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Phytochemical Screening and Separation of Various Extracts of Cyanobacterium *Nostoc muscorum*

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Abstract:

Cyanobacteria is a free-living microorganism surviving in both terrestrial and fresh water habitat. Various strains of cyanobacteria are well known for the production of intracellular and extracellular metabolites with biological activities like antimycobacterial, antifungal, antiviral and antialgal activity. In the present study, we performed phytochemical screening of Cyanobacteria *Nostoc muscorum* to extract and found the presence of alkaloids, flavonoids, saponins, and glycosides; and thin layer chromatography (TLC) of cyanobacteria *Nostoc muscorum*. Afterward, Thin layer chromatography of extract obtained by using different solvents of non-diazotrophically and diazotrophically grown culture of the Cyanobacterium *Nostoc muscorum* were performed.

Keywords: Cyanobacteria, *Nostoc muscorum*, Phytochemical screening.

1. INTRODUCTION

Cyanobacteria is one of the most important organisms having some most important functions like nitrogen fixation, gram-negative and photoautotrophic nature. Cyanobacteria are known for their diversity in terms of morphological properties. Cyanobacteria are also known as blue-green algae and include a highly diverse group of photosynthetic prokaryotic microorganisms. They are widely distributed in nature and can be found in most terrestrial and freshwater habitats (Potts, 2002). In addition, Cyanobacteria can be found in every light Exposed habitat on earth and are a group of gram-negative eubacteria capable of oxygenic photosynthesis same as higher plants and thus they are considered to be the ancestors of higher chloroplast (Rodriguez et al., 2005). Recently, micro algae have become commercially important because of novel compounds and potential medicinal value. Microalgae are known to secrete vitamins, amino acids, siderophores, simple carbon hydrates, and other compounds that are essential or support the growth of other microorganisms. In recent years, microalgae have become an economic source of new drugs and commercially important compounds (Meeting and Pyne 1986). The biologically active compounds isolated from microalgae are known to show antibacterial, antiviral, antifungal, enzyme-inhibiting, immunostimulant, cytotoxic and

antiplasmodial activities (Ghasemi et al. 2004). The secondary metabolites produced by Cyanobacteria are rich sources of novel bioactive compounds applicable for the production of medicines and agriculturally important chemicals. In the natural environment, Cyanobacteria secret some of the extracellular metabolites, function as toxins or allelochemicals (Pflungmacher2002). The antifungal compound namely cryptophycin 1 is known to show potential activity against a large number of agriculturally important fungi (Biondi et al. 2004). The *Cyanobacterium* N*ostoc spongiaeforme* TISTR 8169 synthesizes and releases a violet pigment known as Nostocine A into the medium. Bioactive metabolites from cyanobacteria are also of biotechnological interest, particularly in the field of pharmaceutical industries. Cyanobacteria grow and multiply in minimal culture media on a mass scale. The growth condition can be manipulated to achieve optimum production of desirable bioactive compounds (Dahms et al. 2006). Many species of cyanobacteria are known to produce intracellular and extracellular metabolites. These metabolites show diverse biological activities and are known to inhibit microbial growth (Noaman et al. 2004). The *Cyanobacterium thormidium* has a broad range of antimicrobial activity (Fish and Codd 1994). So that the present study was performed for phytochemical screening of Cyanobacteria *Nostoc muscorum*. After the extraction of different secondary metabolites: alkaloids, flavonoids, saponins and glycosides were extracted and furthermore TLC were performed.

2. MATERIALS AND METHODS

In the present section of the work, the experiment organism was *Nostoc muscorum*. This species of cyanobacteria is a filamentous gram-negative, green brown color and heterocyst forming cyanobacteria. This Cyanobacteria were cultured in chu no.10 as described by (Gerlof et al. 1950). The ideal pH for the growth of this organism is 7.0-8.5. The culture was maintained at 28^0C with the intensity of 14.40 w/m^2 provided by a white fluorescent tube with a light/dark cycle of 16/8 hours.

For the isolation of bioactive compounds, one-week old *Nostoc muscorum* (fully grown) was centrifuged and the pellet was dried in a hot air oven at 60^0C. It was separated using a soxhlet extractor, by using Chloroform, Acetone, Ethanol, Butenol, and Methanol. Chemical test for the screening and identification of bioactive chemicals like alkaloids, carbohydrates, glycosides, phenolic compounds, amino acids, carbohydrates, saponins, and protein were performed.

