Various *in vitro* bioactivities of Secondary Metabolites isolated from sponge collected from Red Sea; Egypt

Asmaa Nabil - Adam, Mohamed A. Shreadah, Nehad M Abd El Moneam, Samy A El-assar

[1]1Marine Biotechnology and Natural products Lab (MBNP), National Institute of Oceanography & Fisheries (NIOF), Alexandria, Egypt

[2]Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

[3]3Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

Abstract

The present study revealed the presence of bioactive constituents in *Hyrtios aff. erectus. sponge* extract (HES) collected from the Red Sea by using skin and scuba diving technique. The Cytotoxicity was tested against hepatocellular carcinoma cell lines as prescreening test. The HES extract had high contents of total phenolic compounds (0.061mg/g), flavonoids (0.2839 mg/g) and carotenoids (1.976 mg/g). Moreover, the HES extract showed high antioxidant capacity with 93.0% and 99% at 1mg using DPPH and ABTS; respectively. Cytotoxic activity against cancerous cell line cleared out that the HES extract could inhibit cell growth effectively with IC50 = 47.5 µg/ml. Furthermore, anticancer activity using tyrosine kinase (PTK) and Sphingosine kinase 1(SHKI) inhibitor screening assays resulted in 71.66 and 85.21% inanition activity; respectively. The anti-inflammatory assays showed that the inhabitation activities against COX_1, COX_2, IL-6 and TNF-α were 71.82, 81.13, 80.89 and 59.74 %; respectively. At the same time, the anti-Alzheimer results showed high activity at 1mg with 83.51%. Additionally, the antiviral activities using reverse transcriptase inanition assay was 91.70%. The marine sponge isolated from the Red Sea showed tremendous activity against many diseases and it is considered as an excellent source for bioactive pharmaceutical compounds.

Keywords: Red Sea, Sponge Secondary Metabolites Activities, Cytotoxic, Anti-oxidant, Anti-Alzheimer, Anti-Cancer, Anti-inflammatory, Anti-Viral

Introduction

Marine habitat is representing a broad different of organisms having variety of biochemical, physiological and ability to adapt their environment. Marine organisms such as sponges, tunicates, fishes, soft corals, nudibranchs, sea hares, Molluscs, echinoderms, bryozoans, prawns, shells, sea slugs, and marine microorganisms are sources of bioactive compounds (Hegazy *et al.,* 2015a,b). Marine sponges belonging to the phylum Porifera (Metazoa), evolutionarily the oldest animals are the single best source of marine natural products. Very recently, marine sponges of the Red Sea have been recognized as a rich source of bioactive secondary metabolites (Aboul-Ela *et al.,* 2012; Abdel Moniem *et al.,* 2017 a, b, c, d; Shreadah *et al.,* 2017). A great number of biologically active compounds as potential anti-tumor, anti-cancer, anti-microtubule, anti-

proliferative, cytotoxic, photo protective, as well as antibiotic and antifouling properties have been isolated.

The main objective of the present study is to investigate the tremendous activities of sponge secondary metabolites collected from the Red Sea as anti-oxidant, cytotoxic, anti-alzheimer, anti-Cancer, anti-inflammatory, and anti-viral.

.

Materials and Methods

Area of study: The Red Sea (Fig. 1a) comprises a wide range of tropical marine habitats, many of which are internationally recognized for their conservation, scientific, economic or recreational value (Aboul-Ela *et al.,* 2012; Hegazy *et al.,* 2015a,b; Abdel Moniem *et al.,* 2017a,b,c,d; Shreadah *et al.,* 2017). It attracts many human activities which in turn impacts its environment (Shreadah *et al.,* 2004, 2008a,b,c, 2011; Okbah *et al.,* 2005; Abdel Ghani *et al.,* 2010; Massoud *et al.,* 2010, 2012, Said *et al.,* 2010), and are likely to affect biological life and disturb the Red Sea's natural ecosystems (Fahmy et al., 2005, 2016; Abdel Halim et al., 2007, 2016; Aboul Khair *et al.,* 2007, 2008, 2016; Gurguess *et al.,* 2009).

Sampling, identification and Prescreening bioassays of the *Hyrtios aff. erectus* sponge: *Hyrtios aff. erectus* sponge sample was collected from Hurghada site at the Egyptian Red Sea coastline during spring, 2014 (Fig. 1b). Samples were collected using skin and Scuba diving techniques, processed, washed with freshwater and were transferred directly to the laboratory in sterile polyethylene bags under reduced temperature (zero °C). Identification of sponge species was kindly done by Dr. Nicole Voogd, at Naturalis Biodiversity Center, Department of Marine Zoology, RA Leiden, The Netherlands. The voucher specimen is incorporated in the collections of the Zoological Museum of the University of Amsterdam under registration number RMNH POR.8633 .

Figure (1). Location of sampling stations at the Red Sea (A) and sponge sample *Hyrtios aff. erectus* RMNH POR.8633 sample (B).

Chemicals and solvents: Potassium ferricyanide, ferric chloride, NaOH, chloroform, glacial acetic acid, ferric chloride solution, H_2SO_4, folin-Ciocalteau, vanillin, methanol, HCl , *n*-hexane, H_2O_2, HNO_3.Se standard, Mn standard, β-carotene, catechin, (+)-quercetin, sodium nitrite, aluminum chloride and gallic acid were purchased from Sigma Aldrich .

Instruments:

Atomic absorption Spectrophotometry (AAS and GFASHIMADZU), GC-MS (Thermo, USA) were applied.

Preliminary bioactive screening of HES **extract:** The ethyl acetate extract of *Hyrtios aff. erectus* sponge were subjected to different chemical tests for the detection of different tannins,

phlobatannins, saponins, alkaloids, flavonoids, quinines, coumarin, terpenoids, and cardiac glycosides phytoconstituents (Thaipong *et al.*,2006).

Quantitative chemotaxonomy profiling:

Determination of total phenolic contents in HES extract: Total phenolic compounds in *Hyrtios aff. erectus* extract was determined by the method of Taga *et al.,* (1984).

Determination of total flavonoids content in HES extract: Total flavonoid content was determined by a colorimetric method of Zhishen *et al.,* (1999).

Determination of total tannins in HES **extract:** Tannins (proanthocyanidins) was determined according to the method of Sun *et al.,* (1998).

Determination of total carotenoids in HES **extracts:** Total carotenoid content was measured using the method of Thaipong *et al.,* (2006).

