Cancer is a group of diseases associated with abnormal cell growth with potentiality to invade or spread to other parts of the body (Rady et al., 2018). It is considered as one of the major ailments threatening the humankind (Rady et al., 2018a). The current available cancer statistics are indicating that the number of new cancer patients has been reached over 10 million cases associated with over 6 million deaths representing roughly 12% of worldwide mortality (Rady et al., 2017, 2018a). Moreover, over 20 million new cancer cases are anticipated to be diagnosed in the year 2025 (Zugazagoitia et al., 2016; Rady et al., 2017) and potentially will be increased to 21.7 million with about 13 million cancer deaths by the year 2030 (Torre et al., 2015; Rady et
In Egypt, according to national incidence rates and proportions, the crude incidence rates on the national level for all sites, excluding nonmelanoma skin cancer (C44) were 113.1/100,000 (both sexes), 115.7/100,000 (males), and 110.3/100,000 (females), where the age-standardized rates (world) were 166.6/100,000 (both sexes), 175.9/100,000 (males), and 157.0/100,000 (females) (Ibrahim et al., 2014). Those high rates of cancer morbidity and mortality are required more ways of cancer control, therapy or management where the current treatment modalities are mainly radiation-based therapy and chemotherapy (Rady et al., 2017, 2018), although their applicability to human is associated with toxic properties causing hair loss with some other serious adverse effects (Tsuda et al., 2004). Natural products have been used to help mankind sustain its health since the dawn of medicine (Moghadamtousi et al., 2015; Rady et al. 2017, 2018). Nowadays, just like in ancient times, utilization of complementary and alternative medicines is gaining more popularity as an important and promising strategy for human diseases prevention and therapy (Peter and Horvath, 2017; Rady et al., 2018). Marine natural products are regarded as a rich and renewable source for novel therapeutics (Jiménez, 2018). Marine derived bioactive compounds are attractive candidates for cancer therapy 50 years ago and in recent past there are like 3000 new marine compounds that have been assessed for their anticancer activity (Young et al., 2006). Beside the anticancer effects of marine organism constituents, a lot of modern studies suggested that some bioactive compounds isolated from marine organisms have been shown to exhibit anti-microbial, anti-fungal, anti-inflammatory, and other pharmacological activities (Venkateswara-Rao et al., 1998; Wali et al., 2019). Likewise, in clinical research, there are many marine anticancer bioactive compounds that have been isolated, characterized, identified, and preclinically assessed; now they are under clinical trials for human use (Wali et al., 2019).

Marine fauna for instance sponges, echinoderms, mollusks, ascidians, and coral reefs are accounting for more than 90% of the total oceanic biomass where those organisms possess a variety of pharmacological bioactive compounds that can be used in drug discovery for cancer control (Francesesco, 1997). Likewise, a variety of bioactive metabolites and compounds have isolated from marine organisms were found to exert antitumor, antimicrobial, and anti-inflammatory (Shnit-Orland et al., 2008). El-Gamal et al. (2004) isolated five bioactive substances from CH2Cl2 extracts of Taiwan soft coral of Nephthea armata, which have showed significant cytotoxicity to human lung adenocarcinoma and human colon adenocarcinoma and mouse lymphocytic leukemia. In addition, the soft corals of the genus Xenia are rich in terpenoids and steroids since six sesquiterpenoids were isolated from CH2Cl2 extracts of the soft coral of Xenia peurto-galera that have founded to induce cytotoxicity against cancer cells (Duh et al., 2002). Also, lemnalol is a bioactive antitumor agent has been isolated from the Japanese soft coral Lemnalia sp. (Kikuchi et al., 1983). As part of research on the bioactive material from marine organisms, the Formosan soft coral Cespitularia hypotentaculata (family: Xeniidae) was studied because its CH2Cl2 extract showed significant cytotoxicity to human lung adenocarcinoma and human colon adenocarcinoma and mouse lymphocytic leukemia cells (Duh et al., 2002a). Moreover, two bicyclic cembranolides have been isolated from Sarcophyton sp. were found to exert cytotoxicity towards MCF7 cancer cells (Gross et al., 2004). Furthermore, among the groups of marine organisms, sponges are the most diverse and abundant, due to their soft bodies and sedentary lifestyles
Marine sponges (Phylum: Porifera) are a large phylum belongs to the animal Kingdom and regarded as prolific factories for bioactive natural products (Blunt et al., 2016, 2017, 2018). Therefore, sponges are suggested to provide a drug discovery for multiple diseases, such as cancer and viruses (Faulkner, 2000; Perdicaris et al., 2013). Red Sea is rich with sponges such as that of genus Negombata, which produce latrunculins that are known for their antimicrobial and antiviral activity (Eid et al., 2011). A variety of natural products from the marine sponges have been found to exhibit remarkable antitumor and anti-inflammatory activities (Edrada et al., 2002). Pawlik and McMurray (2020) have also confirmed that marine sponges are containing variety of natural products than any other marine phylum and those products have bioactivities including anticancer, antimicrobial, and anti-inflammatory activities, and are often applicable for medical use. Moreover, some of the most potential sponge-derived bioactive molecules include the anti-inflammatory compound manoalide from the palauan sponge Luffariella variabilis (De-Silva and Scheuer, 1980). Here, the current study assesses cytotoxicity, viability, and primary apoptotic effects of novel multiple extracts derived from two sponges Callyspongia siphonella and Negombata magnifica collected from Red Sea, Egypt in HepG2, MCF-7, and Caco-2 cancer cell lines.

