ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 6, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

FREE RADICAL SCAVENGING ACTIVITY OF MARINE SPONGES COLLECTED FROM KOVALAM, CHENNAI

YUVARANI THAMBIDURAI[1], SUDARSANAM D[2,3]*, HABEEB SKM[4], ASHOK KUMAR T[5]

[1]Department of Bioinformatics, Bharathiar University, Coimbatore, Tamil Nadu, India. [2]Department of Biotechnology, D.G. Vaishnav College, Arumbakkam, Chennai - 600106, Tamil Nadu, India. [3]Department of Advanced Zoology and Biotechnology, School of Genomics and Bioinformatics, Loyola College, Chennai - 600 034, Tamil Nadu, India. [4]Department of Genetic Engineering, School of Bioengineering, SRM University, Kattankulathur, Chennai - 603 203, Tamil Nadu, India. [5]Naturophathy and Yoga Therapist, Zero Error, Dharmapuri - 636 705, Tamil Nadu, India. Email: dsloy2003@gmail.com

Received: 23 February 2017, Revised and Accepted: 28 March 2017

ABSTRACT

Objective: The main focus of this study is to screen the marine sponges for potent free radical scavenging activity.

Methods: Various methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assay are employed to ascertain the antioxidant properties of marine sponges namely *Dysidea herbacea* and *Sigmadocia pumila.*

Results: On analyzing, the result of ABTS assay *D. herbacea* and *S. pumila* exhibited almost equal antioxidant properties. While calculating the inhibitory concentration 50% value for DPPH assay, the Sample 1 and 2 has an IC of 655.49 and 826.739 µl, respectively, and in FRAP assay, the Sample 1 and 2 has an IC of 67.587 and 74.57 µg, respectively.

Conclusion: Overall from this assay, *D. herbacea* revealed slightly better antioxidant activity when compared to *S. pumila,* also which in future may serve as a better source to fight against various diseases.

Keywords: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), 2,2-diphenyl-1-picrylhydrazyl, Ferric reducing antioxidant power, Antioxidant activity, Marine sponges.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i6.18115

INTRODUCTION

Marine organism serves as an excellent source of bioactive molecules in discovering novel drugs. Marine organisms possess diverse secondary metabolite production. These secondary metabolites have different bioactive properties such as antioxidant, anti-inflammatory, cytotoxic, antimicrobial, and antitumor [1-4]. Pharmaceutical research on sponges was aroused in the year 1950's by the discovery of a number of unknown nucleosides: Spongothymidine and spongouridine in the marine sponge cryptotheca crypta [5,6]. But now, nearly more than 15,000 products of marine species have been described. Marine sponges are champion producers, concerning the product diversity has been found stated that sponges are responsible for more than 5300 different products and every year hundreds of new compounds are being discovered [7-9]. Most bioactive compounds from sponges can be classified as anti-inflammatory, antitumor, antibiotic or antifouling, antiviral, antimalarial, and immune or neurosuppressive. The products of sponges and its chemical diversity are remarkable [10] have been explained that the unusual nucleosides, sterols, bioactive terpenes, alkaloids, fatty acids, peroxides, cyclic peptides, and amino acid derivatives (which are frequently halogenated) have been described from sponges. Marine sponges have a potential to provide future drugs against important diseases, such as malaria, cancer, and a range of viral diseases [11]. Of 10,000 marine sponges, 11 genera are known to produce bioactive compounds, and only three genera (*Haliclona*, *Petrosia,* and Discodemia) are known to produce anticancer, antimalarial, and anti-inflammatory compounds. The marine sponges are rich sources of structurally novel bioactive secondary metabolites.

It is evident that reactive oxygen species (ROS), and many oxidants are responsible for disorders and diseases. This did the researchers to hunt for antioxidants which serve best to maintain a healthy life and prevention of diseases. In fact, our body has its own antioxidative mechanism which has numerous properties such as antimutagenic, anticarcinogenic, and anti-aging responses. Antioxidant helps in stabilizing free radicals before they oxidize cells and cause biological damage. Nowadays, researchers are focussing on discovering natural antioxidants that can be used in food, cosmetic and pharmaceutical products to improve the biological system by rectifying the biological damages.

Antioxidants inhibit the process of oxidation, even at low concentration, they have various physiological roles in the body. Antioxidant acts as free radical scavengers, which helps in converting the radicals to less reactive species [12].

Free radicals are atoms, molecules or compounds which contain one or more unpaired electrons, so it attempts to pair with other molecules to attain stable configuration. Free radicals are reactive chemical species produced by organism's normal use of oxygen [13]. The unstable configuration produces energy which is released on reaction with adjacent molecules such as carbohydrates, proteins, and DNA. Most of the free radicals which damage the biological system are derived from oxygen and hence referred to these radicals as ROS [14]. Even though a small amount (2-3%) of oxygen, which is consumed by respiratory chain is converted to ROS this results in creating toxic effects such as breakdown of lipids, carbohydrates, enzyme inactivation, and induce changes in DNA which results in mutation [15-18].

