CHEMICAL AND BIOLOGICAL INVESTIGATION ON SELECTED RED SEA SPONGES

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دراسة كيميائية و بيولوجية على بعض اسفنجيات البحر الأحمر المختارة

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لاستكمال متطلبات الحصول على

درجة الماجيستير فى العلوم الصيدلية (عقاقير)

من

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GENERAL SUMMARY

Chemical and Biological Investigation on Selected Red Sea Sponges

Marine natural products had received much concern in the past few decades for a number of reasons mainly the great unexplored biodiversity in the world's water , together with the relatively large number of novel compounds with broad biological activities isolated from just a tiny subset of the marine environment , many of these compounds are now in different stages of clinical trials or already approved as drug products in the market.

Red Sea sponges provides a potential source for new lead compounds with interesting biological activities . A considerable number of bioactive unique chemical compounds from different chemical classes had been isolated from Red Sea sponges .

In this thesis two Red Sea sponges belonging to order Poesilosclerida had been selected namely , *Clathria gibbsoa* (Microcionidae) and *Echinodictyum flabelliforme* (Raspailiidae) as no work could be traced for these two species in literature .

The main goal of this thesis is chemical investigation of the biologically active fractions of both sponges in an effort to fractionate and separate hopefully new compounds of interesting chemical structure and effective biological activity.

This study is divided into three chapters:

The first chapter includes: Biological investigation of different extracts of the Red Sea sponges *Clathria gibbsoa* & *Echinodictyum flabelliforme viz* cytotoxic activity.

The second chapter includes: Chromatographic study on the Red Sea sponge *Echinodictyum flabelliforme* .

The third chapter includes: Chromatographic study on the Red Sea sponge *Clathria gibbsoa.*

Chapter 1

Biological investigation of different extracts of the Red Sea sponges *Clathria gibbsoa* & *Echinodictyum flabelliforme* .

The *n*-hexane fraction and the CH_2Cl_2 fractions of both Red Sea sponges *C.gibbsoa* & *E.flabelliforme* were subjected to cytotoxic study at conc. 0-50 µg/ml on human breast carcinoma cell line MCF-7 . It was obvious that the *n*-hexane fraction of both sponges exhibits potent cytotoxic activity .

Two compounds (7- Oxocholesterol & 7-Hydroxycholesterol) isolated from the *n*-hexane fraction of the Red Sea sponge *E. flabelliforme* were also tested for their cytotoxic activity on human breast carcinoma cell lines MCF-7. The two compounds showed moderate cytotoxic activity at IC_{50} 20.3 and 21.5 µg/ml respectively.

By comparing the cytotoxic activity of the *n*-hexane fraction of E. flabelliforme with that of the isolated compounds , it was found that the *n*-hexane fraction exhibited higher cytotoxicty than the isolated compounds, This was attributed to the synrgestic activity of other components present in the *n*-hexane fraction.

Chapter 2

Chromatographic study on the Red Sea sponge *Echinodictyum flabelliforme* .

The sponge material was extracted with MeOH , the methanolic extract (22 gm) was suspended in 90% MeOH and extracted with *n*-hexane , then water is added to the extract to reach 60% and extracted with CH_2Cl_2 .

The *n*-hexane fraction was fractionated over silica gel column to yield 10 fractions . The main fractions and their isolation were displayed in the scheme described in p.(120).

From the 10 fractions , fraction II , IV, VI & VII were fully investigated . These fractions were manipulated through column chromatography , crystallization & preparative TLC , leading to the isolation of isolation of three compounds. Isolated compounds were identified by 1D & 2D ^{1}H NMR & ^{13}C NMR techniques after comparison with reported data.

1: Cholesterol.

2: 7-Oxocholesterol.

3: Epimeric mixture of 7-α & 7-β Hydroxycholesterol.

Chapter 3

Chromatographic study on the Red Sea sponge *Clathria gibbsoa* .

The sponge material was extracted with CH_2Cl_2: MeOH , the extract (35 gm) was suspended in 90% MeOH and extracted with *n*-hexane , then water is added to the extract to reach 60% and extracted with CH_2Cl_2 .

The *n*-hexane fraction was fractionated over silica gel column to yield 8 fractions . The main fractions and their isolation were displayed in the scheme described in p.(121).

