Cytotoxic Potential, Metabolic Profiling, and Liposomes of Coscinoderma sp. Crude Extract Supported by in silico Analysis

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Introduction: Sponge-Coscinoderma sp. (Family: Spongiiidae) is a coastal sponge that possesses a broad variety of natural-products. However, the exact chemical constituents and cytotoxic activity of the extract are still undefinable.

Methodology: In the present study, the metabolomic profiling of Coscinoderma sp. dereplicated 20 compounds, utilizing liquid chromatography coupled with high-resolution mass spectrometry (LC-HRESIMS). Coscinoderma-derived crude extract, before and after encapsulation within nanosized liposomes, was in vitro screened against hepatic, breast, and colorectal carcinoma human cell lines (HepG2, MCF-7, and Caco-2, respectively).

Results: The identified metabolites were fit to diverse chemical classes, covering diterpenes, an indole alkaloid, sesquiterpenoid, sterol, and methylheteropine salt. Comprehensive in silico experiments predicted several compounds in the sponge-derived extract (eg, compounds 1–15) to have an anticancer potential via targeting multiple targets. The crude extract showed moderate antiproliferative activities towards studied cell lines with IC50 values range from 10.7 to 12.4 µg/mL. The formulated extract-containing liposomes (size 141±12.3nm, PDI 0.222, zeta potential 20.8 ± 2.3), significantly enhanced the in vitro anticancer activity of the entrapped extract (IC50 values ranged from 1.7 to 4.1 µg/mL).

Discussion: Encapsulation of both the hydrophilic and the lipophilic components of the extract within the lipid-based nanovesicles enhanced the cellular uptake and accessibility of the entrapped cargo. This study introduces liposomal nano-vesicles as a promising approach to improve the therapeutic potential of sponge-derived extracts.

Keywords: Coscinoderma, sponge, LC-HRESIMS, metabolic profiling, antiproliferative, liposomes

Introduction:
The taxonomic biodiversity of coastal living forms had passed up to 30 x10⁶ species involving greater than 70% of the earth’s surface. However, the total of biologically effective compounds from this enormous origin was restricted to a few thousand.1 Therefore, it was obvious to predict that marine organisms expressed an exceedingly valuable source of novel bioactive materials that can drive the outcome of different drugs.2 Natural products from marine origin as sponges and echinoderms have been explored broadly for their biological activities.3–5

The sponge Coscinoderma sp. (Family: Spongiiidae), fitted to an arrangement of coastal sponges that had a broad variety of natural products, covering diterpenes, sesquiterpene hydroquinones, long-chain aliphatic and acetylenic compounds,
furic, and scalaric sesterterpenes, bromotyrosine alkaloids, hepta- and octaprenylhydroquinones, suvanine analogs, and farnesyquinols, which reported to have antitumor, antimalarial, antibacterial, antifungal, and protein tyrosine phosphatase-1B (PTP1B) inhibitory activities. Despite that Cosinoderma has shown a wide range of biological activities, its chemical constituents are still elusive. Consequently, we selected this sponge in the present to shed some light on its chemical makeup and to investigate its potential as anticancer agent depending on a comprehensive in silico predictive study and a subsequent in vitro validation.

Liposomes are lipid-based bilayer nanovesicles that are capable of entrapment of both hydrophilic and lipophilic drugs either inside its core or within the phospholipid bilayer, respectively. Many studies have reported the impact of formulating natural products within liposomal nano-vesicles in the entrapment of both hydrophilic and lipophilic constituents, improvement of stability of entrapped cargo, improvement of cellular uptake of that natural components and targeted delivery to the definite site of action. Liposomes have the advantages of being biocompatible, non-immunogenic, and flexible dosage form that achieves the controlled delivery of the entrapped active constituents. Several techniques have been adopted for the formation of liposomes including thin-film hydration method, spraying technique and ethanol injection method. The ethanol injection method is a favorable technique because it is simple and enables the nonmixing of the organic phase with the aqueous one producing homogenous nanosized vesicles. Despite the wide range of therapeutic benefits of Cosinoderma sp., its low bioavailability, and leakage of suitable formulation retard the clinical application of such promising marine product. Formulating a convenient dosage form that can guarantee the entire inclusion, enhanced stability, and the cellular delivery of its physicochemically diverse components was a necessity. Consequently, the main purposes of the present investigation are to investigate the chemical profile of the Cosinoderma extract, predict the most probable bioactivity of this extract depending on a comprehensive in silico study of its main components, and test this predicted bioactivity in vitro, with study if the biological activity was enhanced upon entrapment of the extract into nanoformulation.

