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IN-VITRO ANTIOXIDANT AND ANTILIPOLYTIC ACTIVITY OF MARINE ACTINOBACTERIA ISOLATED FROM PULICAT LAKE, TAMILNADU, INDIA

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ABSTRACT

The present study is undertaken to investigate antioxidant and pancreatic lipase inhibitory activity of ethyl acetate extract of actinobacteria isolated from sediment, soil samples of Pulicat Lake, Tamil Nadu, India. The Pancreatic Lipase Inhibitory activity of solvent extract was tested against Pancreatic Lipase enzyme using olive oil as the substrate. To evaluate the antioxidant activity the broth culture of the production medium was used for fermentation process and extracellular metabolites were extracted using the solvent ethyl acetate. The brown color extract obtained was dissolved in methanol and screened for DPPH radical scavenging activity. Out of the 110 isolates tested more than 50% of actinobacteria showed both antioxidant and antilipase activity. The findings of the present study suggested that the metabolites of marine actinobacteria isolated from Pulicat Lake, Tamil Nadu, India may be useful as antioxidants and anti-obesity agents.

KEYWORDS: **Antioxidant, Marine actinobacteria, Olive Oil, Pancreatic Lipase Inhibition, Pulicat Lake.**

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INTRODUCTION

Evolution of life in oceans emerged about 3.5 billion years ago and microbes were the only form of life for two thirds of the planet's existence. Marine environments are basically unexploited sources for the isolation of new micro-organisms with the potential to produce chemically diverse compounds with an extensive range of biological activities. The ocean floor has been recently verified as an ecosystem with many unique forms of actinobacteria [1-2].Among the different microorganisms actinobacteria are undoubtedly the sizeable producers of secondary metabolites. Marine actinobacteria have shown much evidence as a valuable source of various novel bioactive compounds and have the potential to be developed as therapeutic agents[3-4].A number of structurally unique natural products with antitumor [5-6] anti-infective [7]and antimalarial [8] bioactivities have been revealed from marine derived actinobacteria.Free radicals are the routine outcome of normal aerobic cellular metabolism. Despite oxygen is imperative for life, imbalanced metabolism and excess reactive oxygen species (ROS) production end into a range of disorders such as cancer, heart disease, decline in brain function and immune system. Modern research is now heading towards natural antioxidants from plants and microorganisms which serve as safe therapeutics [9]. Recent studies have focused on the response of the antioxidant system of bacteria such as *Streptomyces* growth in various oxidative stress conditions [10].*Nocardiopsis* species are one of the actinobacteria which produce different types of pharmacological compounds with antioxidant, antitumor, anti-inflammatory, antibacterial and antioxidant properties. Lipase inhibitors are substances used to decrease the activity of lipases. The prime function of lipase inhibitors is to reduce the gastrointestinal absorption of fats. These inhibitors could be used for the treatment of obesity, which can therefore lead to Type II diabetes and cardiovascular diseases if not managed. Lipase inhibitors bind to lipase enzymes in the intestine, [11] thus preventing the hydrolysis of dietary triglycerides into monoglycerides and fatty acids [12]. When correlated to the enzyme inhibitors from plants and animal, the microbial inhibitors seize low molecular weight compounds derived from hydrolysis of macromolecular substances [13].The motto of the present investigation describes isolation of marine actinobacteria from Pulicat Lake, and their screening for antioxidant and pancreatic lipase enzyme inhibitory activities.

MATERIALS AND METHODS

(i) Microorganisms

Altogether, 110 actinobacterial isolates were already being isolated using five different media such as Starch Casein Agar (SCA), Potato Dextrose Agar (PDA), Bennet's Agar, Yeast Extract Malt Extract Agar (ISP2) and Glycerol Aspargine Agar (ISP5) from 20 different sediment samples collected from different locations in and around Pulicat Lake, Tamil Nadu, India.

(ii) Fermentation and extraction of secondary metabolites

All the marine actinobacteria were grown in production medium [14]. A total volume of 100 ml of 7-days old cell free supernatant was extracted individually with equal volume of ethyl acetate, resulted organic layer was subjected to evaporation using the rotoevaporator (Buchi, India). After the evaporation of solvent free, the crude extract was kept under refrigeration until further use.