Various methods such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1, 1- diphenyl-2-picryl hydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) are employed to assay the antioxidant activities of compounds that are present in diverse natural sources. In this research, we focused on assaying the antioxidant activity of two marine sponges, namely, *Dysidea herbacea* (Fig. 1) and *Sigmadocia pumila* (Fig. 2) collected from Kovalam. By analyzing the antioxidant activity of these sponges, it may help us to step forward for hunting compounds which will be able to fight against various diseases.

METHODS

Sponge collection

Three species of marine sponges were collected from seashores of Kovalam village in the Kanchipuram district of Tamil Nadu with the help of Dr. Joe K. Kizhakudan, Principal Scientist from Central Marine Fisheries and Research Institute. Taxonomic identification of the samples was done and certified by Dr. Sivaleela, Scientist from Zoological Survey of India (ZSI). The samples were identified as *D. herbacea* (Fig. 1), *S. pumila* (Fig. 2), and *Acanthella elongata*. Samples were preserved in ice boxes and maintained at −20°C until the experimental process. Voucher specimens preserved at 75% methanol was deposited at ZSI.

Extraction procedure

About 5 g of the shade dried powdered sample (Fig. 3) was extracted with methanol (100 ml) at room temperature overnight on soxhlet apparatus as shown in (Fig. 4). The extracts were filtered through filter paper and concentrated in vacuum and were stored at −20°C for further zoochemical analysis.

Fig. 1: *Dysidea herbacea*

Fig. 2: *Sigmadocia pumila*

ABTS assay

Free radical scavenging ability can be ascertained by the use of a stable ABTS radical cation 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid). The scavenging activity of samples is tested using ABTS radical cation decolorization assay [19]. ABTS dissolved in water to get 7 mM concentration. ABTS radical cation ($ABTS^{*+}$) can be produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark room temperature for 12-16 hrs before use. The free radicals (ABTS) are stable for more than 2 days when stored in the dark room temperature. For the study of the test samples, the $ABTS^{*+}$ solution was diluted with absolute ethanol to an absorbance of 0.730 (±0.02) at 734 nm and equilibrated at 30°C. Reagent blank reading was taken (A_0). After addition of 1.0 ml of diluted $ABTS^{*+}$ solution (A734 nm=0.700 (±0.02) to 3 ml of test sample dissolved in methanol, the absorbance reading is taken at 30°C exactly 30 minutes after initial mixing (A_t). Appropriate solvent blanks should run in each assay. All determinations are carried out at least three times. The percentage inhibition of absorbance at 734 nm is calculated using the above formula and decrease of the absorbance between A_0 and At.

$PI = [(AC_0 - AA_t)/AC_0)] \times 100$ where AC_0 is the absorbance of the control at t=0 minute; and AAt is the absorbance of the antioxidant at t=6 minutes [20].

FRAP

Total antioxidant activity is measured by FRAP assay [21]. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. At low pH, reduction of ferric tripyridyl triazine (Fe III 2, 4, 6-tripyridyl-*s*-triazine [TPTZ]) complex to the ferrous form (which has an intense blue color) can be monitored by measuring the change

Fig. 3: Dried and powdered Sample 1 and Sample 2

Fig. 4: Extraction of sample in soxhlet apparatus

in absorption at 593 nm. The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is, therefore, directly related to the combined or "total" reducing power of the electron-donating antioxidants present in the reaction mixture.

Reagents

FRAP reagent

a. Acetate buffer 300 mM pH 3.6: Weigh 3.1 g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water
b. TPTZ (MW 312.34) 10 mM in 40 mM HCl (MW 36.46)
c. $FeCl_3$. $6H_2O$ (MW 270.30) 20 mM.

The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 at the time of use.

Standard

Ascorbic acid (MW 176.13) 1000 µM.

The sample is mixed with 3 ml of working FRAP reagent and absorbance (593 ηm) is measured at 0 minutes after vortexing. Thereafter, samples are placed at 37°C, in a water bath, and absorption is again measured after 4 minutes. Absorbance value can be taken for Sample 1 and 2 at five different concentrations (10, 20, 30, 40, 50 mg W/V) as shown in Figs. 6 and 7. Ascorbic acid standards can be taken at five different concentrations 0.1, 0.2, 0.4, 0.6, 0.8 mM (Fig. 5) and absorbance is read.

