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Original Article

EFFECT OF SOLVENT EXTRACTION SYSTEM ON THE ANTIOXIDANT ACTIVITIES OF ALGAE

TAPAN SEAL[1*], NILU HALDER[2], KAUSIK CHAUDHURI[1], SANKAR NARAYAN SINHA[3]

[1]Plant Chemistry Department, Botanical Survey of India, A. J. C. Bose Indian Botanic Garden, Shibpur, Howrah, India, [2]Department of Botany, Raja Peary Mohan College, Uttarpara, India, [3]Department of Botany, Kalyani University, India.
Email: kaktapan65@yahoo.co.in

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ABSTRACT

Objective: The present study aimed to determine the antioxidant activities of five algae including *Oedogonium globossum, Pithophora oedogonia, Rhizoclonium hieroglyphicum*, *Scytonema ocellatum* and *Spirogyra communis* and the effect of different solvent (benzene, chloroform, acetone and methanol) used for the extraction of active components from algae.

Methods: The five algae were extracted with four different solvents e. g benzene, chloroform, acetone and methanol. The Folin-Ciocalteu procedure was used to estimate the amount of total phenolic content in different extracts of algae. Total flavonoid content was estimated by aluminium chloride colorimetric method. The DPPH radical scavenging assay was used to find out the antioxidant activities of algae under investigation.

Results: The total phenolic content varied from 9.80±0.29 to 44.44±2.26 mg/g, 7.26±0.08 to 21.30±0.17 mg/g, 9.82±0.12 to 50.14±0.99 mg/g and 11.51±0.17 to 54.02±0.58 mg/g dry material in the benzene, chloroform, acetone and methanol extracts of the algae respectively. The flavonoid content was found to be the highest in the acetone extract of *Scytonema ocellatum* (593.81±2.69 mg/g) and flavonol content was found to be the highest in the chloroform extract of *Scytonema ocellatum* (448.22 ± 4.36 mg/g) whereas least amount of flavonoid present in the benzene extract of *Oedogonium globossum* 8.91±0.37 and least amount of flavonol was present in the chloroform extract of *Spirogyra communis* (13.54±1.07 mg/g). The highest DPPH radical scavenging was observed in the methanol extract of *Scytonema ocellatum* with IC_{50} = 0.24±0.003 mg dry material. The reducing power of the extracts of the algae was also evaluated as mg AAE (ascorbic acid equivalent)/g dry material. Chloroform extract of *Rhizoclonium hieroglyphicum* showed the highest reducing power 185.56±2.00 mg AAE/g dry materials.

Conclusion: Thus it could be suggested that these algae can be utilized as natural antioxidant.

Keywords: Algae, Different solvent extracts, Antioxidant activity.

INTRODUCTION

Free radicals and other reactive species are produced as by products in aerobic organism and are responsible for the oxidative damage of amino acids, lipids, proteins and DNA [1].

It has been established that free radicals could induce cellular damage and lead to several degenerative diseases such as atherosclerosis, parkinson's disease, alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancer, AIDS and in aging processes [2].

Antioxidants are molecules which can safely interact with free radicals and terminate the oxidation of other molecules and can prevent or slow oxidative damage in our body. Antioxidant compounds like phenolic acids, anthocyanins, proanthocyanidins, flavonoids, flavonols, tannins etc. scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [3]. Antioxidant compounds decelerates the production of free radical species that are induced by transition metal reactions and inhibit series of free radical reactions [4].

There is a great demand throughout the world in finding new natural sources for antioxidants to prevent oxidative damage to living cells because synthetic antioxidant like, butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA), have restricted use in foods as they are Carcinogenic [5].

The Cyanobacteria (blue-green algae) are Gram negative photosynthetic prokaryotes found in almost all the ecological habitats. Cyanobacteria produce a vast array of different biologically active compounds, some of which are expected to be used in drug development. The fact that some of the active components from cyanobacteria potentially have anticancer, antimicrobial, antiviral, anti-inflammatory, and other effects is being used for marketing purposes.