Preparation and extraction for mineral and metal assessment (Fe, Zn, Co, Mn, Cu, and Se) of HES **extract:** A 0.5 g of dried sample of *Hyrtios aff. erectus* **sponge** marine extract was digested using 5 ml concentrated HNO_3, the mixture was heating using hot plate for 1 h and getting semi dried 5 ml of concentrated HNO_3 and 2 ml of H_2O_2 was added and kept on hot plate for 1 h.The semi dried cooled residue, filtered with the help of wattman filter paper and the residue volume was made up to 25 ml with 2 N HNO_3. Analysis was carried out using atomic absorption spectrophotometer (GFASHIMADZU atomic absorption spectrophotometer AA–6800) according to AOAC (1990) for the determination of for Fe, Zn, Co, Mn, Cu and Se.

Elemental analysis of HES **extract:** The total carbon and hydrogen contents of *Hyrtios aff. erectus* **sponge** marine extract were determined using CHNO Elemental Analyzer.

Prescreening bioassays using *in vitro* cytotoxicity using cell lines: Different concentrations of HES **extract** µg/ml from all samples were tested for each cell line. Samples were dissolved in DMSO and further diluted with cell culture medium. The final DMSO concentration used was 1% of total volume of the medium in all treatments, including the control group. Cells with no treatment were examined as negative and positive controls, respectively (Kosanic *et al.* 2015).

Primary screening assay:

2.2/ - Diphenyl –α-picrylhydrazyl (DPPH) radical scavenging effect of HES **extract:** DPPH radical scavenging assay of the total *Hyrtios aff. erectus* extract was performed using modified previously established methodology by Blois (1958) and Amarowicz *et al.,* (2000). Scavenging ratio of DPPH assay calculated as follows**:**

$$\% \text{ scavenging} = [(A \text{ control} - A \text{ sample}] / A \text{ control} \times 100$$

2,2/-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS radical scavenging activity assay of HES **extract:** The $ABTS^+$ free radical decolorization assay developed according to Chakraborty *et al.,* (2010). The percentage scavenging of $ABTS^+$ was calculated by the following formula:

$$\text{Scavenging activity (\%)} = (A_o - A_x) / A_o \times 100$$

A_x and A_o were the absorbance at 734 nm of samples with and without extract, respectively.

Specialized screening assays:

Acetylcholinesterase inhibition (AChEI) assay of HES extract: Inhibition of AChE by *Hyrtios aff. erectus* extract was evaluated as described by Moyo et al. (2010). Percentage inhibition by extracts were calculated using the below equation

$$\text{Inhibition (\%)} = (1 - \text{Sample reaction rate}) / (\text{Blank reaction rate}) \times 100$$

Determination of tyrosine kinase inhibitory activity (PTK) of HES **extract:**

Sample preparation: The dimethylsulfoxide (DMSO) sample solution of the appropriate extract was diluted with H_2O (1:1 v/v) to yield corresponding sample solutions (1 mg/mL). Tyrosine kinase (TK) inhibitory activity was determined using a commercial test kit (Tyrosine kinase Assay Kit, non-radioactively, Takara Cat.# MK410). PTK activity of sample was calculated based on the prepared standard curve. The color intensity is stable for 1 hr. after addition of stop solution at room temperature in a light room.

$$\text{\% inhibition} = \frac{[\text{Initial activity} - \text{inhibitor}]}{\text{Initial activity}}$$

Determination of sphingosine kinase 1 inhibitor screening assay (SHK1) of HES **extract:** Sphingosine kinase inhibitory activity of the crude extract was determined by using the colorimetric sphingosine kinase 1 inhibitor screening assay kit from Cayamen, The plate was covered and the fluorescence was measured using an excitation wavelength between 530-540 nm and an emission wavelength between 580-590 nm.

$$\text{\% inhibition} = \frac{[\text{Initial activity} - \text{inhibitor}]}{\text{Initial activity}}$$

Determination of cyclooxygenase 1 (COX_1) and cyclooxygenase 2 (COX_2) inhibitor screening assay of HES **extract:** Cyclooxygenase inhibitory activity of the crude extract was determined by using the colorimetric COX (Ovine) inhibitor screening assay kit from Cayamen. The absorbance was measured at 590 nm using a plate reader.

$$\% \text{ inhibition} = \frac{[\text{Initial activity} - \text{inhibitor}]}{\text{Initial activity}}$$

Determination of tumor necrosis factor alpha (TNF-α) assay of HES **extract:** Tumor necrosis factor alpha (TNF-α) inhibitory activity of the crude extract, was determined by using the KOMA BIOTECH INC colorimetric kit. The absorbance was measured at 450nm.

Determination of interleukin 6 (IL-6) assays of HES **extract:** An Interleukin 6 (IL-6) inhibitory activity of the crude extract was determined by using the KOMA BIOTECH INC colorimetric kit**.** The absorbance was measured at 450nm.

Determination of reverse transcriptase enzyme inhibitor screening assay of HES extract: Reverse transcriptase (RT) inhibitory activity of the crude extract against a purified recombinant HIV1-RT, was determined by using Roche colorimetric kit. The assay has been done according to Fonteh *et al.*, (2011). HIV-1 protease enzyme and the substrate which is a synthetic peptide that contains a cleavage site Tyr-Pro for HIV protease as well as two covalently modified amino acids for the detection of cleavage. Acetyl pepstatin (AP) was used as a positive control for HIV-1 PR inhibition. The blank treatment consists of an assay buffer with only the substrate, untreated control of enzyme and substrate was also included. The absorbance was measured at 450nm.

Statistical analysis

All results were analyzed by ANOVA test using prism.

Results and Discussion

The secondary metabolites isolated from HES extract showed high contents of sulfur compounds (Figure 2). The mineral results showed high iron and zinc contents (Figure 3), in addition to polyphenol contents which reflected high tannins and flavonoids. The crude extract of the sponge showed also high carotenoids contents (Figure 4). The bioactive profiling and diversity of natural compounds produced by sponge cleared out the presence of certain chemical classes of steroids chromones, quinones, alkaloids, fatty acids, diketopiperazine, steroid, lactone, quinolone derivatives, anthraquinone, trisindole derivatives, phenol derivatives; dihydropyridine benzoic acids derivatives, terpenoids, macrolactam, ethers, carboxylic acid and terpenes which are responsible for antioxidant, anti-inflammatory, anti-microbial, anti-HIV, anticancer or antitumor activity. The quinolone derivatives are responsible for anti HIV activity; fatty acid esters and fatty acids are responsible for anti-inflammatory activity; pentaketides and alkaloids are responsible for neuroprotective activity (Thomas *et al.,* 2010).

