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Exploring the Antibacterial potency and Phytochemical visibility of the Green Alga *Chara zeylanica* Klein ex Willdenow

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Abstract

Chara zeylanica Klein ex Willdenow a macroscopic green alga, commonly found in the freshwater of North 24 Parganas district, West Bengal, India. Phytochemical analysis and antimicrobial testing were conducted to identify the active components present in this green seaweed. The analysis of phytochemicals indicates the presence of Cardiac glycoside, Cholesterol, Phenol, Quinones, Steroid, and Tannin in the solvents. Anthraquinone and Phlobatannins were discovered to be missing in every solvent tested. Alkaloid was exclusively detected in diethyl ether solution. This particular alga showed strong antibacterial effects against *Escherichia coli* in methanol extracts.

Key word : Phytochemicals, antibacterial activity, *Chara zeylanica*.

Algae are chlorophyll bearing autophytic representatives of thallophytes which can produce their own food. The studies focused on different species of Chara have been done by various workers. Phytochemical screening has been done by Bancova *et al.*[1] & Barger and Schagerl[3] on *Chara globularis* and *Chara aspera* respectively while antibacterial activity of *Chara zeylanica* was studied by Krubha *et al.,*[11]. *Chara zeylanica* Klein ex Willdenow, a common fresh water macroscopic green alga has been taken into consideration in the view that the results may be exploited for its commercial potentials. This alga was studied thoroughly for its morphological, cytological & medicinal properties.

Collection of algal samples :

Algal material were collected from North 24 Prganas District, West Bengal, India within longitude & latitude 22°11'6" N - 23°15'2" N & 88°20' E - 89°5' E, pH 7, temp 29ºC. Freshly collected plant specimen was washed thoroughly and dried in shade at room

temperature. Ecological notes were recorded in the field notebook for documentation & identification. Algal specimens were identified with different standard literature[13].

Preparation of algal extract :

4 sets each of 10 gm dried algal samples were crushed in mortar and pestle to obtain coarse powder. Each dried sample was added with 4 different solvents viz methanol, water, diethyl ether and acetone 100ml each separately and boiled for 2-3 minutes. The solutions were then filtered using Whatman filter paper (Number 40).

Protocol for analysis of phytochemicals of Chara zeylanica :

Alkaloid Test was done following method of Harborne[8] & Trease and Evans[15] as follows: 2ml of algal extract taken and then 2ml of 2N HCl was added to each extract. Shaken vigorously and kept for 5 minutes. Then a few drops of Mayer's reagent ($HgCl_2$ + KI in water) were added when aqueous phase was separated from the liquid phase. A creamy precipitation occurred after the shaking the material.

Anthraquinone Test was done following Trease and Evans[15] as follows: 0.5 gm crude powder of algal extract was taken and 20ml of each solvent was added separately for each solvent. Then boiled for 2-3 minutes and kept for 4 hr. The 10ml of each filtrate was taken and 0.5 ml ammonia solution added which produce violet colour.

Cardiac glycoside Test was done using method followed by Daniel[3]: 5ml algal extract taken and mixed with 2ml glacial acetic acid It was taken along with 0.5 ml of 2% $FeCl_3$. To it 1ml conc. H_2SO_4 was added to form green or brown colour.

Cholesterol Test was done following Harbone *et al.*,[8]: 10ml of algal extract was mixed with 2ml chloroform. 10-11 drops of acetic acid were added and vigorously shaken. 2 drops of conc. H_2SO_4 were added to change the colour from reddish brown to blue green.

Coumarin Test was done following Harbone *et al.*,[8]: At first 0.5ml crude powder taken and 5ml of each solvent were added in a test tube. After that test tube mouth was covered with filter paper which is treated with NaOH (1N). It was then boiled for a few minutes and filtered. Observation was made under UV light whether yellow fluorescence colour was visible.

Flavonoids Test was done following Edeoga *et al.*,[4]: 10ml of each solvent was taken and 0.2gm of powdered sample was added with each solvent in a test tube. Test tube was heated in a steam bath for around 3 minutes. After that solvent was filtered and 4ml of filtrate mixed with 1ml of 1% ammonium solution. It was shaken and observing yellow coloration.

Phenol Test was done following Mace Gorbach[12]: 0.2gm of powdered sample was added to 10ml of each solvent extract. Then 4-5 drops of 2% $FeCl_3$ were added to the solvent extract and observing colour change of the solution.

Phlobatannins Test done following Edeoga *et al.*,[4]: 0.2gm of powdered algal

sample was added to 10ml of each solvent extract and filtered. 2ml of filtrate was mixed with 1ml (1%) HCl and boiled to produce red or brown colouration.

Quinones Test was done following Evans[5]: 1ml of each solvent algal extract filtrate taken and mixed with 1ml of conc. H_2SO_4 to produce red or deep green coloration.

Saponins Test was done following Smolenski *et al*.,[14] & Kapoor *et al*.,[9]: 0.5gm crude algal powder of sample was boiled in a water bath with 15ml of solvent. Intensive foam formation indicates the presence of saponins.