### MATERIALS AND METHODS

1- Sampling and identification of specimens

Specimens were collected during summer 2020 along Gulf of Aqaba at different depths by SCUBA diving. Immediately upon collection, the samples were cleaned with seawater and preserved in ice-box at -20°C. The taxonomy details were studied, and the two voucher specimens were deposited at Marine laboratory, Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt, with a registration number of MZ1047 and MZ1048. The identification of the specimens has been carefully checked based on Porifera morphological characters according to Systema Porifera (Hooper, 2000) associated with the most recent update undertaken in the World Porifera Database (Van Soest et al., 2008) and at the end the taxonomy is directed by Ruggiero et al. (2015).

2- Preparation of extracts

The frozen sponge specimens were left to defrost and then broken down into small pieces. 10 g of macerated tissues were extracted by soaking in 50 ml of different four absolute solvents of (CH₂Cl₂, C₄H₈O₂, C₃H₆O, and CHCl₃) for 24 h at room temperature. Extractions were repeated three times until no color was obtained to ensure complete extraction. The combined extracts were filtered through Whatman no.1 filter paper and dried at 40°C using a rotary evaporator.

3- Cell culture

HepG-2, MCF7, and Caco-2 cancer cell lines and HFB-4 cell line (normal control cells) were obtained from American Type Culture Collection (Manassas, VA, USA) and (VACSERA Co., Cairo, Egypt). HepG-2, MCF7 and Caco-2 cancer cells were cultured in DMEM obtained from Corning Thomas Scientific (Swedesboro, NJ, USA). DMSO was purchased from (Sigma-Aldrich, St. Louis, USA), while FBS was purchased from Hyclone (Pittsburgh, PA, USA) and PSA was obtained from Mediatech Inc. (Herndon, VA, USA). Cancer cells were cultured in DMEM supplemented with 5% heat inactivated
FBS and 1% PSA at 37°C in 5% CO₂ incubator (Chamcheu et al., 2018). Similarly, HFB-4 cells were maintained in RPMI-1640 medium (ThermoFisher Scientific Co., Waltham, MA, USA) supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO₂ (Amin et al., 2012).

4- Measurement of Cytotoxicity by MTT assay

The cytotoxicity of each extract against HepG2, MCF-7, and Caco-2 cancer cells was measured by Cell Proliferation Kit I MTT (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer instructions. Briefly, cells were seeded in 96 well culture plates at 5000/well for 24 h. Then, cells were incubated with different concentrations of each extract (0, 12.5, 25, and 50 μg/ml) for 24 hours in 37 °C and humidified 5% CO₂ incubator. Treated and untreated cells were incubated with MTT powder in the detection day for 2 hours after until violet crystals were formed at different color hue indicating cell metabolic activity. Colorimetric absorbance was measured at 620 nm (A620) and 570 nm (A570) by Synergy™ 2 Multi-Mode Microplate Reader (BioTek Inc., Vermont, USA). The cell viability was calculated as previously reported by Cho et al., (2015) using the following equation:

\[
\text{Percentage of viability} = \frac{A570 - A620 \text{ of UA}}{A570 - A620 \text{ of the Control (0μM)}} \times 100
\]

The IC₅₀ of each extract was calculated by the IC₅₀ calculator (AAT Bioquest, Inc., CA, USA) as had been previously described by Luparello et al., (2019). Treatment and analysis protocols were carried out at least three times.