Cyanobacteria are prokaryotic organism containing a wide variety of antioxidant pigments than the plants and most algal source. Alcoholic extracts of different cyanobacterial isolates including *Oscillatoria salina Synechcococcus, Oscillatoria annae, Oscillatoria chlorina, Spirulina sabsalsa and Spirullina platensis* were screened for their antioxidant property. The alcoholic extract of *Spirullina platensis* showed potent antioxidant activity [6].

The acetone and alcoholic extracts of marine algae *viz. Chaetomorpha linum* (Muller) Kützing, *Grateloupia lithophila* Boergesen, *Sargassum wightii* Greville showed potent antioxidant activities and these seaweeds could be a good source of natural antioxidant [7]. The antioxidant activities are strongly dependant on the plant parts and nature of the solvent used for extraction. The antioxidant activities of methanol, diethyl ether, ethyl acetate, butanol, chloroform and hexane extracts of green algae *Halimeda discoidea* were studied and the non polar chloroform extract was found to exhibit potent antioxidant activity as compared to other extracts [8].

The aim of the present investigation was to evaluate the *in-vitro* antioxidant activities of the four different solvent extracts of five different algae like *Oedogonium globossum, Pithophora oedogonia, Rhizoclonium hieroglyphicum*, *Scytonema ocellatum* and *Spirogyra communis.*

MATERIALS AND METHODS

Plant materials

The five algae e. g *Oedogonium globossum, Pithophora oedogonia, Rhizoclonium hieroglyphicum*, *Scytonema ocellatum* and *Spirogyra communis* were collected from different ponds of Hooghly district, West Bengal and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office. The five algae were shed-dried, pulverized and stored in an air tight container for further extraction.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxy toluene (BHT), ascorbic acid, quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., Folin-Ciocalteu reagent, gallic acid, potassium ferricyanide, aluminium chloride, $FeCl_3$ and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Extraction of Algae (Benzene, Chloroform, Acetone and Methanol extract)

One gram of each algae was extracted with 20 ml each of benzene, chloroform, acetone and methanol at ambient temperature, with agitation for 18 -24 h. The extracts were filtered and diluted to 50 ml and aliquot were analyzed for their total phenolic, flavonoid and flavonol content, reducing power and their free radical scavenging capacity.

Estimation of total phenolic content

The Folin-Ciocalteu procedure was used to estimate the amount of total phenolic content in different plant extracts [9]. The different solvent extracts of algae (20 - 100 µl) were taken into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The extracts and other reagents were mixed well in the test tubes and allowed to stand for 30 min. The absorbance was measured at 765 nm in UV-visible spectrophotometer (Hitachi U 2000 Japan). The total phenolic content was expressed as gallic acid equivalent (GAE) using the equation based on the calibration curve: $y = 0.013x$, $R^2 = 0.9901$, where y was the absorbance and x was the GAE in milligram per gram (mg g^{-1}) of extract.

Estimation of total flavonoid content

Total flavonoids were estimated using the method of Ordonez et al., 2006 [10]. The extracts (0.5 ml) were taken in a test tube and 0.5 ml of 2% aluminium chloride in ethanol solution was added in each tube allowed to stand for one hour at room temperature. The absorbance was measured at 420 nm in a UV-visible spectrophotometer (Hitachi U 2000 Japan). A yellow color indicated the presence of flavonoids. The total flavonoid contents were calculated as rutin (mg/g) equivalent using the equation based on the calibration curve: $y = 0.0182x - 0.0222$, $R^2 = 0.9981$, where y was the absorbance and x was the rutin equivalent in milligrams per gram (mg g^{-1}) of extract.