Materials and Methods
Sponge Material
Cosinoderma sp. sponge was collected from Ahia Reefs. Cosinoderma sp. was kindly identified by El-Sayed Abed El-Aziz (Department of Invertebrates Lab., National Institute of Oceanography and Fisheries, Red Sea Branch, 84511 Hurghada, Egypt). A voucher specimen (2020-BuPD 76) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Egypt.

Chemicals and Reagents
Chemicals and reagents used in this study were described in detail on Supplementary Material and Methods – Chemicals and reagents.

Metabolic Analysis Procedure
Freeze-dry sponge material (8g) was extracted with methanol methylene chloride (1:1). The crude extract, developed at 1mg/mL for mass spectrometry analysis. The recovered ethanolic extract was exposed to metabolic analysis using LC-HRESIMS. The details for the LC-HRESIMS method are described on Supplementary Material and Methods – Metabolomic Analysis Procedure.

Preparation of Cosinoderma sp.-Containing Liposomes
Liposomes were developed by the simple ethanol injection method. The details for the utilized method are described in Supplementary Material and Methods – Preparation of Cosinoderma sp.-containing liposomes.

Characterization of Cosinoderma sp.-Containing Liposomes
Size and polydispersity index of Cosinoderma sp. containing liposomes were assigned with Zetameter Nano ZSP (Malvern Instruments, Malvern, UK). The details for the utilized method described in Supplementary Material and Methods – Characterization of Cosinoderma sp.- containing liposomes.

Transmission Electron Microscopy (TEM)
Prepared liposomes of Cosinoderma sp. were imaged using (JEM-1400, Jeol, Tokyo, Japan) equipped at 80 kV. The liposomal suspension was imaged on a carbon-coated copper grid which was left for 10 minutes at 25°C before examination.
FTIR and TGA of *Coscinoderma* sp.-Containing Liposomes
The effect of temperature on the weight of empty liposomes and *Coscinoderma* extract either free or encapsulated within the prepared liposomes was studied using Thermogravimetric Analysis (TGA). Samples were dried and 20 dried samples were heated from 30°C to 450°C in a platinum pan (heat flow rate of 20 °C/min and nitrogen flow rate 20mL/min). To gain more insight into the probable interaction between lipid S75, cholesterol, and *Coscinoderma* extract, Fourier-transform infrared (FT-IR) measurements were carried out for the *Coscinoderma* extract, blank liposomes, and *Coscinoderma* liposomes over the wavenumber range 4000 to 400 cm ( Nicolet IS 10 FTIR spectrometer, US) after the dispersion of samples in KBr discs.

Ethical Statement
This study was developed under the guidelines of the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) that addressed the Use of cell lines in cancer research.

Cell Culture Conditions
The cancer cell lines HepG2, MCF7, and Caco-2 culture condition described in Supplementary Material and Methods – Cell Culture Conditions.

Antiproliferative Assay
The antiproliferative activity of *Coscinoderma* sp.-containing liposomes and their corresponding empty liposomes were described in detail in Supplementary Material and Methods – Antiproliferative assay.

In silico Biological Activity Predictions
PASS was employed for the prediction of the most possible anticancer metabolites in *Coscinoderma* sp. and to point a probable molecular target for them. The details for PASS were described in Supplementary Material and Methods – In Silico Biological Activity Predictions.

