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Western Ghats terrestrial microalgae serve as a source of amylase and antioxidants enzymes

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Abstract
The purpose of the study was to screen six identified microalga samples from in and around of the Western Ghats Karnataka state for their amylase and antioxidants activity assay. These six collected terrestrial microalgae were grown in plastic tray for multiplication purpose treated with R.O. residual dispense water, pH 8.4 (T_1) and urban waste water (T_2) which reduced the pH and TDS content in that water. Rhizoclonium hieroglyphicum has greater chlorophyll content, which helps in multiplication of microalgae very quickly. Carbohydrate degrading enzymes were analyzed using DNS reagents. Free radical scavenging activities were also determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. Superoxide Dismutase and Peroxidase was the most important antioxidant enzymes that protect against the peroxidation system and maintain the redox state of the cell. Amylase, SOD and POD activities were confirmed by conducting native PAGE.

Keywords: antioxidants, DPPH, free radical, *Rhizoclonium hieroglyphicum*, superoxide dismutase, terrestrial microalgae

Introduction
Microalgae were very effective in reduction of BOD, COD, NH_4, NO^-_3, PO^-_3 and TDS in sewage waste water. Microalgal strains generate oxidative stress triggered by reactive oxygen species (ROS) leading to degradation of cellular macromolecules [5]. All tested microalgae extracts possessed the ability to scavenging DPPH at various degrees. SOD, CAT and POD are important antioxidant enzymes in the algal cell that protect against the peroxidation system and maintain the redox state of the cell [7]. Microalgae contains wide range of biochemical compounds and antioxidant agents; Radical scavenging activity of antioxidants determined by using DPPH [10]. Environmental or artificial stress affects the growth, photosynthesis or other physiological processes by the increase in the production of ROS in cells. SODs catalyze the disproportionation of two molecules of superoxide radical ion to oxygen molecule and hydrogen peroxide molecule and it further scavenged by catalases and peroxidases [15]. Production of algal oil is not only for biofuel but also for high nutritional values of oils. The increase in total lipids in algal cells under various stress conditions consisted primarily of neutral lipids, it's because of the shift in lipid metabolism from membrane lipid synthesis to the storage of neutral lipids [16]. The amylolytic activity of the enzyme extracts was determined using starch as a substrate by the saccharification method, which is based on production of reducing sugars [17]. The Phycoremediation is an alternative methodology for treating waste water at cheaper cost [20, 22]. The powerful antioxidants minimize oxidative damage to living cells and prevent oxidative deterioration [26]. In present study evaluated the activity of antioxidative enzymes such as SOD and POX on different terrestrial microalgae.

Materials and Methods
The experiments were carried out in the laboratory of Agriculture Biotechnology (Agriculture College Hassan), University of Agricultural Sciences, Bengaluru, Karnataka and also in the laboratory of Biochemistry and Biochemical Engineering, JIBB, SHUATS, Allahabad, U.P. India.

Collection of terrestrial microalgae
Terrestrial microalgal samples were collected in autoclaved plastic bags from water bodies like ponds and rivers of Hassan, Kodagu and Udupi district of Western Ghats of Karnataka. Six identified terrestrial microalgae collected from rivers and ponds of fresh water bodies used for analysis includes *Oedogonium sp.*, *Rhizoclonium sp.* and *Spirogyra* collected from the pond of Gadanahalli, Dandiganahalli and Hoovnalli villages, respectively of Hassan district; *Klebsormidium sp.* and *Rhizoclonium hieroglyphicum* from the rivers of Ikola and Kaggodu

