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Phytochemical screening and antioxidant activity of a cyanobacterium, *Oscillatoria limosa* isolated from polythene surface in domestic sewage water

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Running title: Phytochemicals of *Oscillatoria limosa*

Abstract

Carry bags made of polyethylene are widely used commodity in consumer products and packaging. These packaging materials are dumped into landfills and water bodies leading to major contamination of the environment. Phytochemical screening and antioxidant activity of *Oscillatoria limosa* collected from polythene surface in domestic sewage water of Silchar town, Assam (India) is the subject matter of the present work. The carbohydrate, protein, lipid, vitamin C, pigments (chlorophyll a, chlorophyll c, carotenoids, and phycobiliproteins), enzymatic antioxidants, non-enzymatic antioxidants, different radical scavenging, total phenolics, and flavonoids content of *O. limosa*were analysed. The carbohydrate, protein, total phenolics, and flavonoids were found to be 240µgml^{-1}, 378µgml^{-1}, 16.33mgGAE/gDW, and 4.4mgQE/gDW, respectively. The inhibition levels were found to be 63±0.21, 69±0.31, 68±0.21 percent at concentration of 100µg/ml, respectively, for DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity.

Keywords: biochemical; cyanobacterium; sewage water; polythene; phytochemical

Introduction

Widely distributed in fresh, brackish and marine aquatic environments and in moist soil surfaces, cyanobacteria are very unique owing to their capacity to perform photosynthesis, fix nitrogen and grow in almost all types of extreme habitats including wastewater and highly polluted environments (Cuellar-Bermudez *et al*., 2017; Singh and Thakur, 2015; Dubey *et al*., 2011; Akoijam *et al*., 2015). Submerged polythene surfaces, ubiquitous in urban waste water is one such artificial substrate that is known to harbour algae including cyanobacteria (Suseela and Toppo, 2007; Sharma *et al.*, 2014; Kumar *et al.*, 2017; Sarmah and Rout, 2017). Given their ability to acclimatize to extreme environmental conditions, they are considered a rich source of secondary metabolites with potential biotechnological and pharmacological applications (Tan, 2007; Paliwal *et al.*, 2017; Sarmah and Rout, 2018). Such metabolites exhibit diverse biological activities, including antioxidant (Natrah *et al*., 2007), antimicrobial (Mugilan and Sivakami, 2016), anti-inflammatory(Deng and Chow, 2010), anticoagulant (Kim and Wijesekara, 2011), anti-mutagenic (Lahitová *et al*., 1994), antiproliferative (Sergey *et al*., 2013) and anti-cancer activities (Samarakoon *et al*., 2013). Growth of algae on such polythene substrata and their biochemical characterization are also important in the context of biodegradation of polythene (Rai and Rajashekhar 2015; Kumar *et al.*, 2017; Sarmah and Rout, 2018).Biochemical characterization ofrange of natural bioactive metabolites is the key pre-requisite for biotechnological applications. Algae generally considered as potent source of antioxidants due to higher contents of ascorbic acid, glutathione reductase, phenols and flavonoids (Wu *et al.*, 2010). Algal derived antioxidants, such as carotenoids, vitamin E (α-tocopherol), phycobiliproteins, polyphenols have drawn immense interest in health and pharmaceutical industry (Munir *et al.,*2013). The present study therefore addresses the phytochemical screening and antioxidant activity of a cyanobacterium, *Oscillatoria limosa* collected from submerged polythene surface in the domestic sewage water of Silchar town in the state of Assam, India.

Material and methods

Isolation of Oscillatoria limosa

The cyanobacterium was collected from submerged polythene surface in domestic sewage water of Silchar town (Assam, India). The study area lies between latitude 24º49´ North and longitude 92º48´East and altitude of 114.69

meters above sea level on the banks of river Barak. The species was isolated and purified (Rippka *et al.*, 1979). The culture was microscopically examined and identified as *Oscillatoria limosa* (Prescott, 1952; Desikachary,1959) (Fig.1). The BG11 agar petri plates containing the cyanobacterium were incubated for 15 days under continuous illumination (2000lux) at 24±1ºC. The pure colonies of the cyanobacterium were developed in agar petri plates. The pure colonies were diluted in sterilized distilled water and subcultured to 100mL of culture media in 250 mL conical flasks.

Fig. 1. Photomicrograph of *Oscillatoria limosa*

Physico-chemical properties of sewage water

Domestic sewage water was collected in clean polythene bottles, filtered to remove suspended particles, stored at 4°C andwas analysed (APHA, 2005).

