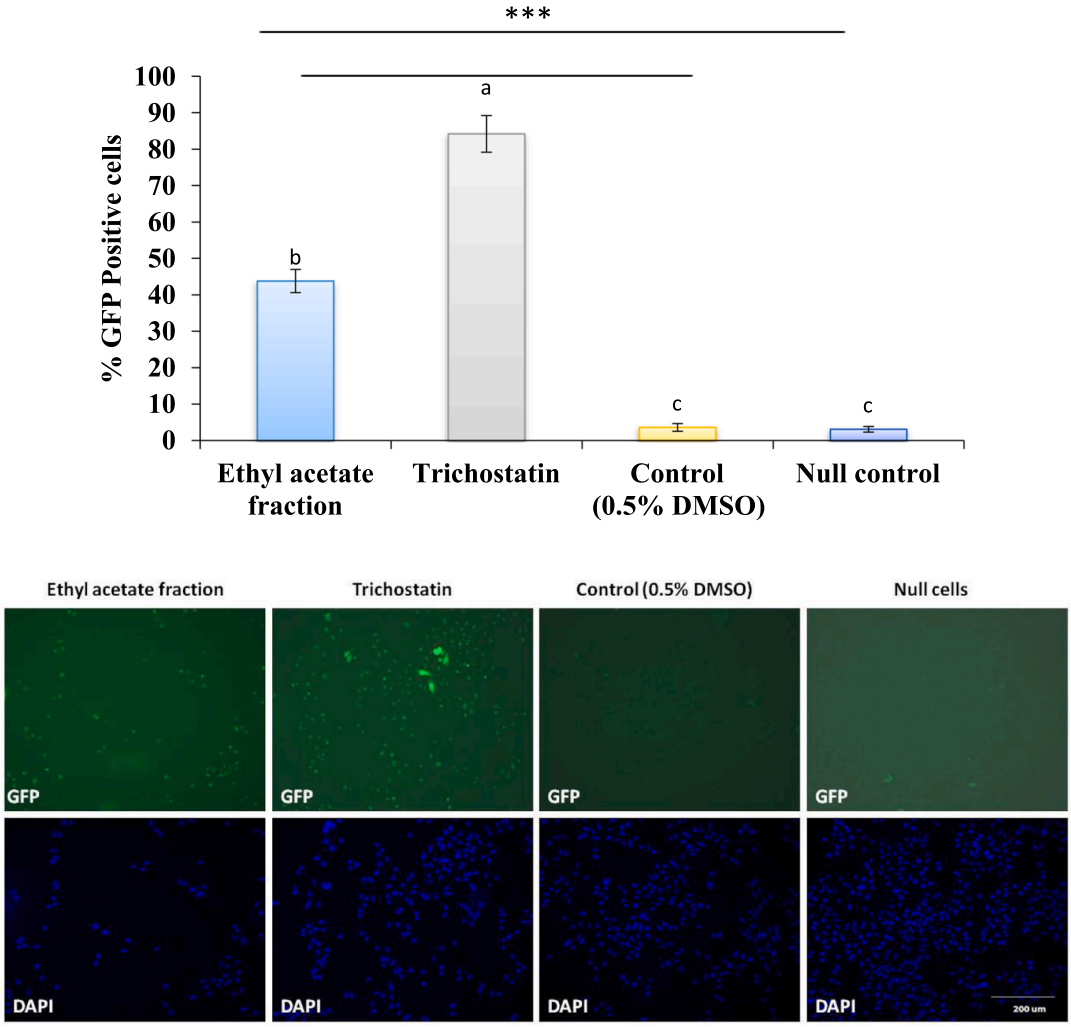


Fig. 6. (a) Effect of the ethyl acetate fraction of *N. exigua* (IC_{50}: 44.34 ± 2.64 μg/mL) on silenced GFP LDR capacity measured in terms of its total mean fluorescent intensity compared to the positive control trichostatin A (10 μmol) and negative controls (one-way ANOVA, $p < 0.05$). Data are presented as mean ± SD ($n = 3$) in three independent experiments and normalised to cell number. Different letters represent significant differences between the sponge fraction, positive and negative controls with the letter a denoting highest LDR activity ($p < 0.05$).***indicates significant difference from the negative control (one-way ANOVA, $p < 0.001$). (b) Fluorescent images showing the effect of the ethyl acetate fraction of *N. exigua* on GFP expression. They were taken at two distinct wavelengths: DAPI (blue), and GFP (green). HeLa/GFP cells contain hypermethylated GFP reporters. X100.

antioxidants.