Molecular Docking Experiments
Molecular docking was carried out utilizing Autodock Vina software. The details were described in Supplementary Material and Methods – Molecular Docking Experiments.
Table 1  Dereplicated Metabolites from LC-HRESIMS Analysis of Coscinoderma sp. Crude Extract

| No. | Metabolites Name                                      | Original Source          | MF               | RT (min) | m/z      |
|-----|------------------------------------------------------|--------------------------|------------------|----------|----------|
| 1   | Ent-13-norisorocaplen-15-al-18-oic acid              | Coscinoderma mathewsi    | C19H32O3         | 7.8187   | 305.2117 |
| 2   | Spongial13(16),14-dien-19-oic acid                   | Coscinoderma mathewsi    | C20H32O3         | 7.6540   | 317.2117 |
| 3   | Coscinamid A; Debromo                                 | Coscinoderma spp.        | C20H11N3O2       | 6.0123   | 330.1243 |
| 4   | 15-Oxospongi-13-en-19-oic acid                       | Coscinoderma mathewsi    | C20H28O4         | 7.58040  | 333.2066 |
| 5   | 15-Hydroxy-16-oxospongi-13-en-19-oic acid           | Coscinoderma mathewsi    | C20H28O5         | 7.56501  | 349.2015 |
| 6   | 15-Methoxy-16-oxospongi-13-en-19-oic acid           | Coscinoderma mathewsi    | C21H34O5         | 7.5908   | 363.2171 |
| 7   | Coscinafurani                                        | Coscinoderma mathewsi    | C21H34O5         | 6.7003   | 369.2794 |
| 8   | Coscinalactone                                        | Coscinoderma mathewsi    | C22H34O5         | 14.5149  | 385.2743 |
| 9   | Coscinamid A                                         | Coscinoderma spp.        | C23H13BrN3O2     | 5.9845   | 408.0348 |
| 10  | 5α,8β-Epidoxycholesta-6-en-3β-ol                     | Coscinoderma spp.        | C23H32O3         | 15.6207  | 415.3212 |
| 11  | 5α,8β-Epidoxycholesta-6,9(11) 24(28)-trien-3β-ol     | Coscinoderma spp.        | C28H42O3         | 15.6109  | 427.3212 |
| 12  | 5α,8β-Epidoxycholesta-6,24(28)-dien-3β-ol            | Coscinoderma spp.        | C28H44O3         | 15.6004  | 429.3369 |
| 13  | (24S)-5α,8β-Epidoxycholesta-6-en-3β-ol               | Coscinoderma spp.        | C28H46O3         | 15.6114  | 431.3525 |
| 14  | Suvanine                                             | Coscinoderma mathewsi    | C25H32O3S        | 4.5508   | 451.2518 |
| 15  | Coscinoquinol                                        | Coscinoderma mathewsi    | C21H46O2         | 6.5731   | 451.3576 |
| 16  | Halisulfate 1                                        | Coscinoderma mathewsi    | C21H32NaO3S      | 4.5440   | 475.2494 |
| 17  | Coscinolactam A                                      | Coscinoderma mathewsi    | C21H34NO3S       | 5.6890   | 524.2682 |
| 18  | Halisulfate 2                                        | Coscinoderma mathewsi    | C21H42O3S        | 4.5409   | 549.3250 |
| 19  | Coscinolactam A; 1’S-isopropyl, 25-deoxo, 19-oxo    | Coscinoderma mathewsi    | C22H32NaO3S      | 5.9013   | 566.3151 |
| 20  | Coscinosulfate                                       | Coscinoderma mathewsi    | C31H34NaO3S      | 7.8758   | 571.3077 |

Abbreviations: MF, molecular formula; RT, retention time; min, minute; m/z, mass-to-charge-ratio.

25-deoxo, 19-oxo 19, that also previously isolated from Coscinoderma mathewsi,28 Another major ion peak with the m/z value of 571.3077 [M+H]+ with molecular formula C31H32NaO3S was detected and dereplicated as coscinosulfate 20, which was isolated earlier from Coscinoderma mathewsi.28

Figure 1  LC-HRESIMS chromatogram of the dereplicated metabolites of Coscinoderma sp. (positive).
Coscinoderma sp.-Containing Liposomes

Morphology of the prepared formulations reveals that vesicular liposomes are successfully prepared (Figure 4A and B). The vesicles are small and homogenously distributed (size=131±12.3, PDI=0.222). The vesicles have a zeta potential of 20.8 ± 2.3.

Thermogravimetric analysis was carried out to evaluate the potential of encapsulating the extract within the formulated liposomes on the enhancement of the physical and chemical stability of the entrapped Coscinoderma extract as a function of temperature. TGA curves for empty liposomes, Coscinoderma and Coscinoderma liposomes are shown in Figure 5. Upon heating from 30°C to 450°C, about 72.5% and 16.3% weight loss was observed at a temperature of 169°C for Coscinoderma extract and Coscinoderma liposomes, respectively. Results show the enhanced thermal stability of the entrapped cargo due to liposomal encapsulation.

