Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

In vitro Cytotoxic Potential of *Nephthea* sp. and its Silver Nanoparticles against Hepatic and Colon Cancer Cells Assisted with Molecular Docking Studies

Omnia Hisham Abdelhafez[1*], John Refaat Fahim[2*], Asmaa M. AboulMagd[3], Usama Ramadan Abdelmohsen[1,2], Samar Yehia Desoukey[2], Mohamed Salah Kamel[1,2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, New Minia 61111, Egypt.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Nahda University, Beni Suef 62513, Egypt.

Received: January 03, 2023; revised: February 02, 2023; accepted: February 05, 2023

Abstract

The current study aimed to evaluate the cytotoxic potential of the total extract of the soft coral *Nephthea* sp. and its derived fractions (petroleum ether, ethyl acetate, *n*-butanol and acetone) in addition to their green synthesized silver nanoparticles (AgNPs) against hepatocellular carcinoma (HepG2) and colon adenocarcinoma (HT29) cell lines. Of these, the petroleum ether fraction exhibited potent inhibitory activity against HepG2 cells, with an IC_{50} value of 1.10 ± 0.03 µg/ml, while the *n*-butanol fraction revealed significant cytotoxic potential against HT29 cells (IC_{50}= 3.69 ± 0.16 µg/ml). On the other hand, the prepared AgNPs of both the total extract and its ethyl acetate fraction demonstrated the maximum inhibitory effects towards HepG2 and HT29 tumor cells, respectively (IC_{50}= 0.35 ± 0.01 and 8.97 ± 0.48 µg/ml, respectively). Moreover, an *in-silico* molecular docking analysis of a group of 14 identified metabolites from the petroleum ether fraction showed the considerable binding affinity of compounds 1 (nephthoside monoacetate), 5 (chabrolobenzoquinone E), 10 (chabrolohydroxybenzoquinone E), and 12 (2-*N*-nonanoyl-4,5-dihydrosphingosine) to the common tyrosine kinase, epidermal growth factor receptor (EGFR), which might suggest their contribution to the observed cytotoxic potential of this fraction of *Nephthea* sp. These results could therefore pave the way towards further search for varied antitumor candidate metabolites from *Nephthea* sp.

Keywords

Cytotoxicity, EGFR, Molecular docking, Nephtheidae, Secondary metabolites, Soft corals

Introduction

Marine ecosystems cover almost 70% of the earth's surface and are inhabited by nearly 80% of the world's plant and animal species [1]. Among them, marine invertebrates such as soft corals are considered a rich source of medicinally relevant metabolites with great structural complexity and diversity [2]. These marine organisms are widely spread from polar to tropical regions of the world's oceans at depths up to 6 km [3]. In this respect, various members of the genus *Nephthea* (family Nephtheidae) have been reported to produce a wide range of secondary metabolites, including steroids, sesquiterpenoids, diterpenes, and quinones, among others, that have been also searched for their biological potential such as anti-diabetic, cytotoxic, antimicrobial, and anti-inflammatory properties [4, 5]. Consequently, in continuation of our research on the chemical and biological attributes of the Red Sea soft coral *Nephthea* sp. [4, 6–8], the current work investigates the possible *in vitro* cytotoxic actions of the crude extract and different fractions of *Nephthea* sp. against hepatic and colon cancer cells in comparison with their green synthesized silver nanoparticles (AgNPs). Finally, the ability of a group of secondary metabolites (previously isolated and identified from *Nephthea* sp. by our research group [8]) to interact with one of the common cellular tyrosine kinases, namely the epidermal growth factor receptor (EGFR) which is implicated in cancer pathogenesis, was also deliberated using the *in silico* molecular docking approach.

2. Experimental

2.1. Soft coral material

Nephthea sp. was collected from the Red Sea at Sharm El-Sheikh using scuba diving at a depth of 10 m. The collected soft coral was identified by Dr. Tarek Temraz, Marine Science Department, Faculty of Science, Suez Canal University, Ismailia, Egypt and immediately kept at −20 °C until investigation. A voucher sample was added to the herbarium section of Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt under the number SAA-26.

