Research Article

Investigation of Bio-Active Compound and *In-vitro* Antioxidant Activity of *Spirastrella pachyspira*

Shabna Roupal Morais*, K Chitra
Sri Ramachandra University, Chennai, Tamilnadu, India.
***Corresponding author's E-mail:** roupal_prabhu@yahoo.com

Accepted on: 26-04-2016; **Finalized on:** 31-05-2016.

ABSTRACT

This paper was designed to study the antioxidant potential of the marine boring sponge *Spirastrella pachyspira* and to screen the compounds for its bio active component. The extracts of increasing polarity were prepared and they were subjected for DPPH scavenging activity and Nitric oxide scavenging activity, out of the four extracts that is hexane, chloroform, ethyl acetate and methanol, the ethyl acetate extract showed a promising activity followed by the hexane extract. The HPTLC fingerprint of all the four extracts were recorded and the phytochemical screening showed the presence of terpenoids, steroids, carbohydrates and amino acids.

Keywords: *Spirastrella pachyspira,* Scavenging activity, marine sponge, Hptlc

INTRODUCTION

Marine sponges for medicinal use were dated back to 2000 years where the marine sponge dipped in vinegar was given to Jesus Christ to relieve his pain on the cross. Marine sponges belonging to the Phylum Porifera, evolutionary the oldest animal are the best source of marine compounds and natural products. Ocean occupies 70% of the earth and contains a large number of marine organisms out of which 15,000 species are marine sponges capable of producing a large number of bioactive compounds there are about.

This study focuses on identifying the various chemicals present in different extracts and to assess the antioxidant potential of the extract by Nitric oxide scavenging technique and Diphenyl Picryl Hydrazyl radical method.

MATERIALS AND METHODS

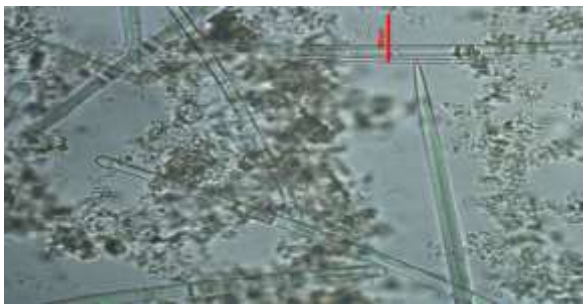
The sponges were collected from the intertidal zones at the coast near Rameswaram. The collected sponges were repeatedly washed with sea water to remove the debris and it with fresh water to remove the impurities. The washed sponges were freezed at a temperature below 4°C and transported to the lab. The sponges were thawed at room temperature and incised into small pieces and soaked in solvent of increasing polarity like hexane, chloroform, ethylacetate and methanol.

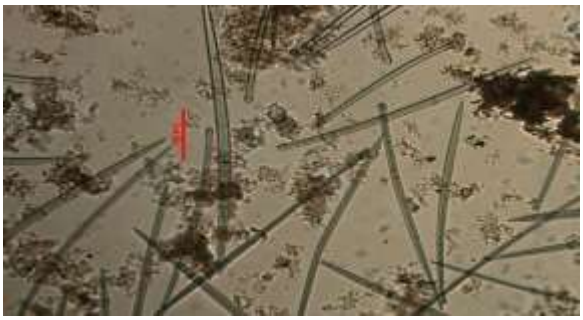
The extraction was carried out until complete exhaustion of constituents. The extract was filtered through Whatmann filter paper (No: 2) and concentrated using vacuum rotary evaporator. The concentrated extract was stored in dessicators for further studies.

Identification and Authentication

The fresh sponges were immediately stored in ethanol for identification. The sponge was identified, registered and deposited in MBRC/ZSI, Chennai by Dr G. Sivaleela Scientist Zoological survey of India with the registration number S-261.

