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Phytochemical studies in blue-green and green algae

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ABSTRACT

A Systematic study of plant crude drugs is embraces through the consideration of both primary and secondary metabolites which are derived from the process of metabolism. The primary metabolites such as carbohydrates, proteins and lipids are used as a food for human beings where as the secondary metabolites such as phenols, alkaloids, flavanoids, lectins, steroids and saponins are used for therapeutic purposes. The chemical composition of algae vary to some extent based on the growth conditions namely temperature, light, P^H and availability of nutrients. In the present investigation the presence of qualitative and quantitative phytochemicals namely Carbohydrates, proteins, Phenols and flavanoids were carried out in Blue green alga Microchaete tenera and Green Algae Nitella tenuissima and Sphaeroplea annulina. The estimated carbohydrate rich in Nitella tenuissima (504mg/100gm) as compared to Sphaeroplea annulina (413mg/100gm) and Microchaete tenera, (301gm/100gm). Protein rich in Nitella tenuissima (624gm/100gm) as compared to Microchaete tenera (496mg/100gm).and Sphaeroplea annulina (350mg/100gm) and Phenol rich in Nitella tenuissima (252mg/100gm) as compared to Microchaete tenera, (204mg/100gm).and Sphaeroplea annulina (186mg/100gm).

Keywords: *Phytochemical studies in blue-green and green algae*

I. INTRODUCTION

Health and diseases are co-evil in human life. The disease and the associated suffering are the bitter experiences of the mankind that might have necessitated remedial measures from the plants around. Many potential algae are currently available to meet the present problems of health and survival. Traditionally algae used as nutrition but also consumed as a nutraceutical that may avail for health (Bagchi, 2006; Hafting et al., 2012). The commercial application of micro algae derived bioactive compounds has received less attention in pharmaceuticals. However, little work has been done to isolate active constituents and their action in vivo. Thus, there is a need of micro algae-based technology for the commercial production of biological active compounds or pharmaceuticals and antibiotics of industrial scale by pharmaceutical companies for the treatment of disease and other ailments.

A large number of algal species were assayed for their primary metabolites whereas only few algal species were screened for the secondary metabolites. It may be considered that biosynthetic industry not only focusing the chemical compounds such as carbohydrates, proteins and lipids that are utilized as a food for human being but also focuses on compounds like phenols, alkaloids, lectins, steroids, flavanoids etc. that exert a physiologic effect. The compounds that are responsible for therapeutic effect are secondary metabolites. A systematic study of crude drugs embraces through consideration of both primary and secondary metabolities which are derived as result of metabolism. The chemical composition of algae varies to some extent depending upon environmental gradients such as temperature, PH, humidity and availability of nutrients.

In the present investigation the qualitative and quantitative phytochemical studies of primary and secondary metabolites was carried out in Blue green alga Microchaete tenera and Green Algae Nitella tenuissima and Sphaeroplea annulina.

II. MATERIAL AND METHODS

For the present study the Blue-green alga *Microchaete tenera* was isolated from the tank of Department of Botany, Gulbarga University, Gulbarga where as Green algae *Nitella tenuissima* and *Sphaeroplea annulina* were isolated from Pala tank, Pala village and Bheema river, Katti sangavi village of Gulbarga district respectively. The collected algal material without any contamination was washed several times in distilled water and its moisture was blotted with a filter paper than it was shade dried. The fully dried material was powdered with the help of pestle and mortar. In the study, cold extract of algal material was used. 10 gm of powdered algal material was extracted with methanol. The extracts were evaporated to dryness on a water bath. Thus, obtained methanolic

extract was subjected to preliminary screening for the detection of various constituents.

Primary metabolites

The preliminary test for primary metabolites such as, protein and carbohydrate were done using methanolic extracts of Blue green alga *Microchaete tenera* and Green Algae *Nitella tenuissima* and *Sphaeroplea annulina* using the method given by Sadasivam and Manickam (1992).

Proteins

*Ninhydrin Test***:** 1 ml of 0.1% freshly prepared Ninhydrin solution was added to 4ml of test solution at neutral pH. The contents were mixed and boiled for a minute and allowed to cool. Observed for the appearance of violet or purple colour solution.