2.2. Extraction and isolation

The freeze-dried soft coral was macerated with methanol–methylene chloride (1:1) [9] and the resulting extract (24.0 g) was suspended in the least amount of distilled water and successively extracted with petroleum ether, ethyl acetate, and *n*-butanol. The organic phase of each step was individually concentrated under reduced pressure to yield three fractions [I (10.0 g), II (3.0 g), and III (3.0 g)], respectively. The left mother liquor was finally desalted on an ion exchange resin which was then re-extracted

**** Correspondence:*** *Omnia Hisham Abdelhafez, John Refaat Fahim*
Tel.: *+2-086-2369075, +2 01227454025*
Email Address: *omnyaheshamhafez@gmail.com, johnrefaat82@yahoo.com*

using acetone; the latter was evaporated to give fraction IV (200.0 mg). The crude soft coral extract and its different fractions were finally stored at 4 °C for biological investigation.

2.3. Synthesis and characterization of AgNPs

The AgNPs of the total extract and different fractions of the soft coral, *Nephthea* sp. were green synthesized according to the method described in [6]. The obtained nanoparticles were then characterized by UV-Visible spectroscopy, Transmission Electron Microscopy (TEM), Fourier-Transform Infrared Spectroscopy (FT-IR).

2.4. Cytotoxic activity

The cytotoxic activities of the total extract and successive fractions of *Nephthea* sp., along with their green synthesized AgNPs were evaluated against hepatocellular carcinoma (HepG2) and colon adenocarcinoma (HT29) cell lines (The American Type Culture Collection (Manassas, VA, USA)) using the MTT assay [10]. Three independent tests were performed and IC_{50} values were calculated by using GraphPad Prism 5 (Version 5.01, San Diego, CA, USA).

2.5. Molecular docking

The crystallographic structure of EGFR in complex with its ligand was obtained from the Protein Data Bank [11]. Molecular docking studies were performed with the Molecular Operating Environment 2019.0101 software (MOE of Chemical Computing Group Inc. (the license is issued to, revised, and renewed annually by Nahda University)) on Core i5 2.2 GHz workstation running on a Windows 10 PC [12]. Compounds' structures were built using ChemDraw Ultra 11.0, then their protonation, the correction of atoms and bond types were defined, hydrogen atoms were added, and finally minimization was performed (MMFF94x, gradient: 0.01) [13]. The docking experiment on EGFR was performed by re-docking the ligand in the PDB file 1M17 after which the ligand was deleted. The default Triangle Matcher Placement method was employed for docking. GBVI/WSA ΔG scoring function which determines the free energy of binding of the ligand from a given pose was chosen to rank the final poses. The ligand complex with the enzyme having the lowest ΔG score was selected. The re-docking of ligand with its target revealed a RMSD of 1.653 Å, which confirms that the ligand binds to the same pocket and assures the dependability of the parameters of docking.

3. Results and discussion

3.1. Synthesis and characterization of AgNPs

The total extract of *Nephthea* sp. as well as its petroleum ether and ethyl acetate fractions were effectively used in the green synthesis of AgNPs as inferred from the formed dark brown color after 48 h, whereas no color change was observed in the control solution of $AgNO_3$ [14, 15]. In contrast, both the *n*-butanol and acetone fractions failed to give AgNPs. Such color transition is due to the reduction of Ag^+ to Ag^0 by some of the secondary metabolites in the total extract, petroleum ether and ethyl acetate fractions [15, 16].

3.2. TEM characterization of AgNPs

The green synthesized AgNPs were first subjected to TEM analysis that unveiled the formation of spherical particles. The total extract-, petroleum ether fraction-, and ethyl acetate fraction-based AgNPs showed mean size ranges of 3.84–13.18, 8.59–11.19, and 5.28–8.34 nm, respectively (supplementary material Figure S1).

3.3. UV–Vis characterization of AgNPs

The formation of AgNPs was depicted by UV-Vis spectrometry as one of the most broadly utilized approaches for the characterization of AgNPs [15, 16]. Therefore, the formation and optimization of AgNPs were observed by measuring their absorbance in the range of 200–600 nm. An absorbance band was observed at 417 nm (supplementary material **Figure S2**), which is similar to that reported in the literature [6, 15, 16].