5- Determination of SI

SI indicates the cytotoxic selectivity (i.e. Safety) of the crude extract against cancer cells versus normal control cells (Prayong et al., 2008). SI= IC₅₀ of the extract in the normal control cells / IC₅₀ of the same extract in the cancer cell line (Bézivin et al., 2003).

6- Determination of the active Caspase-3

Caspase-3 level was measured by using the Invitrogen Caspase-3 (active) Human kit from (ThermoFisher Scientific Co., Waltham, MA, USA). Briefly, after washing the cells with PBS, they collected and lysed by adding the extraction buffer containing 1 mM PMSF (stock is 0.3 M in DMSO) and Protease inhibitor cocktail, e.g., Sigma-Aldrich Cat. # P-2714 (St. Louis, USA). Later, 500 μl per 5 ml cell extraction buffer-protease inhibitors (1 mL per 1 x 107 cells) were added. Next, the lysate was diluted immediately prior to the assay. At the end, OD of each well was determined within 30 minutes using a microplate reader AC3000 (Azure Biosystems, Inc, CA, USA) set at 450 nm.

7- Determination of Bax and Bcl-2

Cancer cells were grown in DMEM containing 5% FBS at 37°C and after treatment with present marine extracts, cancer cells suspension was tested for Bax and Bcl-2 using lysed cell extraction. This cell lysate was diluted in standard diluent buffer over the range of the assay and measured for human active Bax and Bcl-2 content using Bax ELISA (EIA-4487) kit (DRG Instruments, Ma, Germany) and Zymed Bcl-2 ELISA Kit (ThermoFisher Scientific Co., Waltham, MA, USA).
RESULTS

1- Specimens identification

The collected sponges were identified according to Hooper (2000), Van Soest et al. (2008) and Ruggiero et al. (2015) as two marine sponge species; tube-sponge “Callypso siphonella” and finger-sponge “Negombata magnifica”. Both sponge species were classified as follows:

**Superkingdom:** Eukaryota Chatton, 1925

**Kingdom:** Animalia (Metazoa) Linnaeus, 1758

**Subkingdom:** Eumetazoa Buetschli, 1910

**Phylum:** Porifera Grant, 1836

**Class:** Demospongiae Sollas, 1885

**Subclass:** Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault, 2012

**Order:** Haplosclerida Topsent, 1928

**Family:** Callyspongiidae de Laubenfels, 1936

**Genus:** Callypso Duchassaing & Michelotti, 1864

**Species** 1:

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**Order:** Poecilosclerida Topsent, 1928

**Family:** Podospongiidae de Laubenfels, 1936

**Genus:** Negombata de Laubenfels, 1936

**Species** 2:

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Both sponges were collected exactly from Dahap area, Red Sea but differ in depth range where *Callypso siphonella* was between 10 - 37m depth range and *Negombata magnifica* was at 7 - 14 m. *Callypso siphonella* was like tube forming clusters of vertical tubes from a common base. Those tubes can be reach 60cm in height and have a smooth consistency due to the absence of spicules. Color of *Callypso siphonella* is usually pale purple or pink or reddish-brown. It lives on hard surfaces and shelters towards reef slopes. *Negombata magnifica* is Reddish-brown color and narrow crooked branched sponge. It is live and grows between the shallow coral reefs and rocks.

2- Cs and Nm derived extracts, each inhibited cell proliferation, Viability of HePG2, MCF-7, and Caco-2 cancer cells

