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El-Damhougy KA
Zoology Department, Faculty of Science, Al-Azhar University, Cairo

Hussein A El-Naggar
Zoology Department, Faculty of Science, Al-Azhar University, Cairo

Hassan AH Ibrahim
National Institute of Oceanography and Fisheries, Alexandria branch

Mansour AE Bashar
Zoology Department, Faculty of Science, Al-Azhar University, Cairo

Fekry M Abou Senna
Zoology Department, Faculty of Science, Al-Azhar University, Cairo

Correspondence
Mansour AE Bashar
Zoology Department, Faculty of Science, Al-Azhar University, Cairo

Biological activities of some marine sponge extracts from Aqaba Gulf, Red Sea, Egypt

El-Damhougy KA, Hussein A El-Naggar, Hassan AH Ibrahim, Mansour AE Bashar and Fekry M Abou Senna

Abstract
Two marine sponge species (*Grayella cyathophora* and *Negombata magnifica*) were collected during winter 2016 from Gulf of Aqaba, Red Sea, Egypt. The sponge samples were investigated as a promising source of natural products which can be used as antitumor, antiviral, antimicrobial, antioxidant, and anti-inflammatory agents. The present results revealed that the crude extract of *G. cyathophora* showed the high cytotoxic effect to Vero cell with hepatitis A virus which exhibits that Minimum inhibitor concentration was 2.929µg/ml. Also, it showed positive antibacterial activity against *P. aeruginosa* and the antioxidant activity compared to standard ascorbic acid was very weak with a value of 748µg / mL as IC_{50} and the activity of this anti-inflammatory extract was also (89.91%).At the same time, the crude extract of *N. magnifica* showed high antitumor activity with value of 1.09µg/ml and 0.37 µg/ml as IC_{50} against colon cancer (caco-2) and breast cancer (Mcf-7) on cell line, respectively, it does not possess any effect as an antiviral, antibacterial, anti-inflammatory and antioxidant activities. Generally, the present results confirmed that the ethanolic crude extract of *N. magnifica* conducted to promising antitumor agent and that of *G. cyathophora* conducted to promising anti-inflammatory and antiviral agents.

Keywords: Sponges, Red Sea, cytotoxicity, antimicrobial, anti-inflammatory, *Negombata magnifica*, *Grayella cyathophora*

1. Introduction

The sponges are sessile organisms; inhabit every type of marine benthic environment [1]. They are divided into four subclasses mainly according to the composition of their skeletons i.e. Calcarea, Hexactinllida, Sclerospongia and Demospongiae. Among them Demospongiae has the largest number of bioactive compounds [2]. Marine natural products (MNPs) have demonstrated exceptional potency and potential as anticancer therapeutics. *Negombata* sponge was shown to produce potent cytotoxic macrolides called latrunculins (e.g. latrunculins A and B) in addition to other cytotoxic compounds [3]. There are approximately 15,000 species of sponges in the world, of which, 150 species occur in freshwater but only about 17 species are of commercial value [4]. A variety of natural products from marine sponges have been found to exhibit a remarkable antitumor and anti-inflammatory activity [5]. Some compounds originated from marine organisms had been reported to possess in vitro and *in vivo* immune-stimulatory activity [6]. Antitumor studies were conducted with 19 marine natural products in a number of experimental and clinical models proved that sponges act as an excellent source for bioactive compounds [7]. Sponges have evolved chemical defense mechanisms against other invading organisms, which involve the production of secondary metabolites [8]. Recently, studies have suggested that some bioactive compounds isolated from marine organisms have been shown to exhibit anticancer, anti-microbial, anti-fungal, anti-inflammatory and other pharmacological activities [9]. So, the aim of the present study is to screen out the anti-inflammatory and cytotoxicity effects of some sponges *(Grayella cyathophora* and *Negombata magnifica*) collected from the Gulf of Aqaba, Red Sea, Egypt.

2. Materials and Methods

2.1 Sampling and identification of sponge specimens

The collecting of sponge specimens was done by SCUBA diving at different depths along Gulf of Aqaba during winter 2016. The specimen's identification has been carefully checked on the basis of morphological characters according to Systema Porifera [10] and the recent update undertaken in the World Porifera Database [11].

