INTRODUCTION

Natural products derived from marine organisms have been identified as promising sources of anticancer drug candidates in pharmaceutical industries. Steroids are considered as major natural compounds in soft corals [1]. A wide range of pharmacological activity for these compounds such as anti-inflammatory [2], antifouling [3], and anticancer [2,4,5]. In our previous work, the soft coral Litophyton arboreum total extract has been found to show promising anticancer activity in the ovarian (Hela) and leukemia (U937) cell lines, these findings supported the isolation, and the purification of the most active compounds that showed promising cytotoxic activity and high safety margins reported in our previous study [6]. To continue our line of research on the anticancer activities potentially these compounds, we assess for the 1st time, the anticancer activities of these compounds in liver (HepG2), breast (MDA), and lung (A549) cancer cell lines.

METHODS

Marine-derived compounds

Nine compounds, sarcophytol M (1), alismol (2), alismoxide (5), 7β-acetoxy-24-methylcholesta-5,24(28)-diene-3β,7β,19-triol (9), isolated from the soft coral Litophyton arboreum in liver (HepG2), lung (A549), and breast (MDA) cancer cell line.

Methods: Anticancer activities of the compounds were tested using (XTT) 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, Na2) in vitro assay in order to estimate the cytotoxicity and to determine the IC50s. The free radical scavenging activity of the compounds were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•). All compounds were screened at 100 µg/ml while the most potent active compounds were assayed at lower concentrations.

Results: Compounds (7) and (9) showed a strong cytotoxic effect with IC50 of 6.07, 8.5 µg/ml in HepG2, 6.3, 5.5 µg/ml in MDA cells, and 5.2, 9.3 µg/ml in A549 cancer cell lines, respectively. In addition, moderate cytotoxicity was shown by compound (2) (IC50 16.5, 15, and 13 µg/mL) in HepG2, MDA, and A549 cancer cell lines, respectively.

Conclusion: The results obtained in this research work indicated a promising potential cytotoxicity of compounds (7) and (9) compared to its safety margins in Vero cells, and the expected cytostatic effect of compound (2) can be used in drug cocktails for the treatment of the major cancer types’ lung, breast, and liver cancer.

Keywords: Soft corals, Steroids, Anticancer, HepG2, MDA, A549.
and 7.8 µg/ml in HepG2, MDA, and A549 cells, respectively, with high SI. To cells treated with actinomycin D which showed IC

IC 7.02, and 9.3 µg/ml, compound (7) showed strong cytotoxicity with IC

Table

Cytotoxicity IC

The most active compounds (2), (7), and (9) were then subjected to further

<10%) when tested at 25 µg/ml (viability 80%). Compounds (1) and (5) showed strong cytotoxicity when tested at 25 µg/ml, viability of the cells ranged from 30 to 10%. Compounds (2), (7), and (9) showed high toxicity (viability <10%) when tested at 25 µg/ml (Fig. 2).

The most active compounds (2), (7), and (9) were then subjected to further study to determine the IC 50 values in HepG2, MDA, and A549 cell lines.

Cytotoxicity IC 50 determination

Table 1 shows the half inhibitory concentration of the active compounds; compound (2) demonstrated a promising cytotoxicity with IC 50 of 4.5, 7.02, and 9.3 µg/ml. Compound (7) showed strong cytotoxicity with IC 50 of 4.5, 7.02, and 9.3 µg/ml, and compound (9) revealed a strong cytotoxic activity with IC 50 of 6.07, 6.3, and 5.2 µg/ml when compared to cells treated with actinomycin D which showed IC 50 of 17.67, 11.08, and 7.6 µg/ml in HepG2, MDA, and A549 cells, respectively, with high SI.

Free radical scavenging activity

The free radical scavenging activity of compounds was measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) using the method of Shimada et al. [8]. All compounds were screened at 100 µg/ml while the most potent active compounds (gave more 90%) were asayed at 25–75 µg/ml. Briefly, 0.1 mM solution of DPPH in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of compound solution at different concentrations (25–75 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm in microplate reader. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity [9].