The absorption effect was studied on the basis of Thin layer chromatography. In this procedure, the form of mobile phase containing the dissolved solutes moves over the stationary phase. Various solvent extract was subjected to thin layer chromatography. In the matching through the chamber with different solvent systems (toluene: ethyl acetate: formic acid in 5:4:1 ratio) solvent system was used. After pre-saturation with mobile phase for 20 min, the development was achieved. So that, as the run plates were dried, they were sprayed with freshly prepared iodine reagents, to detect the bands on the TLC plates. The movement of the active compounds was expressed by its retention factors (Rf), and values were calculated for different samples.

$$RF = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by the solvent}}$$

3. RESULTS AND DISCUSSION

To Obtain the percentage of yield of extraction is a very important finding in phytochemical extraction. So, firstly the percentage yield of different extracts of the Cyanobacterium N*ostoc muscorum* were found (table no.1). We found 2.7%, 7.3%, 5.5%, 5.3%, and 5.9% yield in non–diazotrophic cultures (Medium containing 1nM NH4Cl) using different solvents n-butanol, chloroform, acetone, ethanol, and methanol, respectively. Chloroform /Methanol extract of non-dizotrophic grown culture exhibited maximum percentage yield in comparison to all solvents. When

compared with non–diazotrophic, diazotrophic extract were also showed similar trends of percentage yield that is 1.2%, 5.5%, 4.2%, and 4.9% using n-butanol, chloroform, acetone, ethanol, and methanol extract, respectively. Chloroform/Methanol extract of diazotrophic–grown culture also exhibited maximum percentage yield in comparison to all solvents. This varying amount of percentage yield is because of the different polarity of solvent used for extraction.

Extract	**Percentage yield (%)**				
	n-Butanol	Chloroform	Acetone	Ethanol	Methanol
Diazotrophic grown culture	1.2 ±0.11	5.5 ± 0.48	4.2 ±0.40	4.6 ±0.39	4.9 ±0.44
Medium containing 5mM KNO_2	1.8 ±0.15	6.6 ±0.52	4.5 ±0.41	4.9 ±0.41	5.2 ±0.48
Medium containing 5mM KNO_3	2.2 ±0.19	6.9 ±0.55	5.1 ±0.49	5.1 ±0.48	5.4 ±0.52
Medium containing 1mM NH4Cl	2.7 ±0.20	7.3 ±0.66	5.5 ±0.51	5.3 ±0.51	5.9 ±0.55

Table 1: The percentage yield of different extracts of Cyanobacterium *Nostoc muscorum (*Non–diazotrophs, and diazotrophs).

Phytochemical analysis

When a small amount of dried extract was subjected to phytochemical analysis to test for the presence of glycoside, saponin, tannins, flavonoids and steroids, the results are presented in table no.2. The results of phytochemical screening were also found most similar in the phytochemicals composition of different solvents (n-butanol, chloroform, acetone, ethanol, and methanol). Alkaloids, flavonoids, carbohydrates, and diterpenes were detected positive in n-butanol extract of non-diazotrophic grown *Nostoc muscorum*, because of less polarity of n-butanol. On the other side, phytochemical screening showed negative findings for glycosides, saponins, phenolics, and proteins. Alkaloids, carbohydrates, and diterpenes were present in the n-chloroform extract of non-diazotrophic grown *Nostoc muscorum*. Because of lower chloroform polarity, Phytochemical screening showed negative findings for glycosides, saponins, phenolics compound, and proteins. The results of phytochemical screening were also showed most similarity in the butanol and chloroform extract. Alkaloids, flavonoids, carbohydrates, and diterpenes were present while glycoside, saponins, phenolics, and proteins were absent in acetone extract. Similar results were found in methanolic and ethanolic extract as alkaloids, alkaloid flavonoids, carbohydrates, and diterpenes were present while glycoside, phenolics, and proteins were absent in acetone extract. Saponins were absent in the ethanolic extract of non-diazotrophically grown *Nostoc muscorum.* Saponins were absent in the ethanolic extract of non-diazotrophically grown *Nostoc muscorum*.