Estimation of total flavonol content

The amount of total flavonols present in the tested extracts was estimated using the method of Kumaran and Karunakaran 2006 [11]. Tested extracts (2.0 ml), equal volume of 2% aluminium chloride in ethanol and 3.0 ml (50 g/L) sodium acetate solutions were taken in a test tube and allowed to stand for 2.5h at 20°C. The absorbance of the solution was read at 440 nm in UV-visible spectrophotometer (Hitachi U 2000 Japan). The total flavonol content in the extracts were calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $y = 0.0049x + 0.0047$, $R^2 = 0.9984$, where y was the absorbance and x was the quercetin equivalent in milligrams per gram (mg g^{-1}) of extract.

Measurement of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu 1986 [12]. Tested extracts (100 µl) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixtures were incubated at 50°C for 20 min. And 2.5 ml 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and a 0.5 ml freshly prepared ferric chloride solution (0.1%). The absorbance was measured at 700 nm in a UV-visible spectrophotometer (Hitachi U 2000 Japan). The reducing power was expressed in ascorbic acid equivalent (AAE) using the equation based on the calibration curve: $y = 0.0023x - 0.0063$, $R^2 = 0.9918$, where y was the absorbance and x was ascorbic acid equivalent in miligram per gram (mg g^{-1}) of extract.

Determination of free radical scavenging activity

The free radical scavenging activity of the extracts and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [13]. The extracts (20 - 100 µl) were placed in the test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed and kept for 30 min in dark. The absorbance was measured at 517 nm in a UV-visible spectrophotometer (Hitachi U 2000 Japan). The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg ml^{-1}) that inhibits the formation of DPPH radicals by 50%. Each value was determined from the regression equation.

Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonol content, reducing power and radical scavenging activities of each plant material were calculated by using Linear Regression analysis.

RESULTS AND DISCUSSION

Total phenol, flavonoid and flavonol content in the different solvent extracts of algae

The total phenolic content of five algae were evaluated as gallic acid equivalents (GAE) in miligram per gram (mg g^{-1}).

The screening of the four different solvent (benzene, chloroform, acetone and methanol) extracts of ten alga revealed that there was a wide variation in the amount of total phenolics ranging from 7.26±0.08 to 50.14±0.99 mg GAE/g dry material (Table 1). The highest amount of phenolic content was found in the acetone extract of *S. ocellatum* (50.14±0.99 mg GAE/g dry material), while the least amount was observed in the chloroform extract of *O. globossum* (7.26±0.08 GAE).

Table 1; Total phenolic content in the algae using different solvent extracts

S. No.	Name of the algae	Total phenolic content (GAE mg / g dry material)			
		Benzene	Chloroform	Acetone	Methanol
1	*Oedogonium globossum*	9.80±0.29	7.26±0.85	9.82±0.12	11.51±0.17
2	*Pithophora oedogonia*	15.64±0.78	15.81±1.46	15.64±0.70	17.92±0.46
3	*Rhizoclonium hieroglyphicum*	15.76±0.07	21.30±0.17	17.04±0.19	19.59±0.11
4	*Scytonema ocellatum*	27.86±1.92	20.03±0.52	50.14±0.99	54.02±0.58
5	*Spirogyra communis*	44.44±2.26	8.15±0.22	45.19±0.55	38.64±0.40

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The benzene, acetone and methanol extract of *S. communis*, chloroform and acetone extract of *R. hieroglyphicum* and *P. oedogonia* were also found to contain a very good amount of phenolic compounds.

Total flavonoid contents in the algae were estimated as rutin equivalent (mg/g) using the following equation based on the calibration curve: y = 0.0182x - 0.0222, R^2 = 0.9981, where y was the absorbance and x was the rutin equivalent (mg/g). The flavonoid contents of the extracts in terms of rutin equivalent were between 8.91±0.37 to 593.81± 2.69 mg/g dry material (Table 2).