From the 8 fractions , fraction II , III & IV were fully investigated . These fractions were manipulated through column chromatography , crystallization, leading to the isolation of isolation of three compounds including cholesterol . Isolated compounds were identified by 1D & 2D ^{1}H NMR & ^{13}C NMR techniques after comparison with reported data.

4: 24-Isopropylcholesterol-3-O-fatty acid ester.

5: 2-O-monoacylglycerol fatty acid ester.

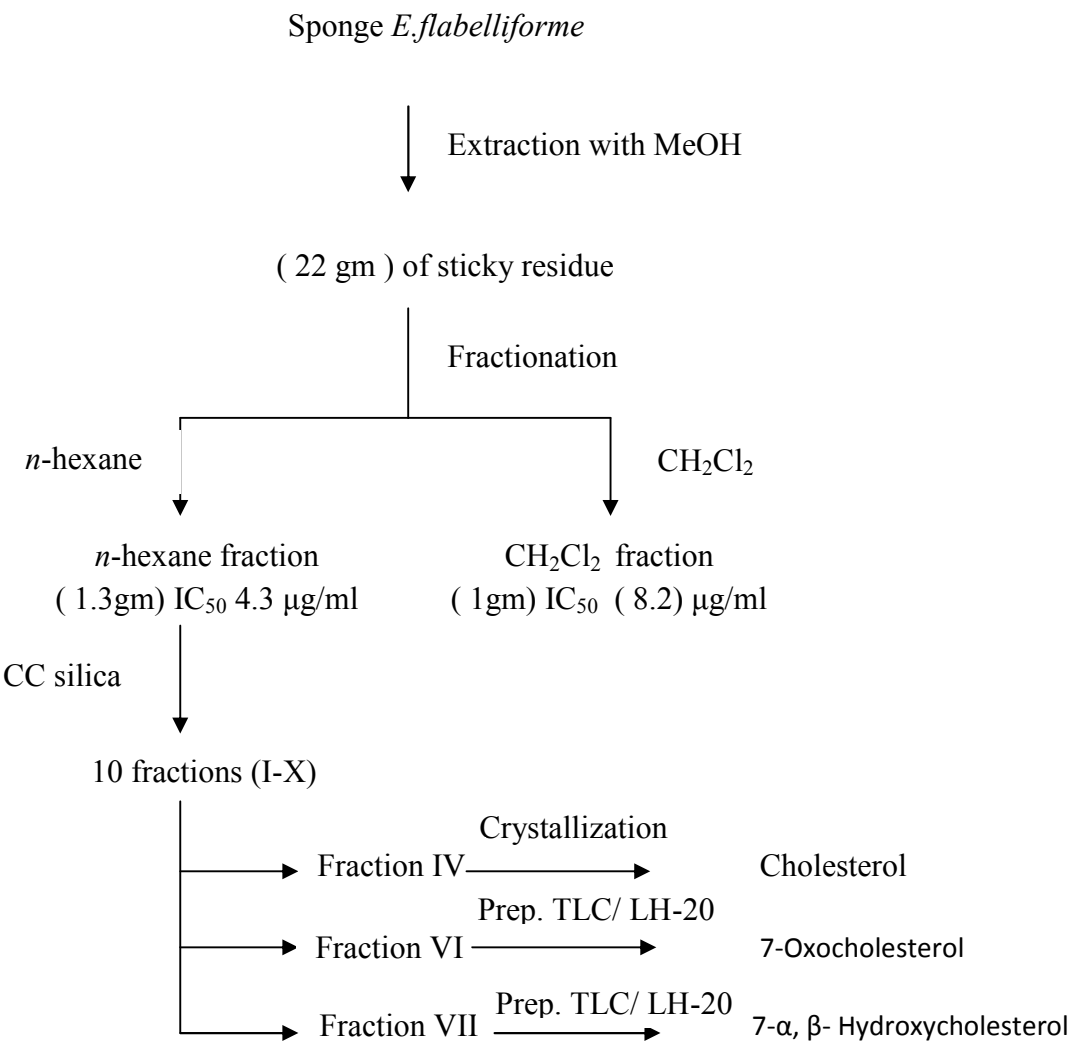


Fig. 39 General scheme for extraction and fractionation of the Red Sea sponge *E.flabelliforme*