A small portion of the sponge was cut and put inside a boiling test tube to which dil nitric acid was added. The solution was boiled and kept aside. The spicules were allowed to settle down and the spicules were viewed under a high end electron microscope. The spicules showed the presence of the following tylostyles and spirasters. The tylostyles was straight with a well developed globular or roimded head. Tips sharply pointed or rarely blunt (6%). There are four different type of spirasters they are (a) small with curved shaft; size0.016mm average (b) typical slender spirasters size 0.042X0.004mm (c) robust spirasters with blunt spines size 0.050X0.016mm and (d) branched spirasters plate like size 0.105X0.063mm. The above parameters identified the sponge as *Spirastrella pachyspira*[1].





Preliminary Screening of Sponges for Chemical Constituents[2]

The freshly prepared sponge extracts were analyzed for the presence of various constituents as described by Harborne (1998).

Table 1: Preliminary screening of sponges for chemical constituents

Test	Observation	Inference
Test for Steroids: To the extract add a minimum amount of $CHCl_3$and three drops of acetic anhydride and two drops of Conc. sulphuric acid	Purple colour solution changing to blue or green	presence of steroids
Test for Triterpenoids To the extract and a piece of tin and three drops of thionyl chloride	Violet or purple colour solution	presence of triterpenoids
Test for Reducing sugar Cold extract+equal volume of Fehling A andB and heat it over a water bath	Red precipitate	presence of reducing sugars
To the extract add Molisch reagent	A violet coloured ring at the junction of two liquids	
Test For Alkaloids To the extract add 2N HCl and 2 drops of mayers reagent	Pale yellow or white precipitate	shows the presence of alkaloids
To the extract add acetic acid and few drops of dragendorffs reagent	Orange or red orange precipitate	
Test for Phenolic Compound To the extract add neutral ferric chloride	An intense blue or violet coloration	shows the presence of phenolic compounds
Test for Saponins To the extract add water and shake vigorously	Formation of foamy layer	shows the presence of saponins
Test for Xanthoproteins To the extract add concentrated nitric acid and excess ammonia	Red orange precipitate	shows the presence of xanthoproteins
Test for Tannins To the extract add basic lead acetate	White precipitate	shows the presence of tannins
Test for Flavonoids To the extract add bit of Mg^{2+}, drops of conc Hcl. Heat and then cool	Red or orange red colour	shows the presence of flavonoids
Test for Aromatic Acids To the extract add saturated sodium bicarbonate	Brisk effervescence	shows the presence of aromatic acids

HPTLC Fingerprint Analysis of the Extracts of *Spirastrella pachyspira*[3]

The chromatographic finger print of hexane, chloroform, ethyl acetate and methanolic extracts of *Spirastrella pachyspira* were studied by HPTLC

Preparation of the sample

The extracts 5mg/ml was dissolved in respective chromatographic grade solvents. The solution is filtered through whatmann filter paper.

The prepared samples of all the four extracts were applied on TLC aluminum silica gel 60F254 sheet. Linomat5 sample applicator is used to apply 4µl sample of each extract of a band length of 6mm.

Developing Solvent System

To get better resolution and maximum number of spots different solvent systems were tried for the successive extracts.

The Toluene: Ethyl acetate: Ammonia in the ratio of 6:3.5:0.5 solvent system for hexane extract and chloroform extract, to get different spots and Hexane: Toluene: Glacial acetic acid solvent system in the ratio of 3:6:1 for ethyl acetate extract, and methanol extract.

Scanning and detection of spots

The bands were scanned using TLC scanner at 256nm.

Nitric Oxide Scavenging Activity

The aqueous solution of sodium nitroprusside at physiological pH spontaneously generates nitric oxide

(NO), which in turn reacts with oxygen to produce nitrate ions which was measured using a spectrophotometer. To 1ml of increasing concentrations of the extracts of *Spirastrella pachyspira* (50-1000µg/ml) 2ml of sodium nitroprusside, (10mM) in phosphate buffered saline (PBS) was added and incubated at 37°C for 4 hours. 0.5mL of Griess reagent was added after incubation. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of extracts. Ascorbic was used as standard.