Figure (2): The elemental analysis result of HES extract.

Figure (3): The mineral profiling for HES extract.

Figure (4): The Polyphenol and carotenoid profiling for HES extract.

The present study revealed that sponge has cytotoxicity against hepatocellular carcinoma (Table 1). This finding agrees well with other research papers (Abdelmohsen *et al*.,2014, Abdelmoniem *et al.,*2017a,b,c, Shreadah *et al.,*2017). The cytotoxic activity is considered as the first parameter in screening for anticancer agent (Abdelmoniem *et al.,*2017a,b,c,d ; shreadah *et al.,*2017), while, the cytotoxicity assay needs to be followed by other experiments to confirm their potential activity as an anticancer and to know the mechanism. It has been reported that the cell death can be induced through three mechanisms: apoptosis, autophagy and oncosis (Elmore, 2007). In the present study the anticancer activity was performed though two different experimental models using tyrosine kinase and sphingosine kinase 1 as anticancer target (Min *et al*.,2005). Sphingolipid-metabolizing enzymes have important role in controlling the balance of the cellular levels of some important bioactive lipids, for example proliferative compound apoptotic compound ceramide and sphingosine 1-phosphate (S1P) (Ponnusamy *et al.*,2011). The discovery of new chemotherapeutic resistance is an urgent and important challenge in oncology. Increased level of sphingosine kinase 1 (SK1) is considered as a poor prognosis, and overexpression of SK1 it mean resistance to chemotherapeutics. The sphingosine kinase has been involved in the development of different cancers and in chemotherapeutic resistance drug. For that, SK1 represent one of the important targets for anticancer drug therapy. Receptor tyrosine kinases (RTKs) are cell surface transmembrane proteins responsible for intracellular signal transduction. They are expressed in several cell types and, after activation by growth factor binding, trigger a series of intracellular pathways, leading to a wide variety of cell responses such as differentiation, proliferation, migration, invasion, angiogenesis, and survival. The Over-expression of protein kinase members is associated to cancers and tumor cells. Therefore, tyrosine kinases are pivotal target drug therapy for cancer. The flavonoids, which are remarkable nontoxic (Rice-Evans *et al.,* 1996) and could inhibit PTK and SKH activity, appear to have a promise bioactivity as anti-cancer agents and are worth to be subjected for further investigation (Huang *et al.,* 1999). Phenolic compounds, especially flavonoids exhibit their effect as anti-inflammatory and anticancer by inhibiting the PTK through several mechanisms. The first one is as an antioxidant and as being competitive inhibitors for the ATP binding sites on a variety of kinase enzymes (Graziani *et al.,* 1983, Santos, 2013). Agullo *et al*., (1997) reported that the effectiveness of flavonoids depends mainly on the position, number and substitution of the hydroxyl group of the β-ring. The saturation of the C_2- C_3 bond is also important factor that affects flavonoids inhibition of phosphatidylinositol 3-kinase. This can be easily found in marine natural products as more rings and chiral centers are there compared to synthetic compounds and drugs. Moreover, marine natural products provide molecules with large molecular weight than synthetic compounds. While on average natural products contain less nitrogen, sulfur, and halogen atoms, they have higher ratios of these constituents compared also to synthetic compounds and drugs (Lahlou, 2013). Another explanation that the pp60src gene product is a

protein tyrosine kinase (PTK) and the activity of which has been shown to be inhibited by phenolic compounds especially flavonoids (Chahar *et al.,* 2011).In the present study, the total polyphenolic assay, i.e. total phenolic and flavonoids cleared out that the *Hyrtios aff. erectus* sponge extract had high polyphenolic contents (Figure 4). Flavonoids are naturally occurring polyphenolic compounds that are present in a variety of natural products, and are the most abundant antioxidants in the human diet (Kumar and Pandey, 2013, Abdel Moniem *et al.,* 2018, Shreadah *et al.,* 2018). While there has been a major focus on the antioxidant properties, there is an emerging view that flavonoids and their *in vivo* metabolites do not act only as conventional hydrogen-donating antioxidants, but also to modulate cell function through actions at protein kinase and lipid kinase signaling pathways (PTK and SHK). These findings are in agreement with many other previous studies (Kasote, *et al*., 2015). In fact, flavonoids, and their metabolites, have been reported to act at PI 3-kinase, Akt/protein kinase B (Akt/PKB), tyrosine kinases, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) signaling cascades. Inhibitory or stimulatory actions at these pathways are likely to affect cellular function profoundly by altering the phosphorylation state of target molecules and by modulating gene expression (Kasote, *et al*., 2015). An understanding of the mechanism of action of flavonoids, either as antioxidants or as modulators of cell signaling, is a key to the evaluate the potency of biomolecules as an inhibitors of oxidative stress in general and neurodegenerative (Williams *et al.,*2004).The flavonoids compounds are characterized by their inhibitory effect on tyrosine kinase. Accordingly, the *Hyrtios aff. erectus sponge* extract revealed highest inhibition activity in PTK and SHK assays. Starok *et al*., (2015) reported that the activity of $pp60^{src}$ gene product which is a protein tyrosine kinase (PTK) has been shown to be inhibited by flavonoids.

Table (1): Inhibitory activities of HES extract against hepatocellular carcinoma cells.

