CATRINA (2014), 9 (1): 01 -06

Anticancer Activities of Some Organisms from Red Sea, Egypt

Enas E. Eltamany[1], Nermeen A. Eltahawy[1], Amany K. Ibrahim[1], Tarek Temraz[2], Hashim A. Hassanean[1], Mohamed M. Radwan[3, 4], Safwat A. Ahmed[1]*

[1]Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt
[2]Department of Marine Science, Faculty of Science, Suez Canal University, Ismailia, Egypt
[3]National centre for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, USA
[4]Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt

ABSTRACT

Encouraged by new drug developments, the present research was investigated on extracts of Red Sea marine resources all from Egypt. Extracts from three sponges *namely Spheciospongia vagabunda* (SAA-14), *Negombata corticata* (SAA-8) and *Negombata magnifica* (SAA-64) in addition to two soft corals *Sarcophyton glaucum* (SAA-33) and *Sarcophyton auritum* (SAA-43) were found to display high potential against $HepG_2$ (liver cancer cell line) and MCF-7 (breast cancer cell line). Cytotoxic potential activities of these five extracts were measured by the Sulpho-Rhodamine-B (SRB) assay. The anticancer activity of SAA-8, SAA-14,SAA-33, SAA-43 and SAA-64 was significantly enhanced in the liver cancer cell line with the values ranged from16.3 to 19.3µg/ml and in the breast cancer cell line with concentrations of 24.7, 19.7, 18.7,21.1and 25.8µg/ml, respectively compared to doxorubicin as positive control. *Negom batacorticata*and *Sarcophyton auritum* based extracts exhibited promising antifungal activity against fungi of genus *Candida, Cryptococcus* and *Aspergillus*, moreover, the five extracts revealed antiprotozoal and antimicrobial activity after *in vitro* assays. Also *Negombata corticata* has good antituberculosis, antimalarial and antilieshmanial potentials.

Key words: Anticancer, Antiprotozoal , Antimicrobial, *Negombata corticata, Negombata magnifica, Sarcophyton glaucum, Sarcophyton auritum* and *Spheciospongia vagabunda*.

INTRODUCTION

Nature has continuously provided mankind with a broad and structurally diverse arsenal of active compounds. These compounds continue to be used as highly effective drugs to combat a multitude of chronic diseases or as lead structures for the development of novel drugs (Jirge *et al*., 2010). Traditionally, since the discovery of the penicillin, higher plants and terrestrial microorganisms have proven to be the richest sources of natural drugs that are indispensable especially for the treatment of human diseases such as cancer (Chin *et al.*, 2006). Marine organisms represent a promising source for natural products of the future due to the incredible diversity of chemical compounds that are mainly accumulated in invertebrates such as sponges, tunicates, bryozoans and molluscs (Jirge *et al*., 2010). The bioactivity profiles of marine metabolites include neurotoxic, antiviral, antitumoral (Wang *et al*., 2011), antimicrobial (Wang *et al*., 2013) or anti convulcent properties (Ahmed *et al*., 2008) and are of considerable biotechnological interest (Bahakuni and Rawat, 2005).