DPPH scavenging effect (%) = 100 - \left( \frac{A0 - A1}{A0} \times 100 \right)

Where, A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample.

Statistical analysis

Mean IC 50 is the concentration of compounds which reduces cell growth by 50% under the experimental conditions and is the average of at least three independent reproducible measurements. The IC 50 values were performed using GraphPad Prism (SanDiego, USA). Data were expressed as means ± standard deviations (SD).

RESULTS

Cytotoxicity

The pure compound where an IC 50 value is <10 µg/ml is reported as strongly cytotoxic [10]; hence, the cytotoxicity of the compounds was screened for their cytotoxicity at a concentration of 25 µg/ml in HepG2 cells.

Results showed weak cytotoxic activity for the compound (8) at 25 µg/ml (viability 80%). Compounds (1) and (5) showed strong toxicity when tested at 25 µg/ml, viability of the cells ranged from 30 to 10%. Compounds (2), (7), and (9) showed high toxicity (viability <10%) when tested at 25 µg/ml (Fig. 2).

The most active compounds (2), (7), and (9) were then subjected to further study to determine the IC 50 values in HepG2, MDA, and A549 cell lines.

Cytotoxicity IC 50 determination

The promising cytotoxic effect has been shown by compound (2) (4.52, 7.02, and 9.23 µg/ml) in HepG2, MDA, and A549 cancer cell lines, respectively. The compound was previously characterized by being cytostatic (antiproliferative) in HeLa cells [6]. Cytostatic compounds are a good candidate in the production of drugs cocktails as it does not kill cancer cells, but it suppresses its proliferating while another drug is treating it.

Free radical scavenging activity

Compounds were tested at the concentrations ranged from 100 to 500 µg/ml; the three compounds exhibited very weak scavenging activities as shown in Table 2.

DISCUSSION

Recent studies reported the strong bioactivities of steroids and triterpenoids [11]. In this study, triterpenoids showed also the highest activities among the different tested compounds. Compound (7) from the soft coral nepthieahabroli and compound (9) from the soft coral Litophyton viridis revealed comparable potential cytotoxic effect against various human cancer cell line such as prostate cancer cell line LNCaP with IC 50 (15.5 and 4.9 µg/ml) [7], HT-29 (0.87 and 0.72 µg/ml), KB (0.38 and 0.58 µg/ml), and P-388 (0.42 and 0.24 µg/ml), respectively [12]. Compound (7) showed superior activity as compared to that of compound (9). Compound (9) showed immunosuppressive action [13] and when mixed with different tissue homogenates in vivo, it revealed preventive activity against lipid peroxidation [14]. The chemical skeleton of compounds (7) and (9), especially at C-19 in addition to the 5-ene B ring, which is noticed in an 8j, 9e-half-chair conformations, implicated effectively with the activity and the mode of interaction with the cell membranes. Meanwhile, the potency of the activity of such compounds stems from the blockage of the hydroxyl group at C-7 of compound (7) which enhances its lipophilicity [15]. This confirms the role of lipophilicity in the pharmacological activity of the compounds as it increases the ability of the drug penetration for different biological membranes and tissues. This constitutes a lead factor in the interaction of drugs with biological systems [16].

According to the National Cancer Institute, it had long been hypothesized that antioxidants can protect against cancer as they scavenge reactive oxygen species that can deteriorate DNA. Despite this fact, a new scope of research confirms that the existence of high levels of antioxidants has been found to prevent a specific free radical damage associated with cancer development, and the antioxidants have been shown to trigger cancer metastasis. In melanoma mouse models, the researcher investigators have demonstrated higher levels of oxidative stress in circulating cancer cells than in cancer cells in primary tumors, and they suggested that the oxidative stress can actually interfere with the development of metastatic tumors. The treatment of these mice with antioxidants suppressed the oxidative stress in the circulating cancer cells and enhanced their capability to metastasize [17]. Another study in mouse models of human lung cancer suggested that supplementing the antioxidants N-acetyl cysteine or Vitamin E in the diet of mice substantially increased the size and stage of the tumors [18]. The weak free radical scavenging activities of the three most cytotoxic compounds...
The authors declare that there are no conflicts of interest.