To estimate the possible interactions between the components of the extract and those of the membrane bilayer of liposomes, FTIR spectra of Coscinoderma extract, empty liposomes, and Coscinoderma liposomes were studied (Figure 6). The FTIR spectrum of Coscinoderma extract contains principle bands at 3409, 1622, 1210, and 1507, and that of empty liposomes contains bands at 2907, 1736, 1459, 1234, and 1060. The FTIR spectrum of Coscinoderma liposomes contains similar bands to those contained in both free extract and empty liposome spectra, indicating that the encapsulation of Coscinoderma extract within the prepared liposomes did not form new linkages.

Target Prediction and Docking Analysis

Neural networks-based biological activity predictions that depend on artificial intelligence and machine learning processing along with other computer-aided drug design approaches have become widely accepted as an integral step during the drug discovery process.30,31

Such in silico–based procedures could be employed in drug discovery from natural sources, where they can register a set of possibly active hits among a complex mixture of other metabolites present in a given-natural crude extract.32

To putatively assign the most probable metabolites that might be associated with the anticancer activity of Coscinoderma sp., we submitted the most abundant metabolites (Figure 3) to a neural network-based prediction software PASS. This software search algorithm depends on the structural analogy of a great number of inhibitors recorded for a broad area of biological targets.23

As shown in Figure 7A, among the detected metabolites in Coscinoderma sp., compounds 1–15 that represented about 76.6% of the detected compounds, were predicted to exhibit
Figure 3 Metabolites putatively identified by LC-HRESIMS analysis of CE. Green metabolites showed the highest scores by PASS-based in silico predictions (anticancer, phosphatase inhibitors, and Pin-1 inhibitors for compounds 3 and 9). Compounds inside blue rectangles were further verified by docking analysis against SHP2. Compounds inside red rectangles were further verified by docking analysis against Pin-1.
Figure 4 (A) TEM images and size distribution of Coscinoderma sp.-containing liposomes, (B) TEM image of empty liposomes.

Figure 5 Thermogravimetric analysis (TGA) of empty liposomes, Coscinoderma extract and Coscinoderma liposomes.
antiproliferative activity (Pa > 0.5). Moreover, human phosphatase was suggested to be the probable target for them except for metabolites 3 and 9 that were predicted to target peptidyl prolyl cis-trans-isomerase NIMA interacting-1 (PIN-1). Accordingly, we searched for human phosphatases that are strongly linked to tumorigenesis. We found the non-receptor protein-tyrosine-phosphatase (SHP2) along with protein-tyrosine-phosphatases (PRL-1, -2, and -3) are currently well established as oncogenic phosphatases.33 These proteins are known to regulate cell survival and proliferation, through activation of the RAS–ERK (extracellular signal-regulated kinase) signaling pathway.33 On the other hand, Pin-1 is a key effector in Ras signaling and is frequently overexpressed in many types of cancers with poor prognosis.34 Consequently, we further assessed the PASS predictions by molecular docking experiments against the oncogenic phosphatases (ie, SHP2, and PRL-1 to -3) together with Pin-1. Among the metabolites that were predicted to mediate an anticancer activity by the inhibition of oncogenic phosphatases (Figures 7B and 8), only compounds 4–6 achieved good binding affinities (Figure 5B; < −5 kcal/mol) toward SHP2, that was also higher than that of the co-crystallized inhibitor (−8.5 kcal/mol for compounds 4–6, and −7.1 kcal/mol for the co-crystal inhibitor). Additionally, the mode of interaction of these metabolites (ie, 4–6) was comparable with this of the reported co-crystallized inhibitor.35 The most important interactions inside the SHP2’s binding site were H-bonding, particularly with ARG-11, PHE-113, and GLU-250, amino acids that were also involved in the interaction with the co-crystallized inhibitor (Figure 8).

