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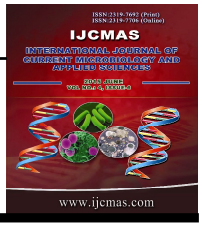

Original Research Article

Free radical scavenging and *In vitro* anticancer properties Breast cancer cell line MCF -7 on Marine Sponge *Spongia tosta*

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Keywords

Antioxidant, anticancer, Marine source and *Spongia tosta*

A B S T R A C T

Marine sponges have been considered as gold mine during the past 50 years, with respect to the diversity of their secondary metabolites *Spongia tosta* is known to posses medicinal uses, but the biological and pharmacological properties are unexplored *Marine sponge* contain active principles which can acts as potent antioxidants. Antioxidants are getting a lot of importance as a panacea for a large number of diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases etc., antioxidants constitute a tissue defense system against aging and stress, caused by free radicals. Natural antioxidants from marine sources are safe, potent and harmless. The *in vitro* free radical scavenging activity was analyzed from the sponge by DPPH, ABTS scavenging assays. The cytotoxic properties were evaluated in breast cancer cell line MCF –7 using MTT colorimetric assay for 24, 48 and 72 hrs the result show that the methanolic extract of *Spongia tosta* possess excellent antioxidant and anticancer potential that may be used for therapeutic purpose of free radical scavenging and cancer treatment with proper evaluation procedures.

Introduction

Cancer is a large group of diseases, all of which have one thing in common i.e., cells growing out of control or fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered (Butter field,2006). Though many diseases (such as heart failure) may have a worst prognosis than most cases of cancer, cancer is the subject of wide spread fear and taboos, there are 200 different types of cancer that afflict humans (Choi et al,2007). The major causes of cancer are smoking, dietary imbalance, hormones and chronic infections inflammation (Cragg GM,2005) .The number is believed to become 9 million 2015 and 11.5 million in 2030(Francis D

and Rita L ,1986).The limited success of clinical therapies including radiation, chemotherapy, immune modulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need for new cancer management several classes of anticancer are currently being used, due to clinical limitation and adverse effects there is critical interest in development of efficient and safe drugs for treatment of cancer.

Marine organisms, which represent approximately one half of the total global biodiversity, are rich reservoirs of biologically active natural products (Kim SK, Sijesekara J, 2010; Malcolm RA). Approximately 15,000 pharmacologically active compounds have been isolated from marine species whose the structure of the most was unique organisms and absent in terrestrial organisms (Mhadhebi L, Chaieb K, Bouraoui A,2012).

In recent years, anticancer drugs from natural sources such as plants, marine organism and microorganism account approximately 60% of all anticancer drugs used (Newman DJ, 2004). However, many anticancer drugs used in chemotherapeutic treatments developed resistance and side effects (Ngo DH, Wijesekara,2011; Panchal RG,1998). Out of these, reactive oxygen species (ROS) and free radicals attack macromolecules such as DNA, proteins and lipids, leading to many death disorders including cancer (Priya et al, 2013;Guha et al,2011). The harmful effect of the free radicals can however, be blocked by synthetic antioxidants, but due to their adverse side effects, search for effective and natural antioxidants has become crucial (Wang et al, 2008).

Hence, the search is still onto find a natural drug possessing anticancer activity and antioxidant properties. The *Marine sponge Spongia tosta(MST)* has become a new marine resource for searching novel bioactive marine natural products as lead compounds in drug development.

Collection of sample

The sponge sample was collected as entangled specimens form a bottom trawl fish net operated off Manoli and hare Islands of Mandapam group of Islands, Gulf of Mannar at Rameshwaram. It was collected by bicatching method. The samples were placed inside sterile ethyl polythene bags under water and transferred to the lab aseptically in the boxes.

Materials and Methods

Preparation of *sponge* extracts

Prior to the extraction, samples were washed with water, cleaned air dried, lyophilized and powdered. They were stored for further use. For the extraction of crude bioactive, 100g of powdered material was exhaustively extracted with 200ml of methanol using soxhelt apparatus and concentrated in a rotary evaporated at reduced pressure

Silver nano particles synthesis using

3 mM solution of silver nitrate was prepared. 20 ml of the plant extract was mixed with 80 ml of 3 mM of silver nitrate solution. The colour changed from yellow to reddish brown colour indicating the formation of silver nanoparticles. The

AgNps thus obtained was purified by repeated centrifugation at 7000 rpm for 10 min. The pellet was collected and dried. The Chemical tests were carried out in AgNPs for Antioxidant and Anticancerproperties . The pH of the solution was also determined.