In parallel, the emergence of multiple drug resistance in human pathogenic microorganisms has lately created a critical momentum to search for new antibiotics [33]. During the past decades, intensive efforts have led to the discovery of interesting antimicrobial leads from marine sponges such as petrosamine B [34] and dysidavarone A [35]. To continue our previous study on the antibacterial and antibiotic potentiating activity of some selected tropical marine sponge extracts [9], we further investigated the antibacterial activity of *N. exigua* against the bacterial strains *C. acnes, S. mutans, P. intermedia* and *M. smegmatis*. As shown in Table 2, the crude extract and fractions displayed MIC values ranging from 0.039 to 2.5 mg/ml. The ethyl acetate fraction recorded the most potent antibacterial activity while the aqueous fraction was unable to inhibit bacterial growth at the highest concentration tested. The diversity in the antimicrobial activity shown by extracts is usually subjected to the differences in their compositions and concentrations of active constituents [36]. Furthermore, extracts with MIC values lower than 1 mg/ml are generally considered as highly active antimicrobial agents worth exploring further for drug development [37]. The ethyl acetate fraction recorded the lowest MIC value of 0.039 mg/ml against *C. acnes* which is the major causative microorganism of acne vulgaris. This is particularly interesting because the fraction also demonstrated high scavenging potential against nitric oxide free radicals (Table 1) which are highly involved in the onset of inflammatory diseases and in the progression of acne. Similarly, noteworthy inhibitory activity against *S. mutans* by the fraction with MIC of 0.078 mg/ml. However, it is striking to note that the highest antibacterial activity against *P. intermedia* was registered by the

crude extract (MIC: 1.25 mg/ml) when compared to its fractions. This may suggest a synergistic effect arising from a mixture of compounds present in the crude extract which could be responsible for its high antibacterial activity. *S. mutans* and *P. intermedia* are the two main causative bacterial species of periodontal diseases and are notorious for their drug resistance [38]. Notable antibacterial effect was also recorded against *M. smegmatis* (MIC: 0.313 mg/ml). This is a non-pathogenic species of *mycobacterium* commonly used as a model in the physiology of mycobacteria such as the pathogenic species *Mycobacterium tuberculosis* which is the causative agent of tuberculosis. Despite the promising bioactivities registered by *N. exigua,* there is no reports on its activity on either of the microorganisms tested in this study, thus providing new knowledge on its antibacterial potential. Nevertheless, this study is in congruence with our previous work where we demonstrated the broad antibacterial activity of the ethyl acetate fraction of *N. exigua* against a panel of bacterial strains including *Escherichia coli, Pseudomonas fluorescens, Pseudomonas aeruginosa, Klebsiella pneumonia, Serratia marcescens, Salmonella enterica, Bacillus cereus* and *Staphylococcus aureus* [9].