The enormous potentials of the marine environment to provide new structural classes with activity against cancer have been reported for bryostatins (Schaufelberger *et al.*, 1991), didemnins (Rinehart *et al.*, 1988) and ecteinascidins (Rinehart *et al.*, 1990). Halichondrin B was first isolated from the sponge *Halichondriao kadai*as the most potent member of a series of related antitumor compounds (Uemura *et al.*, 1985). The chemical and biological diversity of the marine environment is so wide, immeasurable and therefore is an extraordinary resource for the discovery of new anticancer drugs (Petit and Biard, 2013). Recent technological and methodological advances in structure elucidation, organic synthesis, and biological assay have resulted in the isolation and clinical evaluation of various novel anticancer agents (Olano *et al*., 2009). For example, Bryostatin (Hale and Manviazar, 2010) and Didemnin B were considered as promising antitumor drugs. Bryostatin 1 which interferes with protein kinase C is currently in phase II clinical trials (Mackay and Twelves, 2007). Didemnin B, a depsipeptide isolated from the Caribbean tunicate Tridemnim solidum (Rinehart *et al*., 1981) inhibits the synthesis of RNA, DNA and proteins in various cancer cell lines. It shows anti-viral and immunosuppressive activities but also is an effective agent in the treatment of leukaemia and melanoma. Due to its toxicity, it was withdrawn from phase II clinical trials (Faulkner, 2002; Amador *et al.*, 2003). The marine Red Sea environment being one of the most biodiverse in the world offers a potential for producing novel drugs and prototypes. The increasing threat of cancer has initiated a renewal of interest in a research for novel anticancer agents (Martín *et al.*, 2013). Seventy five marine organisms from the Red Sea were tested for their anticancer potentials. The outcomes of these potentials are hereby revealed and discussed.

MATERIALS AND METHODS

Biological materials, collection and identification
Three sponges from different localities were collected

* *Corresponding author: safwat_aa@yahoo.com*

by hand using SCUBA diving method: *Spheciospongia vagabunda* (SAA-14) from Ras Mohamed, *Negombata magnifica* (SAA-64) from Sharm El- Sheikh, and *Negombata corticata* (SAA-8) from Safaga.

The sponge materials were frozen immediately and kept frozen at -20°C until further processing. The voucher specimens were deposited at the Zoological Museum of the University of Amsterdam under registration numbers ZMAPOR19759, ZMAPOR18568 and ZMAPOR 18569, respectively and in the herbarium section of Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt, under registration numbers (SAA-14), (SAA-64) and (SAA-8) respectively. The soft corals *Sarcophyton auritum* (SAA-43) and *Sarcophyton glaucum* (SAA-33) were collected from Safaga, Red Sea, air-dried and stored at low -20ºC until processed. A voucher specimen was also deposited in the herbarium section of the Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt under registration numbers SAA-43 and SAA-33.

Extraction

Studied samples were freeze dried ground and extracted with a mixture of MeOH/CH_2Cl_2 (1:1) (3x 2 L) at room temperature. The extracts were evaporated under vacuum at 40º C to afford 10 g, 12g, 14g, 9g and 11.5 g concentration, respectively.

Biological assays

In-vitro evaluation of the antitumor activity

The potential cytotoxicity of the investigated extracts was measured by the Sulpho-Rhodamine-B (SRB) assay as described by Skehan *et al*, (1990). This was performed on two human cell lines: $HepG_2$ (liver cancer cell line), MCF-7 (breast cancer cell line) which were kindly provided by the National Cancer Institute (Kasr El Ainy Street, Cairo, Egypt). The cells were plated into 96-multiwell plates (104 cells/ well) for 24 hours before treatment with the extracts to allow attachment of cells to the plate's wall. Cells were routinely cultured in Dulbeco's Modified Eagle's Medium (DMEM). Different concentrations of the tested samples (0, 50, 100, 150 and 200 µg/ml in DMSO) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extracts under test for 48 hours, at 37ºC and in atmosphere of 5% CO_2. After 48 hours, the cells were fixed, washed and stained with Sulpho-Rhodamine-B stain. Excess stain was washed with acetic acid and the attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. Moreover, the IC_{50} (Dose of the extract which reduces survival to 50%) was calculated.

