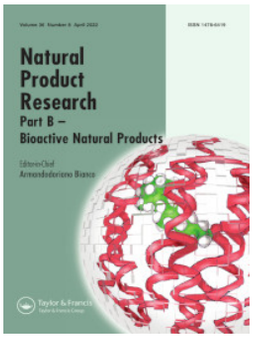

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SHORT COMMUNICATION

Diterpenoids profile of the marine sponge *Chelonaplysilla erecta* and candidacy as potential antitumor drugs investigated by molecular docking and pharmacokinetic studies

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ABSTRACT

The *Chelonaplysilla* genus possesses a numerous bioactive diterpenes with anti-inflammatory and cytotoxic effects. The current study aimed to assess the chemical composition of *C. erecta* crude extract (CECE) based on its metabolomic profile that has been integrated with neural network-based virtual screening and molecular docking using liquid chromatography with high resolution mass spectrometry (LCHR-MS). In addition to the estimation of the antitumor activity of the same extract *via* anti-interleukin-17A (IL-17) action, along with its formulated spanlastics preparation. The CECE markedly displayed growth inhibition for HepG-2 cells at IC_{50} value 16.5 ± 0.8 μg/mL, whereas the spanlastic formulation revealed more eminent antitumor effect against Caco-2 cells ($IC_{50} = 2.8 \pm 0.03$ μg/mL). Among the dereplicated compounds, macfarlandin F (16) and pourewanone (25) demonstrated the highest potential with co-crystallized ligand 63 O within the active site of IL-17A in molecular docking studies. These findings rationalized the antitumor mechanism of marine organism for future chemotherapeutic applications.

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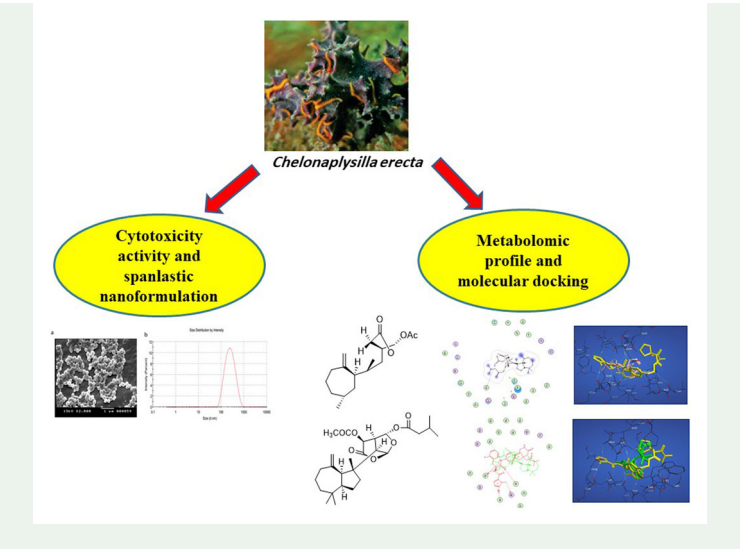

1. Introduction

Chelonaplysilla genus is belonging to family Darwinellidae (Jeon and Sim 2008). The phytochemical investigation of this genus revealed an abundance of diterpenes (Bergquist et al. 1993) and alkaloids (Bobzin and Faulkner 1991), which are likely to be responsible for its biological activities, including anti-tubercular and anti-inflammatory activities (de Oliveira et al. 2020).

Despite the many therapeutic effects of marine natural products, optimal utilization of their bioactivities has been hampered by poor absorption and limited yield of isolated molecules. As a result, various nano-carrier systems have been developed to enhance the bioavailability and biological potential of naturally derived products and their components (Wang et al. 2019). Spans and edge activators were used to prepare span-based nano-carriers; namely spanlastics that have the advantage of being more stable than other nano-carriers, like liposomes (Kakkar and Kaur 2011).

Therefore, the current study investigated the encapsulation effect of *Chelonaplysilla erecta* extract components within spanlastics formulation on the enhancement of its antitumor effect against hepatocellular carcinoma (HepG-2), colon (Caco-2), and breast (Mcf-7) cancer cell lines. In addition, metabolomics based on high-performance liquid chromatography coupled with electrospray ionization high-resolution mass spectrometry (HPLC–ESI–HRMS) was integrated with several *in silico* studies (e.g. neural network-based virtual screening and molecular docking) to highlight the main bioactive compounds responsible for such activity, particularly against IL-17A. In addition, pharmacokinetic investigations were conducted for determination of Lipinski's parameters.

2. Results and discussion

2.1. Metabolomic profiling

The metabolomics analysis of the crude extract of *C. erecta* (CECE) revealed the presence of various rearranged spongiane diterpenes metabolites (**1–27**) along with two

Table 1. Antitumor activities of CECE and its spanlastics represented as IC_{50} (μg/mL) ± SEM against hepatocellular carcinoma (HepG-2), colon (Caco-2), and breast (Mcf-7) cancer cell lines.