Further investigation was done into the *in vitro* cytotoxic effect against the human liposarcoma, SW872. Liposarcomas are malignant tumors that form in fat cells found in deep soft tissue. To-date, traditional treatment measures such as surgery and chemotherapy remain unsatisfactory with local recurrence rates greater than 80% [39]. Hence, this justifies the need for new therapeutic strategies. Herein, the ethyl acetate fraction recorded the highest cytotoxic effect with an IC_{50} value of 44.34 ± 2.64 μg/ml against SW872 cells ($p < 0.05$). However, this activity is regarded as moderate according to the guidelines of the US National Cancer Institute (NCI), which consider extracts exhibiting IC_{50}'s lower than 20 μg/ml as having significant anticancer activity, 21−200 μg/ml as moderate anticancer activity, 201−500 μg/ml as weak anticancer activity and no toxicity towards cancerous cells for values higher than 500 μg/ml [40]. Because this fraction is still a mixture of active and non-active compounds, a higher IC_{50} value is expected and hence, with more refined fractions or pure compounds, lower IC_{50} values can be considered worthy for further investigations. The crude extract recorded low toxicity with an IC_{50} value of 165.87 ± 37.69 mg/ml while an IC_{50} could not be determined for the hexane and aqueous fraction due to poor cytotoxic activity. To the best of our knowledge, the cytotoxic effect of the sponge *N. exigua* against liposarcoma cells has been investigated for the first time. Nonetheless, isolation of several novel compounds, mostly alkaloids with known cytotoxicity, has been reported from this sponge species. Liang et al. [10] reported the isolation of papuamine from the Indonesian sponge, *N. exigua* which displayed cytotoxic activity against glioblastoma SF-295 cells. Chemical investigation of the ethanolic extract of the same sponge species collected in Palau, led to the isolation of several araguspongine alkaloids among which the novel alkaloid araguspongine M displayed a pronounced inhibitory effect against the human leukemia cell line HL-60 [12]. Exiguamine A, another novel alkaloid isolated from *N. exigua* collected in Papua New Guinea was identified as one of the most potent inhibitors of the enzyme indoleamine-2, 3-dioxygenase involved in tumor growth *in vitro* [13]. tumor suppressors are well known for coordinating apoptosis to maintain genomic stability and prevent tumor formation. On the other hand, dysfunction of tumor suppressor genes has been shown to contribute to the development of most human cancers. In many cancers, tumor suppressors are inactivated due to epigenetic aberrations such as DNA hypermethylation of specific promoters of some tumor suppressor genes such as p53 and p21 [41]. Many of the enzymes that mediate these genetic alterations, including deacetylases, histone acetyltransferases, DNA methyltransferases have been identified and are being intensively studied because of their immense potential as therapeutic targets [42]. Natural products known to modulate the functions of these proteins are relatively limited in number, structural diversity, and enzyme class specificity. Based on the encouraging cytotoxic activity of the ethyl acetate fraction, its effect on the derepression of a silenced GFP reporter was investigated with the aim to identify novel molecules that could potentially regulate new targets involved in the epigenetic control of tumor suppressor gene expression. The GFP expression level of the sponge fraction, although lower than the epigenetic drug Trichostatin A, was however significantly higher compared to the negative controls ($p < 0.001$). Therefore, these data suggest that *N. exigua* could be a potential source of epigenetic modulators.

The heatmap analysis (Fig. 7) showed variability in terms of their bioactivities between the crude extract and fractions of *N. exigua* which allowed for easy discrimination. In general, the ethyl acetate fraction was well separated with a red color predominance, characterized by the highest antioxidant, antibacterial, cytotoxic, and epigenetic activity. In this perspective, further research on the ethyl acetate fraction as starting material for the bioassay-guided characterization of the compounds accountable for the excellent bioactivities is actively warranted. It is also important to decipher the underlying mechanisms of action in order to identify potential novel drug targets. Moreover, the selective cytotoxic activity of the fraction should be assessed against normal cell lines in order to evaluate the safety profile.

Conclusions

Overall, this preliminary work highlights the promising bioactivities of the tropical marine sponge *N. exigua*, which considering the emergence of resistant bacteria and side effects of synthetic chemotherapeutic agents, embody a rich niche for the discovery of potential pharmaceutical leads. In particular, its ethyl acetate fraction showed significant antioxidant, antibacterial, cytotoxic, and epigenetic activities which could be useful for pharmaceutical applications. Consequently, this study is considered as a highly important basic work to study the mechanisms of actions underlying the biological activities. However, further investigations are actively warranted to characterize the active ingredients present in the most promising sponge fraction.