Antimicrobial assay

Test strains were obtained from the American Type Culture Collection (Manassas, VA) [*Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *Cryptococcus neoformans* (ATCC 90113), *Aspergillus fumigatus* (ATCC 90906), methicillin-resistant *Staphylococcus aureus* (MR*Sa*) (ATCC 43300) and *Mycobacterium tuberculosis* (ATCC 23068)]. Susceptibility test was performed for all organisms, except *M. tuberculosis* and *A. fumigatus* using modified versions of the CLSI/NCCLS methods, (NCCLS, 2002) – (NCCLS, 2000) while optical density was used to monitor growth. Media supplemented with 5% Alamar Blue was used for growth detection of *M. tuberculosis* (NCCLS, 2000) – (Franzblau *et al.*, 1998) and *A. fumigates* (NCCLS, 1998). Samples dissolved in DMSO were serially diluted in DMSO/saline (20%/0.9%) and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the OD_{630} of cell/spore suspensions in incubation broth RPMI 1640/2% dextrose/MOPS at pH 4.5 for *Candida* spp., Sabouraud Dextrose agar for *C. neoformans*, cation-adjusted Mueller-Hinton at pH 7.3 for MR*Sa*, 5% Alamar Blue in Middlebrook 7H9 broth with OADC enrichment at pH 7.3 for *M. tuberculosis* and 5% Alamar Blue in RPMI 1640/2% dextrose/MOPS at pH 7.3 for *A. fumigates* to afford final target inocula (1×10^4, 1×10^4, 1×10^4, 1×10^5, 5×10^5, 5×10^5, 5×10^5, 2×10^6 and 3×10^4cfu/mL, respectively).

Control antimicrobial agents [fungi: amphotericin B; bacteria: ciprofloxacin (ICN Biomedicals, OH)] were included in each assay. All organisms were scanned using a Biotek Power wave XS plate reader (630 nm) (Bio-Tek Instruments, Winooski, VT) or a Polarstar Galaxy plate reader (excitation wavelength, 544 nm; emission wavelength, 590 nm) (*M. tuberculosis* and *A. fumigatus*) (BMG LabTechnologies, Germany) prior to and after incubation. Percent growth was plotted versus test concentration to afford the IC_{50}. Minimum fungicidal or bactericidal concentrations were determined by removing 5 µL from each clear well, transferring to agar, and incubating until growth was observed. The MFC/MBC defined as the lowest test concentration that kills the organism (allows no growth on agar) was calculated.

In vitro antimalarial assay

The assay is based on the determination of plasmodial LDH activity LDH (Jain *et al.*, 2002; Bharate *et al.*, 2008). A suspension of red blood cells infected with D6 strains of *Plasmodium falciparum* [200 µL, 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and Amikacin (60 µg/mL)] was added to a 96-well plate containing test samples (10 µL each) diluted in medium at various concentrations. The plate was placed in a modular incubation chamber flushed with $N_2/O_2/CO_2$ (90:5:5) and incubated at 37°C for 72 h. Parasitic LDH activity was determined by using Malstat™ reagent (Malker and Hinriches, 1993; Nkhoma *et al.*, 2007). The incubation mixture (20 µL) was mixed with the Malstat™ reagent (100 µL) and incubated (r.t., 30 min). Nitro blue

tetrazolium (NBT)/phenazine methosulfate (PES) (20 µL, 1:1) (Sigma, St. Louis, MO) was added and the plate incubated in the dark for 60 min. The reaction was stopped by the addition of 5% acetic acid (100 µL) and the plate scanned (650 nm) with an EL340 BioKinetics Reader (Bio-Tek Instruments, Winooski, VT). IC_{50} values were computed from the dose-response curves by plotting percent growth *versus* test concentration. Artemisinin was included in each assay as positive control**.**

In vitro antileishmanial assay

The *in vitro* antileishmanial activity was evaluated against a culture of *L. Donovani* promastigotes grown in RPMI 1640 medium supplemented with 10% GIBCO fetal calf serum at 26°C (Jain *et al.*, 2005; Bharate *et al.*, 2008). A three-day-old culture was diluted to 5 × 10^5promastigotes/mL. Drug dilutions (50–3.1 µg/mL) were prepared directly in cell suspension in a 96-well plate, followed by incubation at 26°C for 48 h.