2.2 Sponge crude extracts preparation
After collection, the sponge specimens washed carefully by marine water, then macerated (about one hundred grams of specimens with 200 ml of 70% aqueous ethanol) for a week; the macerated specimen was genitally shaking and filtered through Whatman 542 filter paper. Solvent was evaporated using rotary evaporator to obtain soluble extracts [12].

3. Antimicrobial assay
3.1 Microbial indicator strains
The bacterial indicators were: *Escherichia coli, Pseudomonas aeruginosa* ATCC8739, *Staphylococcus aureus* ATCC6538, *Vibrio damsela,* and *Candida albicans.*

3.2 Media and bacterial cultures
Nutrient broth [13]: composed of (gl^{-1}): yeast extract, 2; beef extract, 1; peptone, 5; sodium chloride, 5. Agar (15-20) was added for obtaining nutrient agar. All pathogenic bacterial strains were maintained on nutrient agar slants. Bacterial inoculate were prepared by inoculating 100 ml of nutrient broth medium, and incubated shaken (250 rpm) at 30˚C for 24h until late logarithmic phase of growth (A_{550}=1). Antibacterial activity was carried out on nutrient agar plates.

3.3 Antimicrobial activity
The well-cut diffusion technique was used to test the ability of the crude extracts of sponges to inhibit the growth of indicator bacteria and fungi (Candida). Fifty millimeters of nutrient agar medium inoculated with indicator microbes were poured into plates. After solidifying, wells were punched out using 0.5 cm cork-porer, and each of their bottoms was then sealed with two drops of sterile water agar. One hundred microliters of crude extract was transferred into each well after sterilizing by ultra-filtration using 0.22 µl sterilized filters. All plates were incubated for 24 - 48 h at appropriate temperature. After incubation period, the radius of clear zone around each well (Y) and the radius of the well (X) were linearly measured in mm, where dividing Y^2 over X^2 determines an absolute unit (AU) for the clear zone. The absolute unit of crude extract, which indicates a positive result, was calculated according to the following equation [14]:
$AU= Y^2\pi/X^2\pi$

3.4 Antitumor activity
Cytotoxicity of extracts at various concentrations (15- 1000 µg/ml-) was assessed for Caco-2 and MCF-7 using the 3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) but with minor modification, following 72 h of incubation. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC_{50}) was determined [15].

Cell viability (%) = Mean OD/ control OD × 100
Mean Abs control, where: Abs absorbance at 490 nm

3.5 Antiviral activity
Vero cells were obtained from American Type Culture Collection (ATCC) continuous cell line established by (Yasumura and kawakita, 1963) [16]. Vero cell line was developed for isolation and propagation of many entero viruses and hepatitis A. In the current study, Vero cells from passage number 76 grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with Hanks salt base, supplemented with 10% fetal calf serum and 50 µg/ml gentamycin antibiotic solution. Rapidly growing virus strains producing cytopathic effect (CPE) in vero cell cultures within 3 days was used during this study and this virus was Hepatitis A virus H-10 strain.
Cytotoxicity of crude extracts of sponges were determined through morphological changes in Vero cells treated with different extracts in comparison with untreated control one. While anti-proliferative activity was measured using MTT assay. The MTT kit was based on dehydrogenase in a viable cell to determine cell viability with a colorimetric method that reduced the coloring reagent. Vero cells were grown as a monolayer in media supplemented with 10% inactivated fetal bovine serum. The monolayers of (10,000) cells were plated (10^4 cells/well) in 96-well tissue culture plate and incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2 before treatment with the extracts to allow attachment of cells to the plate except three well without cells as blank. Different concentrations of crude extracts of sponges (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0 µg/l) were added to the cell monolayer. Triplicate wells were prepared for each concentration as well as another three wells without extract as negative control. The plates were incubated into CO_2 incubator at 37 °C and 5% CO_2 for 48 h. After 48 h the cells were observed under an inverted microscope before completing the assay to observe the difference in morphology between cell controls and treated one at different concentrations of tested substances. The cell culture media containing different concentrations of tested extracts and dead cells was decanted and viable attached cells into the tissue culture plate were left. The plate containing viable cells was washed twice with PBS. Fifty microliters of MTT reagent was added to each well including blank and negative control wells. After addition of MTT reagent the plates were incubated in dark for 4 h for the reduction of MTT into form azan (purple needle color) by dehydrogenase activity in mitochondria of viable cells. One hundred microliters of DMSO was added to each well to solubilize the purple crystals of formazan. Absorbance was measured at (570 nm) with microplate reader. The maximum nontoxic concentration (MNTC) was determined. The percentage of cell survival was calculated by the following equation:

$$\text{Survival rate \%} = \frac{A\ \text{sample} - A_b}{A_c - A_b} \times 100$$

A_c=Negative control

A_b=Blank

3.6 Antioxidant assay
The ability of the extract to scavenge DPPH radicals were determined by the method of (Yen and Duh, 1994) [17], with minor modifications. 20µl of test extract at different concentrations in methanol was mixed with 0.5 ml of 100 mM methanolic solution of DPPH. After 30 min of incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm and the percentage inhibition was calculated using the following formula: Percentage inhibition = (Abs control – Abs sample) X 100/ Abs control.

3.7 Anti-inflammatory assay
The simple economical assay; *in vitro* inhibition of protein (albumin) denaturation technique was used for screening of

the anti-inflammatory producers [18]. Test solution (2500 µl) consisted of 2250 µl of bovine serum albumin (BSA) (1%, w/v aqueous solution) and 250 µl of test solution (supernatant of the culture) was prepared. Test control solution consisted of 2250 µl of bovine serum albumin (BSA) and 250 µl of blank broth was prepared. Standard solution consisted of 2250 µl of bovine serum albumin (BSA) and 250 µl 0f Diclofenac sodium “Voltaren® ampoule Novartis Pharma” (100, 250, 500, and 1000 µg/ml) was prepared. All of the previous solutions were adjusted to pH, 5.5 using a small amount of 0.1N HCl, and or 0.1N NaOH. The samples were incubated at 37 °C for 20min and then transferred to 60 °C water bath for 5 min. The samples at room temperature were cooled, and then 2500 µl of phosphate buffer were added to the above solutions. The absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated using the following formula:

Inhibition of protein denaturation (%) =

$$\left(\frac{\text{absorption of control} - \text{absorption of test}}{\text{absorption of control}}\right) \times 100$$

The control represents 0% inhibition of protein denaturation. The activity of each tested supernatant was compared with the standard commercial anti-inflammatory agent “Diclofenac sodium” using different concentrations (100, 250, 500, and 1000µg/ml).

4. Results

4.1 Sponge identification

The collected sponges were identified according to table (1) and Figure (1).

Negombata magnifica commonly known as toxic finger-sponge, it is reddish-brown narrow crooked branches. it prefers to grow between corals and rocks, or under them. It lives on shallow coral reefs in the northern waters of the Red Sea.

Grayella cyathophora lives in Red Sea and Indian Ocean. It is cream-colored, irregularly shaped, more or less lobed, with large oscula of varying size at the top.

The two identified sponge species were screened as a potential source for bioactive substances. So, antibacterial, antiviral, antioxidant, antitumor, and anti-inflammatory activities were detected.

Table (1): Scientific classification of the sponge samples collected from Gulf of Aqaba.

Scientific classification	Sponge sample	
	S1	S2
Kingdom	Animalia	Animalia
Phylum	Porifera	Porifera
Class	Demospongiae	Demospongiae
Order	Poecilosclerida	Poecilosclerida
Family	Podospongiidae	Crellidae
Genus	Negombata	*Grayella*
Species	*Negombata magnifica*	*Grayella cyathophora*
Common Name	Toxic finger-sponge	irregularly shaped