S. No.	**Constituents**	**N-butanol extract**	**Chloroform Extract**	**Acetone Extract**	**Methanol extract**	**Ethanol extract**
1.	**Alkaloids** Hager's Test:	+	+	+	+	+
2.	**Glycosides** Legal's Test:	-	-	-	-	-
3.	**Flavonoids** Lead acetate Test:	+	+	+	+	+
4.	**Saponins** Froth Test:	-	-	-	+	-
5.	**Phenolics** Ferric Chloride Test:	-	-	-	-	-
6.	**Carbohydrate** Fehling's Test:	+	+	+	+	+
7.	**Proteins** XanthoproteicTest:	-	-	-	-	-
8.	**Diterpenes** Copper acetate Test:	+	+	+	+	+

Table no. 2: Result of Phytochemical Screening of NH4Cl sample.

The result of the phytochemical screening of n-butanol and chloroform extract of diazotrophically grown *Nostoc muscorum* are depicted in table no. 3. The results of phytochemical screening showed similarity in the butanol and chloroform extract. Alkaloids, Flavonoids, carbohydrates, and diterpenes were found to be present while glycoside, saponins, phenolics, and proteins were absent in both the extract. Non-diazotrophically grown *Nostoc muscorum* were also showed similar results in acetone extract where alkaloids, flavonoids and carbohydrates were found to be present while glycoside, saponins, phenolics and protein, and diterpenes were absent in acetone extract. In the methanol extract, Alkaloids, flavonoids, carbohydrates, and diterpenes were present while glycoside, saponins, and protein were absent in acetone extract. In ethanol, Alkaloid, flavonoids, carbohydrates, and diterpenes were found to be present while glycoside, saponins, phenolic, and proteins were absent in acetone extract.

Sr. No.	Constitute	N-butanol extract	Chloroform extract	Acetone extract	Methanol extract	Ethanol extract
1	**Alkaloids** Hager's Test:	+	+	+	+	+
2.	**Glycosides** Legal's Test:	-	-	-	-	-
3.	**Flavonoids** Lead acetate Test:	+	+	+	+	+
4.	**Saponins** Froth Test:	-	-	-	+	-
5.	**Phenolics** Ferric Chloride Test:	-	-	-	-	-
6.	**Carbohydrate** Fehling's Test:	+	+	+	+	+
7.	**Proteins** Xanthoproteic Test:	-	-	-	-	-
8.	**Diterpenes Copper acetate Test:**	+	+	-	+	+

Table no. 3: Result of the phytochemical screening without NH4Cl sample.

3.2 Thin layer chromatography

Thin layer chromatography profile of different extracts of n-butanol, chloroform, methanol, and ethanol non-diazotrophic extract with toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v/) as mobile phase resolved major spots at different Rf at 254nm (short wavelength) and normal light.

Sample extract	n-butanol, chloroform, acetone, methanol, and ethanol non-diazotrophs extract				
Mobile Phase	Toluene: Ethyl acetate: Formic acid (ratio= 5:4:1)				
Plate Tag	NH_4Cl				
Distance traveled by mobile phase	5.0 cm				
Number of spots					
	n-butanol	Chloroform	Acetone	Ethanol	Methanol
Normal Light	4	5	5	2	4
Short Wavelength	4	5	5	2	4
Long Wave Length	4	5	6	4	6
Visibility	Considerable				
Any Match with Quercetin Standard	Yes (0.60cm.)				
R_f Values of each spot (from bottom to top)					

	n-butanol	**Chloroform**	**Acetone**	**Ethanol**	**Methanol**
Normal Light	0.58,0.64,	0.6,0.66,0.4 0.90.0.98	0.6,0.66,0.72, 0.92,0.98	0.6,0.66	0.6,0.66,0.88,0.92
Short Wavelength	0.58,0.64, 0.90,0.98	0.6,0.66,0.4 0.90.0.98	0.6,0.66,0.72, 0.92,0.98	0.6,0.66	0.6,0.66,0.88,0.92
Long Wave Length	0.58,0.64, 0.90,0.98	0.6,0.66,0.4 0.90.0.98	0.6,0.66,0.72, 0.84,0.92,0.98	0.6,0.66, 0.86,0.94	0.6,0.64,0.76,0.88 ,0.92
Spot Sequence (Left to right)					
First	Quercetin				
Second	n-butanol extract				
Third	Chloroform extract				
Fourth	Acetone extract				
Fifth	Ethanol extract				
Sixth	Methanol extract				

Table no. 4: Results of thin layer chromatography of non-diazotrophic extracts.