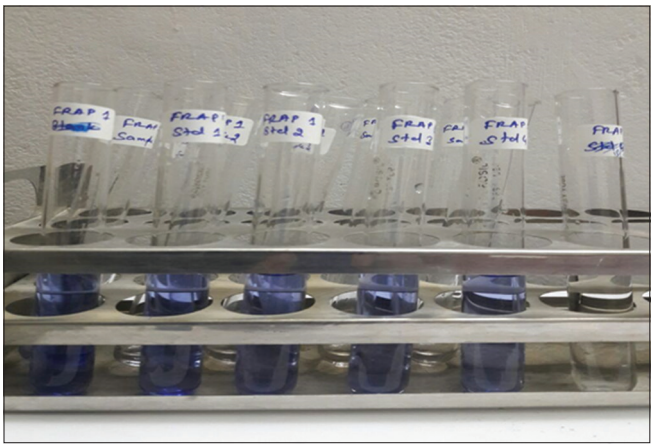


Fig. 5: Ferric reducing antioxidant power assay for standard

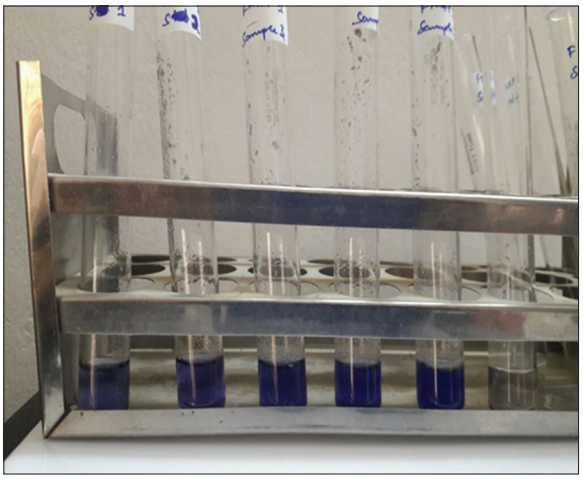


Fig. 6: Ferric reducing antioxidant power assay for Sample 1

DPPH assay

The free radical scavenging activity of the extract is measured by DPPH. In short, 0.1 mM solution of DPPH in ethanol is prepared. This solution (1 ml) is added to 3 ml of extracts in methanol at different concentration (100, 200, 300, 400, and 500 µl/ml (Figs. 9 and 10) dissolved in dimethyl sulfoxide) [19]. The mixture was shaken vigorously and allowed to stand at room temp for 30 minutes. Then, absorbance was measured at 517 ηm using a spectrophotometer (UV-VIS Shimadzu) [22]. Reference standard compound being used is ascorbic acid (Fig. 8) [23]. The inhibitory concentration 50% (IC_{50}) value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, is calculated using log dose inhibition curve. The lower absorbance of the reaction mixture indicated higher free radical activity [24].

The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) or percent inhibition = $A_0 - A_1/A_0 \times 100$.

Where, A_0 was the absorbance of control reaction and,

A_1 was the absorbance in the presence of test or standard sample.

RESULTS

The marine organisms which act as the source of secondary metabolites have shown the radical scavenging activity. The extract of marine sponges revealed potential antioxidant property on subjecting to