Table 2: Total flavonoid content in the algae using different solvent extracts

S. No.	Name of the algae	Total flavonoid content (Rutin equivalent mg / g dry material)			
		Benzene	Chloroform	Acetone	Methanol
1	*Oedogonium globossum*	8.91±0.37	23.60±0.47	12.97±0.42	12.38±0.12
2	*Pithophora oedogonia*	218.49±3.30	415.50±0.61	379.31±3.28	198.35±1.75
3	*Rhizoclonium hieroglyphicum*	18.18±0.14	74.07±1.06	22.76±0.19	27.90±0.10
4	*Scytonema ocellatum*	240.04±4.40	315.15±2.44	593.81±2.69	182.60±2.56
5	*Spirogyra communis*	304.02±5.28	78.02±0.49	305.86±8.25	173.73±6.92

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The highest amount of flavonoid content was observed in the acetone extract of *S. ocellatum* (593.81± 2.69 mg/g) followed by the chloroform and acetone extract of *P. oedogonia* (415.50±0.61 mg/g and 379.31±3.28 mg/g dry materials respectively).

The benzene, acetone and methanol extracts of *S. communis*, *S. ocellatum* and all four extracts of *P. oedogonia* were also found to contain a very good amount of flavonoid.

The flavonol content in the algae was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: y = 0.0049x + 0.0047, R^2= 0.9984, where y was the absorbance and x was the quercetin equivalent (mg/g). In case of flavonol, the highest amount was observed in the chloroform extract of *S. ocellatum* (448.22 ±4.36 mg/g) followed by the acetone extract of the same plant (313.30 ±3.77 mg/g) and the benzene and chloroform extract of *P. oedogonia* (Table 3),

Table 3: Total flavonol content in the algae using different solvent extracts

S. No.	Name of the algae	Total flavonol content (Quercetin equivalent mg / g dry material)			
		Benzene	Chloroform	Acetone	Methanol
1	*Oedogonium globossum*	51.68±1.28	101.11±1.26	41.24±0.98	45.19±0.39
2	*Pithophora oedogonia*	295.57±8.99	278.68±4.53	24.38±0.20	100.66±4.30
3	*Rhizoclonium hieroglyphicum*	29.69±0.69	63.81±2.14	57.20±1.58	40.68±0.10
4	*Scytonema ocellatum*	235.37±7.85	448.22±4.36	313.30±3.77	237.59±8.09
5	*Spirogyra communis*	191.61±11.33	13.54±1.07	105.44±4.20	201.40±6.42

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

Appreciable quantities of flavonol were found in the methanol extract of *S. communis*, *S. ocellatum* and *P. oedogonia*.

It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals [14]. Flavonoids and flavonols are regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that both flavonoids and flavonols show antioxidant activity through scavenging or chelating process [15]. The results strongly suggest that phenolics are important components of these plants. The other phenolic compounds such as flavonoids, flavonols, which contain hydroxyls are responsible for the radical scavenging effect in the plants. According to our study, the high content of these phenolic compounds in the different extracts of *S. ocellatum*, *S. communis* and *P. oedogonia* can explain their high radical scavenging activity.

Reducing power of the different solvent extracts of algae

The reducing power of the algae under investigation were calculated as ascorbic acid equivalent (AAE mg/g) using the equation based on the calibration curve: y = 0.0023x - 0.0063, R^2= 0.9918, where y was the absorbance and x was the ascorbic acid equivalent (mg/g). The reducing powers of the five algae were evaluated as mg AAE/g dry material as shown in Table 4).

Table 4: Reducing power (Ascorbic acid equivalent) in the algae using different solvent extracts

S. No.	Name of the algae	Reducing power (Ascorbic acid equivalent mg / g dry material)			
		Benzene	Chloroform	Acetone	Methanol
1	*Oedogonium globossum*	36.55±1.30	104.30±1.27	58.88±1.74	68.26±0.73
2	*Pithophora oedogonia*	108.62±2.61	74.34±1.67	121.88±3.12	61.71±0.23
3	*Rhizoclonium hieroglyphicum*	170.88±2.37	185.56±3.65	79.24±1.53	54.87±1.26
4	*Scytonema ocellatum*	90.72±3.34	47.71±1.42	127.13±1.61	188.92±1.29
5	*Spirogyra communis*	154.34±4.18	10.79±0.22	123.01±4.79	126.76±3.62