Regarding Pin-1, both metabolites 3 and 9 were predicted to target this oncoprotein, and they were also achieved good binding affinities toward Pin-1 with a mode of interactions convergent to that of the co-crystallized inhibitor (Figures 7B and 9).36 Both compounds 3 and 9 interacted through H-bonding with LYS-63, ARG-69, ASP-112, and SER-154. Moreover, they exhibited two hydrophobic interactions with LEU-122 and PHE-134 (Figure 9). These bis-indole derivatives have been previously identified as anticancer agents.37

**Antiproliferative Activity of the Crude Extract**

According to the results of the in silico analysis, *Coscinoderma* sp.’s crude extract has a great anticancer potential. Consequently, it was in vitro screened for its potential as antiproliferative against hepatic, breast, and colorectal carcinoma cell lines (HepG2, MCF-7, and Caco-2, respectively). Results revealed that the crude extract was able to inhibit the

![Figure 6 FTIR of empty liposomes, Coscinoderma extract, and Coscinoderma liposomes.](image-url)
growth of all tested cell lines moderately with IC\textsubscript{50} values ranged from 10.7±0.05 to 12.4±0.10 μg/mL \((p<0.001)\), respectively (Table 2). Doxorubicin \((\text{IC}_{50} 4.3, 3.8, 3.4 \mu\text{g/mL}, \text{respectively})\) was used as a positive control (Table 2).

To gain more insight into the effect of encapsulation within the liposomal formulation on the improvement of the antiproliferative activity of the components in \textit{Coscinoderma} sp. crude extract, MTT assay was carried out for the extract-containing liposomes. The IC\textsubscript{50} against HepG2, MCF-7, and Caco-2 cell lines was determined for the three investigated cell lines. Results show that the sensitivity of the three investigated cell lines

![Figure 7](image-url)

\textbf{Figure 7} (A) PASS prediction scores of metabolites 1–20. Pa scores >0.5 indicated high-possible experimental activity. Blue columns are for the scores of antiproliferative activity, while the orange columns are for the phosphatase inhibitory activity, and gray columns are for the Pin-1 inhibitory activity. Metabolites 4–6 (assigned by green arrows) showed good binding affinities toward SHP2, while metabolites 3 and 9 (assigned by orange arrows) showed good binding affinities toward Pin-1. (B) Binding affinities of compounds 1–20 against SHP2, PRL-1-3, and Pin-1.
was significantly enhanced after liposomal formulation. Where IC<sub>50</sub> of the crude extract-containing liposomes against HepG2, MCF-7, and Caco-2 has significantly decreased to 2.2±0.31, 4.1±0.25, and 1.7±0.18µg/mL, respectively (p<0.001). Cell viability of the three investigated cell lines was evaluated for Coscinoderma-containing liposomes (at IC<sub>50</sub>) and their corresponding empty liposomes to exclude the cytotoxic effect of the phospholipid membrane (Figure 10). This is consistent with previous studies that reported the impact of nanocarriers on the enhancement of the cellular uptake and accessibility of the entrapped cargo.\textsuperscript{38–40} Nanomaterials with smaller particle sizes are easier to be up taken via endocytosis.\textsuperscript{41} Since the low water solubility of extract components can be an obstacle against availability for absorption and cellular uptake,\textsuperscript{42,43} enhancement of solubilization of the extract components, achieved by encapsulation, may have an important role in the improved cytotoxic effect against the cell lines under investigation.\textsuperscript{44} Favored uptake by interstitial leaky vasculature of tumor tissues can be another scenario for the accelerated cellular internalization.\textsuperscript{45} Besides, the presence of cholesterol contributes to the cellular uptake of liposomes.\textsuperscript{46} Clinically, formulating such cytotoxic payload into a nano-carrier system, that would entrap both the hydrophilic and the lipophilic components with the improvement of cellular uptake, would be of great therapeutic value especially if designed as a long-circulating formulation, which is the scope of our upcoming work.

Figure 8 Binding modes of metabolites 4–6 together with the co-crystallized inhibitor (A–D, respectively) inside the binding site of SHP2.
Conclusion

In the present study, the metabolomic profiling of Coscinoderma sp. crude extract dereplicated 20 compounds, utilizing LC-HRESIMS. The identified metabolites were fit to diverse chemical classes, covering sponging diterpenes, an indole alkaloid, sesterterpenoid, sterol, and methylherbipoline.