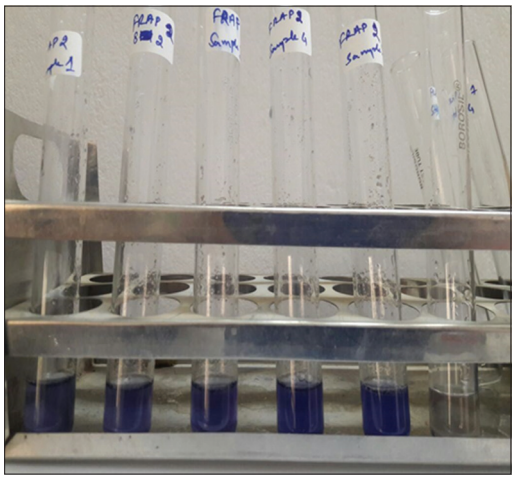


Fig. 7: Ferric reducing antioxidant power assay for Sample 2

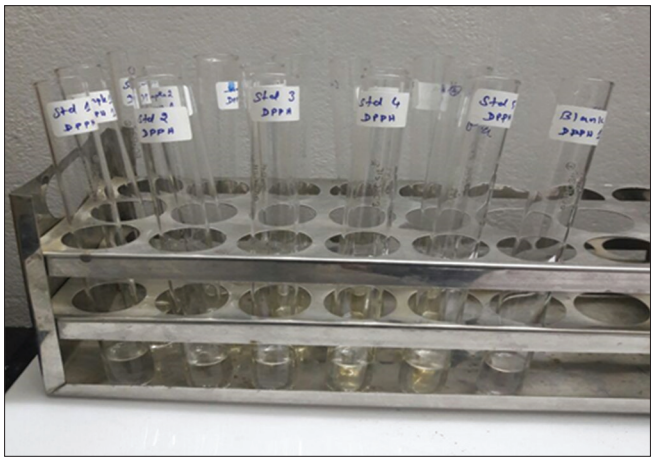


Fig. 8: 2,2-diphenyl-1-picrylhydrazyl assay for standard

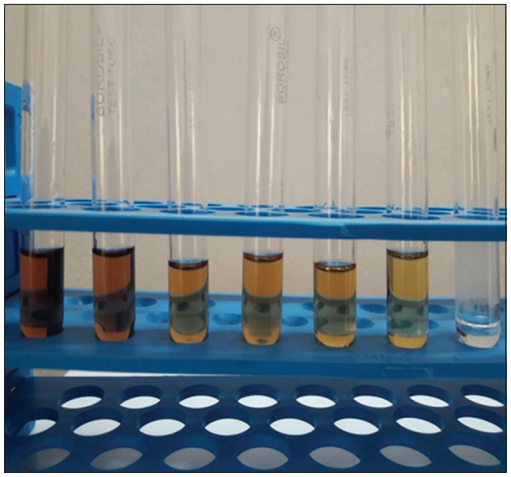


Fig. 9: 2,2-diphenyl-1-picrylhydrazyl assay for Sample 1

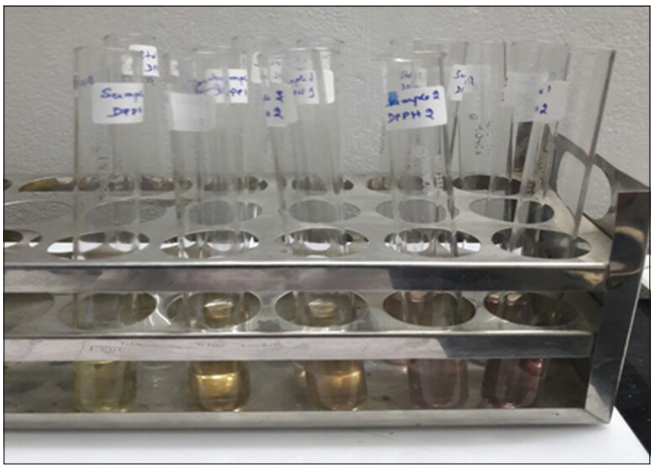
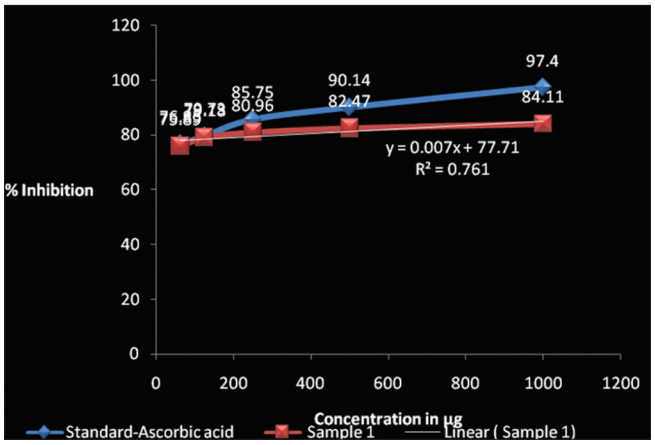


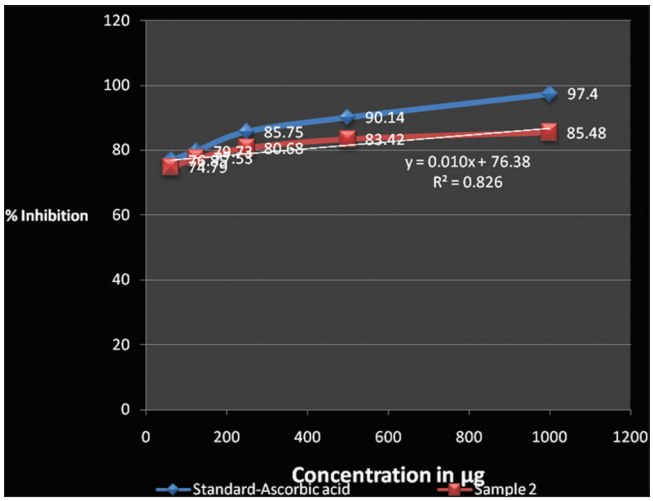
Fig. 10: 2,2-diphenyl-1-picrylhydrazyl assay for Sample 2

ABTS, FRAP and DPPH scavenging assay method. In the ABTS assay, free radical scavenging ability can be ascertained by the use of a stable ABTS radical cation decolorization. The absorbance is read at 734 ηm using the absorbance value the percentage inhibition was calculated for each five different concentrations. As the concentration increases, the percentage inhibition also increased substantially which is mentioned in Table 1. A linear trendline graph is plotted for each sample by comparison with the standard to calculate equation and R^2 value which will aid in calculating IC_{50} values (mentioned in Graphs 1 and 2). ABTS assay displayed almost equally free radical scavenging activity for Sample 1 and 2 in comparison with standard ascorbic acid is displayed in Graph 3.