Growth of leishmanial promastigotes was determined by the Alamar Bue assay (BioSource International, Camarillo, CA) **(**Ma *et al.*, 2004; Mikus and Steverding, 2000; Hamid *et al.*, 2004). Standard fluorescence was measured by a Fluostar Galaxy plate reader (excitation wavelength, 544 nm and emission wavelength, 590 nm). Pentamidine was used as the positive control. Percent growth was calculated and plotted against the tested concentrations in order to determine the IC_{50} and IC_{90} values.

RESULTS AND DISCUSSIONS

***In-vitro* evaluation of the antitumoral activity**

Screening of MeOH/CH_2Cl_2 (1:1) extracts of *Negombata corticata*, *Spheciospongia vagabunda*, *Sarcophyton glaucum*, *Sarcophyton auritum* and *Negombata magnifica* revealed promising anticancer activities against MCF-7 and $HepG_2$ cell lines. The inhibitory properties of these extracts were compared with standard doxorubicin. SAA-8 gave the highest activity against $HEPG_2$ followed by SAA-4$_3$with IC_{50} (µg/ml) of 16.3 and 18.2, respectively. On the other hand, SAA-33 possessed the highest activity against MCF-7 followed by SAA-14 with IC_{50} of 18.7 and 19.7(µg/ml), respectively as shown in (Table 1). Moreover, and the relation between the surviving fraction and the extract concentration was plotted to get the survival curve of each tumor cell line after being treated with the specified extract (Figs. 1 and 2).

Table (1): Half maximum inhibitory concentration of different extracts of red sea marine organisms against two human cell lines; $HepG_2$ and MCF-7.

Extracts MeOH/CH_2Cl_2(1:1)	Human cell lines	
	$HepG_2$ IC_{50} (µg/ml)	MCF-7 IC_{50} (µg/ml)
SAA-8	16.3± 0.24	24.7±0.67
SAA-14	28.1±0.68	19.7±0.3
SAA-33	19.3±0.18	18.7±0.51
SAA-43	18.2±0.49	21.1±0.72
SAA-64	19.2±0.37	25.8±0.9

IC_{50} more than 50 µg/mL indicates weak activity. Each data point represents the mean ±SD of the four independent experiments (significant differences at $p < 0.05$).

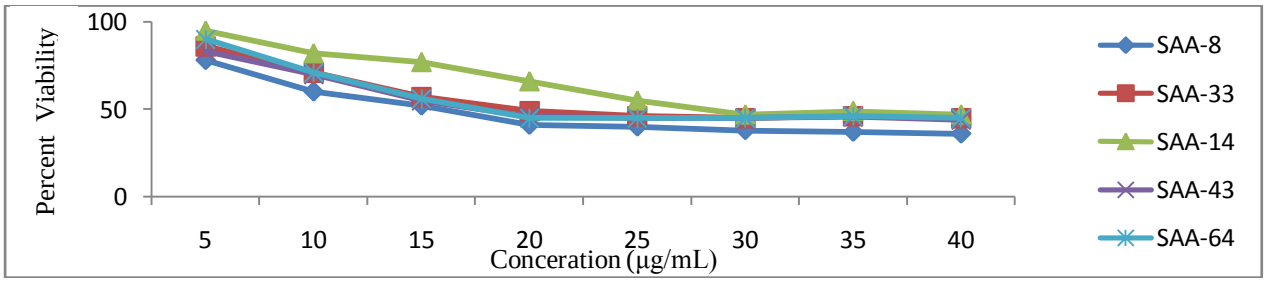

Figure (1): Cytotoxicity: The cytotoxicity of different concentrations of the tested extracts: SAA-8 (◊), SAA-33 (□), SAA-14 (Δ), SAA-43 (x) and SAA-64 (*) against human hepatocellular carcinoma cells ($HepG_2$). The data are represented the mean of four independent experiments.