3.4. FT-IR characterization of AgNPs

FT-IR measurements were performed to identify the functional groups of different biomolecules that are responsible for both the reduction of Ag^+ ions and the capping of AgNPs [15, 16, 17]. In this regard, the obtained spectra (supplementary material **Figure S3**) exhibited several absorption bands (e.g. 3572.2, 3424.9, 3414.0, 3390.9, 2375.9, 2083, 1955.5, 1913.4, 1651.7, 1643.4, 1392.6, 1246, 1076.3, 973.0, 648.0, 694.3, 470.6, and 436 cm^{-1}) corresponding to various functional groups, such as amines, aldehydes, ketones, primary and secondary alcohols, phenols, carboxylic acids, alkenes, and aromatic rings, among others, which implies the contribution of different groups of chemical metabolites to Ag^+ reduction and the stabilization of the produced AgNPs [15, 16, 17].

3.5. Cytotoxic activity

Investigating the potential cytotoxic effects of the total extract of *Nephthea* sp. and its derived fractions against HepG2 and HT29 cell lines indicated that the petroleum ether fraction displayed significant inhibitory activity against HepG2 cells, with an IC_{50} of 1.10 ± 0.03 μg/ml, which was more potent as compared to staurosporine (IC_{50} = 5.00 ± 0.16 μg/ml), followed by the *n*-butanol fraction, the total extract, and the ethyl acetate fraction (IC_{50}= 4.39 ± 0.1, 5.22 ± 0.17, and 6.13 ± 0.2 μg/ml, respectively), whereas the acetone fraction was moderately active (IC_{50}= 15.76 ± 0.5 μg/ml). Furthermore, both the *n*-butanol and the petroleum ether fractions also exerted significant cytotoxic activities against HT29 cells, with IC_{50} values of 3.69 ± 0.16 and 5.59 ± 0.18 μg/ml, respectively. Both fractions were remarkably potent in comparison with the tested reference drug (IC_{50}= 12.07 ± 0.39 μg/ml). However, the acetone fraction, total extract, and ethyl acetate fraction were less active with higher IC_{50} values of 11.04 ± 0.36, 18.25 ± 0.6, and 18.39 ± 0.6 μg/ml, respectively (**Table 1**).

On the other hand, the cytotoxicity of the green synthesized AgNPs using the total extract, petroleum ether, and ethyl acetate fractions of *Nephthea* sp. was also examined for the first time against the previous cell lines in comparison with staurosporine. Results indicated that the AgNPs of the total extract had more potent growth inhibitory activities against HepG2 cells, with an IC_{50} value of 0.35 ± 0.01 μg/ml (**Table 1**), while the ethyl acetate AgNPs exhibited the highest inhibitory properties against HT29 cells with an IC_{50} value of 8.97 ± 0.48 μg/ml, implying the possible helpful impact of nanoparticle preparation on boosting the cytotoxic potential of *Nephthea* sp. samples.

Table 1: *In vitro* cytotoxic activities of the total extract and different fractions of *Nephthea sp*., along with their green synthesized AgNPs in comparison with staurosporine.

Extract/fraction	IC_{50} (µg/ml)	
	HepG2	**HT29**
Total extract	5.22 ± 0.17	18.25 ± 0.60
Petroleum ether fraction	**1.10 ± 0.03**	5.59 ± 0.18
Ethyl acetate fraction	6.13 ± 0.20	18.39 ± 0.60
n-Butanol fraction	4.39 ± 0.10	**3.69 ± 0.16**
Acetone fraction	15.76 ± 0.50	11.04 ± 0.36
AgNPs of the total extract	**0.35 ± 0.01**	72.15 ± 3.81
AgNPs of the petroleum ether fraction	3.45 ± 0.12	24.21 ± 1.52
AgNPs of the ethyl acetate fraction	14.65 ± 0.88	**8.97 ± 0.48**
Staurosporine	5.00 ± 0.16	12.07 ± 0.39

Table 2: A list of binding energy scores of the target compounds–EGFR complex conformations.

No.	Compound	Binding energy score (ΔG; kcal/mol)	Average number of poses per run
1	Nephthoside monoacetate	**–8.088**	10
2	Chabrolosteroid C	–6.385	10
3	Parathyrsoidin E	–5.086	10
4	*O*-Methylisogrifolin	–6.994	10
5	Chabrolobenzoquinone E	**–7.497**	10
6	Chabrolonaphthoquinone A	–6.775	10
7	Dendronesterone C	–5.601	10
8	4*α*-Methyl-ergosta-6,8(14), 22*E*-triene-3*β*-ol	–5.066	9
9	Litophynin C	–4.367	9
10	Chabrolohydroxybenzoquinone E	**–7.740**	10
11	Pacificin H	–5.596	10
12	2-*N*-nonanoyl-4,5-dihydrosphingosine	**–7.143**	10
13	Nebrosteroid O	–5.267	10
14	Ergost-5,25-diene-3*β*,24*S*,28-triol	–4.747	9

[a] The shown score is the mean of three consecutive runs.