% Inhibition was calculated using the following formula

$$\%\ Inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} \times 100$$

DPPH Scavenging activity

A stock solution of 3.9mg DPPH (µM) (1,1-diphenyl-2-picryl hydrazine) was prepared in 100ml of ethanol. To 1ml of extracts of *Spirastrella pachyspira* (50, 100, 200, 400, 600, 800, 1000µg/ml) in DMF (dimethyl formamide), 1ml of DPPH in ethanol was added. Control was prepared without test compound and ethanol was used as blank. The reaction was allowed to complete by keeping in dark for 20mins and absorbance was read at 517nm. Ascorbic acid was used as standard.

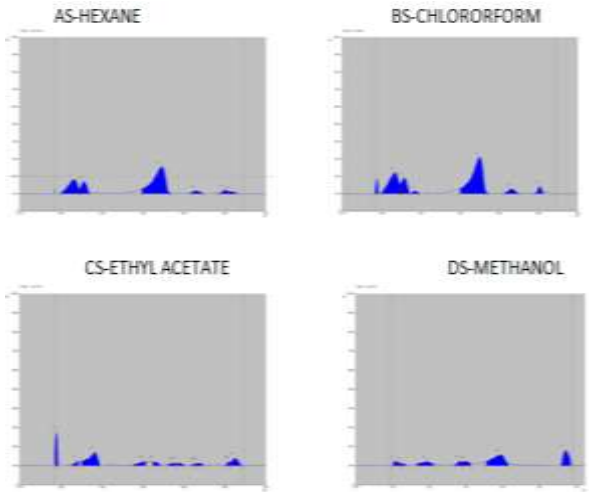
$$\%DPPH\ Inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} \times 100$$

RESULTS AND DISCUSSION

Preliminary screening of *Spirastrella pachyspira* for its chemical constituents

Table 2 shows the presence of the chemical components.

The table 2 indicates that terpenoids and proteins and amino acids are present in hexane and chloroform extracts, steroids and carbohydrates are present in all the three extracts except the methanolic extract and flavonoids, anthraquinones, alkaloids, quinone, phenol, tannins saponins are absent in all the extracts.



The HPTLC fingerprint of hexane, chloroform, ethyl acetate and methanolicextract of *Spirastrella pachyspira* were studied and the fingerprint showed the showed the presence of the number of compounds which can be bioactive.

Anti-Oxidant Activity

Nitric Oxide Radical Scavenging Activity

Nitric oxide a diffusible free radical has different mechanisms which affect the biological activity. The chronic exposure of nitric oxide radical leads to various carcinomas, inflammatory conditions such as arthritis, multiple sclerosis and ulcerative colitis. *In vitro* quenching of NO radical is one of the methods that can be used to determine antioxidant activity. The procedure is based on the principle that at physiological pH aqueous solution of sodium nitroprusside spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. The extracts of *S. pachyspira* were evaluated for their possible regulatory effect on Nitric oxide (NO) levels using sodium nitro prusside as a donor *in-vitro.*

Nitrite radical scavenging assay was carried out on the hexane, chloroform, ethyl acetate and methanolic extracts of *S.pachyspira* from a concentration of 100 to 1000 µg/ml. The extracts exhibited antioxidant activity through competing with oxygen to scavenge for the nitrite radical which was generated from Sodium nitro prusside at physiological pH in an aqueous environment. The antioxidant activity increased with an increase in concentration of the extracts as exhibited in fig 2. The ethyl acetate extract of *S.pachyspira* was the most potent as it removed the nitrite radical at a lower concentration as compared to the other extracts. The ethyl acetate extract had an 80.38% scavenging activity at 1000 µg/ml which showed it to be a more potent extract when compared to other extracts when compared against the standard ascorbic acid.

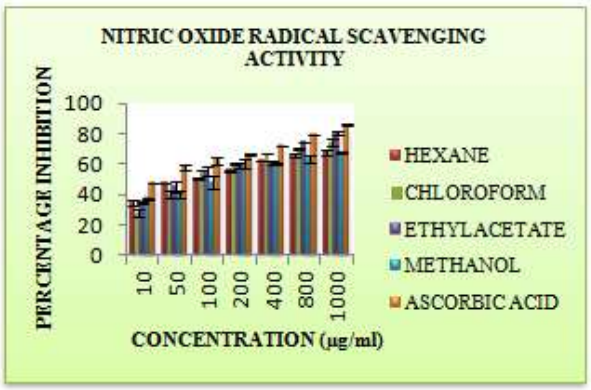


Figure 1: Nitric Oxide Radical Scavenging Activity

Table 2: Preliminary screening of *Spirastrella pachyspira* for its chemical constituents