All extracts inhibited cancer cells growth (HepG2, MCF-7, and Caco-2 cancer cell lines) in dose-dependent manner with remarkable cell viability decrease associated with cytotoxicity increase (Figures 1-12). The IC50 values between all extracts are different in range between 6.18 — 19.48 μg/ml. The IC50 ranges for all extracts on HepG-2, MCF-7, and Caco-2 cancer cells were 7.23 — 19.21 μg/ml, 6.18 — 19.48 μg/ml and 8.37 — 18.35 μg/ml, respectively. Therefore, among all extracts, the lowest IC50 was for Nm-C3H6O2 on MCF-7 cancer cells within a value of 6.18 μg/ml and the highest IC50 was for the Cs-CH2Cl2 on the same cancer cell line within a value of 19.48 μg/ml. Similarly, in HepG2 cancer cells, Nm-CHCl3 inhibited proliferation within the lowest IC50 value of 7.23 μg/ml, while the highest IC50 was for Cs-CHCl3 within a value of 19.21 μg/ml for its proliferation inhibition on HepG-2 cancer cells, whereas, in MCF-7 cancer cells, Nm-C3H6O2 obtained the lowest IC50 value of 6.18 μg/ml and Cs-CH2Cl2 existed the highest IC50 value of 19.48 μg/ml and in Caco-2 cancer cells, Nm-C3H6O obtained the lowest IC50 value of 8.37 μg/ml and Cs-C3H6O exerted the highest IC50 value of 18.35 μg/ml.
Figure (1): Effects of Cs-CH₂Cl₂ on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of Cs-CH₂Cl₂, while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001 vs. control cells.

Figure (2): Effects of Cs-C₄H₈O₂ on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of Cs-C₄H₈O₂ while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001 vs. control cells.
Novel extracts from *Callyspongia siphonella* and *Negombata magnifica* sponges

**Figure (3):** Effects of Cs-C<sub>3</sub>H<sub>6</sub>O on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of Cs-C<sub>3</sub>H<sub>6</sub>O while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001 vs. control cells.

**Figure (4):** Effects of Cs-CHCl<sub>3</sub> on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of Cs-CHCl<sub>3</sub> while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001 vs. control cells.
Figure (5): Effects of Nm-\(\text{CH}_2\text{Cl}_2\) on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of Nm-\(\text{CH}_2\text{Cl}_2\) while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\) and **** \(p < 0.0001\) vs. control cells.

Figure (6): Effects of Nm-C\(_4\text{H}_8\text{O}_2\) on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of Nm-C\(_4\text{H}_8\text{O}_2\) while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\) and **** \(p < 0.0001\) vs. control cells.
Figure (7): Effects of $Nm\text{-C}_3\text{H}_6\text{O}$ on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of $Nm\text{-C}_3\text{H}_6\text{O}$ while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control cells.

Figure (8): Effects of $Nm\text{-CHCl}_3$ on HepG2, MCF-7, and Caco-2 on cell toxicity and viability after 24 h. Cells were incubated with the indicated concentrations of $Nm\text{-CHCl}_3$ while the percentage of cytotoxicity and viability were determined by MTT. Values used for plotting are means of experiments performed three times, with each concentration tested in 7–8 wells. Statistical differences from control cultures are shown as bar graphs with error bars representing the means ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control cells.
Figure (9): Comparative IC\textsubscript{50} for all current investigated extracts on HepG-2, MCF-7, and Caco-2 cancer cells. IC\textsubscript{50} values are shown as bar graphs.

Figure (10): Comparative IC\textsubscript{50} for all current investigated extracts on HepG-2 cancer cells. IC\textsubscript{50} values are shown as bar graphs with error bars representing the means ± SD.
Novel extracts from *Callyspongia siphonella* and *Negombata magnifica* sponges

![Figure 11](image1.png)

**Figure (11):** Comparative IC$_{50}$ for all current investigated extracts on MCF-7 cancer cells. IC$_{50}$ values are shown as bar graphs with error bars representing the means ± SD.

![Figure 12](image2.png)

**Figure (12):** Comparative IC50 for all current investigated extracts on Caco-2 cancer cells. IC$_{50}$ values are shown as bar graphs with error bars representing the means ± SD.

Likewise, the SI results were synergistically matched extremely the same previously mentioned arrangements in HepG2, MCF-7, and Caco-2 cancer cells (Figures 13-16). In addition, SI between all extracts are different in range between 2.23—4.56. The SI ranges for all extracts on HepG-2, MCF-7 and Caco-2 cancer cells were 2.39 — 4.48, 2.23 — 4.56 and 2.47 — 4.16, respectively. Therefore, among all extracts, the highest SI was for *Nm*-C$_4$H$_2$O$_2$ on MCF-7 cancer cells within a value of 4.56 and the highest SI was for the *Cs*-CH$_2$Cl$_2$ on the same cancer cell line within a value of 2.23. Similarly, in HepG2 cancer cells, *Nm*-CHCl$_3$ inhibited proliferation within the highest SI value of 4.48, while the lowest SI was for *Cs*-CHCl$_3$ within a value of 2.39 for its
Figure (13): Comparative SI for all current investigated extracts on HepG2, MCF-7, and Caco-2 cancer cells.