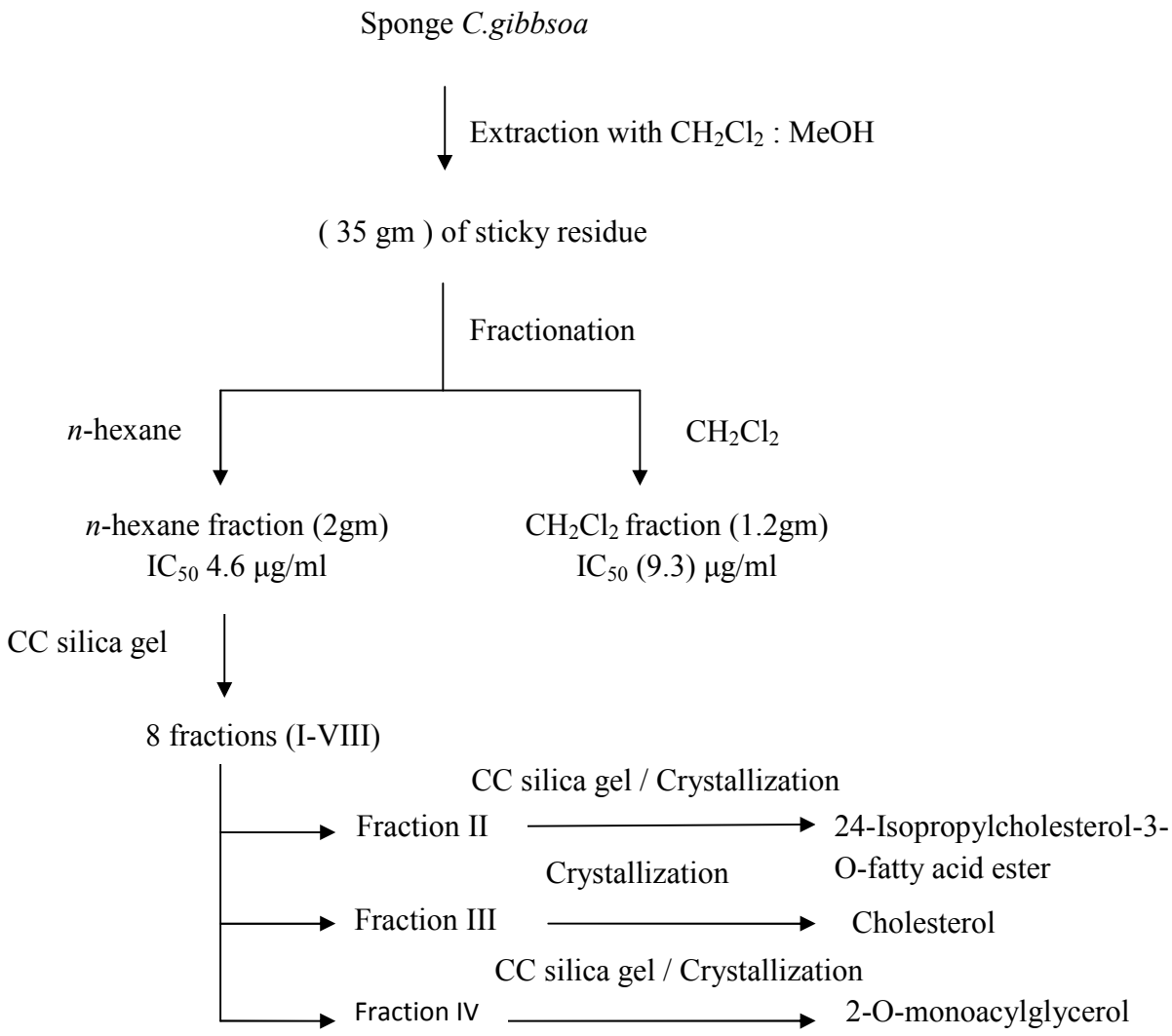


Fig. 40 General scheme for extraction and fractionation of the Red Sea sponge *C.gibbsoa*

INTRODUCTION

Natural products have been the most productive source of lead chemical compounds in drug discovery. Natural products, whether from terrestrial microbes and fungi, vertebrate animals, plants, or from marine fauna and flora have been a major resource for preventing and treating diseases; moreover many natural products are used as biochemical tools for probing fundamental cellular processes.(*Newmann et al. ,2003*).The contribution of natural products or their derivatives was estimated to be 40% of all medicines, about 80% of drugs in clinical use for bacterial infections and 60% of all anti-cancer agents. Natural products are developed by microorganisms, plants , marine organisms , amphibians , animals for different purposes ; for example: for defense against predators , as building blocks , as anti-bacterials , for communication between and within species , pigments or cellular signaling and gene expression .(*John J., 2010).*

Life diversity on terrestrial environment is extraordinary; however , the greatest biodiversity is in the world's oceans, with 34 of the 36 phyla of life represented. The oceans cover more than 70% of the earth's surface and contains more than 300000 described species of plants and animals. (*Pomponi SA, 1999*).