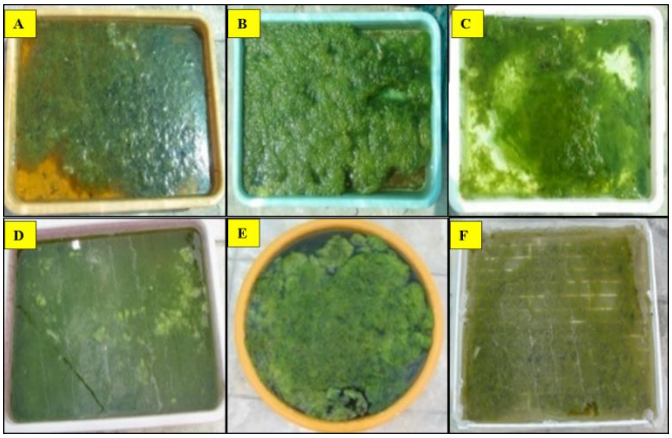
villages of Kodagu district and *Rhizoclonium sp.* collected from the river of Happanadka village of Udupi district.

Multiplication of terrestrial microalgae in plastic tray

Collected terrestrial microalgae were kept for multiplication under direct sunlight in plastic tray using normal tap water (Fig. 1). Evaporation was controlled by kept the algae cultured plastic tray under 50% shade net and maintaining the water level by adding extra water for every 5 days interval.

Effects of terrestrial microalgae on waste water pH and TDS

The pH and TDS was tested by using the portable pH and TDS meter, respectively. During the time of analysis, taken 10ml of water sample from the small plastic trays treated with different water and terrestrial microalgae and noted down the digital value showed in the pH meter and TDS meter (ppm.)



(A) *Rhizoclonium sp.* (Happanadka), (B) *Rhizoclonium sp.* (Dandiganahalli), (C) *Spirogyra sp.* (Hoovnalli), (D) *Klebsormidium sp.* (Ikola), (E) *Rhizoclonium* hieroglyphicum (Kaggodu) and (F) *Oedogonium sp.* (Gadanahalli)

Fig 1: Multiplication of terrestrial microalgae in plastic tray.

Chlorophylls estimation from terrestrial microalgae

The chlorophyll is the essential component for photosynthesis, and occurs in chloroplasts as green pigment in all photosynthetic plant tissues. There are various types of Chlorophyll, Chlorophylls a and b occurs in higher plants, ferns and mosses. Chlorophylls c, d and e are only found in algae and in certain bacteria. Chlorophyll (a, band total) estimation was done by Sadasivam and Manickam (1992) [18] methods. For extraction of chlorophyll ground 1g sample using liquid nitrogen and made fine pulp with 20ml 80% acetone. Centrifuged the sample at 6000 rpm for 10 min and transferred the supernatant to a 100ml volumetric flask. Repeated the same process until the residues becomes colourless. Made up the supernatant 100 ml by adding 80% acetone. Read the absorbance of the solution at 645, 663 and 450 nm against the solvent and 80% acetone used as blank. Chlorophyll c estimation was done according to the method described by Parsons (1963) [13]. For chlorophyll c, first read the sample at 450 nm and noted the absorption value and next added one drop of concentrated HCl to same sample and absorbance was read at 450 nm using spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

Chlorophyll content in terrestrial microalgae was calculated by using the formulae given below:

a. Chlorophyll a/g tissue (mg) = $12.7(A_{663}) - 2.69(A_{645}) \times V/(1000 \times W)$
b. Chlorophyll b/g tissue (mg) = $22.9(A_{645}) - 4.68(A_{663}) \times V/(1000 \times W)$
c. Total chlorophyll /g tissue (mg) = $20.2(A_{645}) + 8.02(A_{663}) \times V/(1000 \times W)$
d. Chlorophyll c/g tissue (mg) = $(A_{450} - A_{450}$ with HCl) × 17.5

Where A = absorption at specific wavelengths, V = Final volume of chlorophyll extracted in 80% acetone (100ml) and W = fresh weight of tissue extracted.