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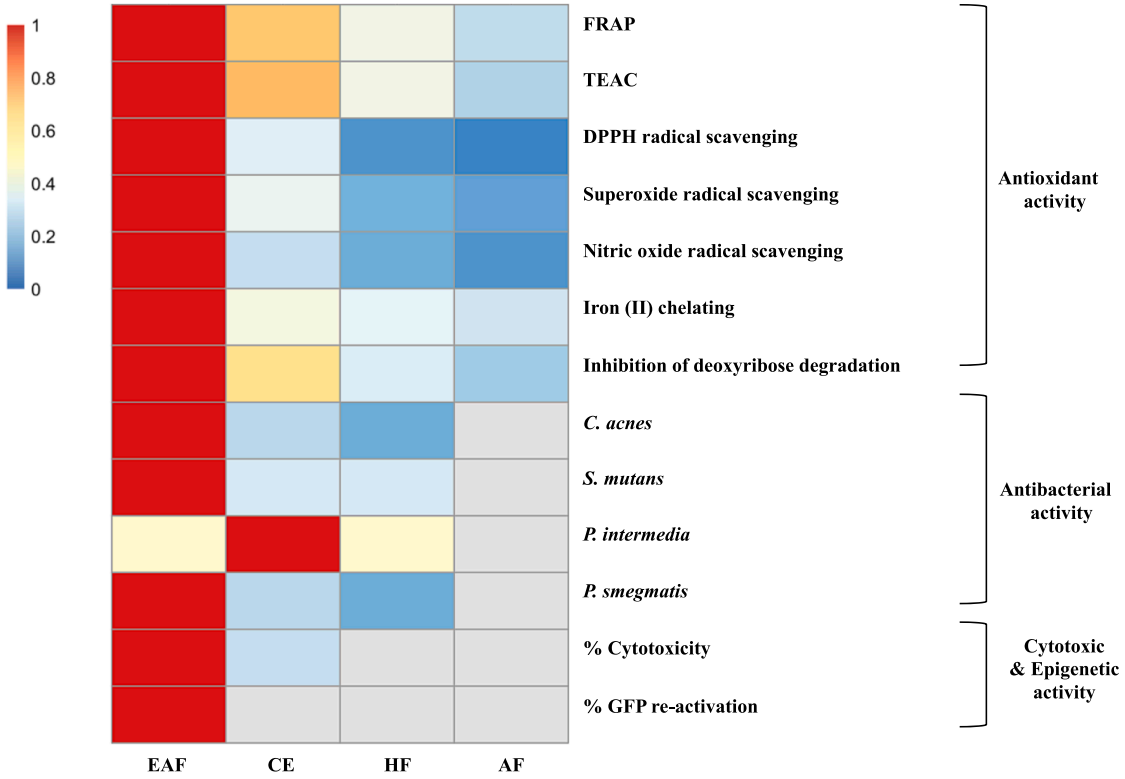

Fig. 7. Heatmap analysis for the biological activities of *N. exigua* crude extract and fractions. The crude extract and fractions are presented on the horizontal axis. The red color presents the highest levels of bioactivity. CE: Crude extract; HF: Hexane fraction; EAF: Ethyl acetate fraction; AF: Aqueous fraction.

Authors' contributions

Rima Beesoo conceptualised and designed the study, conducted the research, analysed the data and drafted the manuscript under the guidance of Ranjeet Bhagooli, Vidushi S Neergheen, Alexander Kagansky, Anna-Mari Reid, Isa Lambrechts, Lydia Gibango, Dikonketso Bodiba, Professor Namrita Lall and Theeshan Bahorun. The latter revised and finalised the manuscript. All authors have read and approved the final manuscript.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information file].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2023.e01867.

References

[1] P.V.R. Snelgrove, An ocean of discovery: biodiversity beyond the census of marine life, Planta Med. 82 (2016) 790–799, https://doi.org/10.1055/s-0042-103934.

[2] M.E. Hay, Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems, Annu. Rev. Mar. Sci. 1 (2009) 193–212, https://doi.org/10.1146/annurev.marine.010908.163708.