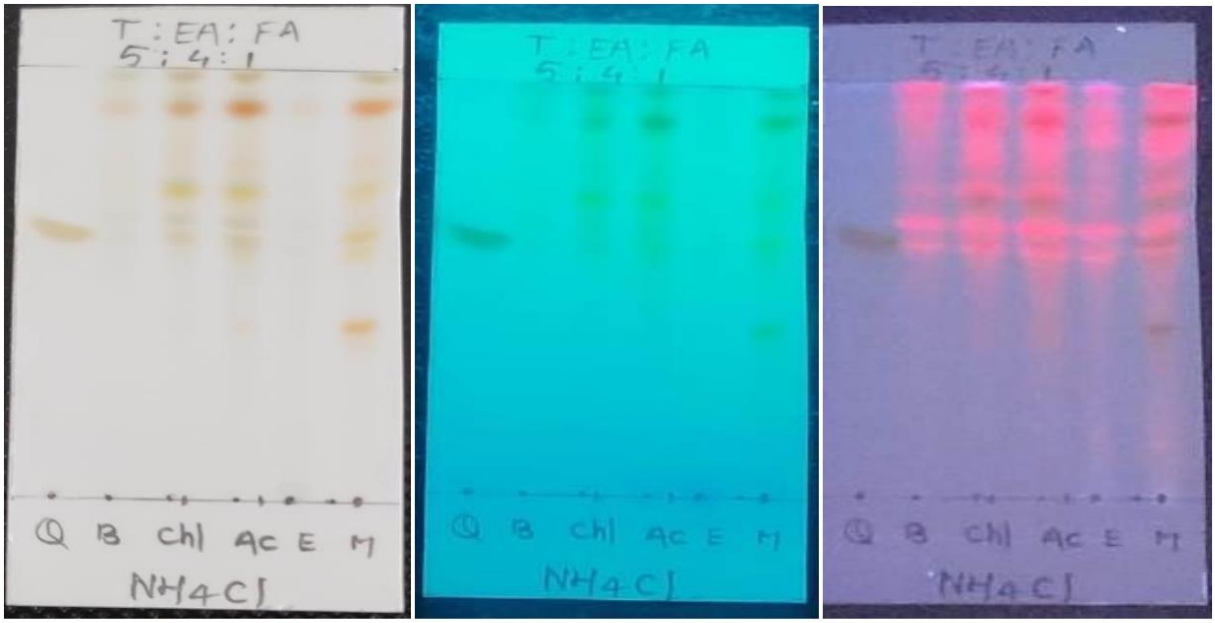

Figure 1: TLC of n-butanol, chloroform, acetone, methanol, and ethanol non-diazotrophs extract.

Sample extract	n-butanol, chloroform, acetone, methanol, and ethanol non-diazotrophs extract				
Mobile Phase	Toluene: Ethyl acetate: Formic acid (ratio= 5:4:1)				
Plate Tag	without NH_4Cl				
Distance traveled by mobile phase	5.0 cm				
Number of spots					
	n-butanol	**Chloroform**	**Acetone**	**Ethanol**	**Methanol**
Normal Light	2	5	5	4	6
Short Wavelength	2	5	4	2	6
Long Wave Length	5	5	6	5	8
Visibility	Considerable				
Any Match with Quercetin Standard	Yes (0.60cm.)				
R_f Values of each spot (from bottom to top)					

	n-butanol	**Chloroform**	**Acetone**	**Ethanol**	**Methanol**
Normal Light	0.6,0.76	0.6,0.66,0.74 ,0.9,0.98	0.58,0.62,0.74 ,0.9,0.98	0.58,0.64, 0.76,0.88	0.58,0.62,0.72, 0.82,0.88,0.96
Short Wavelength	0.6,0.76	0.6,0.66,0.74 ,0.9,0.98	0.58,0.62,0.74 ,0.9	0.58,0.64	0.58,0.62,0.72, 0.82,0.88,0.96
Long Wave Length	0.6,0.66,0.7 6,0.84,0.9	0.6,0.66,0.74 ,0.9,0.98	0.58,0.62,0.74 ,0.8,0.9,0.98	0.58,0.64, 0.86,0.9,0 .98	0.58,0.62,0.72, 0.76,0.82,0.88, 0.9,0.96
Spot Sequence (Left to right)					
First	Quercetin				
Second	n-butanol extract				
Third	Chloroform extract				
Fourth	Acetone extract				
Fifth	Ethanol extract				
Sixth	methanol extract				

Table no. 5: Results of thin layer Chromatography of diazotrophs extracts.