Harvesting

The growth phases of the *O. limosa*were determined interms of their chlorophyll-*a* content (Kobayasi, 1961). The cultures of the cyanobacterium were harvested at exponential growth phase. The cyanobacterial biomass was separated from the BG-11 media by centrifugation at 3500r/min for 10 min.,collected by filtration and transferred to pre-weighed filter paper and oven-dried at 60ºC for 2 hours. The dried biomass was stored in vials at 4°C for further analysis.

Enzymatic and non-enzymatic antioxidants

Standard protocol were followed for catalase activity (Aebi, 1984), peroxidase activity (Kar and Mishra, 1976) andglutathione peroxidase activity (Rotruk*et al.*, 1973). Ascorbic acid content was estimated as per Roe and Keuther (1943). Glutathione reductase was assayed following the method of Scaedle and Bassham (1977).

DPPH free radical scavenging activity, hydroxyl radical scavenging activity, total antioxidant activity

DPPH free radical scavenging activity was measured according to Sanchez-Moreno *et al.*, 1995.
Hydroxyl radical scavenging activity was measured by the method outlined by Ruch*et al.*, 1989and that of total antioxidant activity was assessed by the method of Prieto *et al.*, 1999.

Phytochemical screening

The total phenolic content of the methanol extract was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965).Total flavonoid content of the culture was determined by aluminium chloride method (Jia*et al.*,

1999).Chlorophyll *a*, c and carotenoids were estimated by standard methods(Strickland and Parsons, 1968and Parson, 1984). Vitamin C content was estimated using the method of Roe and Keuther (1943).Total carbohydrate was determined by anthrone method (Spiro, 1966). Total protein was estimated by modified method of Herbert *et al.*, (1971). Phycobiliproteins estimation has been made as per Bennet and Bogorad (1973). Lipid content was estimated by the standard method of Bligh and Dyer (1959).

Results

The physico chemical propertites of domestic sewage water is important to know the natural habitat conditions of *O. limosa*.The temperature of sewage water was 34±0.56ºC. Biological oxygen demand, chemical oxygen demand and dissolved oxygen of water was 600±0.18, 1520±0.18, and 2.1mg/L, respectively. The total alkalinity was 10±1.4mg/L, free CO_2 was 36.98±0.13mg/L and total dissolved solid of sewage water was found to be 500 ± 0.18mg/L. The suspended solid was 50±0.54mg/L. The chloride content and calcium concentration was found to be 60±0.67mg/L, 60±0.13mg/L, respectively. The sulphate, nitrate, magnesium and ammonia of sewage water was present at a concentration of 50 ± 1.6mg/L, 12 ±1.5mg/L, 30± 0.23mg/L and 30±0.45mg/L, respectively.The phosphate concentration found to be 70±0.45mg/L.

The growth rate of *O.limosa* (Fig.2) was found to be 0.158μd^{-1} with generation time 178.25h. The cyanobacterium showed a 28 days life cycle characterized by shorter duration log period with highest pigment production.
The catalase, peroxidase and glutathione reductase activity (Fig. 3) of methanol extract from *O.limosa* were found to be 45.11±0.41%, 87±0.34% and28±0.43% at for 100μg/ml, respectively.
DPPH radical scavenging activity, hydroxyl radical scavenging activity, total antioxidant activity of *O.limosa* (Fig 4) of the extracts were in the range of 5-100 μg/ml concentration. BHT was used as standards at the concentration 5-100 μg/ml concentration. The levels were found to be 63±0.21, 69±0.31, 68±0.21 percent at concentration of 100 μg/ml, respectively, for DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity.
The total phenolic content (Table 1) in methanolic and acetonic extracts were found to be 16.33 and 14mg GAE/g DW, respectively. The total flavonoid content in methanol and acetone extracts were found to be 4.4 and 3.8mg QE/g DW, respectively. The vitamin-C content in the methanol and acetone extracts were found to be 1.2 and 0.9mg/g DW, respectively, in *O.limosa*. The chlorophyll *a* pigment in methanol and acetone extracts were found to be 7.44 and 6.7mg/g DW, respectively. The chlorophyll *c* content in methanol and acetone extracts were found to be 3.44 and 3.32mg/g DW, respectively. The carbohydrate, protein and lipid content in *O.limosa* were found to be 240, 378 and 14.3μgml^{-1}, respectively. The phycocyanin (60.7mg/g DW) and PE content (21.5mg/g DW) of the species were relatively high. Allophycocyanin content was found to be 20.35mg/g DW. Total phycobiliproteins was found to be of 102.55mg/g DW.

