OPEN ACCESS

Int. Res. J. of Science & Engineering, 2017; Vol. 5 (6): 113-118
ISSN: 2322-0015

http://www.irjse.in
UGC Approved Journal No. 63628

RESEARCH ARTICLE

Screening of antioxidant potential of aquatic algae isolated from various aquatic environments

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Manuscript Details
Received : 09.11.2017
Accepted: 05.12.2017
Published: 31.12.2017

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Ratnaparkhe Shraddha*, Mali Devyani and Nabar Bela. Screening of antioxidant potential of aquatic algae isolated from various aquatic environments. *Int. Res. Journal of Science & Engineering,* 2017, 5 (6): 113-118.

ABSTRACT

Algae derived compounds have applications in medicines, cosmetics and food industry. In recent years, use of photosynthetic organisms especially microalgae are getting more attention due to their phytometabolic contents with different chemical structures and biological activities. The phenolic and flavonoid compounds are well known as antioxidants. The organisms adapt themselves with respect to environmental factors and properties of the habitat. Therefore, the organisms from different habitats have diversity in their structure, properties and concentration of such secondary metabolites. The present study is focused on screening of algae for its phenolic and flavonoid compounds and their antioxidant potential. Various algal strains were isolated from different aquatic environments like fresh water, salt water and alkaline soda Lake. Methanolic extracts were studied for phenolic and flavonoid contents. The extracts were studied for their antioxidant potential using DPPH assay. As antioxidant activity is proportional to concentration of phenolic and flavonoid compounds, strains having good antioxidant potential may have promising application in cosmetics and therapeutics.

Keywords: Phenolic, Flavonoids, Antioxidants, Algae

INTRODUCTION

Many biologically and pharmacologically active substances have been isolated by researchers from algae which are difficult to synthesize chemically [1].

Antioxidants are compounds which protect cellular damage by unstable molecules i.e. free radicals. Algae have generally higher antioxidant activity due to presence of non-enzymatic components like ascorbic acid, reduced glutathione, phenols, and flavonoids.

Many of them are difficult to synthesize chemically. These antioxidants have major application as antibacterial, antifungal and anticancer agents. [2] The structure and composition of these metabolites vary with respect to growth conditions, as well environmental factors in different ecosystems. The environmental factors change with respect to their altitude & magnitude and it results in change in the concentration in metabolites. Therefore, the organisms from different habitats have diversity in their structure, properties and concentration.

Mycosporine like amino acids, scytonemin and sporopolleninare are well known for their UV screening ability [3]. Carotenoids are used as food colour, an alternative to synthetic colours. Recent studies are focused on new alternative cost effective natural antioxidants which can replace chemical antioxidants especially used in personal and healthcare industries. Algae possess good potential for these natural antioxidants, as they are easy to scale up than seaweeds so they are focused in this study.

The present work reveals the phytochemical study of the algal species isolated from different ecosystems. Water samples were collected from fresh water ecosystem like Titwala lake, Tapi, Narmada, Krishna, Godavari and Vajreshwari river and sagareshwar lake near Karad. Extreme environments like Lonar Lake and salt pans were also selected for the study.

METHODOLOGY

Collection of samples:
The water samples were collected in sterile container and stored at 4^0C. The 5 ml sample was enriched in 100 ml Bold Basal medium. Incubation was done at 25^0C $\pm$ 2^0Cfor 15 days in natural sunlight for 12 hrs photoperiod and 12 hrs dark. pH and salt concentration was maintained according to the source.

Isolation and purification of samples:
The samples were observed microscopically and different algal strains were isolated. Purification of isolates was done by repeated sub culturing from solid to liquid media and then to solid media. The purity of strains was confirmed by microscopic observation. The cultures were grown using Bold basal medium in natural sunlight at 25^0C + 2^0Cfor 15 days for 12 hrs photoperiod and 12 hrs dark. pH and salt concentration were maintained as per the sample requirement.