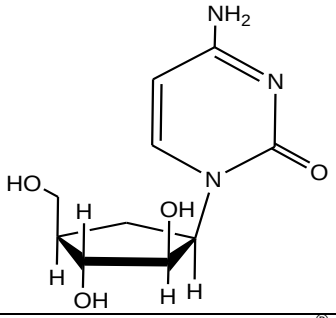
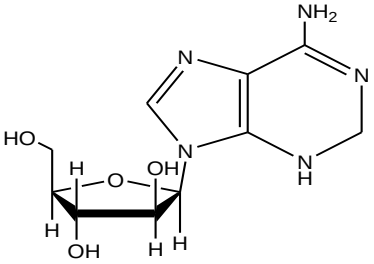
Among all the marine organisms marine invertebrates (e.g. sponges, coral reefs ,mollusks and bryozans) are the primary source of bioactive compounds.
(*Radjasa et al., 2011).*

There is much concern in the last three decades for isolation , structure elucidation as well as estimation of biological activities for the natural products of the marine environment . Up to 2005 approximately 16,000 marine natural products have been isolated from marine organisms and reported in approximately 6,800 publications. In addition to these publications there are approximately another 9,000 publications which cover syntheses, reviews, biological activity studies, ecological studies etc. with hundreds of new compounds still being discovered every year. (*Bhakuni and Rawat ,2005).*

This remarkable yield of novel compounds from marine sources, with a wide range of bioactivities, from a tiny subset of the enormous diversity of life in the marine environment, indicates that an increased effort for the discovery of drugs from marine macro organisms and microorganisms is likely to provide many tens or hundreds of thousands of new leads where research by a small number of academic scientists, limited efforts by major pharmaceutical companies and the work of a few small biotechnology companies (notably, Pharmamar, a Spanish biopharmaceutical company and Nereus Pharmaceuticals in San Diego, USA)

has resulted in the discovery of many thousands of novel compounds, with 961 new compounds reported in 2007 alone. *.(Hill and Fenical , 2010).*
A recent review provided an updated list of selected marine natural products or derivatives thereof in different stages of clinical trials or already approved for use.(Tables 1-4)*(Mayer, et al ,2010)*.

Table 1 . Marine derived products currently in the market.

compound	organism	class	Company	Medicinal value
Cytarabine, Ara –C (Cytosar® , Depocyt®) 	Sponge *Tethya crypta*	Nucleoside	Bedford lab.,U.S.A. , Enzon pharmaceuticals ,USA	*****Anti-metabolite (inhibits DNA polymerase) used in leukemia. *The liposomal preparation (Depocyt®) used in lymphomatous meningitis
Vidarabine ,Ara-A , Vira- A® 	Sponge *Tethya crypta*	Nucleoside	King pharmaceuticals U.S.A	*Anti-metabolite (inhibits viral DNA polymerase and DNA synthesis of Herpes , Vaccinia and Zoster viruses) used in keratoconjunctivitis , epithelial keratitis
Ziconotide , Prilat®	Marine snail Conus magus	Peptide	Elan corporation Ireland	*Analgesic , inhibits presynaptic N-type calcium channels in afferent nerves of spinal cord thus inhibiting the release of excitatory neurotransmitters that increase pain sensation, specially used in AIDs and cancer patients

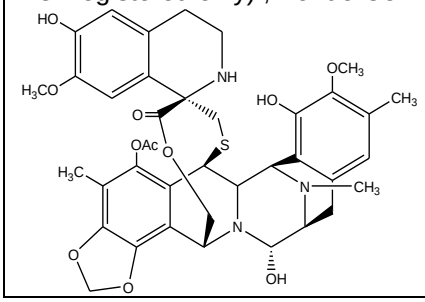
Trabectedin (ET-743) EU- registered only) , Yondelis® 	Marine tunicate *Ecteinascidia turbinata*	Alkaloid	Pharmamar Madrid, Spain	*Anti-cancer (binds with DNA minor grooves and interacts with nucleotide excision repair system ERS) *Used in soft tissue sarcoma and ovarian cancer

Table 2 . Marine derived products currently in clinical trials phase III .