In vitro anticancer activity

Chemicals

3-(4,5- dimethyl thiazol -2-yl)- 5-diphenyl tetrazoluim bromide (MTT). Fetal Bovine serum (FBS), phosphate buffered saline (PBS). Dulbecco's modified Eagle's medium (DMEM) and Trypsin were obtained from sigma Aldrich co, st. Louis, USA, EDTA, Glucose and Antibiotics from Hi media laboratories Ltd., Mumbai Dimethyl sulfoxide (DMSO) and propanol from E. Merck Ltd., Mumbai India. All other reagents and chemicals used in the study were of analytical grade.

Cell lines and culture medium

MCF-7 (Breast Carcinoma) Cell line was procured from National centre for cell science (NCCS). Pune, India. Stock cell is cultured in DMEM supplemented with 10% inactivated fetal Bovine serum (FBS), penicillin (100 Iu/ml), Streptomycin (100µg/ml). and amphotericin β(5µg/ml) in an humidified atmosphere of 5% CO_2 at 37^0C. The cells were dissociated, with TPVG solution (0.2% Trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in $25cm^2$ culture flasks and all experiments were carried out in 96 microtitre plates (Tar sons India Pvt. Ltd) Kolkata, India.

Preparation of test solutions

For cytotoxicity studies, each weighed test drugs was dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filtration serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT assays

The ability of the cells to survive a toxic insult has been the basis of most cytoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazoluim. The assay depends both on the number of cells present and on the mitochondrial activity of the cell. The principle involved in the cleavage of tetrazoluim salt- 3- (4, 5- dimethyl thiazole - 2 –y1)-2, 5- diphenyltetrazoluim bromide (MTT) into a blue coloured products. (Formazon) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells / ml using DMEM containing 10% FBS. To each well of the 96 well micotitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hrs, when a partial monolayer was formed. The supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monoloayer in microtitre plates. The plates were then incubated in 37^0C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted ,every 24hrs interval. After 72hrs the drug solution in the wells were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hrs at 37^0C in 5% CO_2 atmosphere. The

supernatant was removed and 100µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm (Francis and Rita, 1986). The percentage growth inhibition was calculated using the following formula and concentration of the test drug needed to inhibit cell growth by 50% (CTC 50) values are generated from the dose – response curves for each cell line (Yang ,2000).

$$\%\text{Growth inhibition} = 100 - \frac{\text{Mean OD of Individual test group}}{\text{Mean OD of control group}} \times 100$$

Antioxidant activity

Determination of DPPH scavenging activity

The free radical scavenging activity of the methanol extract of marine sponge *Songia tosta* was evaluated by using the stable radical DPPH. The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plates. The find concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.65, 7.812 µg/ml. The plates were incubated at 37^0C for 30min and the absorbance of each solution was measured at 490nm, using a microplate reader.

Determination of ABTS Scavenging Activity

ABTS(54.8mg) was dissolved in 50ml of distilled water to 2 mM concentration and potassium persulphate (17 Mm,0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use. To o.2ml of various concentration of the extracts or standards, 0.1 ml of distelled DMSO and 0.16 ml of ABTS solution was added to make volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm.

Results and Discussion

The cytotoxic effects of methanol extract of *Marine sponge sponge tosta* was shown in table 1. In the presence of investigation *in vitro* anticancer activity of the *Marine sponge sponge tosta* was evaluated against the cancer cell lines viz MCF – 7 (Human breast carcinoma).

The anti-cancer activity displayed by extract of this *Marine sponge sponge tosta* was found 73.24 ± 2.3% at 1000 mg concentration against the MCF-7 (Human breast carcinoma) cell line and the CTC_{50} value was recorded as 226.67 ± 2.4mg/ml. Table 2 the free radical scavenging values are given standard error mean (SEM). The methanolic extract of *Marine sponge Spongia tosta* (MST) showed a maximum DPPH scavenging activity at a concentration of 1000µg/ml with IC_{50} 62.5 µg/ml,whereas ABTS scavenging activity at a concentration of 1000µg/ml with IC_{50} 6.25 µg/ml.Anticancer potential of methanolic extract of the MST against MCF-7 cancer cell lines shown fig:1. Marine sources constitute a common alternative, for cancer prevention and treatment in many countries around the world. Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the marine source worldwide have been reported to posses anticancer properties extracts of these *spongia tosta* in believed to contain a wide array of which might possess cancer preventive and or therapeutic properties.