Figure (14): Comparative SI for all current investigated extracts on HepG2 cancer cells.
proliferation inhibition on HepG-2 cancer cells, whereas, in MCF-7 cancer cells, *Nm*-C₃H₈O₂ obtained the highest SI value of 4.56 and *Cs*-CH₂Cl₂ existed the lowest SI value of 2.23 and in Caco-2 cancer cells, *Nm*-C₃H₂O obtained the highest SI value of 4.16 and *Cs*-C₃H₆O exerted the lowest SI value of 2.47.
3- Cs and Nm derived extracts, each induced apoptosis and increased Bax and caspase-3 expressions in cancer cells

All extracts caused apoptotic effect through the expression of some regulatory proteins. Each extract increased the level of the pro-apoptotic protein Bax and caspase-3, while it decreased the level of the anti-apoptotic protein Bcl2 in HepG2, MCF-7, and Caco-2 cancer cell lines (Figures 17-20). The Bax values between all extracts are different in range between 61.95 — 455.81 ng/ml. The Bax ranges for all extracts on HepG-2, MCF-7 and Caco-2 cancer cells were 83.26 — 436.35 ng/ml, 61.95 — 455.81 ng/ml and 95.47 — 384.63 ng/ml, respectively. Therefore, among all extracts, the lowest Bax expression was for Cs-CHCl3 on MCF-7 cancer cells within a value of 61.95 ng/ml, and the highest Bax expression was for the Nm-C3H8O2 on the same cancer cell line within a value of 455.81 ng/ml. Similarly, in HepG2 cancer cells, Nm-CHCl3 induced cellular apoptosis within the highest Bax value of 436.35 ng/ml (Figure 21), while the lowest Bax expression was for Cs-CHCl3 within a value of 83.26 ng/ml for its apoptosis on HepG-2 cancer cells, whereas, in MCF-7 cancer cells, Nm-C3H8O2 obtained the highest Bax value of 455.81 ng/ml (Figure 22) and Cs-CH2Cl2 existed the lowest Bax value of 61.95 ng/ml and in Caco-2 cancer cells, Nm-C4H8O2 obtained the highest Bax value of 384.63 ng/ml (Figure 23) and Cs-C3H6O exerted the lowest Bax value of 95.47 ng/ml. The Bcl-2 values between all extracts are different in range between 2.47 — 8.81 ng/ml. The Bcl-2 ranges for all extracts on HepG-2, MCF-7 and Caco-2 cancer cells were 2.54 — 8.51 ng/ml, 2.47 — 8.81 ng/ml and 3.58 — 8.24 ng/ml, respectively. Therefore, among all extracts, the highest Bcl-2 expression was for Cs-CH2Cl2 on MCF-7 cancer cells within a value of 8.81 ng/ml and the lowest Bcl-2 expression was for the Nm-C4H8O2 on the same cancer cell line within a value of 2.47 ng/ml. Similarly, in HepG2 cancer cells, Nm-CHCl3 induced cellular apoptosis within the lowest Bcl-2 value of 2.54 ng/ml, while the highest Bcl-2 expression was for Cs-CHCl3 within a value of 8.51 ng/ml for its apoptosis on HepG-2 cancer cells, whereas, in MCF-7 cancer cells, Nm-C4H8O2 obtained the lowest Bcl-2 value of 2.47 ng/ml and in Caco-2 cancer cells, Nm-C3H6O obtained the lowest Bcl-2 value of 3.58 ng/ml and Cs-C3H6O exerted the highest Bcl-2 value of 8.24 ng/ml. The Caspase-3 values between all extracts are different in range between 98.04 — 518.34 ng/ml. The Caspase-3 ranges for all extracts on HepG-2, MCF-7 and Caco-2 cancer cells were 116.45 — 500.08 ng/ml, 98.04 — 518.34 ng/ml and 133.58 — 445.24 ng/ml, respectively. Therefore, among all extracts, the lowest Caspase-3 expression was for Cs-CH2Cl2 on MCF-7 cancer cells within a value of 98.04 ng/ml and the highest Caspase-3 expression was for the Nm-C4H8O2 on the same cancer cell line within a value of 518.34 ng/ml. Similarly, in HepG2 cancer cells, Nm-CHCl3 induced cellular apoptosis within the highest Caspase-3 value of 500.08 ng/ml, while the lowest Caspase-3 expression was for Cs-CHCl3 within a value of 116.45 ng/ml for its apoptosis on HepG-2 cancer cells, whereas, in MCF-7 cancer cells, Nm-C4H8O2 obtained the highest Caspase-3 value of 518.34 ng/ml and Cs-CH2Cl2 existed the lowest Caspase-3 value of 98.04 ng/ml and in Caco-2 cancer cells, Nm-C3H6O obtained the highest Caspase-3 value of 445.24 ng/ml and Cs-C3H6O exerted the lowest Caspase-3 value of 133.58 ng/ml.
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Figure (17): Comparative modulation of Bax protein expressions in HepG-2, MCF-7 and Caco-2 cells were treated with current studied extracts and harvested 24 h after treatments.
Figure (18): Comparative modulation of Bcl-2 protein expressions in HepG-2, MCF-7 and Caco-2 cells were treated with current studied extracts and harvested 24 h after treatments.
Figure (19): Comparative modulation of Caspase-3 protein expressions in HepG-2, MCF-7 and Caco-2 cells were treated with current studied extracts and harvested 24 h after treatments.
Figure (20): Schematic drawing of the mechanism of action of current studied extracts. This carton is based on the current available data throughout the present study.
Figure (21): Comparative modulation of Bax, Bcl-2 and Caspase-3 protein expressions in HEPG-2 cells were treated with current studied extracts and harvested 24 h after treatments.
**Figure (22):** Comparative modulation of Bax, Bcl-2 and Caspase-3 protein expressions in MCF-7 cells were treated with current studied extracts and harvested 24 h after treatments.
**Figure (23):** Comparative modulation of Bax, Bcl-2 and Caspase-3 protein expressions in Caco-2 cells were treated with current studied extracts and harvested 24 h after treatments.
DISCUSSION