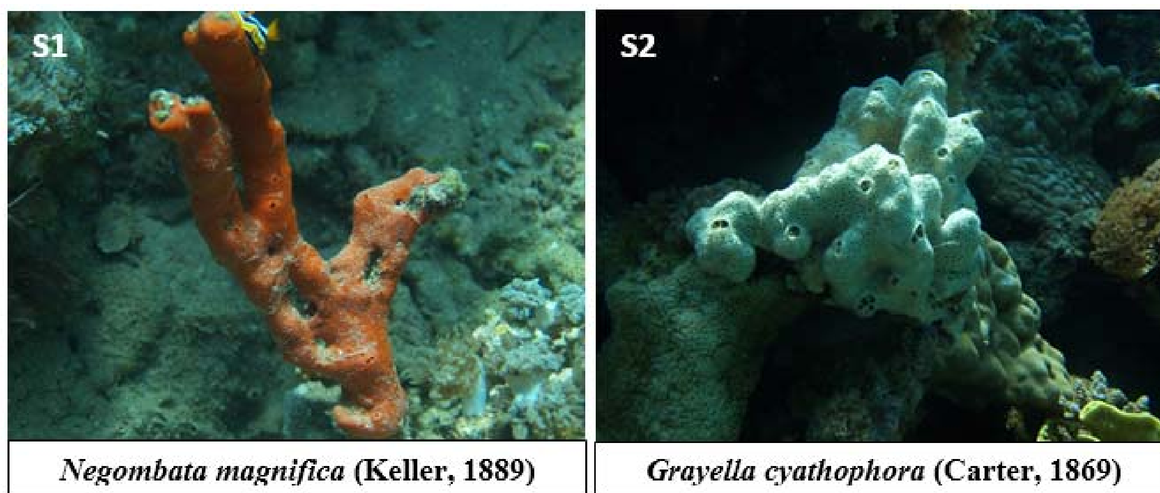
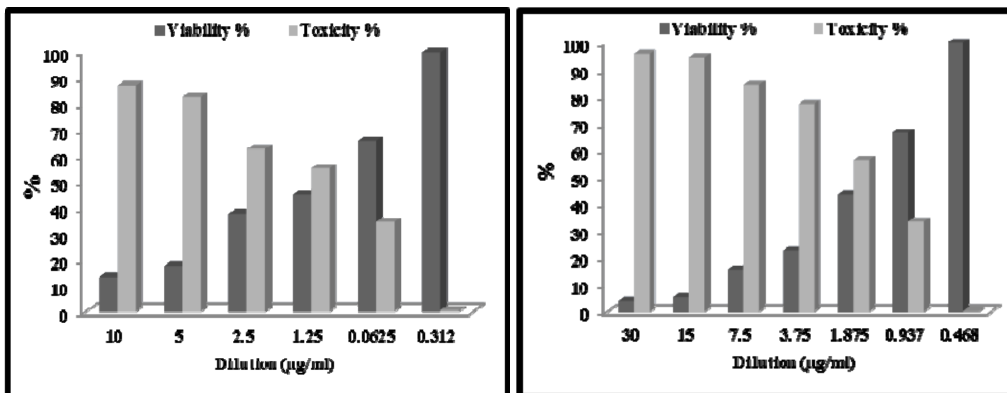


Fig 1: Investigated sponge species.

4.2 Antitumor activity of crude sponge extract

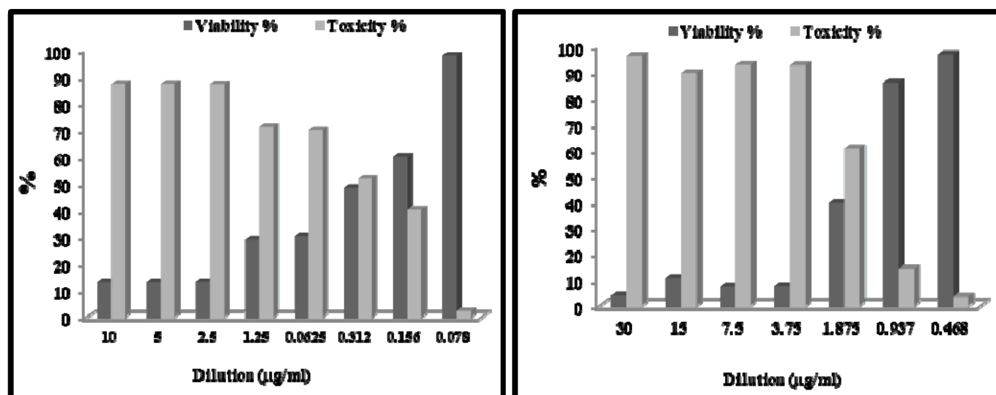
Figure (2A) revealed that the ethanolic extract of *G. cyathophora* showed antitumor activity with value of 2.14µg/ml as IC_{50} which is able to kill the half number of tumor cells of caco-2 while, *N. magnifica* extract showed antitumor activity with value of 1.09µg/ml as IC_{50} which is able to kill the most number of tumor cells of caco-2 Figure (2B). This experiment showed a very strong activity of *N. magnifica* extract against colon cancer cells.