Figure (5): The anti-HIV and anti-cancer profiling of HES **extract.**

Two major types of HIV have been identified so far, HIV-1 and HIV-2. HIV-1 is the cause of the worldwide epidemic and is most commonly referred to as HIV. The basic biological processes in the HIV-1 life cycle are now well established, and natural compounds targeting specific steps in this life cycle can be found (de Souza Barros *et al*.,2015). HIV reverse transcriptase (RT) inhibitors are including nucleotide RT inhibitors and non-nucleotide RT inhibitors. Most clinical anti-HIV drugs are HIV RT (Zhou *et al*., 2013). In the last decade (2002–2011), 132 anti-HIV natural products were obtained from marine organisms. The anti-HIV bioactive marine natural products, before or after 2002, more than half were derived from marine sponges (Zhou *et al,*. 2013). The present study indicated that the highest activities belong to Hyrtios aff. erectus by 91.7, in agreement with previous studies (Subramaniam *et al.,* 2014). Moreover, Simmons *et al.,* (2005) concluded that sessile marine organism (sponge and seaweeds) contain substances capable of potent biological activity which has been also been demonstrated against different types of cancer and HIV/AIDS. Restoring acetylcholine levels by inhibiting AChE has become the primary treatment for the cognitive deficits of AD (Xie *et al*, 2014). The inhibition of AChE is beneficial not only to the enhancement of cholinergic transmission in the brain, but also to reduce the aggregation of β-amyloid and the formation of the neurotoxic fibrils in AD. In recent decades, researchers have been devoted to developing new AChE inhibitors, especially the so-

called “multifunctional AChE inhibitors” with additional efficacy in vascular dementia treatment (Xie *et al*, 2014). There have been plenty of phytochemicals that found to be effective in inhibiting AChE, which mainly consist of alkaloids, cannabinoids, curcumins, stilbenes, and flavonoids (Howes and Perry, 2011). Among them, flavonoids have attracted more and more interest for the high inhibitory activity and low toxicity (Uriarte-Pueyo and Calvo, 2011). Moreover, their diverse activities such as anti-oxidation, inhibition on advanced glycation products, and cardio-cerebrovascular protection give them extra advantages to be the potential multifunctional therapeutic agents for aging related diseases (Gauthier et al., 2013). The anti-Alzheimer results of the present study (Figure 6) using different extracts have been shown highest inhibition ratio by *Hyrtios aff. erectus* **sponge** produces secondary metabolites for their defense against other microorganisms and these secondary metabolites serve as source of bioactive compounds for use in human therapies as they thrive in harsh oceanic climate (Nong *et al.,* 2014, Abdel Moniem *et al.,* 2018, Shreadah *et al.,* 2018). Many studies confirm the high activity of secondary metabolites isolated from marine Hyrtios aff. erectus sponge including alkaloids, esters, fatty acids, glycosides, ketones, lipids, macrolides, alcohols, peptides, peroxides, polyketides, quinones, steroids, sterols, terpenes, terpenoids (Mehbub, *et al*,2014, Abdel Moniem *et al.,* 2018, Shreadah *et al.,* 2018)

Figure (6): The anti-Alzheimer activity of HES **extract sponge.**

Chronic inflammation is believed to play crucial roles in the pathogenesis of various diseases. Several types of drugs are used to treat inflammatory disorders, but they cause adverse side effects. Natural products offer a great hope for discovery of bioactive lead compounds. These compounds can be developed into drugs for treatment of inflammatory disorders. The biological and chemical diversity of marine habitats constitutes a sizeable reservoir of novel compounds. Some of them, like sesquiterpenoids, diterpenes, steroids, polysaccharides, alkaloids, fatty acids, proteins, and other chemical compounds, isolated from marine organisms are found to exhibit anti-inflammatory activity (Fai Cheung et al., 2016). Recently different compounds from sponge have been shown as potent anti-inflammatory (Abdel Moniem et al., 2018, Shreadah et al., 2018). The natural products from marine sponge with different structures such as diterpenes, alkaloids, sulfated polysaccharides, and polyphenols that inhabit different types of pro-inflammatory biomarkers,IL6, TNF, NF-κB, IL-1β, COX1, and COX2 and that through different pathways:

1. The antioxidant effect by inhibition the production of ROS compounds which stimulate the pro-inflammatory biomarkers (Birben et al.,2012)

2. The direct effect by inhibition of prostaglandin and in sequence inhabit the NF-κB cascaded stimuli also the TNF and IL6 (Wojdasiewicz et al.,2014)

Figure (7): The anti-inflammatory profiling of HES extract **.**

When the human body facing a lot of stress, ROS as a result is produced (Lobo, *et al*.,2010). The deficiency of antioxidant agent lead to different degenerative diseases (Lobo, *et al*.,2010),

for example cardiovascular diseases, Alzheimer's and various inflammatory (Leonard *et al.*,2007). Consumption of antioxidant by using natural sources are suitable to reduce oxidative stress. Many studies showed that flavonoids and phenolic constituent have attributed to the antioxidant activities of natural compounds. Furthermore, many studies cited that mineral for example Cu, Zn, Mg, Mn, and Se showed a significant role as antioxidant (Arulselvan *et al*., 2016). Additionally, dietary antioxidants including tocopherols, carotenoids, and ascorbic acid have been investigated (Pertuzatti *et al.,* 2015). Although many synthetic antioxidant have been showed to remediate oxidative stress. But they lack of availability, high cost, and side effects remain as the main challenge in dealing with oxidative stress make the need to discover new antioxidants agent an urgent need. The sponge extract exhibit a potent anti-oxidant as the marine extract contain variety of bioactive compounds known by their effect as antioxidants such as polyphenol (Tannins, Phenolic compounds, and Flavonoids), carotenoids, different mineral (Cu, Fe, Se, Zn, and Mn)

Figure (8): The total antioxidant capacity using DPPH assay.

Figure (9): The total antioxidant capacity using ABTS assay.

Conclusion

The secondary metabolites isolated from sponge *Hyrtios aff. erectus* **collected** from Red sea Egypt have been confirmed to have multi-medicinal effect as anticancer, antiviral, anti-inflammation and anti-Alzheimer. Other investigation should be done to purify the pure compounds.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abdel Ghani, SA, Shobier, AH, Said, TO, and Shreadah, MA (2010). Organotin compounds in Egyptian Mediterranean sediments. Egyptian J.Aqu. Res., 36(2), 221-229.

Abdel-Halim, AM, Aboel-Khair, EM, Fahmy, MA, and Shreadah, MA (2007).Environmental Assessment on the Aqaba Gulf Coastal waters, Egypt.Egyptian J. Aqu.Res., 33(1), 1-14.

Abdel-Halim, AM, Abdel Nabi, MA, Abdel Fattah, LM, Fahmy, MA, Abo-El-Khair, EM, khaled, AM, Abu El-Soud, A, and Shreadah, MA (2016). Environmental studies on the Aqaba Gulf coastal waters during 2011-2013. Journal of Environmental Protection. 7, 1411-1437.