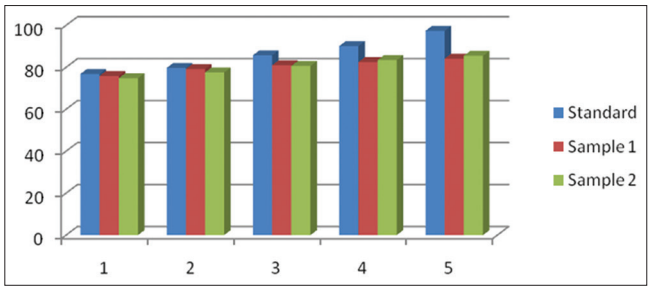
FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method. At low pH, reduction Fe III TPTZ complex in the ferrous form that has an intense blue color can be monitored by measuring the change in absorption at 593 ηm (Table 2). The change in absorbance therefore relates to the total reducing power of the electron-donating antioxidants present in the reaction mixture. Here in this assay, the Sample 1 and 2 are compared with the standard ascorbic acid (Graphs 4 and 5). The Sample 1 revealed slightly higher antioxidant properties in the concentration of 10, 40 and 50 mg, whereas the Sample 2 shown better antioxidant properties in the concentrations of 20 and 30 mg (Graph 6). These sponges exhibited almost equivalent percentage inhibition when compared to standard. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. The IC50 value for Sample



Graph 1: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay graph in comparison with standard and Sample 1



Graph 2: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay graph in comparison with standard and Sample 2



Graph 3: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay bar diagram in comparison with standard and Sample 1

1 has an inhibitory concentration of 67.587 µg, for Sample 2 it has 74.57 µg.

The free radical scavenging activity of the extract is measured by DPPH assay. The absorbance was measured at 517 ηm for the five different sample concentrations (Table 3). Using the absorbance value, the percentage of inhibition was calculated. The percentage inhibition for Sample 1 and 2 is equivalent on comparison with the standard ascorbic acid (Graphs 7 and 8). The Sample 1 revealed slightly higher antioxidant properties in the concentrations of 300, 400 and 500 µl, whereas the Sample 2 shown better antioxidant properties in the concentrations of 100 and 200 µl (Graph 9). The IC_{50} value for Sample 1 is 655.49 µl for Sample 2 it has 826.739 µl.

Table 1: ABTS antioxidant activity for standard, Sample 1 and Sample 2

Sample concentration (µg)	ABTS radical scavenging ability (%)					
	Sample 1		Sample 2		Standard (ascorbic acid)	
	OD	% Inhibition	OD	% Inhibition	OD	% Inhibition
Control	0.730	0	0.730	0	0.730	0
62.5	0.176	75.89	0.184	74.79	0.169	76.85
125	0.152	79.18	0.164	77.53	0.148	79.73
250	0.139	80.96	0.141	80.68	0.104	85.75
500	0.128	82.47	0.121	83.42	0.072	90.14
1000	0.116	84.11	0.106	85.48	0.019	97.40

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), OD: Optical density

Table 2: FRAP antioxidant activity for standard, Sample 1 and Sample 2

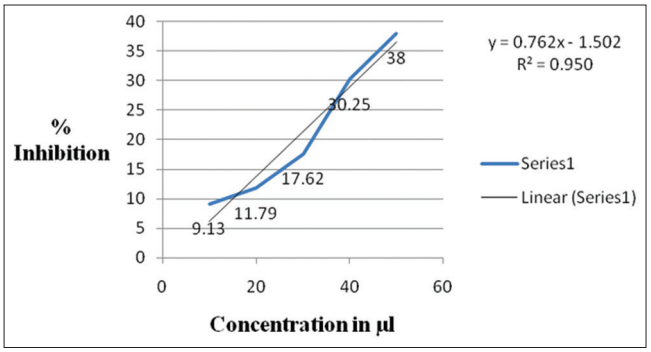
Standard concentration 0.1 mM	Sample in mg W/V	Standard OD at 593	Sample 1 OD at 593	Sample 2 OD at 593	STD % inhibition	FRAP assay for Sample 1 (% Inhibition)	FRAP assay for Sample 2 (%Inhibition)
0.1	10	0.842	0.856	0.871	10.61	9.13	7.54
0.2	20	0.802	0.831	0.741	14.86	11.79	21.34
0.4	30	0.76	0.776	0.767	19.32	17.62	18.58
0.6	40	0.641	0.657	0.672	31.96	30.25	28.66
0.8	50	0.542	0.584	0.612	42.47	38.00	35.03