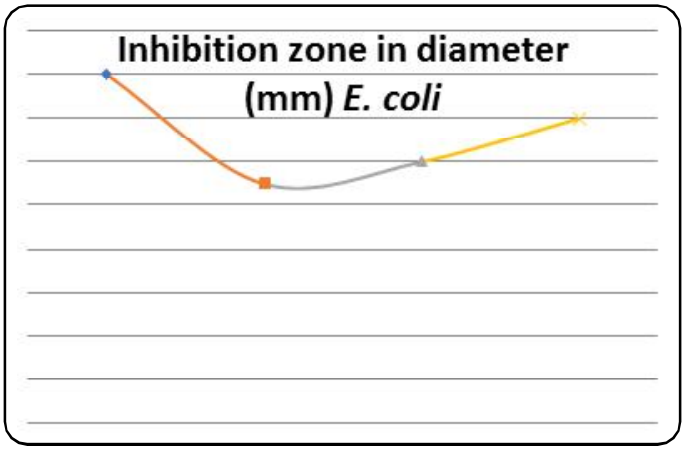
Steroid Test was done following Kolawole[10]: 0.2gm of powdered algal sample was added to 10ml of each solvent and then filtered. 5ml filtrate of each solvent extract was taken in a test tube along with glacial acetic acid added. Then it was cooled for 15 minute in a water bath. 0.5ml chloroform and 1ml conc. H_2SO_4 were added to the test tube and a reddish brown ring formed in between two liquids.

Tannin Test was done following Trease and Evans[16]: 0.5 gm of crude algal powder was add to 20ml of each solvent and boiled then filtered. A few drops of 2% $FeCl_3$ were added to the filtrate to form brownish green to blue black coloration.

Terpenoids Test was done following Evans[5]: 5ml of algal sample extract was taken and 2ml chloroform was added. Then 3ml of conc. H_2SO_4 was added and a layer form which led to green or reddish brown coloration.

Table-1. Phytochemical constituents of *Chara zeylanica* in different solvents

Sl no.	Compound	Methanol	Water	Diethyl ether	Acetone
1	Alkaloid	-	-	+	-
2	Anthraquinone	-	-	-	-
3	Cardiac glycoside	+	+	+	+
4	Cholesterol	+	+	+	+
5	Coumarin	+	+	-	+
6	Flavonoids	+	-	+	+
7	Phenol	+	+	+	+
8	Phlobatannins	-	-	-	-
9	Quinones	+	+	+	+
10	Saponins	-	+	-	-
11	Steroid	+	+	+	+
12	Tannin	+	+	+	+
13	Terpenoids	+	-	+	+



Graph 1: Antibacterial activities of *Chara zeylanica* in different extracts

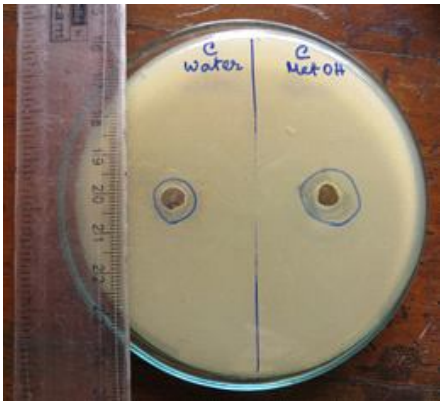


Plate 1. Inhibition of control and methanolic extract against *E. coli*

Plate 2. Inhibition of Acetone and Diethyl ether extract against *E. coli*

Determination of antibacterial activity :

Antibacterial activity of *Chara zeylanica* was determined by disc diffusion method on Muller Hinton agar medium against one bacterial strain *Escherichia coli*. 20 µl of each algal solvent extract was loaded on sterile filter paper discs (5 mm in diameter) and air dried. Muller Hinton agar discs were incubated for at 37ºC for 24 h and inhibition zones were measured. For control discs containing each extracting solvent was used.

Qualitative phytochemical screening shows (Table-1) that Anthraquinone and Phlobatannins were found to remain absent in all solvents. Alkaloid also absent except diethyl ether. Cardiac glycoside, Cholesterol, Phenol, Quinones, Steroid, Tannin were observed in extracts obtained using all the solvents. Saponins were responded only in aqueous extract. Flavonoids and terpenoids were observed in all solvents except aqueous extract. Coumarin was observed in all solvents except diethyl ether.

Effect of different solvent extracts (Graph 1) of *Chara zeylanica* against *Escherichia coli* shows that methanol extract of *Chara zeylanica* (16mm) maximum zone of inhibition over the control (11mm) followed by acetone (14mm), diethyl ether (12mm) as shown on Plate 1 and plate 2.

The phytochemical screening shows that Cardiac glycoside, Cholesterol, Phenol, Quinones, Steroid and Tannin present in solvents. Anthraquinone and Phlobatannins were found to remain absent in all solvents. Alkaloid was only present in diethyl ether solvent. *Chara zeylanica* shows high antibacterial activity under methanol extracts against *Escherichia coli*. This observation reflects that this commonly occurring alga may be used for exploitation after intensive studies.

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