(iii) Antilipase activity of crude extract

Porcine Pancreatic Lipase was purchased Sigma Aldrich (Sigma Aldrich, Bangalore, India). Olive oil amended with rhodamine B agar was used for the detection of lipase inhibition activity. Two units of lipase were dissolved in 1 ml of Tris-HCl buffer (pH 8), the sterilized agar was poured on a Petri plate and 5 mm well was made using sterile cork borer. Different concentration of crude extract was filled in wells separately along with 50 µl prepared lipase enzyme. After 24 h of incubation the plates were seen under UV illumination. The size of the zone of clearance was measured and photographed.

(iv) Free radical scavenging ability by the use of a stable DPPH radical (1, 1-diphenyl-2-picrilhydrazyl)

The effect of the marine actinobacterial crude extracts on DPPH radical was estimated according to the procedure described by Von Gadow[15]. Two ml of 6 $\times 10^{-5}$ M methonolic solution of DPPH were added to 50 µl of a methonolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 minutes at room temperature. Methanolic solutions of crude extract and pure compound [quercetin] were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 minutes duration as follows:All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh[16].

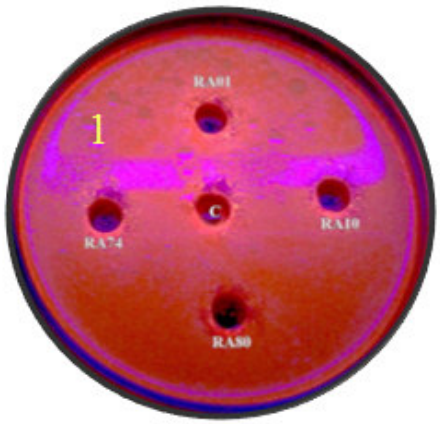
$$IP = [(A_{C\,(0)} - A_{A\,(t)} / A_{C\,(0)})] \times 100$$

Where $A_{C\,(0)}$ is the absorbance of the control at t = 0 minutes; and $A_{A\,(t)}$ is the absorbance of the antioxidants at t = 16 minutes.

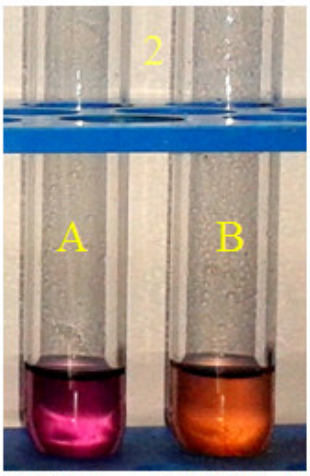
RESULTS

1. Pancreatic Lipase enzyme inhibitory activity of solvent extract

The inhibitory activity of ethyl acetate extract of marine actinobacteria was tested against Pancreatic Lipase enzyme using olive oil as the substrate. It was observed that 50% of the isolates showed lipase activity in primary screening and secondary screening. The inhibitory activity was increased when increasing the concentration of extracts. These results indicated that the inhibition of the enzyme was found to be dose dependent (Fig. 1 (1); Tables: 1& 2).



[1] Pancreatic Lipase Inhibition Assay



[2] (A) Control (B) DPPH Activity

Figure 1
(1) *Pancreatic Lipase Inhibition assay (2) Antioxidant activity by DPPH*

Table 1
Preliminary screening of marine actinobacteria for Anti-lipase Activity by Pancreatic Lipase Inhibition assay

Isolate code	Total Lipase activity
RA01	16.3±0.3
RA02	41.2±0.3
RA03	-
RA04	-
RA05	-
RA06	49.6±0.5
RA07	-
RA08	58.1±0.2
RA09	33.1±0.2
RA10	33.2±0.2
RA11	41.4±0.2
RA12	49.9±0.2
RA13	-
RA14	49.8±0.2
RA15	49.9±0.3
RA16	49.7±0.3
RA17	33.0±0.2
RA18	49.8±0.4
RA19	33.1±0.2
RA20	-
RA21	-
RA22	41.3±0.4
RA23	49.8±0.2
RA24	41.2±0.3
RA25	-
RA26	58.0±0.2
RA27	16.3±0.3
RA28	-
RA29	66.6±0.1
RA30	74.9±0.2
RA31	75.0±0.2
RA32	41.4±0.4
RA33	-
RA34	49.9±0.2
RA35	41.6±0.1
RA36	-
RA37	-
RA38	33.3±0.2
RA39	16.5±0.2
RA40	33.3±0.2
RA41	41.5±0.2
RA42	25±0.1
RA43	75.03±0.2
RA44	41.5±0.3
RA45	16.63±0.2