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The highest reducing power was exhibited by the methanol extract of *S. ocellatum* (188.92 ± 1.89 mg/g AAE) which is also high in flavonoid content (593.81±2.69 mg /g dry material) and flavonol content (448.22±4.36 mg /g dry material). The chloroform extract of *S. communis* showed the lowest activity in terms of ascorbic acid equivalent (10.79± 0.22 mg/g). The different extracts of *R. hieroglyphicum, S. communis*, *S. ocellatum* and *P. oedogonia* showed potent reducing activity. In this assay, the presence of antioxidants in the extracts reduced Fe^{+3}/ferricyanide complex to the ferrous form. This reducing capacity of the extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom [16]

DPPH radical scavenging activity of the different solvent extracts of algae

The evaluation of anti-radical properties of Five algae was performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by the different plant materials was determined (Table 5), a lower value reflected greater antioxidant activity of the sample. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [17]. The antioxidant effect is proportional to the disappearance of the purple colour of DPPH in test samples. Thus antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron

donation and a colorless stable molecule 2,2- diphenyl-1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased.

Hence the more potent antioxidant, more decrease in absorbance is seen and consequently the IC_{50} value will be minimum. In the present study the highest radical scavenging activity was shown by the methanol extract of *S. ocellatum* (IC_{50} = 0.24±0.49 mg dry material), whereas the methanol extract of *O. globossum* showed the lowest activity (IC_{50} = 2.12±0.14 mg dry material). The strong inhibition was also observed for the benzene and acetone extract of *S. communis* (IC_{50} = 0.30±0.01 mg dry material and 0.26±0.01 mg dry material respectively). The high radical scavenging property of *S. ocellatum* and moderate antioxidant activities of the different extracts of *O. globossum*, *P. oedogonia, R. hieroglyphicum, S. communis* may be due to the hydroxyl groups existing in the phenolic compounds that can provide the necessary component as a radical scavenger. Different extracts of all algae under investigation exhibited different extent of antioxidant activities and thus provide a valuable source of nutraceutical supplements. Depending on the values, some plants are more important than some others.

Table 5: Free radical scavenging ability of the algae by the use of a stable DPPH radical (Antioxidant activity expressed as IC_{50}) using different solvents

S. No.	Name of the algae	Free radical scavenging ability IC_{50} mg / g dry material)			
		Benzene	Chloroform	Acetone	Methanol
1	*Oedogonium globossum*	0.51±0.02	0.75±0.04	0.30±0.01	2.12±0.14
2	*Pithophora oedogonia*	0.32±0.01	0.41±0.05	0.31±0.01	1.28±0.05
3	*Rhizoclonium hieroglyphicum*	1.17±0.06	0.73±0.02	0.34±0.01	0.87±0.02
4	*Scytonema ocellatum*	0.63±0.29	0.30±0.01	0.34±0.04	0.24±0.49
5	*Spirogyra communis*	0.30±0.01	1.39±0.23	0.26±0.01	0.37±0.04

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

CONCLUSION

The result of present study showed that the benzene, chloroform and acetone extracts of *S. ocellatum* which contain highest amount of phenolic compounds and appreciable amount of flavonoids and flavonols exhibited the greatest reducing power and also showed strong radical scavenging activity. The highest radical scavenging activity and very strong reducing power of the different extracts of *S. ocellatum* may be due to the presence of a very good amount of total phenolics, flavonoids and flavonols contents in this plant. The antioxidant properties of the five algae under investigation are also well compared with some other marine algae like *Chaetomorpha linum*, *Grateloupia lithophila*, *Sargassum wightii*, *Clitoria ternatea* could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. The radical scavenging activities of the selected algal extracts are still less effective than the commercial available synthetic like BHT. As the plant extracts are quite safe and the use of synthetic antioxidant has been limited because of their toxicity, therefore, these algae could be exploited as antioxidant additives or as nutritional supplements. However, isolation of active components from algae are necessary which are actually responsible for the antioxidant activities.

CONFLICT OF INTERESTS

Declared None

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