[3] N. Papon, B.R. Copp, V. Courdavault, Marine drugs: biology, pipelines, current and future prospects for production, Biotechnol. Adv. 54 (2022), 107871, https://doi.org/10.1016/j.biotechadv.2021.107871.

[4] M.A. Ghareeb, M.A. Tammam, A. El-Demerdash, A.G. Atnasov, Insights about clinically approved and preclinically investigated marine natural products, CRBIOT 2 (2020) 88–102, https://doi.org/10.1016/j.crbiot.2020.09.001.

[5] M.F. Mehbub, P.V. Perkins, W. Zhang, C.M.M. Franco, New marine natural products from sponges (Porifera) of the order Dictyoceratida (2001 to 2012); a promising source for drug discovery, exploration and future prospects, Biotechnol. Adv 34 (2016) 473–491, https://doi.org/10.1016/j.biotechadv.2015.12.008.

[6] D. Sipkema, M. Franssen, R. Osinga, J. Tramper, R. Wijffels, Marine sponges as pharmacy, Mar. Biotechnol. 7 (2005) 142–162, https://doi.org/10.1007/s10126-004-0405-5.

[7] W. Bergmann, R.J. Feeney, The isolation of a new thymine pentoside from sponges, J. Am. Chem. Soc. 72 (1950) 2809–2810, https://doi.org/10.1021/ja01162a543.

[8] P. Proksch, R.A. Edrada, R. Ebel, Drugs from the seas-current status and microbiological implications, Appl. Microbiol. Biotechnol. 59 (2002) 125–134, https://doi.org/10.1007/s00253-002-1006-8.

[9] R. Beesoo, R. Bhagooli, V.S. Neergheen-Bhujun, W.W. Li, A. Kagansky, T. Bahorun, Antibacterial and antibiotic potentiating activities of tropical marine sponge extracts, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 196 (2017) 81–90, https://doi.org/10.1016/j.cbpc.2017.04.001.

[10] Z. Liang, F. Sulzmaier, W. Yoshida, M. Kelly, J. Ramos, P. Williams, Neopetrocyclamines A and B, polycyclic diamine alkaloids from the sponge *Neopetrosia cf exigua*, J. Nat. Prod. 78 (2015) 543–547, https://doi.org/10.1021/np500759r.

[11] G. Carr, M.K.W. Chung, A.G. Mauk, R.J. Andersen, Synthesis of indoleamine 2,3-dioxygenase inhibitory analogues of the sponge alkaloid exiguamine A, J. Med. Chem 51 (2008) 2634–2637, https://doi.org/10.1021/jm800143h.

[12] H. Liu, Y. Mishima, T. Fujiwara, H. Nagai, A. Kitazawa, Y. Mine, H. Kobayashi, X. Yao, J. Yamada, T. Oda, M. Namikoshi, Isolation of araguspongine M, a new stereoisomer of an araguspongine/xestospongin alkaloid, and dopamine from the marine sponge *Neopetrosia exigua* collected in Palau, Mar Drug. 2 (2004) 154–163, https://doi.org/10.3390/md204154.

[13] H. Brastianos, E. Vottero, B. Patrick, R. Van Soest, T. Matainaho, A. Mauk, R. Andersen, Exiguamine A, an indoleamine-2, 3-dioxygenase (IDO) inhibitor isolated from the marine sponge *Neopetrosia exigua*, J. Am. Chem. Soc. 128 (2006) 16046–16047, https://doi.org/10.1021/ja067211.

[14] K.Y. Orabi, K.A. El Sayed, M.T. Hamann, D.C. Dunbar, M.S. Al-Said, T. Higa, M. Kelly, Araguspongines K and L, new bioactive bis -1-oxaquinolizidine N-oxide alkaloids from Red Sea specimens of *Xestospongia exigua*, J. Nat. Prod. 65 (2002) 1782–1785.

[15] T. Venkateshwar Goud, N. Srinivasa Reddy, N. Raghavendra Swamy, T. Siva Ram, Y. Venkateswarlu, Anti-HIV active petrosins from the marine sponge *Petrosia similis*, Biol. Pharma. Bull. 26 (2003) 1498–1501, https://doi.org/10.1248/bpb.26.1498.