OD: Optical density, FRAP: Ferric reducing antioxidant power

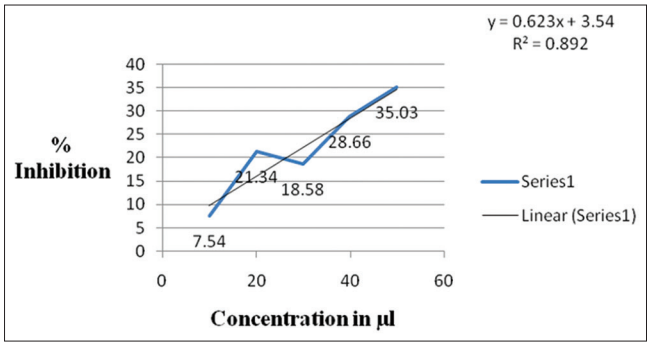
Table 3: DPPH antioxidant activity for standard, Sample 1 and Sample 2

DPPH control at 0.928							
Standard concentration µg/ML	Sample in µl/in ml of DMSO	Standard OD at 517	Sample 1 OD at 517	Sample 2 OD at 517	STD % of inhibition	DPPH assay 1	DPPH assay 2
10	100	0.802	0.810	0.796	13.58	12.71	14.22
20	200	0.740	0.775	0.768	20.25	16.48	17.24
30	300	0.636	0.681	0.712	31.46	26.61	23.27
40	400	0.613	0.650	0.653	33.95	29.95	29.63
50	500	0.525	0.550	0.620	43.42	40.73	33.18

DPPH: 2,2-diphenyl-1-picrylhydrazyl, DMSO: Dimethyl sulfoxide, OD: Optical density



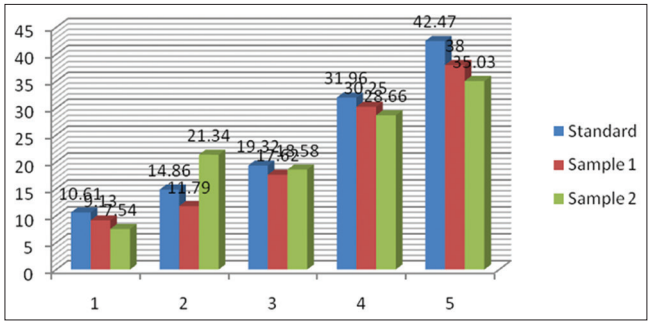
Graph 4: Ferric reducing antioxidant power assay graph in comparison with standard and Sample 1



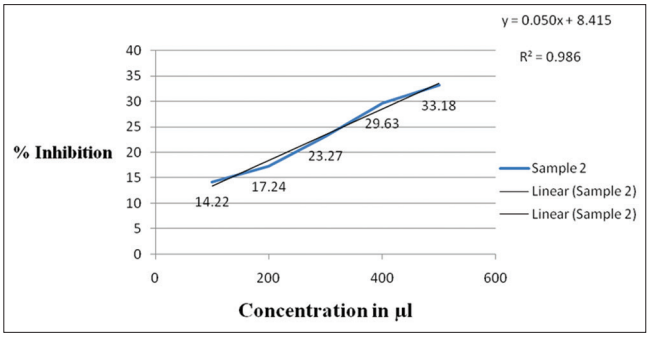
Graph 5: Ferric reducing antioxidant power assay graph in comparison with standard and Sample 2

DISCUSSION

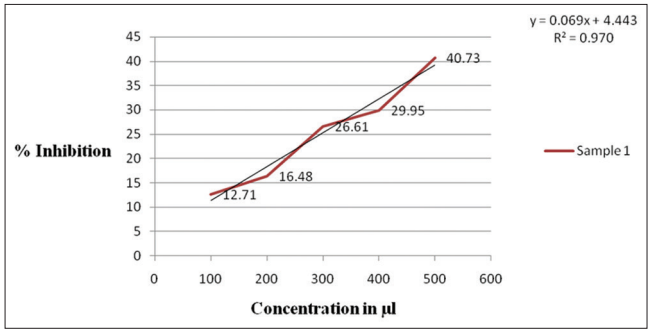
Sponges which are considered to be a great source of novel bioactive compounds serves as a point of interest among researchers [25]. Experiments on sponges raised, which indicates an excellent source for antioxidant, antibacterial, antifungal, anti-inflammatory, and cytotoxicity activities [4,26-32]. Of all, antioxidants are the most admirable one because of free radical scavenging activity as it also serves in treating against cancer, aging and atherosclerosis [33-36]. The extract C. baccifera showed good antioxidant potential also exhibited noticeable cytotoxic activity against Ehrlich ascites carcinoma and HT-29 cell lines. The extract showed a negligible cytotoxic effect against MCF-7 cell lines [37]. The purified L-asparaginase showed good antioxidant activity on DPPH assay and provided a potential anticancer activity against MCF-7 cell line and should be considered for further pharmaceutical use as anticancer agents [38]. The results clearly demonstrate seaweeds *Ulva lactuca* and *Eucheuma cottonii* as promising candidates of new anti breast and anticolorectal cancer agents [39]. Pigmented rice serves as a good sources of antioxidant compounds, red rice varieties showed higher antioxidant properties and has health promoting properties as well as anti-cancer properties [40]. Studies on tropical fruit by-products shown good sources of the antioxidant compound which could be used in the pharmaceutical, food, and feed industries [41].