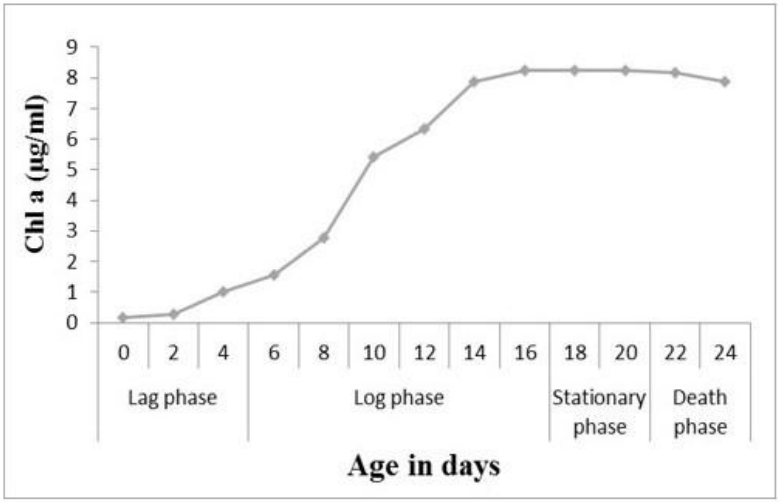

Fig.2. Growth of *Oscillatoria limosa*

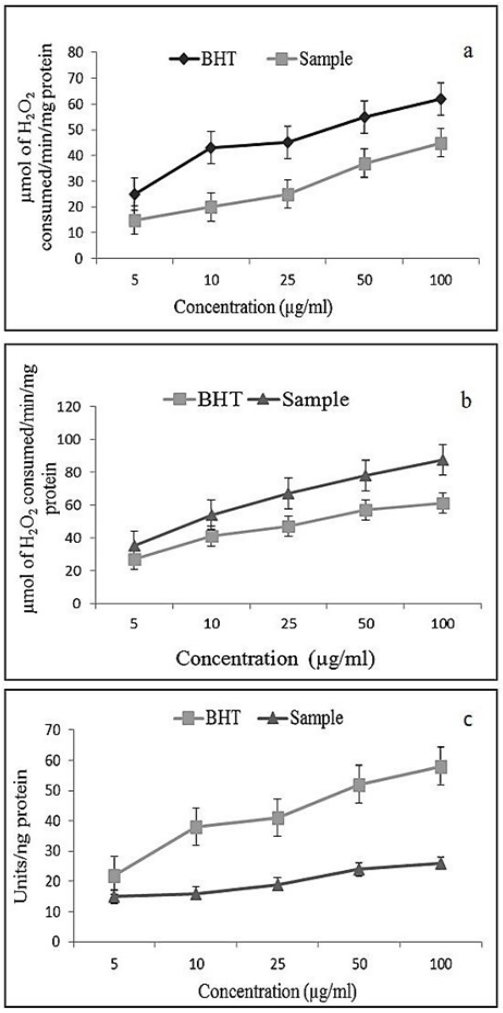

Fig.3. (a) Catalase; (b) Peroxidase; and (c) Glutathione reductase activity of *Oscillatoria limosa*