[b] The docking method was validated by successful pose-retrieval docking experiment of the reference ligand (SYR127063; ΔG= –10.965 kcal/mol).

3.6. Molecular docking

Epidermal growth factor receptor (EGFR) is one of the tyrosine kinases that is overexpressed in a variety of cancer cell types and have been reported to contribute to the pathogenesis of hepatocellular carcinoma and colon adenocarcinoma [18]. Based on the postulation that the inhibition of EGFR is possibly associated with the cytotoxic activities against HepG2 and HT29 cell lines, and in view of the remarkable cytotoxic potential of the petroleum ether fraction of *Nephthea* sp., a group of 14 previously isolated and identified metabolites by our research group from the petroleum ether fraction [8] (supplementary material **Figure S4**) were examined for their interaction with the binding site of EGFR kinase in an attempt to predict the possible mechanism of action (**Table 2**). Searching the literature revealed that the features and binding interactions of EGFR binding site includes the amino acids residue Lys721, Thr766, Met769, Thr830, and Asp831 [19]. The obtained data (**Table 2**) revealed the considerable binding affinity of compounds 1 (nephthoside monoacetate), 10 (chabrolohydroxybenzoquinone E), 5 (chabrolobenzoquinone E), and 12 (2-*N*-nonanoyl-4,5-dihydrosphingosine) to EGFR kinase as inferred from their low binding energy scores (–8.088, –7.740, –7.497, and –7.143 kcal/mol, respectively). The other metabolites also showed moderate to promising interactions within the binding pocket of EGFR, with ΔG scores ranging from –4.367 to –6.994 kcal/mol (**Table 2**) as compared to the co-crystalized reference ligand, SYR127063 (ΔG= –10.965 kcal/mol). Among the studied metabolites, compound 1 formed two important hydrogen bond interactions with Asp831, resulting in a strong binding interaction, while compound 3 (parathyrsoidin E), with a binding energy of –5.086 kcal/mol, showed a hydrogen bond interaction via its carbonyl group with Lys721. Compound 10 (chabrolohydroxybenzoquinone E) also showed binding with Asp831 through its phenolic hydroxyl group, with a bond length of 2.71 Å (supplementary material **Figure S5**). These data might suggest the contribution of the aforementioned metabolites to the observed cytotoxic properties of the petroleum ether fraction of *Nephthea* sp.

4. Conclusion

In conclusion, the present work presented the inhibitory potential of the crude extract of the soft coral *Nephthea* sp. and its derived fractions against hepatic and colon cancers supported with molecular docking studies, of which the petroleum ether and *n*-butanol fractions were the most active against HepG2 and HT29 tumor cells, respectively. Our study also showed the potential of the green synthesized nanoparticles to improve the cytotoxic properties of *Nephthea* sp. extracts, particularly the total extract and its ethyl acetate fraction. These findings could therefore support the search for antitumor candidate metabolites from *Nephthea* sp. as well as their further testing as potential nano-chemotherapeutic agents.