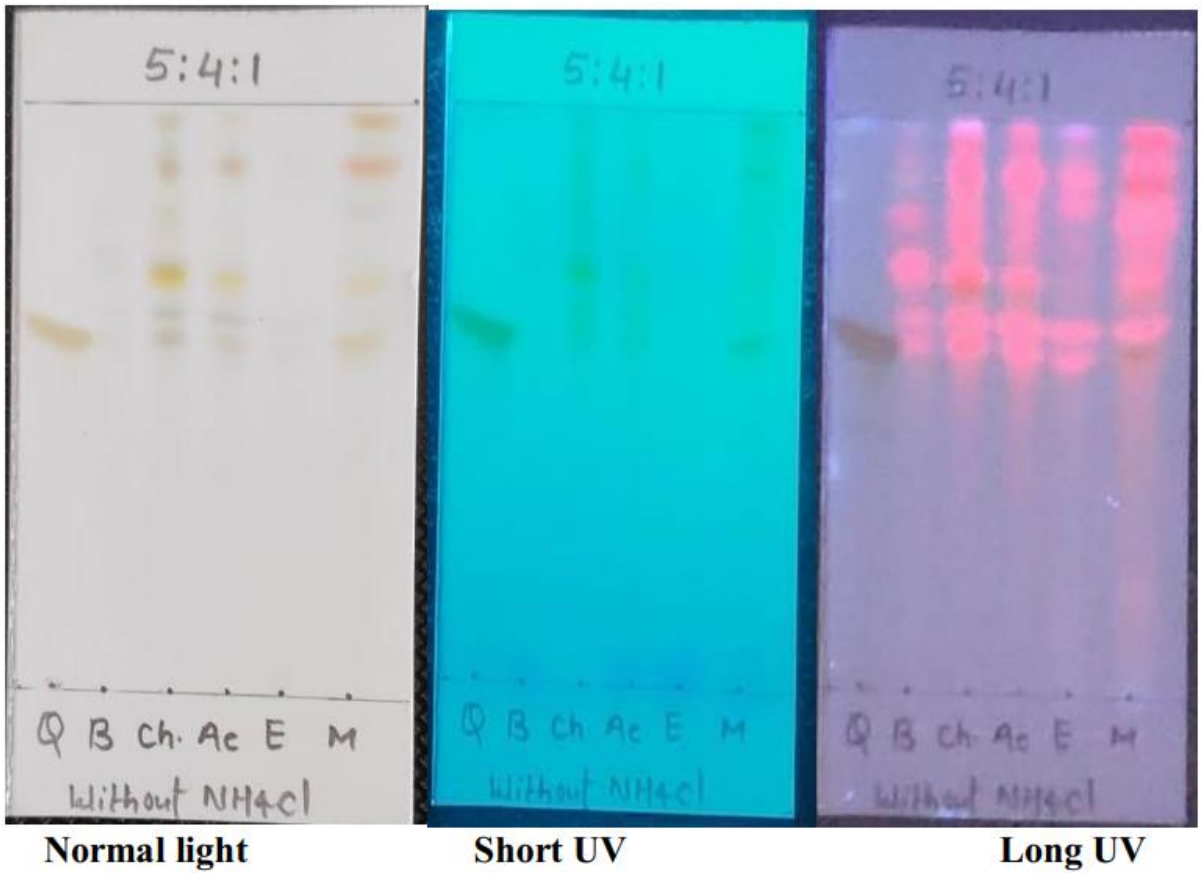

Figure 2: TLC of n-butanol, chloroform, acetone, methanol, and ethanol diazotrophs extract.

4. CONCLUSION

Based on the above findings, it may be concluded that organic solvent serves as the most capable solvent when compare to others. Therefore, Cyanobacterial extract could be seen as an admirable source of pharmaceutical purpose. With this regard, we will be able to develop new drugs from *Nostoc muscorum* and this new drug would be cheap and more effective against pathogens. And thus it will help in removing mycobacterium tuberculosis from this Indian society.

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