Preparation of algal extracts:
The dried samples of algae were extracted in methanol by incubating overnight and the supernatant was removed. The procedure was repeated till colourless pellet was obtained. The extracts were combined and concentrated to 4 ml. The extracts were incubated at 4^0C till further use. [4][5]

Estimation of Total phenolic content:
The total phenolic content was determined by modified Folin-ciocalteu method. 100µg/ml tannic acid was used as standard. 50 microliters of 50% Folin-ciocalteu reagent was added to 100 microliters of extract. The samples were allowed to stand for 5 min at room temperature. After 5 minutes 250 microliters of 2% sodium carbonate was added. The tubes were incubated in the dark at room temperature for 40 min. Distilled water was used as blank. Absorbance was measured at 700 nm on Vidyut kanad 0392 instrument. The total phenolic content was expressed as tannic acid equivalents. The experiments were done in triplicates to ensure repeatability [6].

Estimation of Total flavonoid content:
The total flavonoid content was determined by modified method using aluminium chloride reagent. 200 µg/ml Quercetin was used as standard. 250 microliters of test sample was mixed with 1.25 ml of distilled water followed by addition of 75 microliters of 5% sodium nitrite Incubation was done at room temperature for 6 min. Then, 125 microliters of 10% aluminium chloride was added. After five minutes, 0.5 ml of 1M sodium hydroxide was added in the mixture. The total volume was made up to 2.5 ml with distilled water. Absorbance was measured at 510 nm on Vidyut kanad 0392 instrument. The results were expressed as mg/g Quercetin equivalents. The experiment was performed in triplicates [7].

Estimation of antioxidant activity:
200µL of algal extract was mixed with 2 ml of 0.02% DPPH. Incubated at dark for 30 min. absorbance was measured at 517 nm using (Vidyut kanad 0392 instrument) using methanol as blank. The control was prepared without any extract. The ability to scavenge DPPH radical was calculated by using equation: DPPH scavenging effect (%) = $[(A_C-A_S)/A_C]$

×100; where A_C: absorbance of control reaction and A_S absorbance of sample. The experiment was performed in triplicates [8].

RESULTS AND DISCUSSION

Table 1: Details of sampling locations in Maharashtra region

Location	Altitude & Magnitude	Strains Isolated
Lonar lake	19⁰58'N & 76⁰30'E	22
Narmada river	21°72'N & 73⁰15'E	1
Vajreshwari river	19⁰48'N & 73⁰02'E	1
Tapi river	21⁰04'N & 75⁰80'E	1
Krishna river	16°41'44"N 74°13'54"E	1
Godavari river	19°85'N & 72⁰70'E	3
Sagareshwar pond	17°8'46.42"N 74°22'59.44"E	6
Salt pan Mulund	19°14'N & 72⁰933'E	3
Salt pan Bhandup	19°14'N & 72⁰933'E	2
Fish Tank Bhandup	19°14'N & 72⁰933'E	2

Total 41 strains of algae were isolated from various ecosystems as presented in Table 1. Nature of algal isolates obtained includes both Unicellular and filamentous strains. On microscopic observation microcystis were negatively selected. After repeated sub culturing, strains having luxuriant growth property and good yield were selected for the further study. Maximum numbers of isolates were obtained from Lonar Lake. 22 isolates were obtained from Lonar Lake. 6 strains were isolated from Sagareshwar Lake. 7 isolates were obtained from rivers viz. Narmada, Vajreshwari, Tapi Krishna and Godavari. 7 isolates obtained from salt pan. (Figure 1).

Figure 1: sampling locations

Highest phenol content of 18.22 mg/gm equivalent of tannic acid was observed in isolate from Tapi River TR 2105-01 followed by isolate from LLMS 315-030 i.e.12.28 mg/gm equivalent of tannic acid. The isolates obtained from fresh water of Krishna and Narmada River showed phenolic content 11.57 and 10.45 mg/gm equivalent of tannic acid respectively. (Figure 3)