References

[1] Henson SA, Beaulieu C, Ilyina T, John JG, Long M, Séférian R, Tjiputra J, Sarmiento JL. Rapid emergence of climate change in environmental drivers of marine ecosystems. Nature Communications 2017;8:1–9.
[1] Sang VT, Dat TTH, Vinh LB, Cuong LCV, Oanh PTT, Ha H, Kim YH, Anh HLT, Yang SY. Coral and coral-associated microorganisms: A prolific source of potential bioactive natural products. Marine Drugs 2019;17:468.
[3] Xia JM, Miao Z, Xie CL, Zhang JW, Yang XW. Chemical constituents and bioactivities of starfishes: An update. Chemistry and Biodiversity 2021;8:740–791.
[4] Abdelhafez OH, Fahim JR, Desoukey SY, Kamel MS, Abdelmohsen UR. Recent updates on corals from Nephtheidae. Chemistry and Biodiversity 2019;16:e1800692.
[5] Allam K, Khedr A, Allam A, Abdelkader M, Elkhayat E, Fouad M. Chemical and biological diversity in *Nephthea* soft corals in the current decade: A review. Journal of advanced Biomedical and Pharmaceutical Sciences 2021;4:124–133.
[6] Abdelhafez OH, Ali TFS, Fahim JR, Desoukey SY, Ahmed S, Behery FA, Kamel MS, Gulder TAM, Abdelmohsen UR. Anti-Inflammatory potential of green synthesized silver nanoparticles of the soft coral *Nephthea* sp. supported by metabolomics analysis and docking studies. International Journal of Nanomedicine 2020;15:5345–5360.
[7] Abdelhafez OH, Fahim JR, Rateb ME, Ngwa CJ, Pradel G, Abdelmohsen UR, Desoukey SY, Kamel, MS. Cytotoxic potential of *Nephthea* sp.-derived actinomycetes supported by metabolomics analysis. Natural Product Research 2022;36:6464–6469.
[8] Abdelhafez OH, Fahim JR, Mustafa M, AboulMagd AM, Desoukey SY, Hayallah AM, Kamel MS, Abdelmohsen UR. Natural metabolites from the soft coral Nephthea sp. as potential SARS-CoV-2 main protease inhibitors. Natural Product Research 2022;36:2893–2896.
[9] Hsiao TH, Sung CS, Lan YH, Wang YC, Lu MC, Wen ZH, Wu YC, Sung PJ.. New anti-inflammatory cembranes from the cultured soft coral *Nephthea columnaris*. Marine Drugs 2015;13:3443–3453.
[10] Rasheed T, Bilal M, Iqbal HMN, Li C. Green biosynthesis of silver nanoparticles using leaves extract of *Artemisia vulgaris* and their potential biomedical applications. Colloids and Surfaces B, Biointerfaces 2017;158:408–415.
[11] Stamos J, Sliwkowski MX, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. Journal of Biological Chemistry 2002;277:46265-46272.
[12] Nagai J, Shi H, Sezaki N, Yoshida N, Bandow K, Uesawa Y, Sakagami H, Tomomura M, Tomomura A, Takao K, Sugita Y. Quantitative structure–cytotoxicity relationship of 2-arylazolylchromones and 2-triazolylchromones. Anticancer Research 2019;39:6479-6488.
[13] Tosco P, Stiefl N, Landrum G. Bringing the MMFF force field to the RDKit: implementation and validation. Journal of Cheminformatics 2014;6:37.
[14] Moosa A, Ridha A, Allawi M. Green synthesis of silver nanoparticles using spent tea leaves extract with atomic force microscopy. International Journal of Current Engineering and Technology 2015;5:3233–3241.
[15] Haggag EG, Elshamy AM, Rabeh MA, Gabr NM, Salem M, Youssif KA, Samir A, Bin Muhsinah A, Alsayari A, Abdelmohsen UR. Antiviral potential of green synthesized silver nanoparticles of *Lampranthus coccineus* and *Malephora lutea*. International Journal of Nanomedcine 2019;14:6217–6229.
[16] Ahmed SST, Fahim JR, Youssif KA, Amin MN, Abdel-Aziz HMH, Brachmanne AO, Piel J, Abdelmohsen UR, Hamed ANE. Cytotoxic potential of *Allium sativum* L. roots and their green synthesized nanoparticles supported with metabolomics and molecular docking analyses. South African J. Botany 2021;142:131–139.
[17] Hembram KC, Kumar R, Kandha L, Parhi PK, Kundu, CN, Bindhani BK. Therapeutic prospective of plant-induced silver nanoparticles: application as antimicrobial and anticancer agent. Artificial Cells, Nanomedicine, and Biotechnology2018;46(sup3):S38–S51.
[18] Huang SZ, Wei MN, Huang JR, Zhang ZJ, Zhang WJ, Jiang QW, Yang Y, Wang HY, Jin HL, Wang K, Xing ZH, Yuan ML, Li Y, He XS, Shi Z, Zhou Q. Targeting TF-AKT/ERK-EGFR pathway suppresses the growth of hepatocellular carcinoma. Frontiers in Oncology 2019;9:150.
[19] Hegazy MF, Elshamy AI, Mohamed TA, Hamed AR, Ibrahim MAA, Ohta S, Paré PW. Cembrene diterpenoids with ether linkages from *Sarcophyton ehrenbergi*: An anti-proliferation and molecular-docking assessment. Marine Drugs 2017;15:192.