Biuret Test: 2ml of 10% Sodium hydroxide was added to 2ml of test solution, mixed well and two drops of 0.1% Copper sulphate solution was added. Observed for the appearance of violet or pink colour.

Test for Carbohydrates

Molisch's Test: 2 drops of Molisch's reagent was added to 2ml of test solution and mixed well. Incline the tube and 1ml of Conc.Sulphuric acid was added along the side of the test tube and observed for the appearance of red cum violet colour ring at the junction of the 2 liquids.

Iodine Test: A few drops of iodine solution were added to 1ml of the test solution and observed the formation of deep blue colour.

Quantitative estimation of protein (Lowry *et al.*, 1951)

Reagents required

A-2% Sodium carbonate in 0.1 NaOH
B-0.5% Copper sulphate mixed with 1% Sodium potassium tartarate (1:1).
C-Alkaline copper solution- Mix 50 ml of A and 1.0ml B prior to use.
D-Folin-Ciocalteu solution -Dilute with equal volume of water just before use.

Extraction of protein from algal plant material

1g of plant material was taken in a clean pestle and mortar, homogenated with 5ml of distilled water and centrifuged at 2000rpm for 30 minutes. To the supernatant, 2ml of 30% Trichloroacetic acid was added. The precipitation formed was allowed to settle down. It was again centrifuged for about 10 min. and the supernatant liquid layer was used for protein estimation.

Procedure

To 1ml of the extract, 5ml of alkaline copper solution was added and allowed to stand at room temperature (28±2ºC) for 10 minutes. Then, added 0.5ml of Foline- Ciocalteau reagent with immediate mixing. After 30 minutes, read the absorbance at 660nm. Calculated the amount of protein in the sample with the help of the standard curve prepared using Bovine Serum Albumin (BSA).

$$\text{Protein Content mg/100gm} = \frac{\text{Graph value X Total volume of extract X 100}}{\text{Plant material taken X volume taken for reading}}$$

Quantitative estimation of total carbohydrates (Hedge and Hofreiter,1962)

Reagents required

Anthrone reagent: Dissolved 200mg Anthrone in 100ml of ice cold 95% H_2SO_4. Prepare fresh before use.

Extraction of total carbohydrate from the plant material

100mg of the plant material was hydrolysed by keeping it in a boiling water bath for 3h along with 5ml of 2.5N HCl and cooled to room temperature. Neutralized by adding solid Sodium carbonate until the effervescence ceases. Made the total volume 100ml with distilled water and centrifuged. The supernatant was collected and used for the quantitative estimation of total carbohydrates.

Procedure

0.1ml of aliquot of the supernatant was taken in a clean test tube and made it 1ml by adding distilled water. 4ml of Anthrone reagent was added and heated for 8 min. in a boiling water bath. Cooled rapidly and measured the absorbance at 630nm. Using the standard graph of glucose, calculated the amount of total carbohydrates present in the extract.

$$\text{Carbohydrate Content mg/100gm} = \frac{\text{Graph value X Total volume of extract X 100}}{\text{Plant material taken X volume taken for reading}}$$

Secondary metabolites

The preliminary tests for secondary metabolites such as, alkaloids, phenols, steroids, flavonoids, saponins, tannins and lignins were done using methanolic extract of Microchaete sp., Nitella sp. And Sphaeroplea sp. (Gibbs, 1974; Kleipool, 1952).

Test for alkaloid

Various extracts were mixed well with 5ml of 1% HCl and filtered. The filterate thus obtained was used in the following tests.

Mayer's reagent- 1 ml of filterate was mixed with a few drops of Mayer's reagent and kept for the formation of creamy white precipitate.

Wagner's reagent- 1 ml of filterate was mixed with a few drops of Wagner's reagent and observed the appearance of reddish brown precipitate.

Test for phenols

Phenol Test: 1ml of plant extract was mixed with a few drops of ferric chloride solution and observed the formation of intense colour.