Marine organisms are a rich source of structurally novel and biologically active metabolites (El-Damhougy et al., 2017; Hasaballah and El-Naggar, 2017; Ibrahim et al., 2017; El-Damhougy et al., 2019). Recently, studies have suggested that some bioactive compounds isolated from marine organisms have been shown to have anticancer activity (Borowitzka and Borowitzka, 1992; Mayer and Hamann, 2005; Blunt et al., 2005; Somnath and Ghosh, 2010). According to many previous studies, those bioactive constituents of marine organisms are attractive candidates for cancer chemoprevention and therapy. Therefore, investigations are still required to gain more details about the biological activity of marine organism compounds although scientists have gained a lot about nutritional and economical importance of many marine organisms. Here, eight marine organism extracts Cs-CH$_2$Cl$_2$, Cs-C$_6$H$_5$O$_2$, Cs-C$_3$H$_6$O, Cs-CHCl$_3$, Nm-CH$_2$Cl$_2$, Nm-C$_4$H$_8$O$_2$, Nm-C$_3$H$_6$O, and Nm-CHCl$_3$ are introduced to study their anticancer effects such as many marine organism derived compounds and extracts that have shown to exert multiple anticancer effects, for instance, Sphingolipids/glycosides of sea cucumber Stichopus japonicus (Kariya et al., 2004), TBL12 (Chari et al., 2018), Holothuria parva methanolic extract (Salimi et al., 2017). Apostichopus japonicus extracts (Kim et al., 2017) Phlinopside E (Tian et al., 2005, 2007), Acaudina molpadioides cerebrosides and Asterias amurensis cerebrosides (Du et al., 20012). The present study has utilized HepG-2, MCF7, and Caco-2 cancer cell lines, each was subjected to Cs-CH$_2$Cl$_2$, Cs-C$_6$H$_5$O$_2$, Cs-C$_3$H$_6$O, Cs-CHCl$_3$, Nm-CH$_2$Cl$_2$, Nm-C$_4$H$_8$O$_2$, Nm-C$_3$H$_6$O, and Nm-CHCl$_3$ dose independent treatments differ from the other previously investigations that have used Skin melanoma cancer cell line-2 (Kim et al., 2017), murine sarcoma cancer cell line-180 (Tian et al., 2005, 2007; Du et al., 20012) and hepatoma 22 cancer cells (Tian et al., 2005, 2007) that have been exposed to previous bioactive compounds of different prior studied marine organisms. Compared to the anticancer effect of the ethanolic crude extract of the same sponge studied here Callyspongia siphonella in two cancer cellines MCF-7 and Caco-2 (Ibrahim et al., 2017), the present CsEs showed lower anticancer activity where the ethanolic Callyspongia siphonella crude extract inhibited MCF-7 and Caco-2 cancer cells with IC$_{50}$ values of 0.37 and 5.57 µg/ml, respectively. Therefore, ethanol is completely more efficient than current CsEs to induce better Callyspongia siphonella antiproliferative activity output on MCF-7 and Caco-2 cancer cells. Similarly, the ethanolic crude extract of Negombata magnifica (El-Damhougy et al., 2017) induced more intense anticancer activity with lower IC$_{50}$ values of 1.39 and 2.09 µg/ml against MCF-7 and Caco-2 cell lines, respectively, compared to current NmEs, but the differences between IC$_{50}$ values overall indicated that both ethanolic and current crude extracts of Negombata magnifica are extremely potential for anticancer properties although ethanolic extract remain require more information about its effects on cancer cell induced apoptosis.