## Table 1: The half inhibitory concentration of the active compounds in HepG2, MDA, A549, and Vero cell lines as well as their SI

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Compound 2</th>
<th>Compound 7</th>
<th>Compound 9</th>
<th>Actinomycin D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀±SD (µg/ml)</td>
<td>IC₅₀±SD (µg/ml)</td>
<td>IC₅₀±SD (µg/ml)</td>
<td>IC₅₀±SD (µg/ml)</td>
</tr>
<tr>
<td>HepG2 cells</td>
<td>4.52±0.1113</td>
<td>2.93</td>
<td>6.07±0.09286</td>
<td>8.5±0.1989</td>
</tr>
<tr>
<td>MDA cells</td>
<td>7.02±0.08407</td>
<td>1.88</td>
<td>6.3±0.1909</td>
<td>5.5±0.2104</td>
</tr>
<tr>
<td>A549 cells</td>
<td>9.23±0.1062</td>
<td>1.43</td>
<td>5.2±0.1465</td>
<td>9.3±0.9093</td>
</tr>
<tr>
<td>Vero cells</td>
<td>13.23±0.1062</td>
<td>7.2±0.1465</td>
<td>12.3±0.9093</td>
<td>7.5±0.201</td>
</tr>
</tbody>
</table>

*Values are expressed as mean±standard deviation of the three replicates. SI: Selectivity indices

## Table 2: The scavenging percentage of the tested compounds compared to the positive controls Vitamin C and BHA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Scavenging (%)</th>
</tr>
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<tbody>
<tr>
<td>Compound 2</td>
<td>2.2</td>
</tr>
<tr>
<td>Compound 7</td>
<td>2.2</td>
</tr>
<tr>
<td>Compound 9</td>
<td>14.5</td>
</tr>
</tbody>
</table>

*The IC₅₀ value of the positive control, Vitamin C, was measured as 12±3.5 µg/ml. The IC₅₀ of the positive control, BHA, was measured as 53±3.1 µg/ml. BHA: Butylated hydroxyanisole

Determination of antioxidant activity by one method cannot identify all possible mechanisms. Therefore, different free radical scavenging methods should be considered in future research [19].

## Conclusion

The current approach provides important insights into marine steroids which are considered as the most active chemical class for the discovery of bioactive anticancer drugs. In this work, we report for the first time, the cytotoxic effect of 7ß-acetoxy-24-methylcholesta-5,24-diene-3β,7β,19-triol (9) on the major cancer types (liver, lung, and breast cancer cells).

Polyhydroxylated steroids, especially those derived from the marine organism, showed a potent cytotoxic impact on cancer cell lines. This sheds light on the importance of this chemical skeleton in developing active anticancer drugs from marine organisms. The mechanism of cytotoxic activity with the weak antioxidant activity of the compounds has more to be explained in further studies, but it can be considered as an advantage as it will not interfere with the metabolic mechanism of the cells. The polyhydroxysterols skeleton of our compounds is similar to the human sterols, so it can be accounted as promising lead compounds for the development of new, safe, and effective chemotherapy.

## Authors’ Contribution

Dr. Mona Ellithey participated actively in the invention of the scientific idea, arranging and preparing the experimental step, preparing the compounds, conducting the necessary cell culture assays, analyzing the data, and writing the paper. Prof Hanaa Hamdy participated actively in the invention of the scientific idea, conducting the necessary biochemical analyses, revising the paper critically, editing and approving the final version, and submission of the manuscript to the journal.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## References