Ellagic acid Test: A small quantity of plant material is macerated with ethanol. The ethanolic extract is separated and treated with a few drops of 5% acetic acid and a few drops of 5% Sodium nitrate solution. Observed the formation of muddy yellow/olive green/niger brown/deep chocolate colour.

Test for flavonoids

Ferric chloride Test: A few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract and observed the formation of a blackish green colour.

Lead acetate Test: A few drops of lead acetate solution (10%) were added to the alcoholic solution and observed the formation of yellow precipitate.

Shinoda Test: A few drops of alcoholic extract mixed with a few fragments of magnesium turnings and concentrated HCl and observed the formation of magenta colour after a few minutes.

Flavonoid Test: To the plant extract added few pieces of Magnesium turnings and a few drops of concentrated H_2SO_4. Observed the formation of scarlet colour.

Test for Steroids

Salkowaski Test: Few drops of concentrated Sulphuric acid were added to the plant extract, shaken well and allowed to stand for the appearance of red colour in lower layer.

Leibermann-Burchardt Test: To the plant extract, a few drops of acetic anhydride were added and mixed well. 1ml of concentrated Sulphuric acid was added from the side of the test tube and observed the formation of a reddish brown ring at the junction of two layers.

Test for Saponins

Foam Test- Small amount of extract was shaken well with a little amount of water and kept for the formation of foam.

Test for tannins.

Ferric chloride Test: 1% Ferric chloride solution mixed with the extract and observed the appearance of blue green or brownish green colour.

Test for Lignins

Labat Test: Gallic acid was added to iml of methanolic algal extract. If the extract develops olive green colour, indicates the positive reaction for liognans.

Furfuraldehyde Test: To 1ml of methanolic algal extract and 1ml of 2% furfuraldehyde was added the development of red colour indicate Positive reaction to lignans.

Quantitative estimation of phenols by Folin-Ciocalteu method (Malick and Singh, 1980)

Reagents required

80% Ethanol, Folin-Ciocalteu reagent, 20% Na_2CO_3

Extraction of total phenols

500mg of the algal material was homogenated using pestle and mortar in 80% methanol. The homogenized solution was centrifused at 10,000 rpm for 20 minutes. The supernatant was retained and the extraction was repeated with the residue for 5-7 times. All the supernatants were mixed and evaporated to dryness. The residue thus obtained was dissolved in 5ml of distilled water and used for the estimation of total phenols.

Procedure

1ml of the extract was mixed with 1ml of Folin-Ciocalteu reagent and 2ml of Sodium carbonate solution. Shaken the tube and heated in a boiling water bath for 1 min. and then cooled under running tap water. Diluted the solution to 25ml by adding distilled water and measured the absorbance at 650nm. With the help of standard curve obtained using different concentrations of catechol, calculated the amount of phenol present in the sample.

III. RESULTS AND DISCUSSION

The primary and secondary metabolites present in Microchaete tenera, Nitella tenuissima and Sphaeroplea annulina are estimated and presented. (Fig. 1,2,3).

Fig.-1- ***Microchaete tenera,***

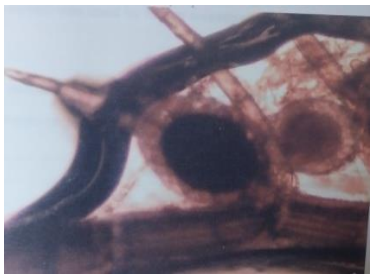


Fig.-2-*Nitella tenuissima*

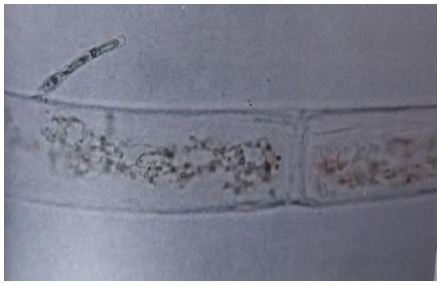


Fig.-3- ***Sphaeroplea annulina***

Preliminary phytochemical analysis of primary metabolites
The presence of proteins and carbohydrates were tested

Test for proteins
Ninhydrin test showed positive reaction by developing violet colour of methanolic extracts of Microchaete sp. Nitella sp. and Sphaeroplea sp. Biuret test showed positive reaction by developing violet colour of methanolic extracts of Microchaete sp, *Nitella tenuissima,* and Sphaeroplea sp.

