Phytochemical analysis of freshwater cyanobacteria *Anabaena sp.*PCC550, *Anabaena sp.*PCC574 and *cylindrospermum sp.*PCC518, *cylindrospermum sp.*PCC 567

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ABSTRACT:

The present study involves culturing, extraction and phytochemical analysis of *Anabaena sp.*PCC550, *Anabaena sp.PCC574* and *Cylindrospermum sp.*PCC518, *Cylindrospermum sp.*PCC 567. A qualitative phytochemical analysis of *Anabaena* PCC550, *Anabaena* PCC574 and *Cylindrospermum sp.*PCC518, *Cylindrospermum sp.*PCC 567 extract in organic solvents like; methanol, ethanol and Hexane was performed for the presence of glycosides, alkaloids, saponins, flavonoids, terpenoids, steroids, tannin and phenolic compounds. Observation revealed the presence of maximum biological compounds in ethanol extract. Therefore, the result shows that the crude extract of *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum sp.*PCC*518, Cylindrospermum sp.*PCC *567* serves as a good source of useful pharmaceutical drugs.

Key Words: *Anabaena sp.*PCC550, *Anabaena sp.PCC574* and *Cylindrospermum sp.*PCC518, *Cylindrospermum sp.*PCC 567, Phytochemical analysis, Cyanobacteria.

1. INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that have an oxygenic photosynthesis comparable to plants (Adams and Duggan, 1999) and a cellular organisation similar to gram-negative bacteria (Stanier, 1988). Because of their lengthy existence and widespread distribution in terrestrial, freshwater, and marine settings, cyanobacteria constitute a big and morphologically diverse group of unique photosynthetic organisms of enormous value. They are well-known for providing crucial insights into the beginning of life, photosynthesis, nitrogen fixation, and fundamental metabolism as a basic research tool (Chinnu et al 2014). Cyanobacteria have natural features that make them an attractive organism for a variety of biotechnological applications (Mukund et al 2014).Cyanobacteria have emerged as important sources of wholesome food materials, fixed atmospheric nitrogen, natural colourants, bioplastics, biofuels, fine chemicals, bioactive substances, and other common and fine chemicals such as lipids, pigments, enzymes, polysaccharides, glycerol, and other novel biologically active compounds (Thajuddin and Subramanian 2005, Mishra et al 2010). Cyanobacteria, sometimes known as blue-green algae, is a gram-negative bacteria assemblage found all over the world. Cyanobacteria are a rich source of physiologically active and structurally unique compounds. A recent study of biologically active secondary metabolites from Cyanobacteria revealed a diverse set

of molecules with antibacterial, antiviral, antineoplastic, and toxic activities (Falch et al 1995, Moore 1996, Namikoshi and Rinehart 1996).

One of the most important categories of proteins found in seaweed is phycobiliproteins. The presence of a tetrapirrolic ring covalently connected to the structure of these water-soluble proteins, which are mostly found in blue-green and red algae, distinguishes them. This pigment, which can be phycocyanobilin (blue-green algae) or phycoerythrobilin (red algae), is responsible for some of the functional capabilities of these proteins, including hepatoprotective, anti-inflammatory, and antioxidant effects (Bhat et al 1998; Romay et al 2003; Bhat and Madyastha 2000).

2. MATERIALS AND METHODOLOGY:

2.1 Preparation of cyanobacterial extract:

The strains of Anabaena and Cylindrospermum species were procured from IARI, New Delhi, and cultures were introduced in conical flasks with algal culture medium and incubated in a culture chamber at 30±2ºC with fluorescent tubes saving 4 Klux at the vessel surface. The flask was shaken three to four times per day during the growing period. The light is provided for 16 hours per day, i.e., 16 hours of light and 8 hours of darkness are maintained. The experiment was repeated three times. All manipulations involving the transfer of culture in liquid media were done in an aseptic condition with a laminar air flow. The cultures were sub-cultured and extracts were prepared using methanol, ethanol and hexane as a solvent from soxhlet appratus.

Figure1: Culturing of *Anabaena sp.*PCC550, *Anabaena sp.*PCC574 and *Cylindrospermum sp.*PCC518, *Cylindrospermum sp.*PCC 567

2.2 Phytochemical screening:

Phytochemicals such as tannins, flavonoids, terpenoids, steroids, saponins, glycosides, and alkaloids were screened using hexane, methanol and ethanol extracts obtained from all four cyanobacteria (Sanjeet et al 2010).

A] Test for Tannins: Tannins were determined by combining 1-2 ml of cyanobacterial extract with 50 mL of distilled water and adding 5 percent ferric chloride drop by drop. The presence of tannins was revealed by the formation of a dark green solution.

B] Test for Flavonoids: Flavonoids were determined by dissolving 2-3 ml of cyanobacterial extract in 1 ml of 10% NaOH and adding a few drops of strong HCl. The loss of yellow colour suggested the existence of flavonoids.

C] Test for Terpenoids: Terpenoids were tested by adding 0.5 ml of chloroform to 1 ml of cyanobacterial extract and mixing it thoroughly. A few drops of sulfuric acid concentration were added. The presence of terpenoids was suggested by the formation of a reddish -brown interface.

D] Test for Steroids: Steroids were detected by adding 2 ml of acetic anhydride and a few drops of strong sulfuric acid to 2ml of cyanobacterial extracts. The presence of steroids was shown by the creation of a brown ring.