Assay for amylase enzyme in terrestrial microalgae

Amylase was extracted by grinding 0.5 gm of the acetone defatted algal sample with 2.5 ml of 0.1M phosphate buffer (pH 7.4) and at 4 ℃ centrifuged the sample for 10 min at 6000 rpm. Added equal amount of chilled acetone to supernatant and kept at -20 ℃. After 30min, centrifuged at 12000 rpm for 15 min, supernatant was discarded and pellet was dissolved in 1 ml phosphate buffer (0.1M, pH 7.4) and used for amylase estimation. Pipetted out 1 ml starch solution and 1ml of properly diluted enzyme (1:5) (extracted sample) in a test tube, incubated at 27 ℃ for 15 min and the reaction was stopped by adding 2 ml of DNS reagent. Heated solution in a boiling water bath until color developed *i.e.* 8 min. While the tubes were warm, added the 1 ml of potassium sodium tartarate solution, cooled it and made the volume to 10 ml by adding 6 ml of distilled water and measured the absorbance at 560 nm using spectrophotometer. Standard graph with 200-1200 µg maltose was prepared [8, 14]. Amylase activities was calculated by below formulae

$$\frac{\text{Absorbance at 560} \times \text{Final volume including DNS}}{\text{Time of hydrolysis} \times \text{Extinction coefficient} \times \text{Enzyme used (ml)} \times \text{cuvette diameter(1cm)}}$$

Native PAGE was done by using Electrophoresis (Life technologies mini gel tank), which contains 10% resolving gel and 5% stacking gel (Table 1). Run the gel by connecting the cathode and anode to the power bank and set the 60 v, 450 Amp for 2 hrs [19]. Washed the gel in distilled water and incubated in a mixture containing soluble starch (2%) and sodium acetate buffer (0.1M, pH 5.0) at room temperature for 2 hrs. Once again washed the gel in distilled water to remove excess starch solution and kept the gel in a container for 2hr in distilled water. Gel was stained by HCl (0.2 M) containing I_2 (5.7 mM) and KI (43.3 mM). The activity band was observed after 10 min of incubation with stain. The whitish band under violet blue background was observed after washing the stained gel in distilled water 2-4 times [9].

Table 1: Native - PAGE recipes for antioxidant enzymes

Particles	Gel Resolving (30 ml)	Staking Gel (10 ml)
Water	11.9 ml	3.4 ml
30% acrylamide mix	10 ml	0.83 ml
Tris buffer (1.5M, pH8.8)	7.5 ml	-
Tris buffer pH(1.0M, 6.8)	-	0.63 ml
10%APS	0.3 ml	0.05 ml
TEMED	0.012 ml	0.005 ml

Assay for antioxidants activity in terrestrial microalgae

DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity

Ground 0.2 gm of the acetone defatted algal sample with 2 ml of 0.1M acetone in a pestle and mortar and ground properly at 4 ℃. Incubated the sample for 1 hour at room temperature, shakes occasionally and centrifuged it at 6000 rpm for 10 min at 4 ℃. Collected supernatant used as enzyme. For estimation

taken 0.2 ml of aliquot sample in a 2 ml glass vial covered with aluminum foil, which prevent the direct expose of bright light. Added 80 µl of 7.4 pH Tris-HCl buffer and mixed well. Then added 100 µl DPPH to the reaction mixture and made the volume to 1ml by adding remaining water. Mixed vigorously in dark for 20 minute and measured the absorbance at 517 nm [25]. Mixture of acetone, Tris-HCl and water was used as blank. DPPH along with acetone, Tris-HCl and water treated as control. Radical scavenging activity by DPPH method was calculated by using the formulae given below:

$$\text{DPPH activity} = 1 - \left(\frac{\text{Absorption reading of sample}}{\text{Absorption reading of control}}\right) \times 100$$

Peroxidase activity

$$\text{Guaiacol} + H_2O_2 \xrightarrow{\text{POD}} \text{Oxidized Guaiacol} + 2H_2O$$