Test for Carbohydrates
Molisch's Test showed positive reaction by developing red cum violet colour ring at the junction of the 2 liquids of methanolic extracts of Microchaete sp. *Nitella tenuissima* and *Sphaeroplea annulina* Iodine Test did not showed any positive reaction for methanolic extracts of *Microchaete tenera* Nitella sp. and *Sphaeroplea annulina*.

Preliminary phytochemical analysis of Secondary metabolites

Test for alkaloid
The alkaloids are commonly identified by the formation of precipitate and turbidity of the solution with mayers, wagners reagents. Both these reagents did not show any turbidity of the solution in the methanolic extracts of selected three algal species.

Test for phenols
Elagic acid test showed positive reaction by developing muddy yellow colour in the methanolic extract of *Microchaete tenera* and *Nitella tenuissima,* and niger brown in the methanolic extract of *Sphaeroplea annulina*

Test for Steroids
The methanolic extract of selected algae did not show any positive reaction for the presence of steroid.

Test for Flavonoids
Shinoda test showed positive reaction by developing yellowish brown colour in the methanolic extract of selected three algal species.

Test for Saponins
The methanolic extract of selected algae did not show any positive reaction for the presence of saponins.

Test for Tannins
The methanolic extract of selected algae did not show any positive reaction for the presence of Tannins

Test for Lignans
The methanolic extract of selected algae did not show any positive reaction for the presence of Tannins

Quantitative estimation of proteins
In the present investigation it was estimated that Protein rich in *Nitella tenuissima,* (624gm/100gm) as compared to Microchaete Sp. (496mg/100gm).and *Sphaeroplea annulina* (350mg/100gm) (Table-1)
Quantitative estimation of Carbohydrates

In the present investigation it was estimated that carbohydrate rich in *Nitella tenuissima,* (504mg/100gm) as compared to *Sphaeroplea annulina* (413mg/100gm) and *Microchaete tenera* (301gm/100gm) (Table-2)
Quantitative estimation of Phenol

In the present investigation it was estimated that Phenol rich in *Nitella tenuissima,* (252mg/100gm) as compared to *Microchaete tenera* (204mg/100gm) and *Sphaeroplea annulina*(186mg/100gm) (Table-3) .

Sl. No	Standard Bovine Serum Albumin solution in ml	Distilled water in ml.	Alkaline copper solution (Lowry's reagent) in ml	Allow to stand for 10 minutes	Folin-Ciocalteu reagent in ml	Incubation at room temperature in dark for 30 minutes	standard	Optical density (OD) at 650 nm		
								Microchaete tenera,	*Nitella tenuissima,*	*Sphaeroplea annulina*
1	0.0Blank	1.0	5.0		0. 5		-----			
2	0.2	0.8	5.0		0. 5		0.056			
3	0.4	0.6	5.0		0. 5		0.116			
4	0.6	0.4	5.0		0. 5		0.183			
5	0.8	0.2	5.0		0. 5		0.224			
6	1.0	0.0	5.0		0. 5		0.291			
7	1.0 Plant extract	-	5.0		0. 5		-	0.090	0.114	0.072

Table-1: Quantitative estimation of Protein in *Microchaete tenera,* *Nitella tenuissima* and *Sphaeroplea annulina*