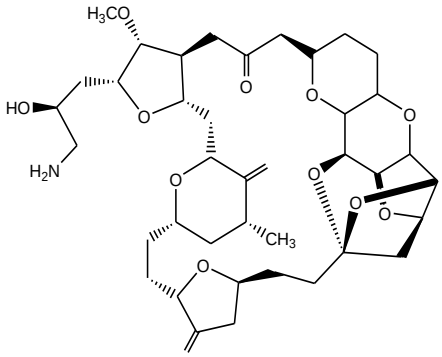
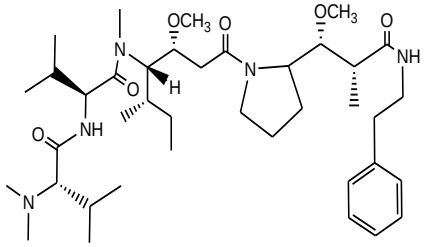
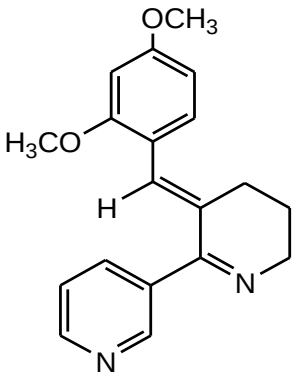
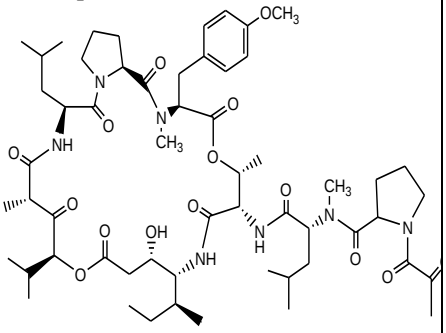
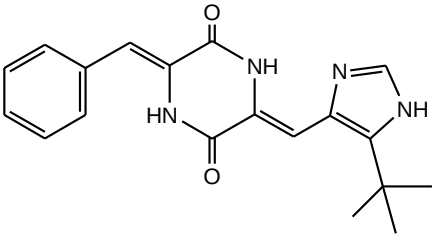
compound	organism	class	Company	Medicinal value
Eribulin mesylate (E7389) 	Sponge *Halichondria sp.*	Macrolide	Eisai Inc, Tokyo , Japan	*Anti-cancer agent (inhibits microtubule dynamics) , most promising response is against breast cancer.
Soblidotin (TZT 1027) 	Bacterium	Peptide	Aska pharmaceuticals , Tokyo , Japan.	*Anti-cancer agent (vascular disrupting agent VDA that collapsing vasculature inside tumor cell beside its tubulin inhibiting activity.

Table 3 . Marine derived products currently in clinical trials phase II.

compound	organism	class	Company	Medicinal value
DMXBA (GTS-21) 	Marine worms (Phylum Nemertea)	Alkaloid	CoMentis Inc., South San Francisco, U.S.A	*Improves cognition and displays neuroprotective effects through stimulation of α7 nicotinic acetylcholine receptors in CNS and macrophages . *promising effect in schizophrenic patients.
Plitidepsin , Aplidin® 	Tunicate *Aplidium albicans*	Depsipeptide	Pharmamar	*Anti-cancer agent (potent inducer of apoptosis through depletion of GSH and activation of Rac-1) *most promising response is against multiple myloma and T cell lymphoma.
Plinabulin (NPI-2358) 	Marine fungus *Aspergillus sp.*cultured from the marine algae *Halimeda lacrimosa*	Alkaloid	Nereus pharmaceuticals San Diego, ,U.S.A.	*Anti –cancer agent (potent inhibitor of tubulin polymerization thus disrupting vasculature of tumor cells VDA) *Most promising response is against non-small cell lung cancer.
Elisidepsin , Irvalec®	Marine mollusk	Cyclic peptide	Pharmamar ,	*Anti-cancer agent with no established mechanism of action but most probably work through oncolytic rather than apoptotic activity