Ground 0.5 gm of the acetone defatted algal sample with 2.5ml of phosphate buffer (0.1M, pH 7.1) at 4 ℃and centrifuged for 15 min at 6000 rpm by maintaining 4 ℃. Collected the supernatant and used it as enzyme. Prepared the solution A and solution B reagent mixtures separately. Solution A contains combination of 1.5 ml of phosphate buffer solution (pH 6.1), 0.5 ml Guaiacol solution, 0.1 ml of enzyme and make up 3 ml reaction mixture by adding distilled water. Solution B contains all reaction mixture of Solution A along with 0.5 ml H_2O_2. Solution A containing cuvett was kept in reference place and used as blank. Solution B showed the peroxidase activity at 470 nm in spectrophotometer (CECIL CE 7400), set for enzyme kinetics to show reading every 10 s up to 120 s. Peroxidase activity was calculated based on 30 s difference between any two values [7]. Native PAGE was also carried out based on Sambrook and Russell (2001) [19]. PAGE gel was stained by incubating the gel inside the staining mixture containing 100 ml of Phosphate buffer (0.1M, pH 6.1), 0.2 ml of Guaiacol solution and 0.1 ml of H_2O_2. Slowly shook the gel along with staining mixture. Brown coloured bands were observed after 5 min of staining.

Superoxide dismutase

Superoxide Dismutase is a metal containing enzyme with a vital role in scavenging superoxide (O_2^-) radicals.

$$O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

So hydrogen peroxide is eliminated by peroxidases and catalases. Ground 0.5 gm of the acetone defatted algal sample with 2.5 ml of 0.1M phosphate buffer in a pestle and mortar. Centrifuged at 6000 rpm for 15 min at 4 ℃and collected the supernatant and used it as enzyme. Prepared the 3ml reaction mixture by adding 200µl methionine, 100 µl EDTA, 100 µl NBT, 100 µl sodium carbonate solution, 1.5 ml phosphate buffer (0.1M), sample volume 100 µl, riboflavin100 µl and make up the volume to 3 ml by distilled water. One reaction mixture kept at dark and another kept under sunlight until the colour of the reaction mixture turned to blue colour i.e. 10 min. Prepared one set of reaction mixture without enzyme for both light and dark reaction and it used as control. It was known as 100% inhibition. Read the absorbance at 560 nm immediately, dark reaction sample used as a blank for their respective sample [18]. SOD activity was calculated by below formulae:

$$\text{Superoxide Dismutase activity} = \frac{\text{Without Enzyme absorbance (Control)}}{\text{With enzyme Absorbance (Sample)}} \times 100 - 100$$

$$50\%\ \text{inhibition of SOD activity (U/mg protein)} = \frac{\text{Concentration of sample (µg)}}{\text{SOD activity of sample}} \times 50$$

Native PAGE was also carried out based on Sambrook and Russell (2001) [19]. For staining the gel prepared solution A which contains 20 ml of NBT (0.1%), 500 µl of EDTA (0.2 M), 50 µl of phosphate buffer (100 mM, pH 7.4) and 330 µl of TEMED; Solution B as 30 µl of riboflavin (0.005%). Staining mixture was prepared by adding both solution A and Solution B simultaneously in plastic gel tray. Incubate the gel inside the plastic gel tray containing combination of staining mixture for 15 min in dark without expose to direct light. After 15 min gel was exposed to the light for 3-5 min. Whitish coloured bands were observed with dark bluish background.

Results and Discussion

Highest decreasing percentage of TDS was observed in *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum.* It is because of the absorbing salt contents in the treated water and used as nutrients for their multiplication of cell and yielding good quantity of biomass. 50th day change in percentage of decreasing TDS content was observed in currently studied terrestrial microalgae are 8.34, 41.76, 14.97, 12.11, 38.70 and 16.93%, respectively on R.O. residual dispense water whose pH 8.4 (T_1) and 8.36, 28, 12.27, 10.85, 28.07 and 8.94%, respectively on urban waste water (T_2). The pH content in the water T_1 and T_2 was gradually decreasing after 10th days in all present studied (Table 2). These results indicates that all selected species are converting the alkaline water into acidic. And it was the clear indication that the selected species are well adapted to acidic water of pH 5.48 - 6.4.