So, beside the proliferation inhibitory effect for the present CsEs and NmEs in HepG-2, MCF7, and Caco-2, CsEs and NmEs were also founded to induce apoptosis in the same cancer cell lines while some other marine bioactive compounds that had previously studied in vitro suppressed the angiogenesis and osteoclastogenesis and also rose up the cytotoxicity (Kariya et al., 2004). Similarly, cerebrosides of both Asterias amurensis and Acaudina molpadioides inhibited in vitro cell proliferation and induced
apoptosis in murine sarcoma cancer cell line-180 (Chari et al., 2018). Holothuria parva methanolic extract induced in vitro apoptosis in Chronic lymphocytic leukemia B-lymphocytes (Salimi et al., 2017). Apostichopus japonicus extracts from low-temperature ultrasonification process was in vitro inhibited skin melanoma cell line-2 proliferation and metastasis (Kim et al., 2017) and philinopside E was fund to in vitro inhibit the murine sarcoma cancer cell line-180 and hepatoma proliferation and induced apoptosis and anti-angiogenic activity (Tian et al., 2005, 2007).

However, the in vitro anticancer effects of current CsEs and NmEs were supported by Bcl-2 decrease along with Bax and caspase-3 increase in HepG-2, MCF7, and Caco-2 cancer cells, the anticancer effects of the other previously investigated bioactive compounds of marine organisms were resulted in Bcl-2, STAT3, and MMP-9 decrease (Kim et al., 2017). Additionally, there is an in vivo prior study mentioned have used quantitative real-time PCR analysis which revealed that the administration of cerebrosides of either Asterias amurenensis or Acaudina molpadioides decreased the expression of both Bcl-2 and Bcl-XL while increased Bax, Cyt c, caspase-3, and caspase-9 of the murine sarcoma cancer cell line-180 ascites (Du et al., 20012) compared to the present CsEs and NmEs, which were studied in vitro, where it suppressed the expression of Bcl-2 and increased the expression Bax and caspase-3 in HepG-2, MCF7 and Caco-2 cancer cell lines, unlike another in vivo investigation indicated philinopside E anti-angiogenic activity associated with downturn of VEGFR2 signaling in murine sarcoma cancer cell line-180 (Tian et al., 2005, 2007). Despite of the clinical research about the anticancer efficacy of bioactive compounds of marine organisms is very limited, there is an available investigation of a total 20 patients with high risk asymptomatic multiple myeloma were given TBL12, the TBL12 was well tolerated and 9 (45%) patients remain on treatment with one minimal response noted (Chari et al., 2018).

Altogether, the data generated during the current study is emphasize our hypothesis of Cs-CH$_2$Cl$_2$, Cs-C$_4$H$_8$O$_2$, Cs-C$_3$H$_6$O, Cs-CHCl$_3$, Nm-CH$_2$Cl$_2$, Nm-C$_4$H$_8$O$_2$, Nm-C$_3$H$_6$O, and Nm-CHCl$_3$, each has anticancer properties that can be promising and developed in the future as anticancer drug for cancer therapy outcome.