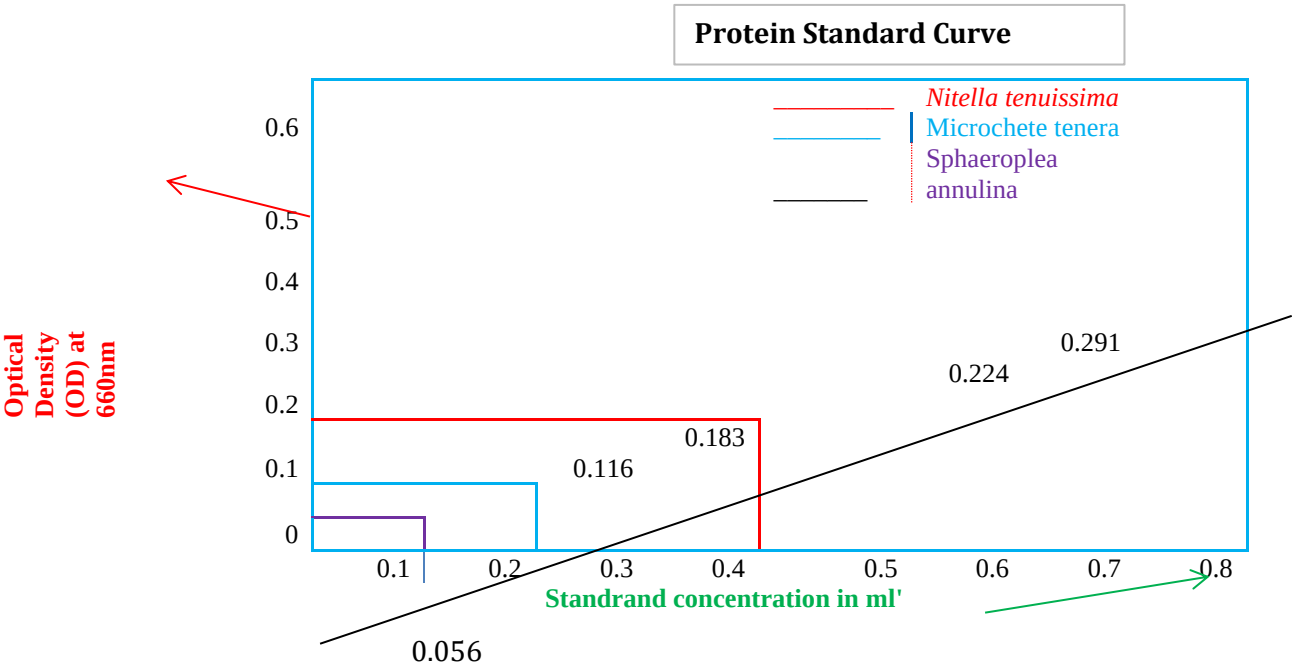


Table-2: Quantitative estimation of Carbohydrates in *Microchaete tenera, Nitella tenuissima* and *Sphaeroplea annulina*

Sl. No	Standard Glucose solution in ml	Distilled water in ml.	Anthrone reagent in ml	Heat for 8 minutes in boiling water bath	standard	Optical density (OD) at 650 nm		
						Microchaete tenera,	*Nitella tenuissima,*	*Sphaeroplea annulina*
1	0.0Blank	1.0	4.0		-----			
2	0.2	0.8	4.0		0.125			
3	0.4	0.6	4.0		0.231			
4	0.6	0.4	4.0		0.246			
5	0.8	0.2	4.0		0.494			
6	1.0	0.0	4.0		0.584			
7	1.0 Plant extract	0.0	4.0		-	0.341	0.415	0.247