Table 2: Responses of terrestrial microalgae on waste water

RO residual dispense water whose pH 8.4 (T_1)							
		A	**B**	**C**	**D**	**E**	**F**
TDS	0th	695	680	681	685	682	685
	50th	637	396	579	602	418	569
pH	0th	8.28	8.24	8.32	8.40	8.29	8.20
	50th	6.40	5.76	5.94	6.20	5.40	5.60
Urban waste water (T_2)							
TDS	0th	825	825	823	820	830	830
	50th	756	594	722	731	597	753
pH	0th	8.51	8.38	8.48	8.50	8.46	8.65
	50th	6.38	5.74	5.99	6.00	5.70	6.05

(A)*Rhizoclonium sp.* (Happanadka), (B) *Rhizoclonium sp.* (Dandiganahalli), (C) Spirogyra sp. (Hoovnalli), (D) *Klebsormidium sp.* (Ikola), (E) *Rhizoclonium hieroglyphicum* (Kaggodu) and (F) *Oedogonium sp.* (Gadanahalli).

Chlorophyll content in terrestrial microalgae

The chlorophyll is the important components and it contains chloroplasts green pigment in photosynthetic plant tissues. The maximum chlorophyll a, b, c and total chlorophyll content was found in *Rhizoclonium hieroglyphicum* collected from Kaggodu (Kodagu district) as 14.82 ± 0.039, 12.42 ± 0.007, 7.11 ± 0.002 and 13.85 ± 0.087 mg/g tissue, respectively. The minimum chlorophyll a, b and total chlorophyll content was seen in *Rhizoclonium sp.* (Dandiganahalli) as 3.31 ± 0.002, 3.55 ± 0.151 and 3.78 ± 0.011 mg/g tissue, respectively. The minimum chlorophyll c

of 0.93 ± 0.014 mg/g tissue was observed in *Rhizoclonium sp.* (Happanadka). In current study chlorophyll a, b, c and total chlorophyll content in *Rhizoclonium hieroglyphicum* has 77.63, 71.41, 80.78 and 72.68% more than *Rhizoclonium sp.* (Happanadka) (Table 3).

Table 3: Chlorophyll content of terrestrial microalgae studied

Sample	Chlorophyll a (mg/g tissue)	Chlorophyll b (mg/g tissue)	Chlorophyll c (mg/g tissue)	Total chlorophyll (mg/g tissue)
A	7.36 ± 0.008	5.54 ± 0.008	0.93 ± 0.014	6.32 ± 0.005
B	3.31 ± 0.002	3.55 ± 0.151	1.37 ± 0.004	3.78 ± 0.011
C	4.14 ± 0.011	4.38 ± 0.122	1.94 ± 0.019	4.68 ± 0.014
D	7.81 ± 0.011	6.49 ± 0.011	4.03 ± 0.005	7.25 ± 0.045
E	14.82± 0.039	12.42± 0.007	7.11 ± 0.002	13.85± 0.087
F	3.97 ± 0.005	4.10 ± 0.001	2.15 ± 0.003	4.39 ± 0.010
All the values are MEAN ± SD of three replicates				

(A)*Rhizoclonium sp.* (Happanadka), (B) *Rhizoclonium sp.* (Dandiganahalli), (C) *Spirogyra sp.* (Hoovnalli), (D) *Klebsormidium sp.* (Ikola), (E) *Rhizoclonium hieroglyphicum* (Kaggodu) and (F) *Oedogonium sp.* (Gadanahalli).

The results of present study are in accordance with the similar study done by Benemann *et al.* (1978) [2] and Ismaiel *et al.* (2016) [7] who observed 10.6 mg/g of Chl a. The reaction centers of PSI and PSII in chloroplast thylakoids are the major generation site of reactive oxygen species (ROS). The photo production of ROS is largely affected by physiological and environmental factors [1].