**REFERENCES**


### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>AaC</em></td>
<td>[<em>Asterias amurensis</em> cerebrosides]</td>
</tr>
<tr>
<td><em>AjEs</em></td>
<td>[<em>Apostichopus japonicus</em> extracts]</td>
</tr>
<tr>
<td><em>AmC</em></td>
<td>[<em>Acaudina molpadioides</em> cerebrosides]</td>
</tr>
<tr>
<td><em>ASx</em></td>
<td>Asymptomatic</td>
</tr>
<tr>
<td><em>Bax</em></td>
<td>B-cell lymphoma 2 associated X protein</td>
</tr>
<tr>
<td><em>Bcl-2</em></td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td><em>Bcl-xL</em></td>
<td>B-cell lymphoma extra-large</td>
</tr>
<tr>
<td><em>C₃H₆O</em></td>
<td>Acetone</td>
</tr>
<tr>
<td><em>C₄H₈O₂</em></td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td><em>Caspase-3</em></td>
<td>Cysteine proteases with aspartate specificity 3</td>
</tr>
<tr>
<td><em>Caspase-9</em></td>
<td>Cysteine proteases with aspartate specificity 9</td>
</tr>
<tr>
<td><em>CH₂Cl₂</em></td>
<td>Methylene chloride</td>
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<tr>
<td><em>CHCl₃</em></td>
<td>Chloroform</td>
</tr>
<tr>
<td><em>CLLB</em></td>
<td>Chronic lymphocytic leukemia B-lymphocytes</td>
</tr>
<tr>
<td><em>CsE</em></td>
<td>[<em>Callyspongia siphonella</em> crude extract]</td>
</tr>
<tr>
<td><em>Ctrl</em></td>
<td>Control</td>
</tr>
<tr>
<td><em>Cyt C</em></td>
<td>Cytochrome complex</td>
</tr>
<tr>
<td><em>DMEM</em></td>
<td>Dulbecco’s modified Eagle’s medium</td>
</tr>
<tr>
<td><em>DMSO</em></td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td><em>FBS</em></td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td><em>HepG-2</em></td>
<td>Human hepatocellular carcinoma cell line</td>
</tr>
<tr>
<td><em>HFB-4</em></td>
<td>Normal human melanocytes cell line</td>
</tr>
<tr>
<td><em>HpE</em></td>
<td>[<em>Holothuria parva</em> methanolic extract]</td>
</tr>
<tr>
<td><em>IC₅₀</em></td>
<td>50% inhibitory Concentration</td>
</tr>
<tr>
<td><em>MCF7</em></td>
<td>Mammary gland breast cancer cell line</td>
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<tr>
<td><em>MM</em></td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td><em>MMP-9</em></td>
<td>Matrix metalloproteinase-9</td>
</tr>
<tr>
<td><em>MTT</em></td>
<td>3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide</td>
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<tr>
<td><em>NmE</em></td>
<td>[<em>Negombata magnifica</em> crude extract]</td>
</tr>
<tr>
<td><em>OD</em></td>
<td>Optical density</td>
</tr>
<tr>
<td><em>PE</em></td>
<td>Philinopside E</td>
</tr>
<tr>
<td><em>PMSF</em></td>
<td>Phenylmethylsulfonyl fluoride</td>
</tr>
<tr>
<td><em>PSA</em></td>
<td>Penicillin-streptomycin-amphotericin B</td>
</tr>
<tr>
<td><em>rPCR</em></td>
<td>Real-time polymerase chain reaction</td>
</tr>
<tr>
<td><em>RPMI-1640</em></td>
<td>Roswell Park Memorial Institute series no. 1640</td>
</tr>
<tr>
<td><em>S180</em></td>
<td>Murine Sarcoma cancer cell line-</td>
</tr>
</tbody>
</table>
Novel extracts from *Callyspongia siphonella* and *Negombata magnifica* sponges

180

**SCUBA**  Self-contained underwater breathing apparatus

**SI:**  Selectivity index

**SjSG:**  Sphingolipids/glycosides of sea cucumber *Stichopus japonicus*

**SK-MEL-2:**  Skin melanoma cancer cell line-2

**STAT3:**  Signal transducer and activator of transcription 3

**TBL12:**  Drug extract of sea cucumber

**VEGFR2:**  Vascular endothelial growth factor-2

**Xp-CH₂Cl₂:**  *Xenia peurto-galera* methylene chloride extract

**Xp:**  *Xenia peurto-galera*