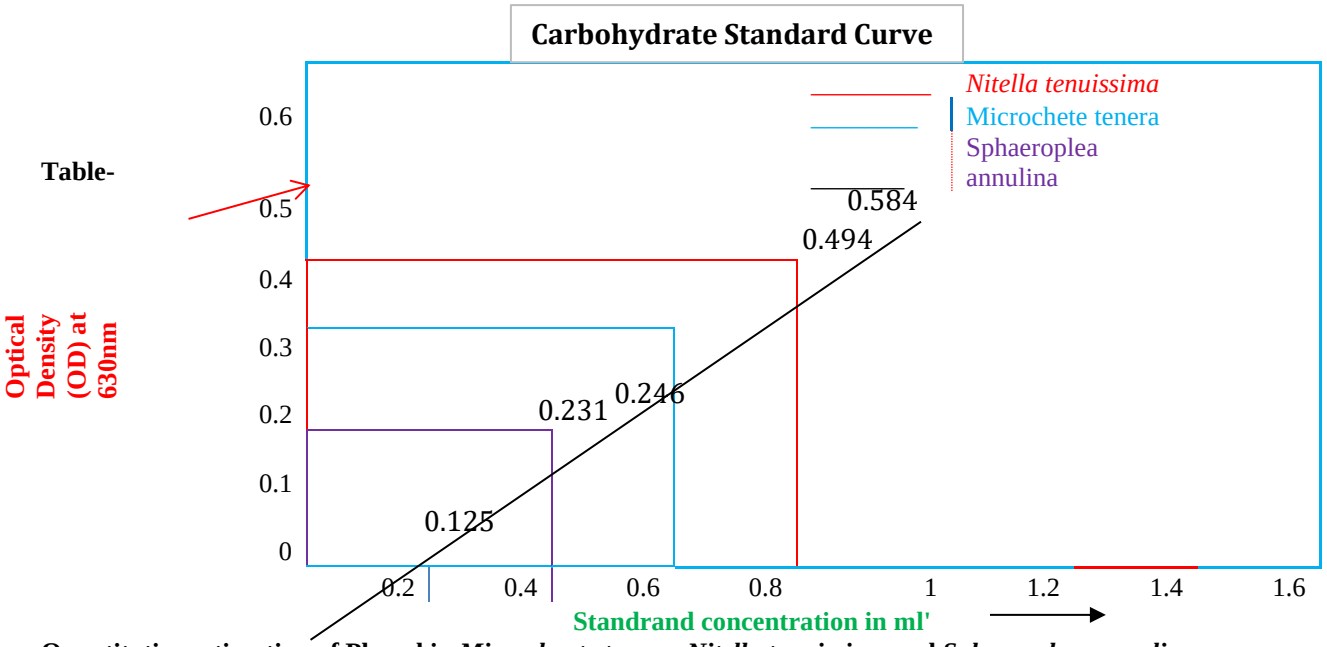
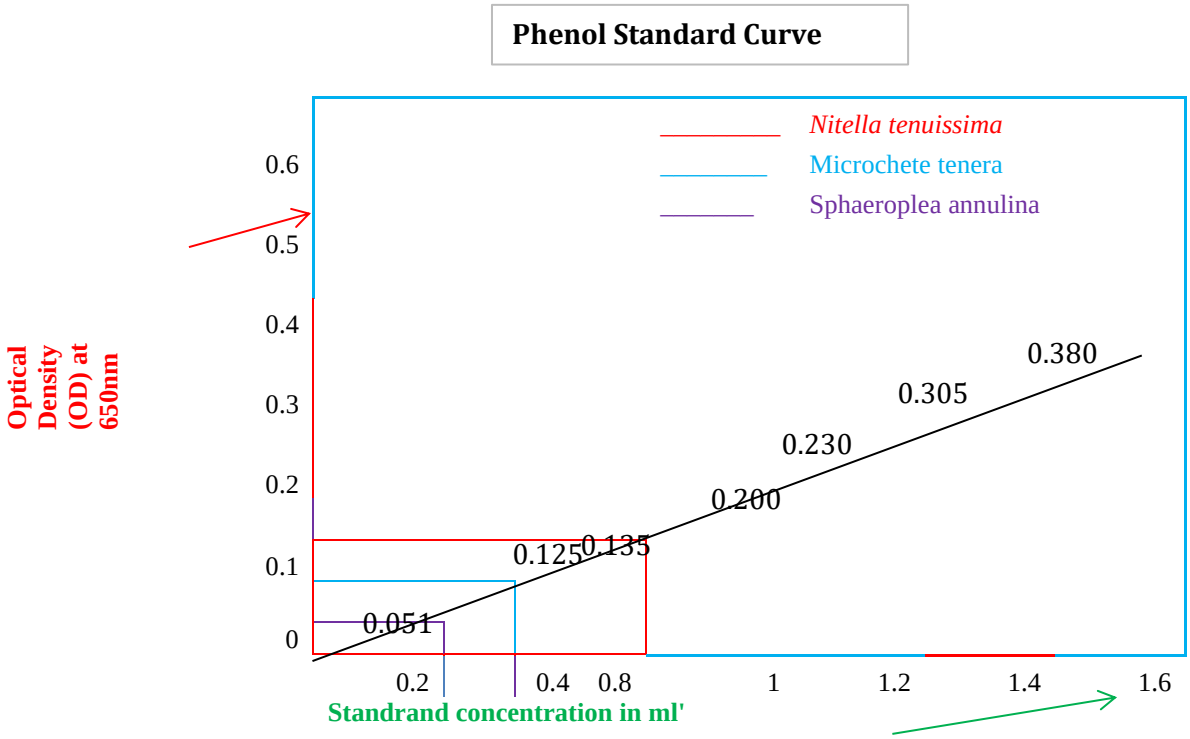