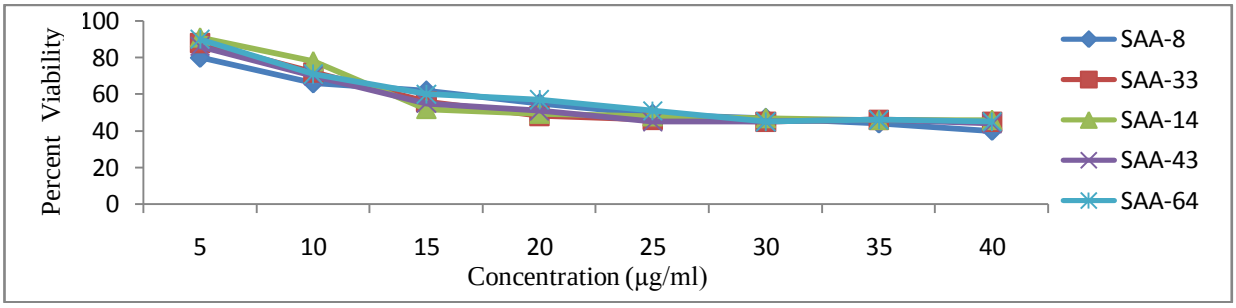

Figure (2): Cytotoxicity: The cytotoxicity of different concentrations of the tested extracts: SAA-8 (◊), SAA-33 (□), SAA-14 (Δ), SAA-43 (x) and SAA-64 (•) against human breast cancer cell line (MCF-7). The data are represented as the mean of four independent experiments.

Table (2): Antimicrobial activity of the tested extracts of marine organisms.

Sample	% Growth Inhibition at Concentration (40ug/mL) *					
	Aspergillus fumigatus	***Candida glabrata***	***Candida krusei***	***Cryptococcus Neoformans***	***Candida albicans***	**Methicillin-resistant *Staphylococcus aureus***
SAA-8	100	100	100	97	12	44
SAA-14	0	2	19	6	0	0
SAA-33	0	18	24	34	11	13
SAA-43	0	96	100	3	88	9
SAA-64	48	11	14	23	6	36

* Samples showing % growth inhibition <50 are considered inactive at 40 ug/mL.

Antimicrobial assay

Antifungal activity

The *in vitro* antifungal activity of the investigated marine organisms extracts of SAA -8 and SAA-43 indicated strong activities against *C. glabrata* and *C. krusei*. In Addition, SAA -8 had potential antifungal activity against *C. neoformans* and *A. fumigatus* while SAA-43 possessed strong activity towards *C. Albicans* (Table2).

Antibacterial activity

The *in vitro* antibacterial assay of the extracts showed almost complete inactivity to a panel of bacteria except SAA-8 which had promising anti-tuberculosis activity as shown in (Tables 2 and 3).

Table (3): Antituberculosis activity of the tested marine organisms extracts.

Sample	% Inhibition at (128ug/mL)
SAA-8	82.4
SAA-14	33.3
SAA-33	50
SAA-43	21
SAA-64	35

Antimalarial activity

The *in vitro* antimalarial activity of the extracts under investigation against *Plasmodium falciparum* [chloro-quine-sensitive (D6)] was established based on the determination of plasmodial LDH activity.

Only SAA-8 showed antimalarial activity with 74% inhibition, (IC50 = 12 µg/mL) (Tables 4 and 5).

Table (4): Antimalarial Screening Assays of the tested marine organisms extracts (primary).

Sample	***Plasmodium falciparum* (D6 Clone)**
	% Inhibition*
SAA-8	74
SAA-14	16
SAA-33	4
SAA-43	17
SAA-64	13

* Samples showing % inhibition <50 are considered inactive at 15.9 ug/mL. *P. falciparum* (D6 clone)*:* chloroquine - and quinine-susceptible derived fromCDC Sierra Leone.

Antileishmanial activity

The three major forms of human leishmaniasis are normally differentiated as cutaneous, mucocutaneous and visceral, with the latter being potentially lethal. Leishmaniasis is caused by various species of the protozoan parasite *Leishmania*, which is transmitted by female sand flies (Singh *et al.*, 2006; Maltezou, 2008).