Protein content in terrestrial microalgae

In the current study total protein were estimated by Lowry method. Terrestrial microalgae *Rhizoclonium sp.* (Happanadka), *Rhizoclonium sp.* (Dandiganahalli), *Spirogyra sp., Klebsormidium sp., Rhizoclonium hieroglyphicum* and *Oedogonium sp.* had 9.93 ± 0.03, 22.24 ± 0.03, 6.64 ± 0.03, 5.12 ± 0.16, 19.51 ± 0.04 and 5.73 ± 0.02 mg/g of protein, respectively**.**

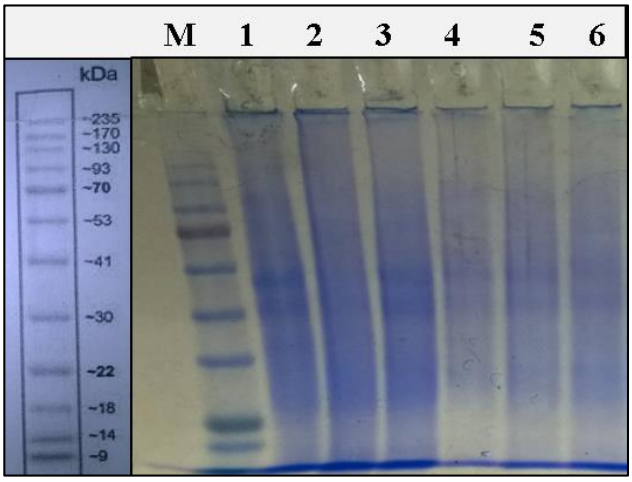


Fig 2: SDS-PAGE for protein content of terrestrial microalgae

Where M. Marker (9-235 kDA); 1,2,3,4,5 and 6 indicate the sample in that well are *Rhizoclonium sp.* (Happanadka), *Rhizoclonium sp.* (Dandiganahalli), *Spirogyra sp., Klebsormidium sp., Rhizoclonium hieroglyphicum* (Kaggodu) and *Oedogonium sp.*, respectively.

In SDS- PAGE, bands for all the samples were observed in 41 kDa, 30 kDa and 22 kDa region in the gel indicating the terrestrial microalgae proteins as 41kDa, 30kDa and 22kDa of molecular weight (Fig. 2). The results of present study are in accordance with the similar study done by Peter *et al.*(2010) [14] who correlated higher protein content with high lipid content in the microalgae.

Carbohydrate degrading enzyme (Amylase enzyme)

The amylases are common hydrolytic enzymes which hydrolyze or processing the starch [6, 27]. Endoamylases, Exoamylases, Debranching enzymes and Transferases are the four types of starch converting enzymes. Most starches are mixture of two polymers such as amylose and amylopectin [12, 24]. Ratio of these two polymers in starch varies due to origin, plant species, variety within plants, plants organs, age of organ, and growth conditions [11, 23]. In present study amylase enzyme was estimated in terrestrial microalgae of *Rhizoclonium sp.* (Happanadka), *Rhizoclonium sp.* (Dandiganahalli), *Spirogyra sp., Klebsormidium sp., Rhizoclonium hieroglyphicum* and *Oedogonium sp.* are 30.049 ± 0.015, 30.620 ± 0.022, 30.217 ± 0.042, 26.383 ± 0.004, 30.553 ± 0.015, 30.116 ± 0.065 and 27.939 ± 0.031 U/mg of maltose, respectively. The maximum amount of amylase enzyme was observed in *Rhizoclonium sp.* (Dandiganahalli) 30.620 ± 0.022 U/mg of maltose and minimum in *Klebsormidium sp.* 26.383 ± 0.004 U/mg of maltose (Table 4).The carbohydrate digestive amylase enzyme activities in microalgae were confirmed by native- PAGE appearing whitish band under violet blue background (Fig. 3). In that native-PAGE 2nd well Rhizoclonium *sp.* (Dandiganahalli) sample showed bright band compared to other indicating higher amylase activity that *Rhizoclonium sp.* (Dandiganahalli) as more amylase enzyme activity. The result of present study is in accordance with the similar study done by Rodrigues (2017) [17].