On the other hand, the same extract of *G. cyathophora* showed antitumor activity on Mcf-7 cell line with value of 1.66µg/ml as IC_{50}which able to kill about half number of tumor cells of Mcf-7(Figure, 3A) but *N. magnifica* extract showed antitumor activity with value of 0.37µg/ml as IC_{50} which is able to kill the more number of tumor cells of Mcf-7 (Figure, 3B). This experiment showed a very strong activity of *N. magnifica* extract against breast cancer cells.



A B

Fig 2: Effect of two sponge crude extracts on Caco-2 cells.



A B

Fig 3: Effect of the two studied species crude extracts on Mcf-7 cells

4.3 Antiviral activity of crude sponge extracts

N. magnifica extract showed cytotoxic effect to Vero cell in all dilutions except the last ones; this experiment showed that MIC of it is 0.312µg/ml. The incubation period increases the effect of its extract on Vero cell. Finally, MNTC was detected as 0.156µg/ml for it at further studies by MTT assay. The *G. cyathophora* extract showed cytotoxic effect to Vero cell in all dilutions except the last two ones; its cytotoxic effect on Vero exhibits that MIC was 2.929µg/ml. also the incubation period increases the effect of extract on Vero cell. MNTC was detected as1.464 µg/ml for it at further studies by MTT assay.

According to figure (4, 5), The maximum non-toxic concentrations of both extracts (*N. magnifica* and *G. cyathophora*) were 2.9 and 0.3 µg/ml, respectively, they subsequently investigated to evaluate their potentially against HAV (hepatitis A virus), so, according to method applied, HAV was injected to Vero cell causing toxicity for 60% of them which represents 100% of its actual virulent power. By application of *N. magnifica* crude extract the toxicity of virus to Vero cell lowered to 61.7% which represented 101.9% of its actual power that means that *N. magnifica* crude extract not possessed an antiviral activity. While, by using *G. cyathophora* crude extract the toxicity of virus to vero cell became 8 which represent 21.2% of viral activity so, *G. cyathophora* crude extract exhibited antiviral activity in percentage of 78.8%. Entirely, the *G. cyathophora* showed a relatively higher activity than *N. magnifica*.

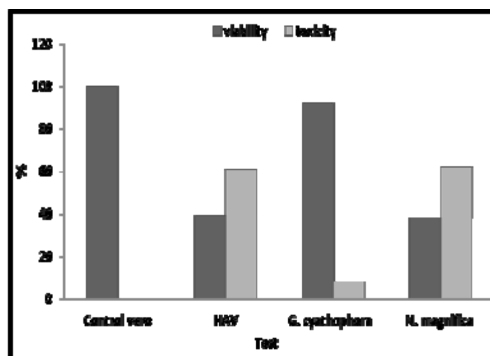


Fig 4: Antiviral activity of the two studied species crude extract and HAV against Vero cell

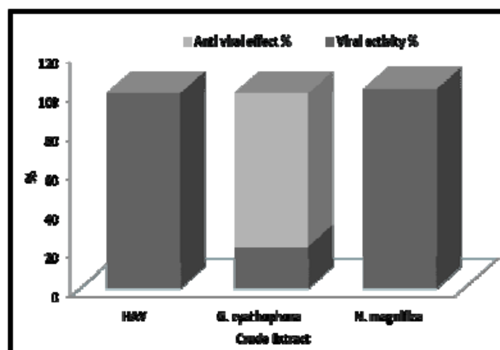


Fig 5: Antiviral activity of the two studied species crude extracts against HAV

4.4 Antimicrobial activity of *N. magnifica* crude extract.

The antimicrobial activity produced by different crude extracts of sponge species was screened against several human and fish pathogens (*E. coli, P. aeruginosa* ATCC8739, *S. aureus* ATCC6538, *V. damsela,* and *C. albicans*).