Abdel Monein, NM, Yacout, GA, Aboul-Ela, HM, and Shreadah, MA (2017a). Hepatoprotective Activity of Chitosan Nanocarriers Loaded With the Ethyl Acetate Extract of Astenotrophomonas sp. Bacteria Associated with the Red Sea Sponge Amphimedon Ochracea In CCl_4 Induced Hepatotoxicity in Rats. Advances in Bioscience and Biotechnology (ABB), 8(1), 27-50

Abd El Moneam, NM, Al-Assar, SA, Shreadah, MA, and Nabil-Adam, A (2017b). Isolation, Identification and Molecular Screening of Psudomance Sp Metabolic pathways NRPs and PKS

associated with the Red Sea sponge, Hyrtios aff. Erectus, Egypt. Journal of pure & Applied Microbiology. 11(3), 1299-1311.

Abd El Moneam, NM, Shreadah, MA, Al-Assar, SA, and Nabil-Adam, A (2017c). Protective role of antioxidants capacity of Hyrtios aff. Erectus sponge extract against mixture of persistent organic pollutants (POPs)-induced hepatic toxicity in mice liver: biomarkers and ultrastructural study" Environmental Science and Pollution Research. DOI 10.10071/s 11356-017-9805-8, 1-12.

Abd El Moneam, NM, Al-Assar, SA, Shreadah, MA, and Nabil-Adam, A (2017d). Isolation, Identification and Screening of Pseudomonas sp. Metabolic Pathways NRPs and PKS associated with the Red sea Sponge, Hyrtios aff. Erectus, Egypt. Journal of Pure and Applied Microbiology,11(3), 1299-1311.

Abd El Moneam, NM, Shreadah, MA, Al-Assar, SA, De Voogd, NJ, and Nabil-Adam, A (2018). Hepatoprotective effect of Red Sea sponge extract against the toxicity of a real-life mixture of persistent organic pollutants. Biotechnology & Biotechnological Equipment 32 (3), 734-743.

Abo-el-Khair, EM, Abdel Halim AM, Shriadah, MA and Fahmy, MA (2007). Environmental Conditions of the Suez Gulf and the Red Sea Coastal Waters, Egypt. Proceedings of the 8thInternational Conference on the Mediterranean Coastal Environment .MEDCOAST 2007. E. Ozhan (Editor). 13 – 17 November 2007. Alexandria. Egypt. 517-526.

Abo-El khair, EM, Abdel Halim, AM, Fahmy, MA, and Shreadah, MA (2008). Environmental Impact Assessment of Northern Red Sea Regions during 2005 – 2007. Egyptian J. Aqu. Res., 34(2), 20-30.

Abo-El-Khair, EA, Abdel Fattah, LM, Abdel-Halim, AM, Abdel Nabi, MA, Fahmy, MA, Ahdy, HH, Hemeilly, A, Abu El-Soud, A, and Shreadah, MA (2016). Assessment of the hydrochemical characteristics for the coastal waters of the Suez Gulf during 2011-2013. Journal of Environmental Protection, 7, 1497-1521.

Aboul-Ela, HM, Shreadah, MA, Abdel-Monem, NM, Yakout, GA, and Van Soest, RWM (2012). Isolation, cytotoxic activity and phylogenetic analysis of Bacillus sp. bacteria associated with the red sea sponge *Amphimedonochracea*. Advances in Bioscience and Biotechnology, 3 (7),815-823.

Agullo G., Gamet-Payrastre L., Manenti, S. (1997). Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. Biochem Pharmacol, 53, 1649-1657.

Amarowicz R., Naczk M., Zadernowski R., Shahidi F. (2000). Antioxidant activity of condensed tannins of beach pea, Canola hulls, evening primrose, and faba bean. Journal of Food Lipids, **7**: 195–205. (2000).

AOAC. (1990). Official Methods analysis of association of offcialanaltical chemists 15 th End., Association of official analytical chemists .Washington DC., USA. (1990).

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of Antioxidants and Natural Products in Inflammation. *Oxidative Medicine and Cellular Longevity*, *2016*, 5276130. http://doi.org/10.1155/2016/5276130

Blois, MS. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. Nature 181: 1199 – 1200; doi:10.1038/1811199.

Chahar M.K., Sharma N., Dobhal M.P., Joshi, Y.C. (2011). Flavonoids: A versatile source of anticancer drugs. Phcog Rev., 5,1-12

Chkraborty, K, Lipton, AP, Paul Raj, R, Vijayan, KK. Antibacterial labdane diterpenoids of Ulva fasciata Delile from southwestern coast of the Indian Peninsula. Food Chem. 119: 1399–1408. (2010).

Elmore, S. (2007). Apoptosis: A Review of Programmed Cell Death. *Toxicologic Pathology*, *35*(4), 495–516. http://doi.org/10.1080/01926230701320337

Fahmy, MA, Shriadah, MA, AbulSoud, A, Abdel Rahman, SM, and Shindy, M (2005). Hydrography and Chemical Characteristics of the Coastal Water along the Gulf of Suez. Egyptian J. Aquatic Res., 31, 1-14.

Fahmy, MA, Abdel Fattah, LM, Abdel-Halim, AM, Abdel Nabi,MA, Abo-El-Khair, EM, Ahdy, HH, Hemeilly, A, Abu El-Soud, A, and Shreadah, MA (2016). Evaluations of the Coastal Water Quality of the Egyptian Red Sea during 2011-2013. Journal of Environmental Protection, 7(12), 1810-1834.

Fonteh, P.N., Keter, F.K. Meyer, D. (2011). New bis(thiosemicarbazonate) gold(III) complexes inhibit HIV replication at cytostatic concentrations: potential for incorporation into virostatic cocktails. J Inorg Biochem ,105:1173–1180.

Gurguess, SM, Shreadah, MA, Fahmy, MA, Aboul El Kheir, EM, and Abdel Halim, A (2009).Assessment of Water Quality in the Red Sea using in Situ Measurements and Remote Sensing Data .Egyptian J. Aqu. Res. 35 (2), 1-13.

Hegazy, MF, Mohamed, TA, Elshamy, AI, Hassanien, AA, Abdel Azim, NS, Shreadah,MA, Abdelgawad, II, Elkady, EM (2015). A New Steroid from the Red Sea Soft Coral *Lobophytum Lobophytum.* Natural Products Research, 30, 340-344.

Hegazy, MF, Gamal-Eldeen, AM, Mohamed, TA, Alhammady, MA, Hassanien, AA, Shreadah, MA, Abdelgawad, II, Elkady, EM (2015). Cytotoxic Constituents from the Red Sea Soft Coral *Nephthea* Sp., Natural Products Research, 30, 1266-1272.