[16] N. Oku, S. Matsunaga, R.W.M. Soest, N. Fusetani, Renieramycin J, a highly cytotoxic tetrahydroisoquinoline alkaloid, from a marine sponge *Neopetrosia* sp, J. Nat. Prod. 66 (2003) 1136–1139, https://doi.org/10.1021/np030092g.

[17] Y. Nakao, T. Shiroiwa, S. Murayama, S. Matsunaga, Y. Goto, Y. Matsumoto, N. Fusetani, Identification of renieramycin A as an anti-leishmanial substance in a marine sponge *Neopetrosia* sp, Mar Drug. 2 (2004) 55–62. PMCID: PMC3783859.

[18] Y. Li, S. Qin, Y.W. Guo, Y.C. Gu, R.W. Soest, 9'-Epi-3beta, 3'betadimethylxestospongin C, a new macrocyclic diamine alkaloid from the Hainan sponge *Neopetrosia exigua*, Planta Med. 77 (2011) 179–181, https://doi.org/10.1055/s-0030-1250164.

[19] X. Wei, K. Nieves, A.D. Rodriguez, Neopetrosiamine A, biologically active bis-piperidine alkaloid from the Caribbean Sea sponge *Neopetrosia proxima*, Bioorg. Med. Chem. Lett. 20 (2010) 5905–5908, https://doi.org/10.1016/j.bmcl.2010.07.084.

[20] H. Fujiwara, K. Matsunaga, M. Saito, S. Hagiya, K. Furukawa, H. Nakamura, Y. Ohizumi, Halenaquinone, a novel phosphatidylinositol 3-kinase inhibitor from a marine sponge, induces apoptosis in PC12 cells, Eur. J. Pharmacol. 413 (2001) 37–45, https://doi.org/10.1016/s0014-2999(00)00944-4.

[21] P.L. Winder, H.L. Baker, P. Linley, E.A. Guzman, S.A. Pomponi, M.C. Diaz, J.K. Reed, A.E. Wright, Neopetrosiquinones A and B, sesquiterpene benzoquinones isolated from the deep-water sponge *Neopetrosia cf. proxima*, Bioorg. Med. Chem. 19 (2011) 6599–6603, https://doi.org/10.1016/j.bmc.2011.09.026.

[22] R.W.M. Van Soest, N. Boury-Esnault, J.N.A. Hooper, K. Rützler, N.J. de Voogd, B. Alvarez, E. Hajdu, A.B. Pisera, R. Manconi, C. Schönberg, M. Klautau, M. Kelly, J. Vacelet, M. Dohrmann, M.C. Díaz, P. Cárdenas, J.L. Carballo, P. Ríos, R. Downey, C.C. Morrow, World Porifera Database 2021. *Neopetrosia exigua* (Kirkpatrick, 1900). http://www.marinespecies.org/porifera (accessed 04 April 2021).

[23] I.F. Benzie, J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power: the FRAP assay, Anal. Biochem. 239 (1996) 70–76, https://doi.org/10.1006/abio.1996.0292.

[24] A. Campos, E. Lissi, Kinetics of the reaction between 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) derived radical cations and phenols, Int. J. Chem. Kinet. 29 (1997) 219–224, https://doi.org/10.1002/(SICI)1097-4601(1997)29:3<219::AID-KIN9>3.0.CO;2-X.

[25] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol. 28 (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.

[26] M. Nishikimi, N.A. Rao, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem. Biophys. Res. Commun. 46 (1972) 849–854, https://doi.org/10.1016/S0023-6438(95)80008-5.

[27] A.M. Leone, P.L. Francis, P. Rhodes, S. Moncada, A rapid and simple method for the measurement of nitrite and nitrate in plasma by high performance capillary electrophoresis, Biochem. Biophys. Res. Commun. 200 (1994) 951–957, https://doi.org/10.1006/bbrc.1994.1542.