E] Test for Saponins: Saponins were determined by adding a few drops of 1 percent ferric chloride to 1 ml of cyanobacterial extracts. Saponins were detected by frothing or the appearance of a creamy mass of tiny bubbles.

F] Test for Glycosides: Glycosides were determined by aqueous 1M NaOH was added to the cyanobacterial extract with 1ml of distilled water, appearence of yellow colour indicates presence of Glycosides.

G] Test for Alkaloids: To test for alkaloids, a few drops of Wagner's reagent (iodine in potassium iodide) were added to the algal extract. The presence of alkaloids was suggested by the appearance of a reddish-brown precipitate.

H] Test for Phenols: In 5 ml of distilled water, the cyanobacterial extract was dissolved. A few drops of neutral ferric chloride solution (0.5% ferric chloride) were added to this. The presence of phenolic compounds is indicated by a dark green colour.

3. RESULTS:

Table1: Test compounds treated for presence or absence of phytochemicals.

Phytochemicals	Test Compounds from cyanobacteria											
	A1 E	A2 E	C1 E	C2 E	A1 H	A2 H	C1 H	C2 H	A1 M	A2 M	C1 M	C2 M
Flavonoids	-	+	+	+	-	-	-	-	-	+	+	+
Glycosides	-	+	+	+	-	-	-	+	-	-	-	-
Phenols	-	-	-	-	+	-	-	-	-	-	-	-
Saponins	-	-	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	-	+	+	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	-	+	+	+	+	-	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+

A1E- Ethanol extract of ***Anabaena sp.*****PCC550**
A2E- Ethanol extract of ***Anabaena sp.*****PCC574**
C1E- Ethanol extract of ***Cylindrospermum sp.*****PCC518**
C2E- Ethanol extract of ***Cylindrospermum sp.*****PCC 567**
A1H- Hexane extract of ***Anabaena sp.*****PCC550**
A2H- Hexane extract of ***Anabaena sp.*****PCC574**
C1H- Hexane extract of ***Cylindrospermum sp.*****PCC518**
C2H- Hexane extract of ***Cylindrospermum sp.*****PCC567**
A1M- Methanol extract of ***Anabaena sp.*****PCC550**
A2M- Methanol extract of ***Anabaena sp.*****PCC574**
C1M- Methanol extract of ***Cylindrospermum sp.*****PCC518**
C2M- Methanol extract of ***Cylindrospermum sp.*****PCC567**

Table2: Test showing presence or absence of phytochemicals.

Test	**Inference**	**Result**

Flavonoids	Change of colour from yellow to colourless	
Glycosides	Presence of yellow colour	
Phenols	Presence of green colour	
Saponins	Presence of foamy layer	
Sterols	Presence of red chloroform layer and greenish yellow acid layer	
Tannins	Presence of dark green colour	
Terpenoids	Presence of reddish brown colour	
Alkaloids	Presence of orange - brown colour	
Carbohydrates	Presence of brick red precipitate	

4. DISCUSSION:

Plant extracts were subjected to phytochemical analysis, which demonstrated the existence of elements with medicinal and physiological properties (Sofowra 1993). Phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids were found in the cyanobacterial extracts. As free radical terminators, phenolic substances have been widely researched for their antioxidant capabilities (Namikoshi 1993). Because of the hydrogen-donating ability of their hydroxyl groups as well as their ability to donate electrons to stop the generation of free radicals as a result of oxidative stress, polyphenols are effective antioxidants (Afroz et al 2014).
Flavonoids are the most common type of polyphenol, with a diphenylpropane structure (C6–C3–C6) that consists of two aromatic rings connected by three carbons. Flavonoids' methods of action are based on scavenging or chelating activities (Schillig et al 2005). As free radical terminators, phenolic substances have been widely researched for their antioxidant effects (Namikoshi et al 1993). In contrast, antioxidant-rich dietary molecules scavenge free radicals and block the radical chain reaction of oxidation, delaying or suppressing the oxidation process. The cancer chemopreventive and therapeutic benefits of phenol derivatives are due to their antioxidant function (Dai et al 2010). Flavonoids have been shown to have anti-inflammatory and antibacterial properties, as well as the ability to suppress platelet aggregation and mast cell histamine production (Koley et al 2011).

Cyanobacteria may provide significant health benefits in this aspect. Through morphological changes such as cell shrinkage, chromatin condensation, and nuclear fragmentation (Lowe 2000), the several phenol derivatives detected in these extracts arrest the cell cycle and induce apoptosis, thus serving as an important defence mechanism preventing cancer cell proliferation. This study, like others looking into the anticancer properties of Dendrobium species (Bao 2008), discovered that phenol derivatives cause cancer cells to die. Extracts containing phenol derivatives have antioxidant and anticancer properties (Cai 2004). Flavonoids, tannins, saponins, alkaloids, and terpenoids are phytochemicals with a variety of biological effects, including antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer capabilities (Starlin et al 2019). Phytosterol mixtures can be used to condition the skin in

cosmetic items (creams and lipstick), and they are gaining popularity in pharmaceutics for the generation of therapeutic steroids (Fernandes 2007) (Saini and Kium et al 2019). Carotenoids are widely employed in food, feed, nutraceuticals, and cosmetics. A diet high in carotenoids has been linked to improved skin health, cancer prevention, cardiovascular, neuronal, and gastrointestinal protection, as well as vision and immune system enhancement [Saini and Kium et al 2019].

5. CONCLUSION:

Cyanobacterial extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids which will be potential antimicrobial and anticancer drug in future.

Conflicts of interest -None

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