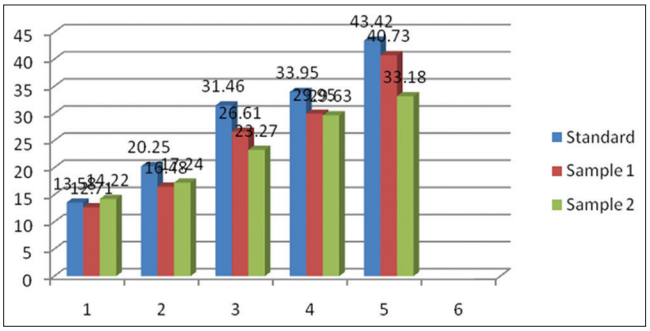
Graph 6: Ferric reducing antioxidant power assay bar diagram in comparison with standard and Sample 1 and 2



Graph 8: 2,2-diphenyl-1-picrylhydrazyl assay graph in comparison with standard and Sample 2



Graph 7: 2,2-diphenyl-1-picrylhydrazyl assay graph in comparison with standard and Sample 1



Graph 9: 2,2-diphenyl-1-picrylhydrazyl assay bar diagram in comparison with standard and Sample 1 and 2

In reviewing, it is evident that the biosource which has potent antioxidant activities can be very fruitful in treating against cancer, after gaining that idea we can better predict that these sponges may also fight against cancer.

CONCLUSION

On analyzing, the result of ABTS assay both these sponges, i.e., *D. herbacea* and *Sigmadocia pumila* exhibited almost equal antioxidant properties in comparison with a standard, While calculating the IC_{50} value for DPPH assay; the Sample 1 has an inhibitory concentration of 655.49 µl, for Sample 2 it has 826.739 µl, and for FRAP assay the Sample 1 has an inhibitory concentration of 67.587 µg, for Sample 2 it has 74.57 µg. Overall from this assay, *Dysidea herbacea* resulted slightly better antioxidant activity when compared to *S. pumila,* also which in the future, both these sponges may serve as a better source to fight against various diseases.

ACKNOWLEDGMENT

This research was done with the help of Dr. Sivaleela, Scientist from Zoological Survey of India for helping in taxonomic identification of the sponge samples.

REFERENCES

1. Higa T, Tanaka JI, Kitamura A, Koyama T, Takahashia M, Uchida T. Bioactive compounds from marine sponges. Pure Appl Chem 1994;66:2227-30.
2. Haefner B. Drugs from the deep: Marine natural products as drug candidates. Drug Discov Today 2003;8(12):536-44.
3. Belarbi H, Contreras Gómez A, Chisti Y, García Camacho F, Molina Grima E. Producing drugs from marine sponges. Biotechnol Adv 2003;21(7):585-98.
4. Sipkema D, Franssen MC, Osinga R, Tramper J, Wijffels RH. Marine sponges as pharmacy. Mar Biotechnol (NY) 2005;7(3):142-62.
5. Faulkner DJ. Marine natural products. Nat Prod Rep 2000;17(1):7-55.
6. Faulkner DJ. Marine natural products. Nat Prod Rep 2001;18(1):1-49.
7. Faulkner DJ. Marine natural products. Nat Prod Rep 2002;19(1):1-48.
8. Tramper J. Cultivation of marine sponges. Detmer Sipkema. The Netherlands: Process Engineering Group, Wageningen University; 1999. p. 184.
9. Aneiros A, Garateix A. Bioactive peptides from marine sources: Pharmacological properties and isolation procedures. J Chromatogr B Analyt Technol Biomed Life Sci 2004;803(1):41-53.
10. Müller WE, Schröder HC, Wiens M, Perovic-Ottstadt S, Batel R, Müller IM. Traditional and modern biomedical prospecting: Part II-the benefits: Approaches for a sustainable exploitation of biodiversity (secondary metabolites and biomaterials from sponges). Evid Based Complement Alternat Med 2004;1(2):133-44.
11. Thakur NL, Hentschel U, Krasko A, Anil AC, WE Müller. Antibacterial activity of the sponge suberites domuncula and its primmorphs: Potential basis for chemical defense. Aquat Microbial Ecol 2003;31:77-83.
12. Mandal S, Yadav S, Yadav S, Nema RK. Antioxidants: A review. J Chem Pharm Res 2009;1(1):102-4.
13. Tiwari AK. Antioxidants: New - Generation therapeutic base for treatment of polygenic disorders. Curr Sci 2004;86:1092-102.
14. Shinde A, Ganu J, Naik P. Effect of free radicals and antioxidants on oxidative stress. J Dent Allied Sci 2012;1:63-6.
15. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979;59(3):527-605.
16. de Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology 1994;41(4):328-32.
17. Nakazawa H, Genka C, Fujishima M. Pathological aspects of active oxygens/free radicals. Jpn J Physiol 1996;46(1):15-32.
18. Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int 1999;49(2):91-102.
19. Warier VP. Indian Medicinal Plants: A Compendium of 500 Species, I. Kottakkal-India: Orient Longman Publishing House; 2002. p. 146.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
21. Benzie FF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999;299:15-23.
22. Ahmed M, Saeed F, Mehjabeen, Jahan N. Evaluation of insecticidal and antioxidant activity of selected medicinal plants. J Pharm Phytochem 2013;2(3):153-8.