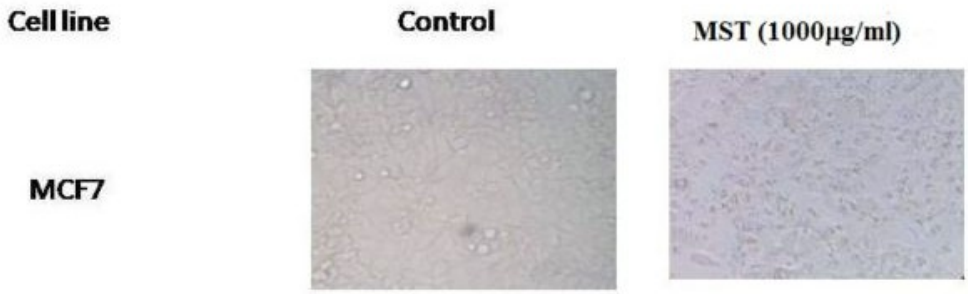
Table.1 Cytotoxic properties of Methanolic extract of MST against MCF-7 cell line

S. No	Name of Test sample	Test Concn. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	***MST***	1000	73.24±2.3	226.67±2.4
		500	64.39±1.3	
		250	53.23±3.3	
		125	38.42±0.3	
		62.5	30.67±4.6	

Table.2 In vitro antioxidant properties of methanolic extract of MST

Samples	**IC_{50} values µg/ml by methods**	
	DPPH	**ABTS**
MST	<62.5	<6.25
Standard	Rutin	Rutin
	3.16±0.08	195.12

Fig.1 Anti cancer potential of methanolic extract of the MST against MCF-7 cancer cell lines



The result of our study revealed that methanol extract of *Spongia tosta* has a potent cytotoxic effect on MCF-7. Human breast adeno carcinoma cell line in concentration department manner. Morphological studies also confirmed that the methanol extract of *Spongia tosta* has got potential cytotoxic effect.

DPPH is a stable free radical, on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products(Jun

M,2004).Insitu, free radicals like ROS, reactive nitrogen species and polyaromatic hydrocarbon cations induces biochemical alterations in cell that have been directly induces biochemical alternations (or) indirectly linked with carcinogenesis, arthritis and or in cardiovascular disorders(Yen G C and Chen H Y, 1995).

ABTS is a green chromophore produced by the reaction between ABTS and Methhaemoglobin (Katalinic and coworkers 2006) found that in ABTS assay, ABTS radical cation was generated directly instable form using potassium persulfate.Generation of radical before the antioxidants added prevents interference of compounds, which affect radical formation.

In today's world the percentage of people using chemicals and drugs are increasing with their side effects. "The boon given to our earth is the marine system", which needs to be utilized in sustainable manner many of today's drugs are derived from marine sources. The cytotoxicity assay indicated the potential of the methanol extract of *spongia tosta* could be a source of anticancer therapeutic agent against MCF-7 cell lines. Further it is reasonably concluded that isolation and characterization of the antioxidant component through in-vivo studies will help in understanding their mechanisms of action as a better antioxidant and anticancer.

Radical scavenging activity of MST and Rutin against DPPH radical increase at increasing concentration was shown figure 2. And the radical scavenging activity of MST and Rutin against ABTS radical increase at increasing concentration was shown figure 3.