RA46	41.6±0.2
RA47	66.2±0.3
RA48	49.9±0.2
RA49	41.2±0.3
RA50	41.2±0.4
RA51	-
RA52	58.1±0.2
RA53	-
RA54	33.0±0.3
RA55	66.2±0.4
RA56	-
RA57	49.7±0.3
RA58	-
RA59	-
RA60	16.2±0.4
RA61	58.0±0.3
RA62	33.0±0.3
RA63	33.2±0.3
RA64	-
RA65	33±0.3
RA66	49.7±0.4
RA67	49.9±0.4
RA68	41.3±0.4
RA69	-
RA70	74.7±0.3
RA71	66.2±0.4
RA72	58.0±0.3
RA73	41.2±0.5
RA74	33.0±0.3
RA75	-
RA76	49.9±0.4
RA77	58.2±0.4
RA78	-
RA79	41.0±0.5
RA80	33.2±0.4
RA81	49.8±0.4
RA82	41.0±0.6
RA83	41.1±0.5
RA84	33.2±0.4
RA85	-
RA86	58.4±0.3
RA87	-
RA88	58±0.4
RA89	24.8±0.2
RA90	66.1±0.4
RA91	58±0.3
RA92	41.2±0.4
RA93	-
RA94	41.1±0.5
RA95	-
RA96	33.1±0.4
RA97	-
RA98	16.2±0.4
RA99	41.4±0.4
RA100	49.9±0.1
RA101	-
RA102	-
RA103	25.0±0.2
RA104	75.6±1.0
RA105	50.1±0.2
RA106	16.3±0.3
RA107	33.1±0.2
RA108	66.1±0.4
RA109	25 ± 0.1
RA110	41.2±0.4

(-) No Activity
**** Values are mean of triplicates with standard deviation***

Table 2
Secondary screening of marine actinobacteria for Anti-lipase Activity by Pancreatic Lipase Inhibition assay

Isolate code	Total Lipase activity
RA03	50±0.2
RA13	49.9±0.4
RA20	41.4±0.2
RA21	50.0±0.3
RA28	50±0.3
RA36	41.3±0.3
RA38	50.1±0.2
RA43	16.5±0.3
RA47	33.2±0.3
RA48	49.9±0.1
RA51	49.9±0.4
RA57	33.3±0.2
RA58	33.2±0.2
RA64	33.1±0.3
RA66	41.5±0.3
RA68	50.2±0.2
RA75	33.4±0.2
RA78	41.3±0.3
RA86	41.4±0.2
RA87	41.5±0.4
RA89	50.1±0.2
RA90	41.5±0.5
RA92	49.9±0.2
RA93	49.8±0.2
RA95	50±0.2
RA96	-
RA97	33.3±0.2
RA98	41.4±0.2
RA99	50±0.2
RA101	33.4±0.1
RA102	33.2±0.1

(-) No Activity
**** Values are mean of triplicates with standard deviation***

2. DPPH scavenging activity

The DPPH free radical scavenging activity (%) of ethyl acetate extracts in primary and secondary screening is shown in (Fig. 1 (2); Tables: 3& 4). DPPH is the reduction of purple colored DPPH in the presence of hydrogen donating antioxidants, by the formation of yellow colored non radical form of DPPH. The degree of stable DPPH decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. DPPH activity was dose dependent.

$$\text{Free radical scavenging activity (\%)} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100.$$

In both primary and secondary screening of marine actinobacterial ethyl acetate extract showed prominent antioxidant activity. The potential isolates which shows more antioxidant activity was selected for further studies.

Table 3
Preliminary screening of marine actinobacteria for Antioxidant Activity by DPPH assay

Isolate code	Total DPPH %
RA01	0.7±0.1
RA02	-
RA03	1.1±0.2
RA04	-
RA05	1.03±0.2
RA06	-
RA07	-
RA08	1.9±0.1
RA09	1.7±0.2
RA10	1.06±0.3
RA11	-
RA12	-
RA13	10.1±0.4
RA14	-
RA15	-
RA16	2.7±0.1
RA17	6.2±0.4
RA18	-
RA19	1.03±0.2
RA20	4.6±0.2