Name of the extract	Terpenoids	Flavonoids	Steroids	anthraquinones	Carbohydates	Alkaloids	Quinone	Phenol	Tannins	Saponins	Proteins and amino acids
Hexane	Present	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present
Chloroform	Present	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present
Ethyl acetate	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent
Methanol	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

The HPTLC fingerprints showed the presence of the following components

Table 3: HPTLC fingerprints

Parameters	Hexane-AS	Chloroform-BS	Ethyl acetate-CS	Methanol-DS
No of components	6	7	11	7
Rf values	0.15 Rf 0.20 Rf 0.60 Rf 0.75 Rf 0.88 Rf 0.92 Rf	0.04 Rf 0.15 Rf 0.20 Rf 0.25 Rf 0.60 Rf 0.76 Rf 0.88 Rf	0.05 Rf 0.14 Rf 0.25 Rf 0.47 Rf 0.54 Rf 0.62 Rf 0.66 Rf 0.72 Rf 0.76 Rf 0.89 Rf 0.94 Rf	0.06 Rf 0.09 Rf 0.24 Rf 0.40 Rf 0.44 Rf 0.64 Rf 0.98 Rf
Area%	22.87% 11.25% 57.35% 3.41% 3.60% 1.52%	3.25% 25.68% 11.07% 1.49% 51.10% 4.09% 3.33%	17.55% 4.67% 35.78% 8.11% 5.20% 4.07% 3.50% 2.11% 2.91% 3.58% 12.52%	6.22% 2.32% 12.44% 6.17% 7.13% 41.16% 24.56%

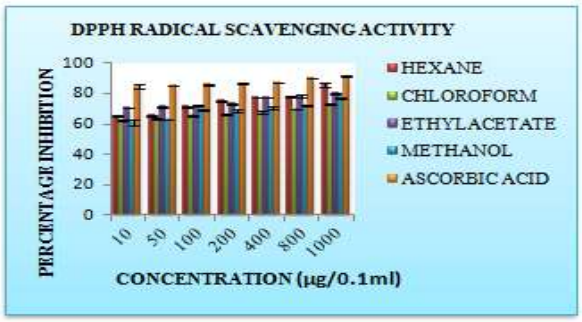


Figure 2: DPPH Radical Scavenging activity

DPPH Radical Scavenging activity

DPPH radical unlike other free radicals such as the hydroxyl radical and superoxide anion has the advantage of being unaffected by certain side reactions such as metal ion chelation and enzyme inhibition and also that DPPH is a stable synthetic radical that does not disintegrate in water, methanol and ethanol. The DPPH solution at its absorption maximum of 517nm exhibit a deep purple colour but in the presence of an antioxidant the purple colour fades or disappears. The four extracts of *S.pachyspira* shows free radical scavenging property

(fig 2) at all the seven concentration (10µg/ml-1000µg/ml). There was a reduction in the concentration of DPPH due to the scavenging ability of the extracts of *S.pachyspira*. At the highest concentration the scavenging ability of the hexane, chloroform, ethyl acetate and methanolic extract is as follows 85.72%, 73.17%, 80.41% and 77.16% when compared with standard ascorbic acid of 91.44%. When compared with other extracts hexane extract and ethyl acetate extract showed a promising activity when compared with that of the standard.

CONCLUSION

This study is the first report on the antioxidant activity exhibited by the different extracts of *Spirastrella pachyspira* by DPPH assay and nitric oxide scavenging activity. The chemical identification and HPTLC fingerprint reveals that terpenoids, steroids, proteins and amino acids might be responsible for the antioxidant activity. The hexane and ethyl acetate fraction showed promising antioxidant activity.

Acknowledgement: The authors would like to thank Dr Sivaleela Scientist-C Zoological survey of India for the authentication of sponges.

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Source of Support: Nil, **Conflict of Interest:** None.