Kosanic, M, Rankovic, B, Stanojkovic, T. (2015). Biological activities of two macroalgae from Adriatic coast of Montenegro. Saudi J Biol Sci, 22(4): 390-397.

Lahlou, M. (2013). The Success of Natural Products in Drug Discovery. Pharmacology & Pharmacy, 4, 17-31. doi.org/10.4236

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, *4*(8), 118–126. http://doi.org/10.4103/0973-7847.70902

Masoud, MS, Said, TO, El- Zokm, GM, and Shreadah, MA (2010). Speciation of Fe, Mn and Zn in Surficial Sediments from the Egyptian Red Sea Coasts. Chemical Speciation andBiodiversity,22(4), 257-269.

Masoud, MS, Said, TO, El-Zokm, GM, and Shreadah, MA (2012). Assessment of Heavy Metals Contamination in Surface Sediments of the Egyptian Red Sea Coasts. Australian Journal of Basic and Applied Sciences, 6, 44-58.

Mehbub, MF, Lei, J, Franco, C, and Zhang, W (2014).Marine Sponge Derived Natural Products between 2001 and 2010: Trends and Opportunities for Discovery of BioactivesMar Drugs. 2014 Aug; 12(8): 4539–4577. doi: 10.3390/md12084539

Moyo, S.J., Aboud, S., Kasubi, M., Lyamuya, E.F., Maselle S.Y. (2010). Antimicrobial resistance among producers and non-producers of extended spectrum beta- lactamases in urinary isolates at a tertiary Hospital in Tanzania, A Short Report. BMC Research Notes, 3:348, doi:10.1186/1756-0500-3-348. (2010).

Min, J., Traynor, D., Stegner, A. L., Zhang, L., Hanigan, M. H., Alexander, H., & Alexander, S. (2005). Sphingosine Kinase Regulates the Sensitivity of *Dictyostelium discoideum* Cells to the Anticancer Drug Cisplatin. *Eukaryotic Cell*, *4*(1), 178–189. http://doi.org/10.1128/EC.4.1.178-189.2005

Okbah, MA, Shata, MA, and Shriadah, MA (2005). Gochemical forms of trace metals in mangrove sediments-Red Sea (Egypt). Chemistry and Ecology, 21, 23-36.

Ponnusamy, S., Meyers-Needham, M., Senkal, C. E., Saddoughi, S. A., Sentelle, D., Selvam, S. P., Ogretmen, B. (2010). Sphingolipids and cancer: ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. Future Oncology (London, England), 6(10), 1603–1624. http://doi.org/10.2217/fon.10.116

Rice-Evans CA, Miller NJ, Paganga G (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933-56.

Said, TO, Shreadah, MA, AbdelGhani, SA, andAhmed, AM (2010). Alkyltin and alkayllead compounds in coastal water of Suez Gulf, Egypt. Egyptian J. Aqu. Res., 36(1), 33-42.

Santos C.C., Salvadori M.S., Mota V.G., Costa L.M., de Almeida A.A., de Oliveira G.A., Costa J.P., de Sousa D.P., de Freitas R.M., de Almeida R.N. (2013). Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. Neurosci J. , 949452. doi: 10.1155/2013/949452.

Shreadah, MA, Said, TO, El Zokm, GM, and Masoud, MS (2008a). Physico-Chemical Characteritics of the Surficial Sediments along the Egyptian Red Sea Coasts. Egyptian J. Aqu.Res., 34(4), 16- 34.

Shreadah, MA, Said, TO, Abd El Ghani, SA, and Ahmed, AM (2008b). Alkyllead and Alkyltin Species in different fishes collected from the Suez Gulf, Egypt. Proceedings of the 2nd International Conference on Aquatic Res, Egyptian J. Aqu.Res., 34(4), 64-73.

Shreadah, MA, Masoud, MS, Said, TO, and El Zokm, GM (2008c). Application of IR, X-Ray, TGA and DTA to determine the mineral composition of the Sediments and study of reaction kinetics along the Egyptian Red Sea Coasts. Egyptian J. Aqu.Res., 34(2), 83- 95.

Shreadah, MA, Said, TO, Abdel Ghani, SA, and Ahmed, AM (2011). Distribution of Different Organotinand Organolead Compounds in Sediment of Suez Gulf. Journal of Environmental Protection, 2(5), 545- 554.

Shreadah, MA, Abd El Moneam, NM, Al-Assar, SA, and Nabil-Adam, A (2017). The Ameliorative Role of a Marine Sponge Extract against Mixture of Persistent Organic Pollutants induced Changes in Hematological Parameters in Mice. Expert Opinion Environmental Biology, 6(2), DOI: 10.4172/2325-9655.1000143b.

Shreadah, MA, Abd El Moneam, NM, Al-Assar, SA, and Nabil-Adam, A (2018). Phytochemical and pharmacological screening of Sargassium vulgare from Suez Canal, Egypt. Food Science and Biotechnology, 1-17.

Shriadah, MA, Okbah, MA, and El-Deek, MS (2004). Trace metals in the water columns of the Red Sea and the Gulf of Aqaba, Egypt. Water, Air and Soil Pollut., 153, 115-124.

Sun, B, Richardo-Da-Silvia, JM, Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *J Agric Food Chem*, 46, 4267–4274. (1998).

Taga, MS, Miller, EE, Pratt, DE. (1984). Chia seeds as a source of natural lipid antioxidants. Journal of the American Oil Chemists' Society, 61(5), 928–931. (1984).

Thaipong,K, Boonprakob,U, Crosby, K, Cisneros-Zevallos, L, Byrne, DH. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, 19 (6–7): 669-675. (2006).

Zhishen, J., Mengcheng,T. and Jianming,W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 64(4),555–559.