RA21	-
RA22	5.3±0.1
RA23	2.7±0.2
RA24	4.2±0.3
RA25	-
RA26	-
RA27	-
RA28	2.3±0.3
RA29	-
RA30	-
RA31	-
RA32	1.2±0.3
RA33	-
RA34	-
RA35	-
RA36	-
RA37	-
RA38	-
RA39	0.09±0.01
RA40	-
RA41	1.7±0.2
RA42	-
RA43	-
RA44	12±0.2
RA45	-
RA46	-
RA47	29.0±0.3
RA48	-
RA49	4.2±0.1
RA50	3.6±0.3
RA51	2.7±0.2
RA52	-
RA53	-
RA54	3±0.2
RA55	-
RA56	1.4±0.3
RA57	22.5±0.5
RA58	4.4±0.4
RA59	-
RA60	2.6±4.6
RA61	-
RA62	-
RA63	-
RA64	-
RA65	5.5±0.3
RA66	-
RA67	1.7±0.2
RA68	-
RA69	-
RA70	-
RA71	2±0.3
RA72	-
RA73	-
RA74	-
RA75	14.9±0.2
RA76	1.1±0.2
RA77	-
RA78	-
RA79	-
RA80	-
RA81	-
RA82	-
RA83	-
RA84	-
RA85	-
RA86	-
RA87	-
RA88	-
RA89	3.3±0.3
RA90	-
RA91	-
RA92	22±0.1
RA93	-
RA94	0.1±0.1
RA95	-
RA96	-
RA97	-
RA98	9.8±0.2

RA99	4.5±0.2
RA100	3.6±0.2
RA101	18.1±0.2
RA102	-
RA103	-
RA104	-
RA105	-
RA106	-
RA107	3.5±0.2
RA108	-
RA109	0.1±0.1
RA110	-

(-) No Activity
**** Values are mean of triplicates with standard deviation***

Table 4
Secondary screening of marine actinobacteria for Antioxidant Activity by DPPH assay

Isolate code	Total DPPH %
RA03	59±0.1
RA13	51.7±0.3
RA20	46.3±0.3
RA21	43.1±0.2
RA28	49.3±0.3
RA36	44.3±0.1
RA38	44.1±0.2
RA43	42.6±0.2
RA47	48.3±0.3
RA48	46.5±0.2
RA51	48.5±0.3
RA57	33.3±0.2
RA58	37±0.2
RA64	47.4±0.2
RA66	42.4±0.4
RA68	53.3±0.2
RA75	44.8±0.2
RA78	47.1±0.1
RA86	55.4±0.2
RA87	45.7±0.2
RA89	47.4±0.2
RA90	48.4±0.2
RA92	40.4±0.2
RA93	48.7±0.2
RA95	46.7±0.2
RA96	44.5±0.2
RA97	45.4±0.2
RA98	47.4±0.1
RA99	46.7±0.2
RA101	46.2±0.2
RA102	47.1±0.1

(-) No Activity
**** Values are mean of triplicates with standard deviation***

DISCUSSION

There are only few reports available on antioxidant activity of marine actinobacteria. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [17]. There are several methods available to assess antioxidant activity of the compounds. The last few years have seen an increasing amount of knowledge about the important role of free oxygen radicals in various diseases. These pathological and clinical backgrounds have prompted to investigate novel and potent antioxidant compounds from marine actinobacteria which are ultimately of therapeutic use. Out of these 110 isolates of marine actinobacteria more than 50% showed potent antioxidant activity in both primary and secondary screening. In the present study, the antioxidant activity of the isolated marine actinobacterial ethyl acetate extract was checked for DPPH scavenging activity. Radical scavenging activity was calculated using the following formula:[18] In the vision of pharmaceutical industry utility of the antioxidant, a cost effective media formulation becomes a primary concern [19]. Understanding the roles of various antioxidants and their action to various diseases is challenging because it can act as a several mechanisms like donating hydrogen to radicals, reducing power, free radicals scavenging activity, metal chelating ability, inhibition of β- carotene bleaching and quenching single oxygen [20]. One of the most important strategies in the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption, in an attempt to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanisms [21-22] Pancreatic lipase inhibition is one of the most widely studied mechanisms used to determine the potential efficacy of

natural products as anti- obesity agents. The present study highlighted the pancreatic lipase inhibitory potential of crude solvent extract of marine actinobacteria.

CONCLUSION

The results of this study indicated that more than 50 % of isolated marine actinobacteria possess significant DPPH free radical scavenging activity and antilipolytic activity too. Based on the results it can be concluded that the isolate produces extracellular secondary metabolites capable of scavenging DPPH free radicals hence, they may be resulted for further characterization. The metabolites of isolated actinobacteria from Pulicat Lake, Tamil Nadu may prove to be useful as antiobesity agents. Further studies are needed to be carried out to the promising isolates for active principles present in their crude solvent extract and determine its biological activity.

CONFLICT OF INTEREST

We declared that we have no conflict of interest.

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