Figure.2 DPPH radical scavenging activity of methanolic extract MST

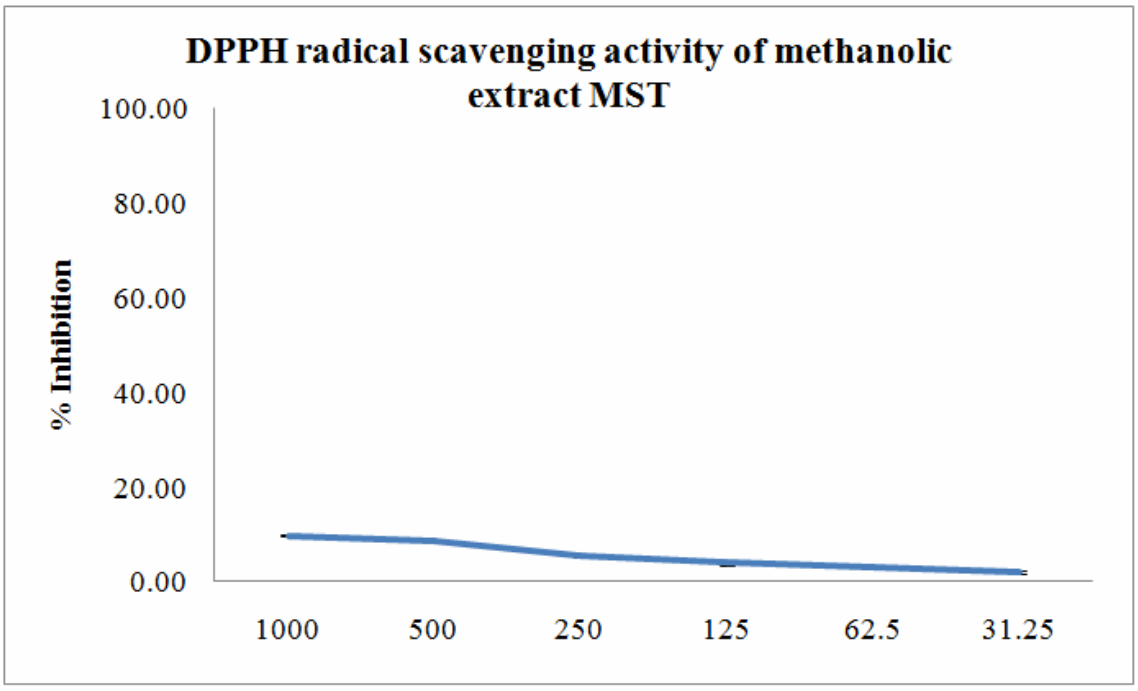
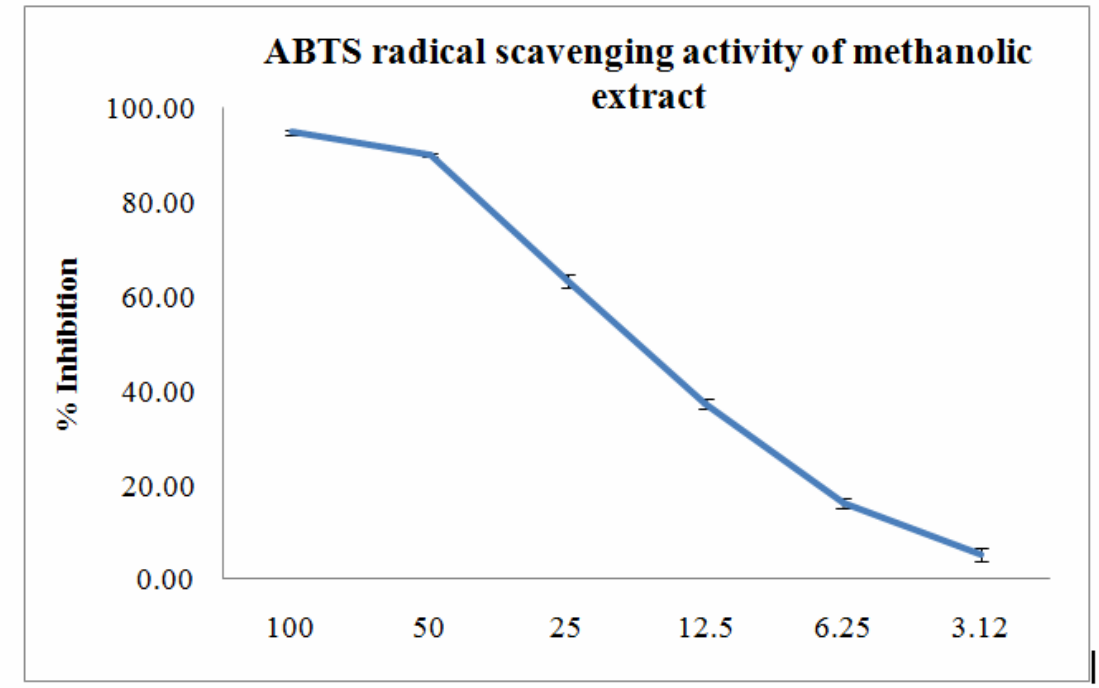


Figure.3 ABTS radical scavenging activity of methanolic extract MST



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