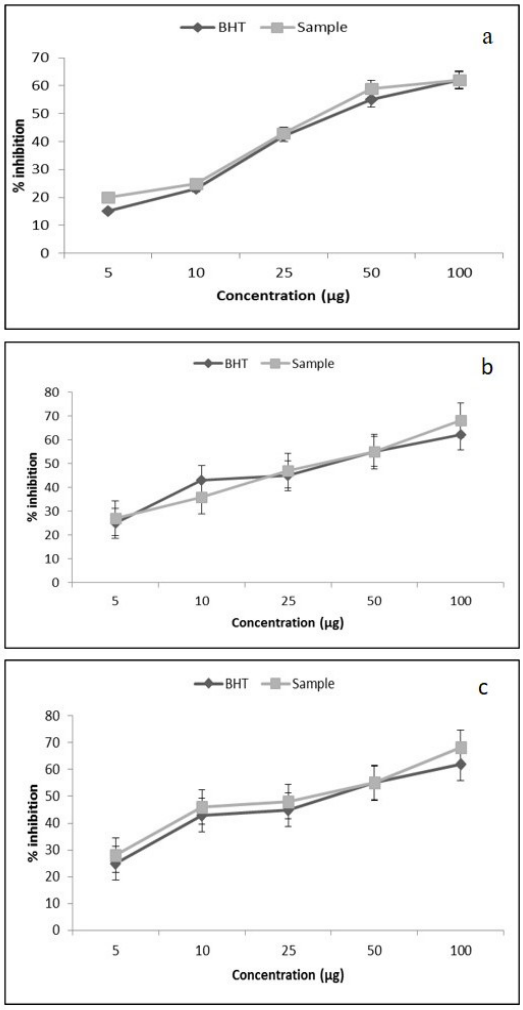

Fig. 4. (a) DPPH free radical scavenging activity; (b) Hydroxyl radical scavenging Activity; and (c) Total antioxidant activity of *Oscillatoria limosa*

Phytochemical											
Total phenolics (mg GAE/g DW)		**Total flavonoids (mg QE/g DW)**		**Vitamin C (mg/g DW)**		**Chlorophyll-a (mg/g DW)**		**Chlorophyll-c (mg/g DW)**		**Carotenoid (mg/g DW)**	
Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone
16.33 ± 0.12	14± 0.13	4.4± 0.16	3.8± 0.12	1.2± 0.11	0.9± 0.12	7.44± 0.18	6.7± 0.12	0.56 ± 0.02	0.43± 0.03	3.44± 0.01	3.32± 0.03

Discussion

The analysis of phytochemical constituents such as pigments, phycobiliproteins (PBP), protein, carbohydrate, vitamin C, lipid, total phenolics, and total flavonoids of the *O. limosa* revealed the relative concentration to be rather similar to those recently reported for *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum*, and *Cylindrospermum muscicola* isolated from the domestic sewage water of Silchar (Sarmah and Rout, 2018).

The presence of enzymatic and non-enzymatic antioxidants in *O.limosa* clearly demonstrated its role against oxidant and other free radicals. The occurrence of enzymes viz., catalase, peroxidase and glutathione reductase in the cyanobacterium are key factors to its adaptation to extreme environmental conditions (Mukund *et al*., 2014).The carotenoid content of methanol and acetone extracts were 3.44mg/g DW and 3.32mg/g DW, respectively. It is pertinent to mention herein that carotenoid play an important role in protecting the chloroplasts against photodamage, by scavenging several active oxygen species (ROS) such as $^1O_2$, O_2^-, H_2O_2, hydroxyl radicals (HO^-) and peroxy radicals (Burton, 1989; Krinsky, 1989; Munir *et al*., 2013).

The phenolic contents of methanolic and acetonic extracts of the cyanobacterium were 16.33 and 14mgGAE/g DW, respectively. Phenolic entity can donate a hydrogen atom or an electron in order to form stable radical intermediates (Jimenez-Escrig *et al*., 2001). The inhibition level in the methanolic extract of the *O.limosa*were found to be 68.11±0.21, 62.11±0.11, 69±0.31 percent at concentration of 100µg/ml, respectively, for DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity. The cyanobacterium is believed to have developed defense against photo-oxidative damage by various antioxidative mechanisms to detoxify and remove highly reactive oxygen species (ROS) by producing several oxidative and radical stressors such as phenolic compounds and carotenoids (Tsao and Deng, 2004). As the cyanobacterium was collected from submerged polythene surface in sewage water, it is anticipated that it might have gradually developed a system of either accumulating or releasing intra- or extracellular compounds to cope with the stress (Grossman *et al*., 1993; Ward and Singh, 2005, Sarmah and Rout 2017; Paliwal *et al*., 2017).

The total phycobiliproteins (PBPs) of *O. limosa* was found to be of 102.55mg/g DWin in the methanolic extract. The PBPs are believed to be a strong antioxidant which have antiviral, antitumor, anti-inflammatory and antifungal activities (Rai and Rajashekhar, 2015). The natural habitat of the *O. limosa* is rich in inorganic nutrients such as nitrate, phosphate, calcium etc. and presumed to play an important role in growth and metabolic processes by transferring energy to cells, nucleic acid biosynthesis, phospholipid biosynthesis and membrane development (Reitan *et al.*, 1994;Khozin-Goldberg and Cohen, 2006; Sang *et al*., 2012). The species is found to be the natural source of several bioactive compounds which have potential for pharmaceutical applications. The luxuriant growth of the species in polluted habitat is relevant in the context of pollution abetment and monitoring (Cairns and Dickson, 1971; James and Evison, 1979) including biodegradation of polythene (Sharma *et al*., 2014; Kumar *et al*., 2017).

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