23. Patel RM, Patel NJ. *In vitro* antioxidant activity of coumarin compounds by DPPH, superoxide and nitric oxide free radical scavenging methods. J Adv Pharm Educ Res 2011;1:52-68.
24. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal 2002;13(1):8-17.
25. Bhakuni DS, Rawat DS. Bioactive Marine Natural Products. New Delhi, India: Springer and Anamaya Publishers; 2005.
26. Newbold RW, Jensen PR, Fenical W, Pawlik JR. Antimicrobial activity of caribbean sponge extracts. Aquat Microbial Ecol 1999;19:279-84.
27. Faulkner DJ. Highlights of marine natural products chemistry (1972-1999). Nat Prod Rep 2000;17(1):1-6.
28. Rangel M, Sanctis B, Freitas JC, Polatto JM, Granato AC, Berlinck RG, *et al*. Cytotoxic and neurotoxic activities in extracts of marine sponges from southeastern Brazilian coast. J Exp Biol Ecol 2001;262(1):31-40.
29. Touati I, Chaieb K, Bakhrouf A, Gaddour K. Screening of antimicrobial activity of marine sponge extracts collected from Tunisian coast. J Med Mycol 2007;17(3):183-6.
30. Ferreira M, Cabado AG, Chapela MJ, Fajardo P, Atanassova M, Garrido A, *et al.* Cytotoxic activity of extracts of marine sponges from NW Spain on a neuroblastoma cell line. Environ Toxicol Pharmacol 2011;32(3):430-7.
31. Lakshmi V, Mishra SK, Srivastava S, Chaturvedi A, Srivastava MN, Shukla PK. Antifungal activity of marine sponge haliclonaexigua (krikpatrick). J Med Mycol 2010;20:31-3.
32. Li H, Shigeki S, Fusetani N. Simple antifungal metabolites from a marine sponge, *Halichondria* sp. Comp Biochem Physiol B Comp Biochem 1994;107(2):261-4.
33. Chairman K, Ranjit Singh AJ, Alagumuthu G. Cytotoxic and antioxidant activity of selected marine sponges. Asian Pac J Trop Dis 2012;2:234-8.
34. Wickens AP. Ageing and the free radical theory. Respir Physiol 2001;128(1):379-91.
35. Ozyürek M, Bektasoglu B, Güçlü K, Apak R. Hydroxyl radical scavenging assay of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method using catalase for hydrogen peroxide degradation. Anal Chim Acta 2008;616(2):196-206.
36. Halliwell B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet 1994;344(8924):721-4.
37. Rajani P, Kotaiah MR, Jayaveera KN, Sekhar KB. Evaluation of antioxidant and anticancer activities of cipadessa baccifera. Asian J Pharm Clin Res 2015;8(5):312-5.
38. Rani SA, Sundaram L, Vasantha PB. *In vitro* antioxidant and anticancer activity of l-asparaginase from *Aspergillus flavus* (kufs20). Asian J Pharm Clin Res 2011;4 Suppl 2:174-7.
39. Arsianti AA, Fadilah F, Yazid F, Wibisono LK, Suid K, Azizah NN, *et al.* Phytochemical composition and anticancer activity of seaweeds ulva lactuca and *Eucheuma cottonii* against breast Mcf-7 and colon Hct-116 cells. Asian J Pharm Clin Res 2016;9(6):115-9.
40. Vichit W, Saewan N. Antioxidant activities and cytotoxicity of thai pigmented rice. Int J Pharm Pharm Sci 2015;7(7):329-4.
41. Fitria A, Andriania M, Sudarmanb A, Toharmatb T, Yonekurac L, Tamurac H, *et al*. Screening of antioxidant activities and their bioavailability of tropical fruit byproducts from Indonesia. Int J Pharm Pharm Sci 2016;8(6):96-100.