The extracts were tested against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as controls table (6) (Jain *et al.*, 2005; Bharate *et al.*, 2008). This test indicated that both SAA-8 and SAA - 33 have antileishmanial activity. SAA -8 was the most active with IC_{50} of 74ug/mL. Results are shown in (Table 6).

Table (5): Antimalarial screening assays of the tested marine organisms extracts(Secondary)**.**

Sample	***Plasmodium falciparum* (D6 Clone)**		***Plasmodium falciparum* (W2 Clone)**		**Cytotoxicity (Vero)**
	IC_{50} (µg /mL)	S.I.	IC_{50} (µg /mL)	S.I.	TC_{50} (µg /mL)
SAA-8	12	0.6	24	0.3	7.3

P. falciparum (D6 clone)*:*chloroquine- and quinine-susceptible derived from CDC Sierra Leone., *P. Falciparum* (W2 clone)*:*mefloquine-susceptible derived from CDC Indochina III., S.I.: Selectivity index., IC50:Concentration causing 50% growth Inhibition.

Table(6): Antileishmanial Screening Assays of the tested marine organisms extracts.

Sample	IC_{50} (ug/mL)	IC_{90} (ug/mL)
SAA-8	74	>100
SAA-14	NA	NA
SAA-33	100	>100
SAA-43	NA	NA
SAA-64	NA	NA

IC_{50}: concentration causing 50% growth Inhibition. IC_{90}: concentration causing 90% growth Inhibition, NA: not active.

CONCLUSION

From the tested bioassays for the anticancer activity it can be concluded that the sponges *Spheciospongia vagabunda*, *Negombata magnifica*, *Negombata corticata* and soft corals *Sarcophyton auritum* and *Sarcophyton glaucum* possess moderate anticancer properties against breast cancer cell line (MCF-7) and $HepG_2$. The *in vitro* antifungal activity of the investigated marine organism extracts indicated that *Negombata corticata* and *Sarcophyton auritum* have promising antifungal activity against different strains of fungi. *Negombata corticata* has good anti tuberculosis, antimalarial and antileishmanial potentials. Further chemical analysis on these marine organisms would definitely reveal the important chemical and structural components responsible for these biological activities.

ACKNOWLEDGMENTS

The authors are grateful to R.W.M. van Soest, Faculty of Science, Zoological museum Amsterdam for taxonomic identification of the sponge samples. Thanks are also due to the Egyptian Environmental Affairs Agency (EEAA) for facilitating sample collection along the coasts of the Red Sea.

REFERENCES

AHMED, S. A., S.I. KHALIFA, AND M.T. HAMANN. 2008. Antiepileptic ceramides from the Red Sea sponge *Negombata corticata*. Journal of Natural Product **71**(4): 513-515.

AMADOR, M. J., J. JIMENO, L. PAZ-ARES, H. CORTES-FUNES, AND M. HIDALGO. 2003. Progress in the Development and Acquisition of Anticancer agents from Marine sources. Annals of Oncology **14**: 1607-1615.

BHAKUNI, D.S., AND D. S. RAWAT. 2005. Bioactive Marine Natural Products. Springer-Verlag, New york.

BHARATE, S.B., S.I. KHAN, B.L. TEKWANI, M. - JACOB, I.A. KHAN, AND I.P. SINGH. 2008. S-Euglobals: Biomimetic synthesis, Antileishmanial, Antimalarial, and Antimicrobial Activities. Bioorganic and Medicinal Chemistry **16**: 1328-1336.

CHIN, Y. W., M.J. BALUNAS, H.B. CHAI, AND A.D. KINGHORN. 2006. Drug Discovery from Natural Resources. American Association of Pharmaceutical Scientists journal (AAPS J.) **8**(2): 239-253.

FAULKNER, D.J. 2002. Marine Natural Products. Natural Products Reports **19**(1): 1-48.