Table 4: Amylase enzyme estimated from terrestrial microalgae

Samples	Amylase (U/mg maltose)
A	30.049 ± 0.015
B	30.620 ± 0.022
C	30.217 ± 0.042
D	26.383 ± 0.004
E	30.553 ± 0.015
F	30.116 ± 0.065
G	27.939 ± 0.031
All the values are MEAN ± SD of three replicates	

(A) *Rhizoclonium sp.* (Happanadka), (B) *Rhizoclonium sp.* (Dandiganahalli), (C) *Spirogyra sp.* (Hoovnalli), *(D) Klebsormidium sp.* (Ikola), (E) *Rhizoclonium* hieroglyphicum (Kaggodu) and (F) *Oedogonium sp.* (Gadanahalli)

Antioxidant activities in terrestrial microalgae

DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity

DPPH radical is the stable organic nitrogen free radicals, which used to determine the free radical scavenging ability of the various samples [3]. DPPH is a purple color dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple color fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance[21]. In present study DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity were estimated in terrestrial microalgae of *Rhizoclonium sp.* (Happanadka), *Rhizoclonium sp.* (Dandiganahalli), *Spirogyra sp.,Klebsormidium sp.*, *Rhizoclonium hieroglyphicum* and *Oedogonium sp.* had 28.97 ± 2.069, 40.00 ± 3.006, 41.38 ± 2.068, 38.62 ± 1.379, 44.14 ± 1.194 and 42.07 ± 0.689% of radical scavenging activity (% RSA) respectively (Table 5). Free radicals scavenging potential was assayed by DPPH

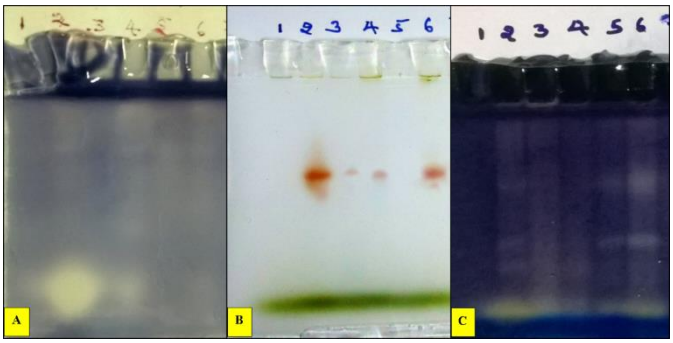
method and were expressed in % radical scavenging activity (% RSA). DPPH method gives antioxidant activity which helps to understand the electron abstraction potential of microalgae. The highest % of RSA was found in *Rhizoclonium hieroglyphicum*. The antioxidant activities (the radical scavenging activity, reducing power and chelating activity) were enhanced under stress conditions and at the pH level that supports optimal growth. The % RSA observed in present study are statistically significant. The results of present study are in accordance with the similar study done by Maadane *et al.* (2015) [10] and Trabelsi *et al.* (2016) [25] on microalgae of different species.

Peroxidase activity

In microalgae an increase in the activity of these enzymes not only promotes ROS removal, but also increases ATP synthesis *via* the Mehler peroxidation reaction which is the source of additional ATP for Na^+ expulsion at high salinity. POD activities were estimated in terrestrial microalgae of present studied samples are 0.01 ± 0.003, 0.21 ± 0.004, 0.19 ± 0.008, 0.19 ± 0.002, 0.21 ± 0.004 and 0.01 ± 0.001 µmole/min/g weight of sample, respectively (Table 5). The antioxidants produced by algae alleviate the harmful effects of ROS. In present study monitored the enzyme activities of algae in various present studied species. The highest peroxidase activity were observed in *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum* are 0.21 ± 0.004 µmole/min/g and lowest in *Oedogonium sp.* are 0.01 ± 0.001 µmole/min/g weight of sample. POD activities in microalgae were confirmed by conducting native-PEGE for POD and brown coloured bands were observed. *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum* (Kaggodu) samples shows bright bands in native-PAGE gel because of high POD activity *Spirogyra sp.* and *Klebsormidium sp.* samples showed light band because of low POD activity (Fig. 3). The result of present study is in accordance with the similar study done by Chakraborty *et al.* (2010) [4].