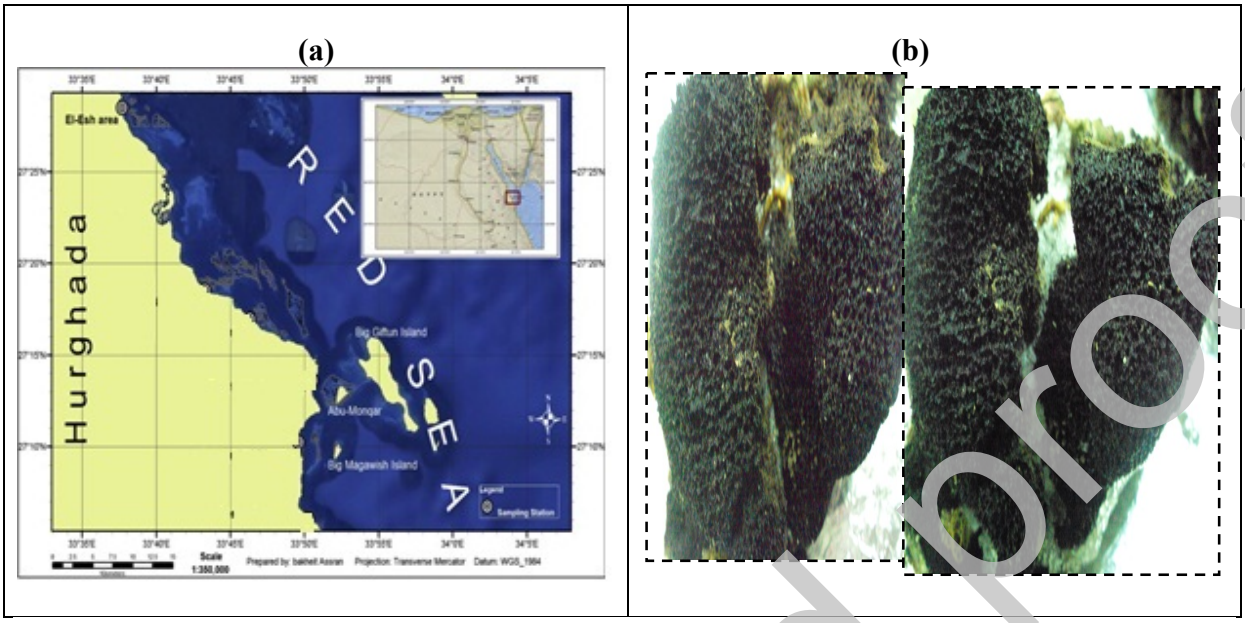
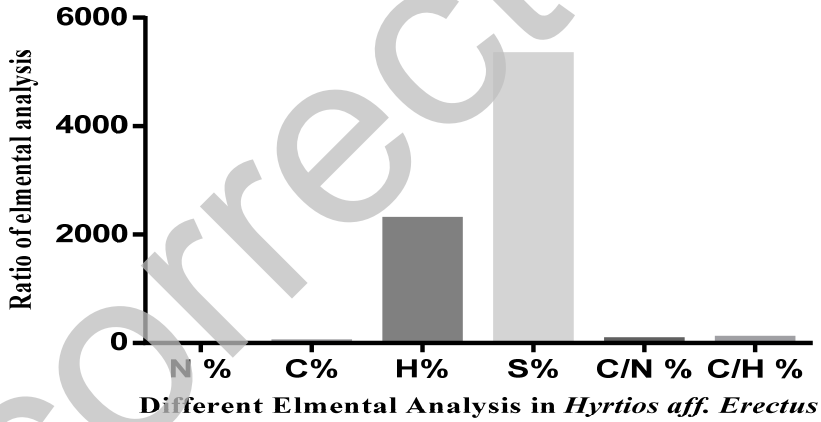
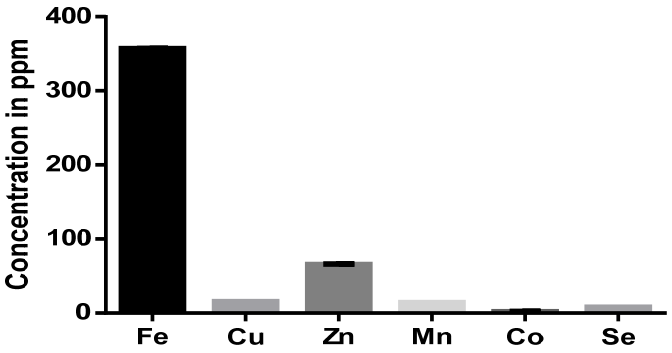


Figure (1). Location of sampling stations at the Red Sea (A) and sponge sample Hyrtios aff. erectus RMNH POR.8633 sample (B).



	N %	C%	H%	S%	C/N %	C/H %
Mean	3.490	7.680	2269	5301	48.73	74.25
Std. Deviation	0.01000	0.1200	0.06006	0.01001	0.2750	0.05508

Figure (2): The elemental analysis result of Hyrtios aff. erectus.



Different Minerals and elements *of Hyrtiosaff. Erectus* Extract

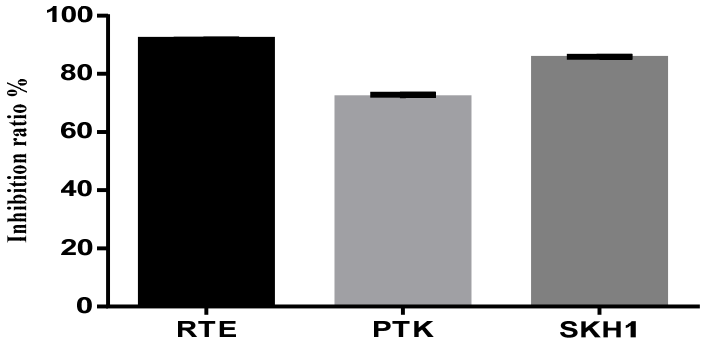
	Fe	Cu	Zn	Mn	Co	Se
Mean	356.8	15.54	65.55	14.10	1.990	7.886
Std. Deviation	0.2050	0.06506	0.4500	0.005774	0.01000	0.01445

Figure (3): The mineral profiling for Hyrtios aff. erectus.

Different Phytochemical paramters of *Hyrtiosaff. Erectus* Extract

	b-Caroten	Taninns	Phenolic	Flavanoids
Mean	0.2733	0.4100	0.1700	1.035
Std. Deviation	0.005773	0.02000	0.01000	0.01500

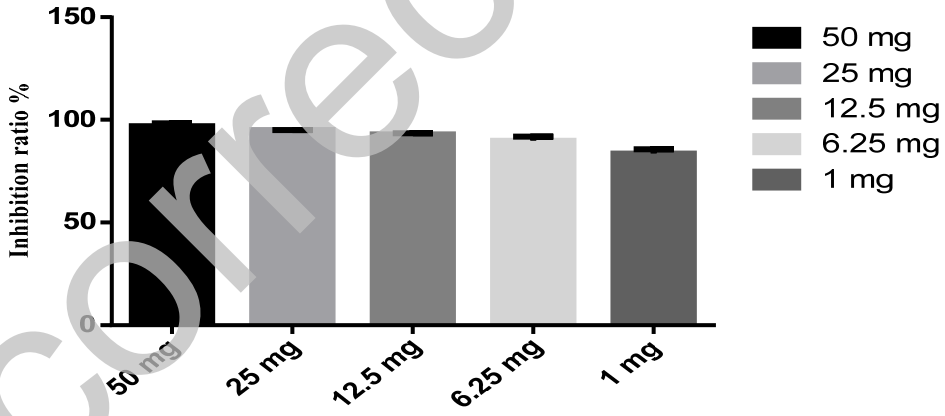
Figure (4): The Polyphenol and carotenoid profiling for HES extract .