FRANZBLAU, S. G., R. S. WITZIG, J. C. MCLAUGHLIN, P. TORRES, G. MADICO, A. HERNANDEZ, M. T. DEGNAN, M. B. COOK, V. K. QUENZER, R. M. FERGUSON, AND R.H. GILMAN. 1998. Rapid, Low-technology MIC determination with Clinical *Mycobacterium tuberculosis* isolates by using the Microplate Alamar Blue assay. Clinical Microbiology **36**: 362-366.

HALE, K. J., AND S. MANVIAZAR. 2010. New approaches to the Total Synthesis of Bryostatin Antitumor Macrolides. Asian Journal of Chemistry **5** (4): 704–754.

HAMID, R., Y. ROTSHTEYN, L. RABADI, R. PARIKH, AND P. BULLOCK. 2004. Comparison of Alamar Blue and MTT assays for High through-put screening. Toxicology *in Vitro* **18**: 703-710.

JAIN, M., S.I. KHAN, B.L. TEKWANI, M.R. JACOB, S. SINGH, P.P. SINGH, AND R. JAIN. 2005. Synthesis, Antimalarial, Antileishmanial, and Antimicrobial activities of some 8-Quinolinamine analogues. Bioorganic Medicinal Chemistry **13:** 4458-4466.

JIRGE, S., AND C. YOGESH. 2010. Marine: The Ultimate Source Of Bioactives And Drug Metabolites. International Journal of Research in Ayurveda and Pharmacy **1** (1): 55-62.

MA, G., S.I. KHAN, M.R. JACOB, B.L. TEKWANI, Z. LI, D.S. PASCO, L.A. WALKER, AND I.A. KHAN.

2004. Antimicrobial and Antileishmanial Activities of Hypocrellins A and B. Antimicrobial Agents and Chemotherapy **48**: 4450-4452.

MACKAY, H.J., AND C.J. TWELVES. 2007. Targeting the protein kinase C family: are we there yet?. Nature Reviews Cancer **7** (7): 554–562.

MAKLER, M.T., AND D.J. HINRICHS. 1993. Measurement of the Lactate Dehydrogenase Activity of *Plasmodium falciparum* as an Assessment of Parasitemia. American Journal of Tropical Medicine and Hygiene **48**: 205-210.

MALTEZOU, H.C. 2008. Visceral Leishmaniasis: Advances in Treatment. Recent Patent on Anti-infective Drug Discovery **3**: 192-198.

MIKUS, J., AND D. STEVERDING. 2000. A Simple Colorimetric Method to screen Drug Cytotoxicity against *Leishmania* using the dye Alamar Blue®. Parasitology International **48**: 265-269.

MARTÍN, M. J., L. COLLO, R. FERNANDEZ, F. REYES, A. RODRIGUEZ, C. MURCIA, M. GARRANZO, C. MATEO, F. SANCHEZ-SANCHO, S. BUENO, C. DE EGUILIOR, A. FRANCESCH, S. MUNT, AND C. CUEVAS. 2013. Isolation and First Total Synthesis of PM050489 and PM060184, Two New Marine Anticancer Compounds. Journal of American Chemical Society **135** (27): 10164–10171.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS (NCCLS) (WAYNE, PA.). 2002. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard – Second Edition Document M27-A2, **22**: 1-51.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS (NCCLS). 1998. Reference method for Broth Dilution Antifungal Susceptibility testing of Conidium Forming Filamentous fungi. Proposed Standard Document M38-P, **18**:1-39.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS (NCCLS). 2000. Susceptibility testing of Mycobacteria, Nocardia, and other Aerobic Actinomycetes. Tentative Standard Document M24-T2, **20**: 1-81.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS (NCCLS). 2000. Methods for Dilution Antimicrobial Susceptibility tests for Bacteria that grow aerobically. Approved Standard – Fifth Edition Document M7-A5, **20**: 1-58.