Table- 3:

Quantitative estimation of Phenol in *Microchaete tenera, Nitella tenuissima* and *Sphaeroplea annulina*

Sl. No	Standard catechol solution in ml	Distilled water in ml.	Folin-Ciocalteu reagent in ml		Sodium carbonate in ml	Standard	Optical density (OD) at 650 nm		
							Microchaete tenera,	*Nitella tenuissima,*	*Sphaeroplea annulina*
1	0.0Blank	3.0	5.0	Allow to stand for 3 minutes	2.0	-----			
2	0.2	2.8	0.5		2.0	0.051			
3	0.4	2.6	0.5		2.0	0.125			
4	0.6	2.4	0.5		2.0	0.135			
5	0.8	2.2	0.5		2.0	0.200			
6	1.0	2.0	0.5		2.0	0.230			
7	1.2	1.8	0.5		2.0	0.305			
8	0.4	1.6	0.5		2.0	0.380			
9	1.0 Plant extract	2.0	0.5		2.0	-	0.085	0.105	0.074



In the present investigation the phytochemical studies on one Blue-green alga and two Green algae were carried out. The preliminary test is very important to identify the presence of primary and secondary metabolites. The primary metabolites such as protein and carbohydrates and secondary metabolites such as phenols and flavanoids are present in the selected algae namely *Microchaete tenera, Nitella tenuissima, Sphaeroplea annulina.*

Carbohydrates and Proteins ment for good health and nutrition. Apart from *Microchaete tenera, Nitella tenuissima,* and *Sphaeroplea annulina carbohydratwes* and proteins are isolated from Green alga *Ulva linza*, Brown alga *Sargassum vulgare* and Red alga *Pterocladia capillacea* (Dalia M. S. A. Salem et al., 2018).

Phenols are the compounds which contains aromatic ring in their structure with ahydroxyl group attached to it are known to be effective against antiseptic, anthelmintic, diuretic and other problems. Apart from *Microchaete tenera, Nitella tenuissima* and *Sphaeroplea annulina* the phenol isolated from red algae *Polysiphonia lanosa* (Karl-Werner Glombitza and Gisela Gerstberger, 1985) which has shown the antifungal activity.

Flavonoids are the organic compounds known for pharmacological and antibiotic properties. Apart from *Microchaete tenera, Nitella tenuissima* and *Sphaeroplea annulina* the flavonoid isolated from *Chlorella pyrenoidosa which* have been patented as potentially useful antitumor and immunostimulating supplements (Joanna Slusarczyk *et al* 2021). Both Phenols and Flavanoids are expected to be therapeutic by nature.
Therefore the present investigation warrants to the detail phytochemical studies of these three selected algae.

IV. CONCLUSION

There is very much less phytochemical work has been carried out on the selected algae namely *Microchaete tenera, Nitella tenuissima* and *Sphaeroplea annulina* as compared to the other algae namely *Spirulina, Chlorella, Scenedesmus and Prophyridium*. But it is a very good attempt to explore the active constituents present in the selected algae which can receive greater attention of the phytochemists to produce fine chemicals for various purposes. Systematic screening of the algae for specific products would probably lead to a greater commercial potential.

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