Different Anti-HIV and Anti-cancer biochemical paramters of *Hyrtiosaff. Erectus* Extract

	RTE	PTK	SKH1
Mean	91.70	71.66	85.21
Std. Deviation	0.1250	1.190	0.7050

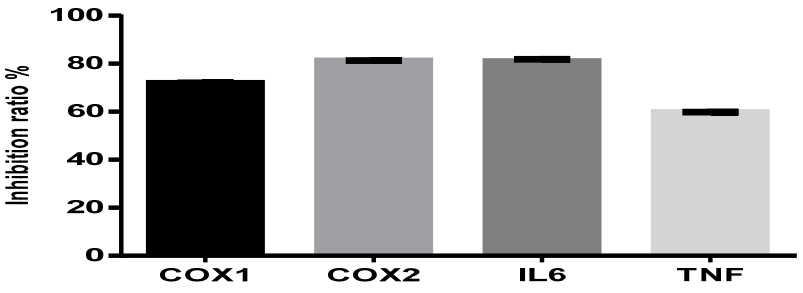
Figure (5): The anti-HIV and anti-cancer profiling of HES extract



Different concentrations of *Hyrtiosaff. Erectus in Acetylecholineestrase inhabtor assay*

	50 mg	25 mg	12.5 mg	6.25 mg	1 mg
Mean	96.99	95.02	92.89	89.82	83.51
Std. Deviation	1.365	0.02828	0.5515	2.001	2.135

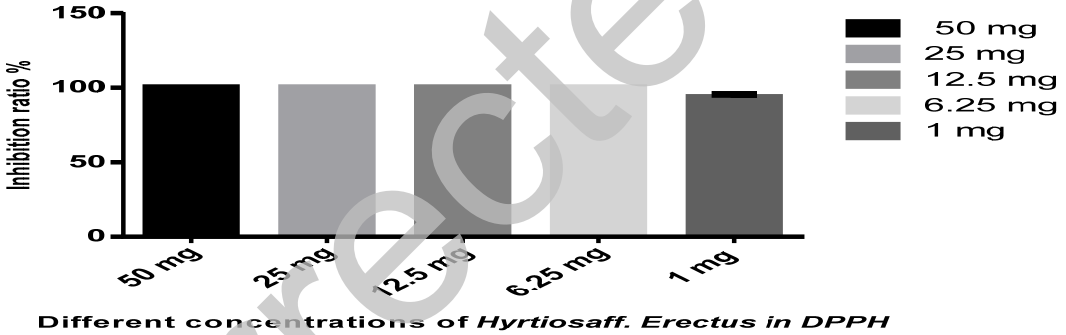
Figure (6): The Acetylecholinesterase inhibitor activity of HES extract.



Different anti-inflammatory biochemical paramter of *Hyrtiosaff. Erectus* Extract

	COX1	COX2	IL6	TNF
Mean	71.82	81.13	80.89	59.74
Std. Deviation	0.1850	0.1250	0.8850	0.06000

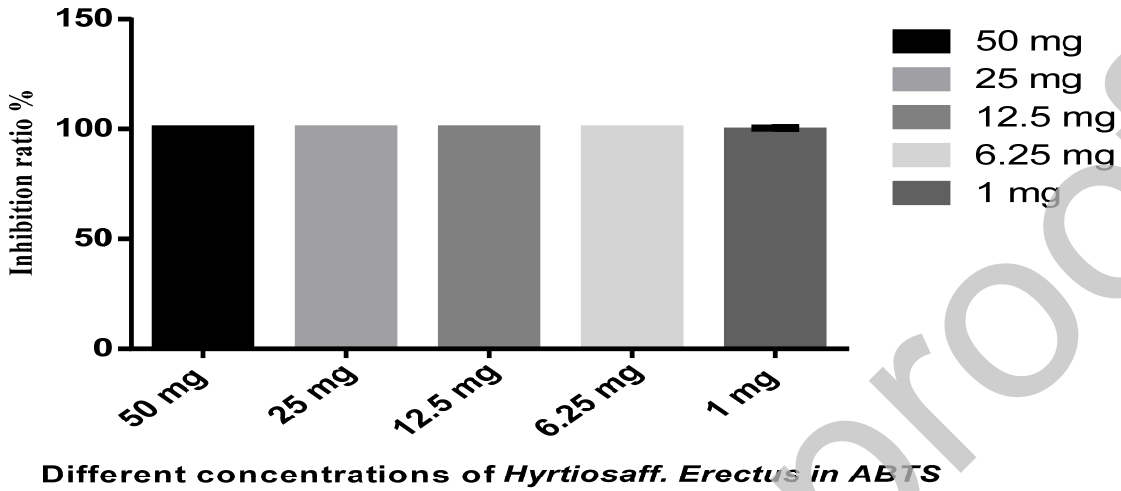
Figure (7): The anti-inflammatory profiling of HES extract.



Different concentrations of *Hyrtiosaff. Erectus in DPPH*

	50 mg	25 mg	12.5 mg	6.25 mg	1 mg
Mean	100.0	100.0	100.0	100.0	93.50
Std. Deviation	0.0	0.0	0.0	0.0	2.121

Figure (8): The total antioxidant capacity using scavenging (%) of DPPH assay.



	50 mg	25 mg	12.5 mg	6.25 mg	1 mg
Mean	100.0	100.0	100.0	100.0	99.00
Std. Deviation	0.0	0.0	0.0	0.0	1.414

Figure (9): The total antioxidant capacity using scavenging (%) ABTS assay.

Table (1): Inhibitory activities of HES extract *against* hepatocellular carcinoma cells.

Sample concentration (µg)	Viability %
50.00	47.83
25.00	69.17
12.50	80.24
6.25	93.62
3.125	97.89
1.56	100
0 .00	100.00