NKHOMA, S., M. MOLYNEUX, AND S. WARD. 2007. In vitro Antimalarial Susceptibility Profile and PRCRT/PFMDR-1 genotypes of *Plasmodium falciparum* field isolates from Malawi. American Journal of Tropical Medicine and Hygiene **76**: 1107-1112.

OLANO, C., C. MENDEZ, AND J.A. SALAS. 2009. Antitumor compounds from marine actinomycetes. Marine Drugs **7**(2): 210–248.

OLANO, C., C. MENDEZ, AND J.A. SALAS. 2009. Antitumor compounds from marine actinomycetes. Marine Drugs **7**(2): 210–248.

PETIT, K., AND J.F. BIARD. 2013. Anticancer Agents. Medicinal Chemistry **13**(4):603-631.

RINEHART, K.L., J.B. GLOER, AND J.C. COOK. 1981. Structures of the Didemnins, Antiviral and Cytotoxic Depsipeptides from a Caribbean Tunicate. Journal of American Chemical Society **103**: 1857-1859.

RINEHART, K.L., T.G. MMHOLT, N.L. FREGEAU, P.A. KEIFER, G.B. WILSON, T.J. PERUN, R. SAKAI, A.G. THOMPSON, J.G. STROH, L.S. SHIELD, D.S. SEIGLER, L.H. LI, D.G. MARTIN, C.J. GRIMMELI-KHUIJZEN, AND G. GÄDE. 1990. Bioactive Compounds from Aquatic and Terrestrial Sources. Journal Of natural Products **53**: 771-792.

RINEHART, K.L., V. KISHORE, K.C. BIBLE, R. SAKAI, D.W. SULLINS, AND K.M. LI. 1988. Didemnins and Tunichlorin: Novel Natural Products from the Marine Tunicate *Tridemnum solidum*. Journal Of natural Products **51**: 1-21.

SCHAUFELBERGER, D.E., M.P. KOLECK, J.A. BEUTLER, A.M. VATAKIS, A.B. ALVARADO, P. ANDREWS, L.V. MARZO, G.M. MUSCHIK, J. ROACH, J.T. ROSS, W.B. LEBHERZ, M.P. REEVES, R.M. EBERWEIN, L.L. ROGERS, R.P. TESTERMAN, K.M. SNADER, AND S.J. FORENZA. 1991. The Large-Scale Isolation of Bryostatin 1 from *Bugulaneritina* Following Current Good Manufacturing Practices. Natural Product **54**: 1265-1270.

SIMMON, T.L., E. ADRIANASOLO, K. MCPHILL, P. FLATT, AND W.H. GERWICK. 2005. Marine Natural Product as Anticancer Drugs. Molecular Cancer Theraputics **4**(2): 333-342.

SKEHAN, P., R. STORENG, D. SCUDIERO, A. MONKS, J. MCMAHON, D. VISTICA, J.T. WARREN, H. BOKESCH, S. KENNEY, AND M.R. BOYD. 1990. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. Journal of National Cancer Institute **82**(13): 1107-1112.

Uemura, D., K. TAKAHASHI, T. YAMAMOTO, C. KATAYAMA, J. TANAKA, Y. OKUMURA, AND Y. HIRATA. 1985. Norhalichondrin A: an Antitumor Polyether Macrolide from a Marine sponge. Journal of American Chemical Society **107**: 4796-4798.

WANG, H., H. HUANG, J. SU, C. HUANG, C. HSU, Y. KUO, AND J. SHEU. 2011. Sarcocrassocolides M–O, Bioactive Cembranoids from the Dongsha Atoll Soft Coral *Sarcophyton crassocaule*. Bioorganic & Medicinal Chemistry **21**: 7201–7204.

WANG, Z., H. TANG, P. WANG, W. GONG, M. XUE, H. ZHANG, T. LIU, B. LIU, Y. YI, AND W. ZHANG. 2013. Bioactive Polyoxygenated Steroids from the South China Sea Soft Coral *Sarcophyton* Sp. Marine Drugs **11**: 775-787.