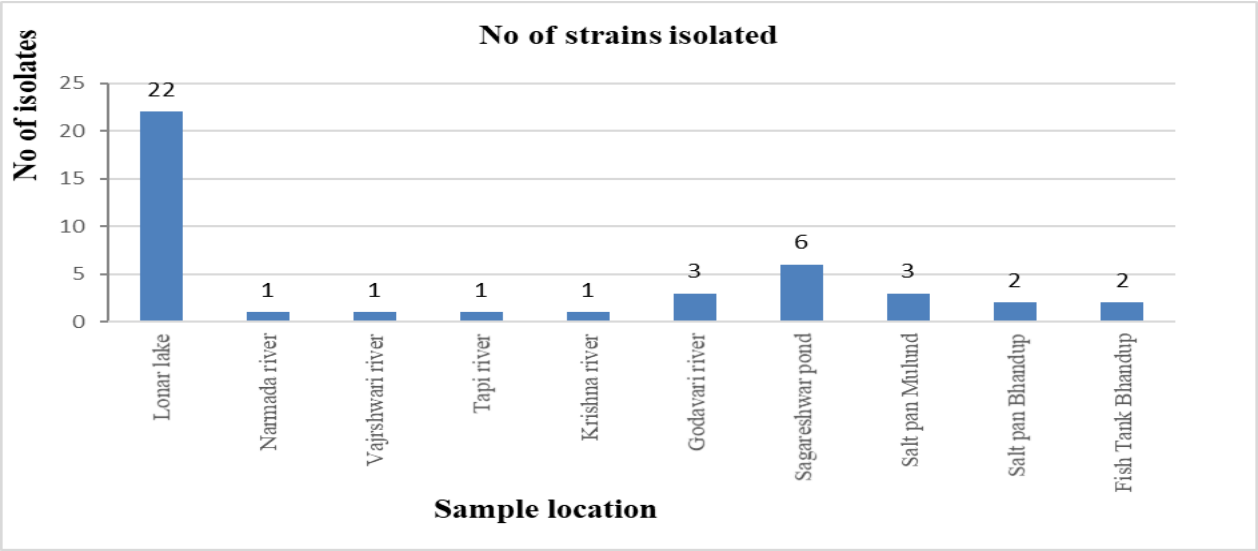

Figure 2: No of isolates obtained per sample

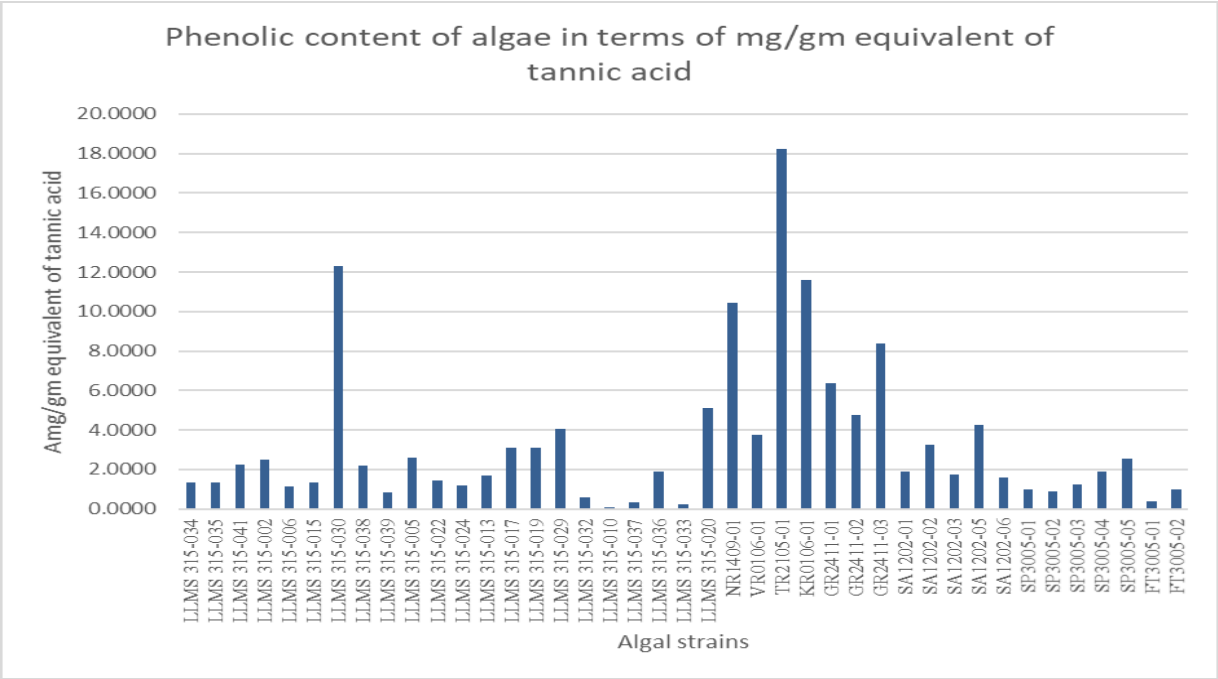

Figure 3: Phenolic contents of algal isolates in mg/g equivalents of tannic acid

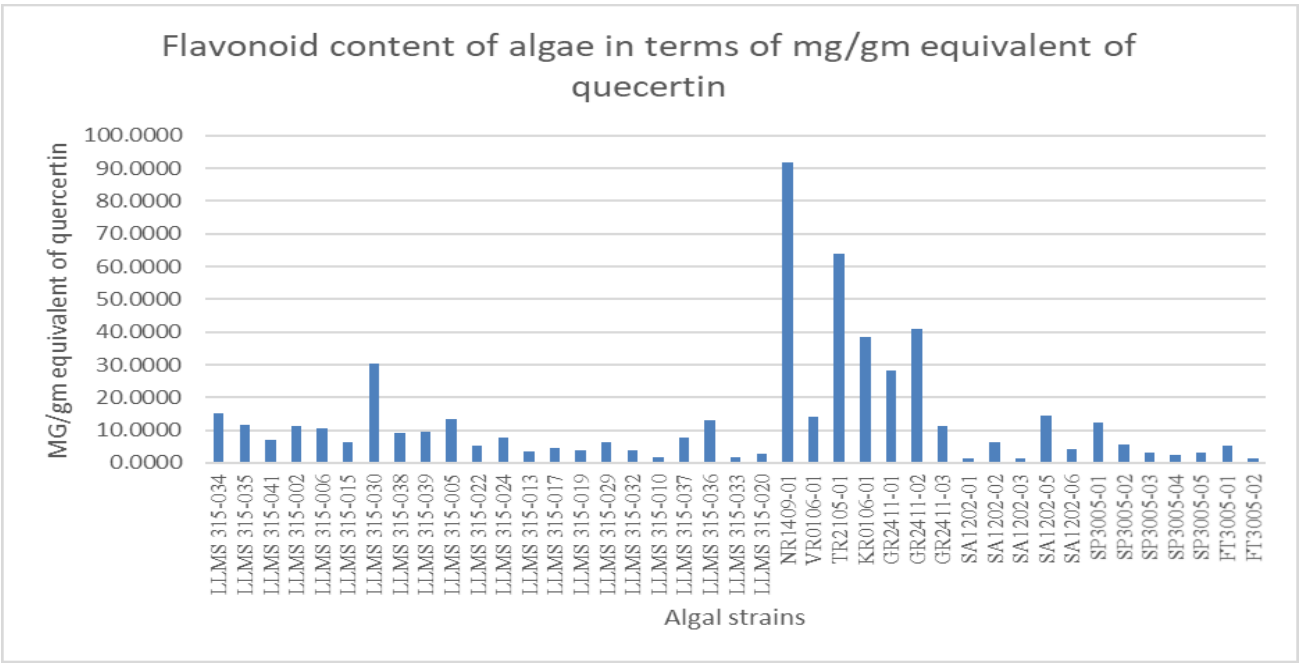

Figure 4: Flavonoid contents of algal isolates in mg/g equivalents of quercetin

Table 2: Comparative data of Godavari strains with respect to Phenolics, flavonoids content and Antioxidant potential

Isolate	Phenolic content mg equivalence Tannic acid/gm	Flavonoid content mg equivalence Quercetin/gm	DPPH radical Scavenging activity (%)
GR2411-01	28.0997	6.3746	44.05
GR2411-02	40.9518	4.7581	59.18
GR2411-03	11.1157	8.4059	41.54