IC_{50} Values (μg/mL) ± SEM			
Sample	HepG-2	Mcf-7	Caco2
CECE	22.31 ± 0.03	17.34 ± 0.15	16.46 ± 0.76
CECE-containing spanlastics	8.12 ± 0.15	4.91 ± 0.04	2.78 ± 0.03
Doxorubicin*	1.32 ± 0.06	1.72 ± 0.03	2.12 ± 0.04

*Positive control drug

alkaloids, named chelonin C (**28**) and chelonin A (**29**). Among the identified diterpenes, 26 compounds were reported for the first time in *C. erecta*. Most of these metabolites had been isolated and structurally elucidated previously from *C. violacea*. Moreover, they exhibited anti-inflammatory activities (Bergquist et al. 1993). The total ion chromatograms (TIC) in the negative and positive ionization modes are demonstrated in Figure S1 & S2, respectively. In addition, the chemical names of the tentatively identified metabolites are listed in Table S1 and their chemical structures are traced in Figure S3 (Supplementary materials).

2.2. The spanlastics preparation and characterization of Chelonaplysilla erecta crude extract

The spanlastics of *C. erecta* crude extract (CECE) were efficiently prepared, resulting in small and homogeneous particle size distribution. Characterization by SEM images of the formulated spanlastics revealed a vesicular structure with a bi-layered membrane with almost no un-entrapped extract (Figure S4a, b, Supplementary materials).

2.3. Cytotoxic activity

The cytotoxic activity of CECE was investigated by means of the MTT assay using doxorubicin as a benchmark. As shown in Table 1, CECE attenuated the growth of HepG-2, Mcf-7, and Caco-2 cells with IC_{50} values of 22.3 ± 0.03, 17.3 ± 0.2, and 16.5 ± 0.8 μg/mL, respectively. CECE-containing spanlastics, by contrast, displayed more prominent cytotoxic potentials towards the investigated cell lines as demonstrated in Table 1. In particular, the cytotoxic activity against Caco-2 cells gave an IC_{50} at 2.7 ± 0.03 μg/mL, which was remarkably as effective as doxorubicin (IC_{50} = 2.1 ± 0.04 μg/mL), emphasizing the valuable role of formulated spanlastics in improving the cytotoxic ability of CECE. These findings were in agreement with many studies that revealed the role of nano-carriers in improvement of the cellular uptake and approachability of the entrapped payload (Musa et al. 2021, Refaat et al. 2019). Reduced energy for endocytosis achieved by the small particle size of formulated spanlastics might be associated with more facilitated cellular uptake (Salatin et al. 2015). Moreover, encapsulation of CECE within the formed spanlastics enhanced the solubilization of the components of the extract, making them more available for absorption and cellular uptake (Hajizadeh et al. 2019).

From the clinical point of view, such nano-carriers are very promising in the treatment of cancer due to the enhanced permeation and retention effect in tumor tissue

enabled by the small particle size of the carrier and the increased permeability of the tumor cells (Alaaeldin et al. 2017, Alaaeldin et al. 2021).

2.4. Molecular docking

In-silico molecular docking simulations were performed within the active site of IL-17A to gain some insight about possible interactions of the tested identified metabolites with various amino acids of the active site of IL-17A. Interestingly, twelve compounds out of 29 tested molecules showed better interaction profile compared to co-crystallized ligand and this was indicated by their lower binding free energy (S) value. Although 13 molecules were found to have higher binding free energies than 63 O, their docking accuracy (RMSD) values were to a great extent better than the RMSD of 63 O (1.8627 Å), indicating how good their docking is within the active site of IL-17A. On the other hand, metabolites **2**, **4**, **6**, and **18** failed to fit within the active site of IL-17A (Table S2 & S3, Supplementary materials). Moreover, the 2 D-interaction diagrams of molecules **9**, **10**, **11**, **16**, and **25** were demonstrated in Figure S5 (Supplementary materials). They showed also great overlay with the co-crystallized ligand (63 O) within 5HI3 active site and this explains their good docking score (S = −7.5712, −5.5264, −6.0461 and −5.9475 kcal/mol, respectively) and high docking accuracy (RMSD <2.0 Å), as shown in Figure S6 (Supplementary materials).

2.5. Physicochemical and pharmacokinetics studies

The results showed that most of the isolated molecules obeyed Lipinski's rule of five (RO5), except for five molecules, i.e. spongian-16-one (**1**), 15a,16a-diacetoxyspongian (**2**), chelodane (**21**), barekoxide (**22**), and zaatirin (**23**) (Table S4, Supplementary materials).

3. Conclusion

The crude extract of underexplored sponge *C. erecta* (CECE) revealed a remarkable anti-proliferative capacity against colon, breast, and liver tumor cell lines, which was attributed to several metabolites, mainly diterpenoid compounds. HPLC–ESI–HRMS has succeeded the identification of 27 diterpenoids, in addition two alkaloids. Among them, 26 diterpene compounds were reported for the first time in *C. erecta*. Docking analysis of the dereplicated compounds, particularly macfarlandin F (**16**) and pourewanone (**25**), showed moderate to substantial binding interactions with the active site of IL17A as a candidate mechanism of their antitumor action. Moreover, the current study succeeded to improve the potency of the crude extract by using a spanlastics formulation. Based on the promising results, chromatographic isolation of the most bioactive metabolites and structural elucidation using 1- and 2-D NMR techniques are planned with the goal of complete characterization, in-depth pharmacological investigation, and production of potential novel anticancer drugs.

Disclosure statement

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