[28] O.I. Aruoma, Nutrition and health aspects of free radicals and antioxidants, Food Chem. Toxicol. 32 (1994) 671–683, https://doi.org/10.1016/0278-6915(94)90011-6.

[29] V. Neergheen, M. Soobrattee, T. Bahorun, O. Aruoma, Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activities *in vitro*, J. Plant. Physiol. 163 (2006) 787–799, https://doi.org/10.1016/j.jplph.2005.09.009.

[30] N. Lall, C. Henley-Smith, M. De Canha, C. Oosthuizen, D. Berrington, Viability reagent, prestoblue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assays, Int. J. Microbiol. (2013), 420601, https://doi.org/10.1155/2013/420601.

[31] M. Partolina, H.C. Thoms, K.G. MacLeod, G. Rodriguez-Blanco, M.N. Clarke, A.V. Venkatasubramani, R. Beesoo, V. Larionov, V.S. Neergheen-Bhujun, B. Serrels, H. Kimura, N.O. Carragher, A. Kagansky, Global histone modification fingerprinting in human cells using epigenetic reverse phase protein array, Cell Death Discov. 3 (2017) 16077, https://doi.org/10.1038/cddiscovery.2016.77.

[32] M. Alkan, H. Yüksek, F. İslamoğlu, S. Bahçeci, M. Calapoğlu, M. Elmastaş, H. Akşit, M. Özdemir, A Study on 4-Acylamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones, Molecules 12 (2007) 1805–1816, https://doi.org/10.1016/j.cis.2020.102122.

[33] World Health Organisation (WHO), 2021. Fact sheets: Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed 11 April 2021).

[34] A. Carroll, S. Duffy, V. Avery, A. Citronamides, B, Tetrapeptides from the Australian sponge *Citronia astra*, J. Nat. Prod. 72 (2009) 764–768, https://doi.org/10.1021/np800832w.

[35] B. Schmalzbauer, J. Herrmann, R. Müller, D. Menche, Total synthesis and antibacterial activity of dysidavarone A, Org. Lett. 15 (2013) 964–967, https://doi.org/10.1021/ol400156u.

[36] N. Aktas, Y. Genç, B. Gozcelioglu, B. Konuklugil, U. Harput, Radical scavenging effect of different marine sponges from Mediterranean coasts, Rec. Nat. Prod. 7 (2013) 96–104.

[37] S. Van Vuuren, Antimicrobial activity of South African medicinal plants, J. Ethnopharmacol. 119 (2008) 462–472, https://doi.org/10.1016/j.jep.2008.05.038.

[38] C. Popova, V. Dosseva-Panova, V. Panov, Microbiology of periodontal diseases, a review, Biotechnol. Equip. 27 (2013) 3754–3759, https://doi.org/10.5504/BBEQ.2013.0027.
[39] J.A. Livingston, D. Bugano, A. Barbo, H. Lin, J.E. Madewell, W.L. Wang, A.J. Lazar, W.W. Tseng, C.L. Roland, B.W. Feig, R. Pollock, A.P. Conley, R.S. Benjamin, S. Patel, N. Somaiah, Role of chemotherapy in dedifferentiated liposarcoma of the retroperitoneum: defining the benefit and challenges of the standard, Sci. Rep. 7 (2017) 11836, https://doi.org/10.1038/s41598-017-12132-w.
[40] A. Goldin, J.M. Venditti, J.S. Macdonald, F.M. Muggia, J.E. Henney, V.T. Devita, Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute, Eur. J. Cancer 17 (1981) 129–142, https://doi.org/10.1016/0014-2964(81)90027-x.
[41] S.B. Baylin, P.A. Jones, Epigenetic determinants of cancer, Cold Spring Harb. Perspect. Biol. 8 (2016), https://doi.org/10.1101/cshperspect.a019505.
[42] R. Johnson, W. Huang, A. Jadhav, C. Austin, J. Inglese, E. Martinez, A quantitative high-throughput screen identifies potential epigenetic modulators of gene expression, Anal Biochem. 375 (2008) 237–248, https://doi.org/10.1016/j.ab.2007.12.028.