Superoxide dismutase (SOD) activity

One unit of SOD activity were defined as the corrected amount of enzyme (by the negative control) required to result in a 50% inhibition of the Nitroblue tetrazolium (NBT) reduction measured at 560 nm in comparison with the positive control under the assay conditions described. The activities were expressed in U/mg protein. In present studied sample as SOD activities of 124.40 ± 0.211, 97.98 ± 1.025, 186.29 ± 2.205, 168.66 ± 0.131, 92.23 ± 1.029 and 282.95 ± 0.036 U/mg proteins, respectively was noted (Table 5). In present study the highest SOD activity was observed in *Rhizoclonium hieroglyphicum* because it needs only 92.23 ± 1.029 U/mg protein for 50% inhibition of enzyme (IC_{50}) and lowest was observed in *Oedogonium sp.*, it was needed 282.95 ± 0.036 U/mg protein for $IC_{50.}$ SOD, CAT and POD are most important antioxidant enzymes in the algal cell that protect against the peroxidation system and maintain the redox state of the cell.[7]The scavenging of ROS not only protects chloroplasts from the direct effects of ROS, but also relaxes the photon (electron) excess stress [1]. The SOD activity in microalgae was confirmed by native-PEGE for SOD and observed whitish bands under dark bluish background (Fig. 3).



(A) Amylase, (B) Peroxidase and (C) SOD activity. 1, 2, 3, 4, 5 and 6 indicate the sample in that well are *Rhizoclonium sp.* (Happanadka), *Rhizoclonium sp.* (Dandiganahalli), *Spirogyra sp.*, *Klebsormidium sp.*, *Rhizoclonium hieroglyphicum* (Kaggodu) and *Oedogonium sp.*, respectively.

Fig 3: Native-PAGE for amylase and antioxidant activity

Table 5: Antioxidant activities in terrestrial microalgae

SAMPLES	DPPH (% RSA)	POX (µmole/min/g)	SOD (U/mg protein)
A	28.97 ± 2.069	0.01 ± 0.003	124.40± 0.211
B	40.00 ± 3.006	0.21 ± 0.004	97.98 ± 1.025
C	41.38 ± 2.068	0.19 ± 0.008	186.2 ± 2.205
D	38.62 ± 1.379	0.19 ± 0.002	168.66 ± 0.131
E	35.86 ± 3.160	0.07 ± 0.002	178.91± 0.096
F	44.14 ± 1.194	0.21 ± 0.004	92.23 ± 1.029
G	42.07 ± 0.689	0.01 ± 0.001	282.95 ± 0.036
All the values are MEAN ± SD of three replicates			

(A) *Rhizoclonium sp.* (Happanadka), (B) *Rhizoclonium sp.* (Dandiganahalli), (C) *Spirogyra sp.* (Hoovnalli), (D) *Klebsormidium sp.* (Ikola), (E).*Rhizoclonium* hieroglyphicum (Kaggodu) and (F) *Oedogonium sp.* (Gadanahalli)

Conclusion

Terrestrial microalgae collected from Western Ghats were adjusted to grow in RO residual dispense water with pH 8.4 and urban waste water. *Rhizoclonium sp.* and *Rhizoclonium hieroglyphicum* collected from Dandiganahalli and Kaggodu, respectively grown very quickly and which reduces the pH of waste water from alkaline to neutral or acidic and also reduces the TDS content of water. On the basis of above investigation it can be concluded that the terrestrial microalgae can be used for bioremediation purpose of alkaline water. Amylase enzyme activity was high in *Rhizoclonium sp.* (Dandiganahalli) which can be used in industries for carbohydrate degrading purpose in future. *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum* (Kaggodu) showed good response to antioxidant properties to survive in stress. Based on study conducted, SOD, POD and DPPH were the scavenger of ROS protecting the chloroplast from direct effect of ROS and also protecting the cell from aging effects. This may be the reasons for microalgae were able to survival in various environmental factors.

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