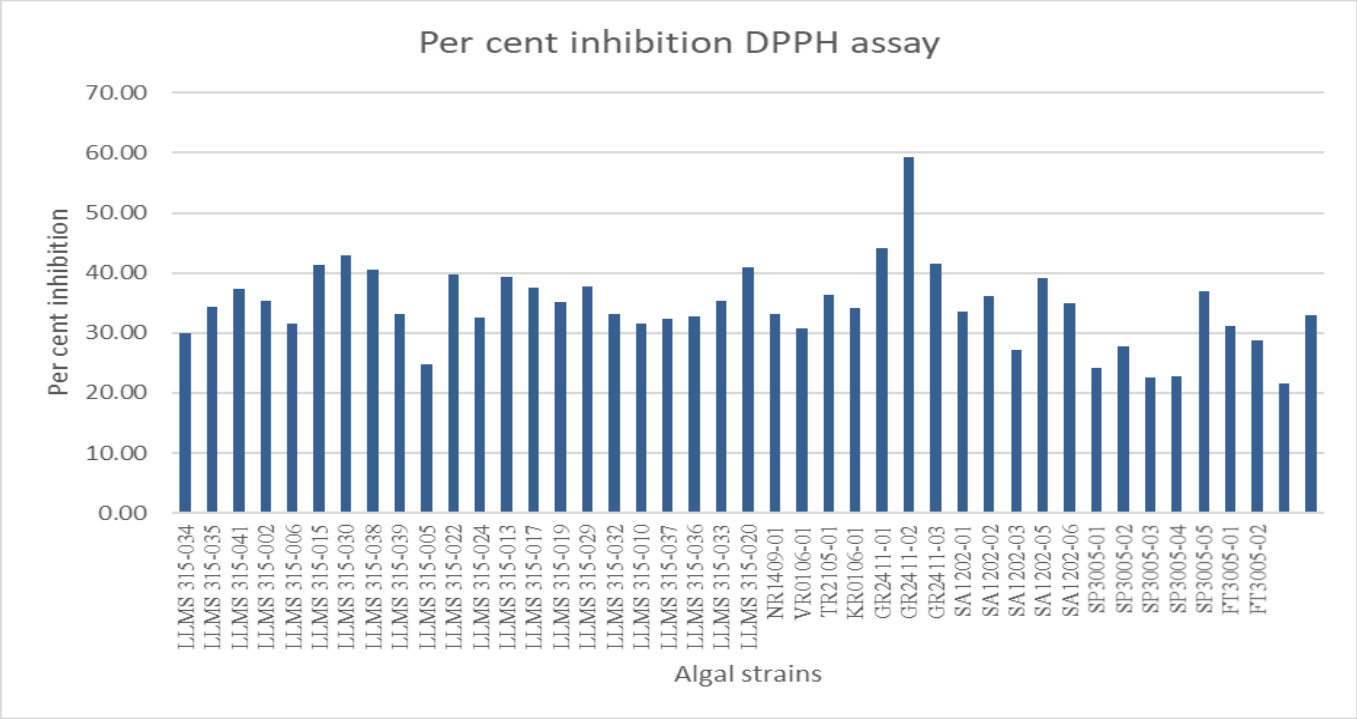

Figure 5: Antioxidant value of algal isolates in terms of percent inhibition of DPPH

Highest flavonoid content of 91.98 mg equivalents of quercetin/gm of algae belonged to NR1409-01 strain followed by 63.76 mg equivalents of quercetin/gm of algae in isolate from Tapi River TR 2105-01

Highest antioxidant activity belonged to Godavari isolate GR2411-02 i.e. 59.18% followed by another Godavari isolate GR2411-01 44.04%.

In present study, 3 isolates of Godavari GR2411-01, GR2411-02 and GR2411-03 are having good antioxidant property.(Table 2) They are having good phenolic and flavonoid contents. These strains were selected for further studies.

CONCLUSION

Algae produce phytochemicals as their protective measures. Phenolic and flavonoid compounds are the main bioactive substances which contribute in antioxidant activity The algal extracts were analysed for phenolic content, flavonoid content and antioxidant potential. Out of 41 strains, 3 isolates from Godavari were selected for further study. They may have significant contribution in therapeutic and cosmetic formulations.

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