Table 2 In vitro Antiproliferative Activity of Coscinoderma sp. Crude Extract and Its Liposome Form Against HepG2, MCF7, and Caco-2 Cancer Cell Lines, Expressed as IC$_{50}$ ± (SEM) µg/mL

|        | HepG2     | MCF7      | Caco-2    |
|--------|-----------|-----------|-----------|
| Code   |           |           |           |
| Crude  | 12.4±0.10 | 11.3±0.21 | 10.7±0.05 |
| Crude containing liposomes | 2.2±0.31* | 4.1±0.25* | 1.7±0.18* |
| Doxorubicin | 4.2±0.05 | 3.8±0.10 | 3.4±0.31 |

Notes: The IC$_{50}$ value of compounds against each cancer cell line, which was defined as the concentration (µg/mL) that caused a 50% inhibition of cell growth in vitro, data were expressed as mean±SEM (n = 3). One-way analysis of variance (ANOVA) followed by Dunnett’s test using PASW Statistics® version-18 (Quarry Bay, Hong Kong) was applied. GraphPad Prism software version-6 (La-Jolla, CA, USA) was used for statistical calculations. *Statistically significant at p < 0.001. Doxorubicin a positive control.
Salt. *Coscinoderma* sp. crude extract showed moderate antiproliferative activities against HepG2, MCF-7, and Caco-2. The improved delivery to the studied cell lines was achieved by the entrapment of *Coscinoderma* sp. crude extract within liposomal vesicles. Although our results mainly denote the in vitro MTT experiments, liposomal entrapment of the extract seems to be a promising approach to enhance the antiproliferative potential of the extract components. PASS in silico predicted compounds 1–15 as antiproliferative which target both SHP2, and Pin-1. Further isolation of the active components from the crude extract together with the in vivo studies are in progress to find out the applicability of such formulation as an anticancer therapeutic approach.

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**Disclosure**

The authors declared no conflict of interest for this work.

**References**

1. Henseler C, Nordström MC, Törnroos A, et al. Coastal habitats and their importance for the diversity of benthic communities: a species-and trait-based approach. Estuar Coast Shelf Sci. 2019;226:106272. doi:10.1016/j.ecss.2019.106272
2. Farag MA, Fekry M, Al-Hammady M, et al. Cytotoxic effects of Sarcophyton sp. soft corals—Is there a correlation to their NMR fingerprints? Mar Drugs. 2017;15:211. doi:10.3390/md15070211
3. Alves A, Sousa E, Kiijoo A, Pinto M. Marine-derived compounds with potential use as cosmeceuticals and nutricosmetics. Molecules. 2020;25:2536. doi:10.3390/molecules25112536
4. Avila C, Angulo-Preckler C. Bioactive compounds from marine heterobranchs. Mar Drugs. 2020;18:657. doi:10.3390/md18120657
5. Musa A, Al-muaikel N, Abdel-Bakky M. Phytochemical and pharmacological evaluations of ethanolic extract of *Bassia Eriophora, Bassia Eriophora* Der Pharma Chem. 2016;8:169–178.
6. Mioso R, Marante F, Bezerra R, et al. Cytotoxic compounds derived from marine sponges. A review (2010–2012). Molecules. 2017;22:208. doi:10.3390/molecules22020208
7. Meisner D, Mezei M. Liposome ocular delivery systems. Adv Drug Delivery Rev. 1995;16:75–93. doi:10.1016/0169-409X(95)00016-Z
8. Refaat H, Naguib YW, Elsayed M, Sarhan HA, Aalaeedln E. Modified spraying technique and response surface methodology for the preparation and optimization of propolis liposomes of enhanced anti-proliferative activity against human melanoma cell line A375. Pharmaceutics. 2019;11:558. doi:10.3390/pharmaceutics11110558
45. Tran MA, Watts RJ, Robertson GP. Use of liposomes as drug delivery vehicles for treatment of melanoma. Pigm Cell Melanoma Res. 2009;22:388–399. doi:10.1111/j.1755-148X.2009.00581.x

46. Xu L, Wempe MF, Anchordoquy TJ. The effect of cholesterol domains on PEGylated liposomal gene delivery in vitro. Ther Delivery. 2011;2:451–460. doi:10.4155/tde.11.13