Table (2) revealed that the most effective extract was of *G. cyathophora* against *P. aeruginosa.* On the other hand, the extract of *N. magnifica* exhibited no antibacterial activities**.**

Table 2: Antimicrobial activity of the two studied species crude extracts

Crude extract	*E. coli*	*P. aeruginosa*	*S. aureus*	*V. damsela*	*C. albicans*
N. magnifica	-ve	**-**ve	-ve	-ve	-ve
G. cyathophora	-ve	+**ve**	-ve	-ve	-ve

4.5 Antioxidant activity of of the two studied species crude extracts

Data in Table (3) showed that *G. cyathophora* extract conducted an antioxidant activity in corresponding to ascorbic acid standard with value of 748µg/ml as IC_{50} which was larger than IC_{50} of ascorbic acid and that means the antioxidant power of ascorbic acid is larger than this sample. On the other hand, the extracts of *N. magnifica* had no antioxidant activity at any dilutions.

Table 3: DPPH % of the two studied species crude extracts.

Extract	Concentration (µg/ml)						
	0	25	50	100	200	400	800
Ascorbic acid	0.0	77.41	93.6	100	100	100	100
N. magnifica	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G. cyathophora	0.0	2.8	10.9	15.4	20.4	31.8	52.7

4.6 Anti-inflammatory activity of the two studied species crude extracts

The ethanolic crude extracts of the investigated sponge species were examined as anti-inflammatory agents. Table (4) exhibited that there was only one crude extract had anti-inflammatory activity; it was of *G. cyathophora* (89.91%). Data revealed that the crude extract of *G. cyathophora* conducted to promising anti-inflammatory agent. The extract of *N. magnifica* had no any anti-inflammatory activity at all.

Table 4: Anti-inflammatory activity of the two studied species crude extracts

Sponge crude extract	% inhibition of protein denaturation	Anti-inflammatory %
Blank	0.00	0.00
N. magnifica	-319.27	0.00
G. cyathophora	89.91	89.91

5. Discussion

Negombata magnifica was collected form Dahab (laguna) at 10-20 m depth. Gab-Alla *et al.* (2000) [19] recorded it at Gulf of Aqaba in Ras Um EI Sied site and Kelman *et al.* (2000) [20] found it from the northern Red Sea Gulf of Aqaba (Eilat) at 1-30 m depth. On the other hand, *Grayella cyathophora* was collected from laguna of Dahab at 3-20 m depth, Also, this species recorded in Ras Nusrani site "Gulf of Aqaba" and in the northern Red Sea Gulf of Aqaba at Eilat from 1 to 30 m depth(Gab-Alla *et al.*, 2000; Kelman *et al*., 2000) [19-20].

Marine sponges have been ranked very high in the priority of natural product research because the discovery of a wide range of bioactive chemical components and secondary metabolites with potential pharmaceutical applications gave promising results [21]. Marine sponge *Theonella* spp. show in vitro cytotoxity and *in vivo* antitumor activity in many leukemia and solid tumor model systems [22]. The toxicity of sponges has been well-documented, which could be ascribed to the diverse and potent cytotoxic compounds [23].

Indeed marine sponges have the potential to provide future drugs against important diseases, such as cancer, a range of viral diseases, and inflammations [24-21].

Results obtained during this study revealed that the crude extract of *N. magnifica* showed the highest antitumor activity with value of 1.09µg/ml and 0.37 µg/ml as IC_{50} against colon cancer (caco-2) and breast cancer (Mcf-7) on cell line, respectively. In case of its mesohyl IC_{50} was 5 µl/ ml against colon cancer (caco-2) this means that this selected sponge species significantly decreased the proliferation of Caco-2 cells in a dose dependent manner [25]. The present results are similar to who reported that the IC_{50} of *Halicolona exigua* was approximately 0.31mg/ml for MCF7 the cell cytotoxicity assay demonstrates that the extract exhibited the highest potency in inhibiting cell growth [26]. *Negombata* sponge was shown to produce potent cytotoxic macrolides called latrunculins in addition to other cytotoxic compounds [27].

Also, new compounds with anti-tumor activity a class of DNA intercalating (Plakinidines) were isolated from the marine sponge, *Crella spinulata* [27]. Rady (2014) [25] has tested the anticancer activity of mesohyl of four sponge species against colon cancer cell line (Caco-2) *in vitro* using MTT assay and cell cycle analysis was evaluated using flow cytometry. All the four selected sponge species significantly decreased the proliferation of Caco-2 cells in a dose-dependent manner. Results also, confirmed that the mesohyl of *Negombata magnifica* sponge, IC_{50} was 5 µl/ ml, while *Crella spinulata* exhibited cytotoxicity with IC_{50} = 8 µl/ ml.

The sponge crude extracts seems to have effective cytotoxic property that was detected by Brine shrimp assay.Sponges of the subclass Demospongiae are known to produce the largest number and diversity of secondary metabolites isolated from marine invertebrates, most of them with medically relevant biological activities and important ecological roles [28].

Red Sea sponges offer a potential for production of novel drugs and prototypes. The genus *Negombata* is a type of sponges abundant in the Red Sea. This sponge produces latrunculins that have antimicrobial and antiviral effects [29].

Our results indicated that the *Grayella cyathophora* extract showed positive antibacterial activity against *P. aeruginosa* but it hasn't any effects as antifungal. While, the ethanol extract of *Negombata magnifica* exhibited no antimicrobial activities.

Actually, the extracts of a large number of sponges exhibited broad spectrum of antibacterial activity. Some of these

extracts were especially active against *Staphylococcus, Pseudomonas*, acid fast bacteria and pathogenic yeasts, such as *Monila* [30]. Abou-Elela *et al.*, (2009) [31] tested crude extracts of marine sponge against different bacterial pathogens by well-cut agar diffusion method. They found that extracts of *Spongia officinalis* (Alam El Roomat 50m) showed the highest inhibiting activity. Activity units ranged from 4.59 against *S. aureus, S. faecalis and P. aeruginosa* to 6.61against *E. coli*. Also, they observed that chloroform crude extracts were superior to the ethanolic crude extracts. Also, chloroform crude extracts of sponge inhibited the growth of all bacterial pathogens.

The antibacterial and anticandidal activities of sponges collected from the southern part of the Gulf of Aqaba were investigated. Methanol extracts of ten sponge species were tested against six test microorganisms: Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli* and *Proteus vulgaris*, the yeasts *Candida albicans* and *C. tropicalis*. Three species only named; *Acanthella carteri, Ircinia felix* and *Ircinia strobilinia* had broad spectrum antimicrobial activity. On the other hand, growth promotion was stimulated by *Ircinia felix* towards *Escherichia coli.* Investigating different bioactivity of sponges may open new avenues for introducing novel marine compounds into pharmaceutical industry. Also, screening the inhibitory or promoting activities of sponge extracts may reflect the ecological mechanisms of fouling organisms settled on the sponge substratum [19].

The present extract of *Grayella cyathophora* exhibited high cytotoxic effect to Vero cell with HAV; which exhibits that MIC was 2.929µg/ml. Also, its crude extract exhibited antiviral activity in percentage of 78.8%. Isolation of natural components and metabolites from sponges and screening bioactive substances led to the discovery of numerous chemicals with antiviral properties [32].

These bioactive molecules are often secondary metabolites, whose main function is to enable and/or modulate cellular communication and defense. They are usually produced by functional enzyme clusters in sponges and/or their associated symbiotic microorganisms. Several of them have successfully been approved as antiviral agents for clinical use or have been advanced to the late stages of clinical trials. The most important antiviral lead of marine origin reported thus far is nucleoside Ara-A (vidarabine) isolated from sponge *Tethya crypta*. It inhibits viral DNA polymerase and DNA synthesis of herpes, vaccinica and varicella zoster viruses [33]. The Hepatitis C virus causes chronic infections in humans, which can develop to liver cirrhosis and hepatocellular carcinoma. Recent research on antiviral compounds isolated 128 molecules from marine invertebrates and microorganisms. The best results were obtained from the extracts produced from the *Bacillus* sp. isolated from the sponge *Petromica citrina*. These studied organisms lead to the development of drugs which ensure an alternative therapy for the treatment of hepatitis C [34].

Kohn *et al.,* 2012 [35] have isolated Norbatzelladine L from a marine sponge of the genus *Monanchora* displayed antiviral activity against *Herpes Simplex* virus type (HSV-1), with 97% of inhibition in the viral adsorption phase.

In the present investigation, extract of *Grayella cyathophora* showed an antioxidant activity with value of 748µg/ml as IC_{50}. Shaaban *et al.*, (2012) [36] have collected four marine sponges, *Smenospongia*, *Callyspongia*, *Niphates*, and *Stylissa* from the Red Sea at Egyptian coasts. The sponges' extracts exhibited diverse inhibitory effects on oxidative stress indices and carbohydrate hydrolyzing enzymes in linear relationships to some extent with concentration of inhibitors (dose dependant). The extracts of sponges (*Niphates*, *Smenospongia*, and *Callyspongia*,) showed, respectively, potent-reducing power. Seradj *et al*., (2012) [37] have isolated some compounds from natural sources of sponges collected from Persian Gulf. These compounds have capable of protecting against reactive oxygen species (ROS) mediated damage. Therefore, there is a growing interest in novel substances exhibiting antioxidant properties.

They evaluated the effects of different concentrations of the dichloromethane and methanolic extracts of six sponges on scavenging DPPH and OH free radicals. The activities of these extracts were compared with those of commercial antioxidants such as gallic acid. The maximum level of DPPH radical scavenging (0.234± 0.033 mg/ml) was observed for the methanolic extract of *Pseudosaberites clavatus* in the reaction mixture. Also, most sponge extracts exhibited medium to high hydroxyl radical scavenging activity. The results of their study suggest that marine sponges of the Persian Gulf are promising sources of antioxidants [37].

Grayella cyathophora extract had anti-inflammatory activity (89.91%). Mayer *et al.* (2005) [38] have concluded a structure-activity relationship study to investigate the anti-neuroinflammatory properties of the indole derived alkaloids manzamines isolated from the marine sponges; *Haliclona* sp., *Amphimedon* sp., and *Xestospongia* sp.

In this context, Youssef *et al*. [39] have found that bioassay-guided fractionation of the anti-inflammation fractions of the Red Sea sponges *Scalarispongia aqabaensis* and *Callyspongia siphonella* yielded two new sterols from chloroform fractions of methanol extracts, namely scalaristerol (5a,8adihydroxycholest-6-en-3ß-ol) (1) from *Scalarispongia aqabaensis*, and callysterol (ergosta-5,11-dien-3ß-ol) (2) from *Callyspongia siphonella*. Results indicated that the anti-inflammatory activity of compounds 1 and 2 was assessed that compound 2 has a strong anti-inflammatory activity, which is close to that of cortisone, while compound 1 showed moderate anti-inflammatory activity.

6. Conclusion

1. The crude extract of *N. magnifica* showed high antitumor activity against colon cancer (caco-2) and breast cancer (Mcf-7) on cell line.
2. The *G. cyathophora* extract showed high cytotoxic effect toVero cell with HAV, it showed positive antibacterial activity against *P. aeruginosa,* and conducted an antioxidant activity in corresponding to ascorbic acid standard with value of 748µg/ml as IC_{50}. Also it had anti-inflammatory activity.
3. Generally, the ethanolic crude extract of *N. magnifica* and *G. cyathophora* conducted to promising antitumor agent and *G. cyathophora* conducted to promising anti-inflammatory and antiviral agents.

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