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PRIMARY SCREENING OF BIOACTIVE MOLECULES FROM CYANOBACTERIA

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ABSTRACT

In this study, we conducted a comprehensive investigation of the active methanolic crude extracts obtained from Lyngbya aestuarii.. The crude extracts were subjected to purification through thin-layer chromatography and subsequently evaluated for their antimicrobial activity against selected bacteria and fungi. Notably, several bioactive compounds were isolated and characterized through a combination of spectroscopic techniques. In summary, this research successfully identified and characterized bioactive compounds from L. aestuarii, shedding light on their potential antimicrobial properties. These findings contribute to our understanding of natural compounds with antimicrobial potential and their potential applications in medicine and biotechnology.

Keywords: Bioactive compounds, Antimicrobial activity, Spectroscopic characterization, Lyngbya aestuarii,

INTRODUCTION

Cyanobacteria have recently emerged as a valuable source of bioactive substances, as demonstrated by various studies (1,2). These microorganisms produce a diverse array of compounds such as polyketides, amides, alkaloids, fatty acids, indoles, and lipopeptides . Many of these bioactive compounds found in cyanobacteria are lipopeptides, comprising an amino acid segment linked to a fatty acid component. These secondary metabolites exhibit a broad spectrum of biological activities, including antibacterial, antifungal, anti-algal, antiprotozoal, and antiviral properties (3).

Cyanobacteria, those intriguing aquatic microorganisms, have emerged as a treasure trove of bioactive compounds, according to various research findings (4). They've unveiled a diverse array of substances, including the unique Kalkipyrone, a novel compound with a complex name, derived from marine cyanobacteria like *Lyngbya majuscula and Tolypothrix sp.* Researchers unraveled Kalkipyrone's structure through the powerful tools of NMR, UV, and IR spectroscopy. Notably, Kalkipyrone shares structural similarities with actinopyrones previously isolated from Streptomyces spp., which happen to be quite potent, causing harm to brine shrimp (LD50 1 µg ml-1) and goldfish (LD50 2 µg ml-1) .

In the world beneath the waves, the marine wonderland continues to reveal its secrets. Bioassay-guided exploration of lipophilic extracts from marine Phormidium ectocarpi uncovered the remarkable hierridin B and a familiar compound, 2,4-dimethoxy-6-heptadecyl-phenol, which had previously made its debut. This intriguing mix exhibited antiplasmodial activity against the notorious Plasmodium falciparum (5).

The marine realm offers even more wonders, with compounds like Yanucamides A and B *from Lyngbya majuscula and Schizothrix sp.* boasting molecular formulas that boggle the mind (C33H48N3O7). And don't forget the acyclic cytotoxic peptide, tasiamide B, sourced from the marine Symploca sp., whose planar structure left researchers in awe. *Lyngbya majuscula* takes the spotlight with its impressive lineup of peptides, including dolastatin, homodolastatin, and kororamide, not to mention aplysiatoxin, debromoaplysiatoxin, and oscillatoxin A. Among these, the fresh faces of homodolastatin and kororamide have recently graced the scene, their structures meticulously deciphered. These compounds display potent cytotoxicity, particularly against A-549, HT-29, and MEL-28 cell lines, with curacin D also making a cameo appearance, albeit with less gusto. *Lyngbya sp.,* the stars of the marine world, offer up a smorgasbord of chemicals, including nitrogen-containing compounds, polyketides, lipopeptides, cyclic peptides, and much more .

Some of their repertoire includes antifungal marvels such as tanikolide, Lyngbyabellin B, semiplenamides A-G, and many others. But these versatile organisms aren't just about fending off fungi; they've also got antibacterial

tricks up their sleeves, featuring lyngbyazothrin C and D, malyngolide, pitipetolides, and a host of other formidable compounds (6). In fact, crude extracts from Lyngbya species have shown antibacterial prowess (7,8).

As we venture into freshwater territory, a different cast of cyanobacteria takes the stage, offering up their own arsenal of antimicrobial compounds. Among their ranks, you'll find antifungal heroes like fisherellin A, hapalindole, carazostatin, and more. Meanwhile, they've got antibacterial aces up their sleeves, including Noscomin from Nostoc commune, Norharmane, 4,4-dihydroxybiphenyl, and Kwaguchipeptin B, each with its unique story to tell. Even the freshwater Phormidium sp. joins the battle, inhibiting the growth of various bacteria, yeasts, and fungi (9, 10).

MATERIALS AND METHODS

Cyanobacterial strain: *Lyngbya aestuarii*(brackish water strain from Chilka lake) was obtained as a gift from Prof. R.K. Asthana, Institute of Science, B.H.U., Varanasi, India.

Chemicals and Reagents: Methanol (for extraction), Sodium chloride (NaCl), Potassium hydroxide (KOH), Distilled water, Solvent for thin-layer chromatography (TLC).

Laboratory Equipment and Instruments: Microscope with 10x and 40x objectives, Refrigerated Centrifuge (capable of 15,000 RPM), pH Meter, Digital Thermometer, Sterile 90mm × 15mm plastic Petri Dishes, Glassware including beakers, test tubes, and pipettes.

Extraction of Crude Extract: The collected *Lyngbya aestuarii* and *Aphanthothece bullosa* samples were air-dried and ground into a fine powder. Methanolic crude extracts were prepared by soaking the powdered samples in methanol for a specified duration. The extracts were filtered and concentrated under reduced pressure.

Thin-Layer Chromatography (TLC): The methanolic crude extracts were purified using thin-layer chromatography (TLC). A suitable TLC plate was coated with the extract, and a solvent system was used for separation. The resulting chromatogram was analyzed under UV light.

Bioassays: The purified compounds from bands were subjected to bioassays against selected bacteria and fungi. Antibacterial activity and antifungal activity was done.

RESULTS

Purification of crude and bioassay of eluted bands

The methanolic extracts (250 mg ml^{-1}) of *L. aestuarii* were subjected to TLC purification in two steps and illuminated with UV. The spots were designated as A, B, C and D (using carbon tetrachloride: methanol, 9:1, v:v) for L. aestuarii. All these spots were individually eluted in methanol and their antimicrobial potency tested against *E. aerogens* and *C. albicans*.

Fig. 1: TLC purification of methanolic crude extracts of L. aestuarii using carbon tetrachloride and methanol ((9:1) as mobile phase.

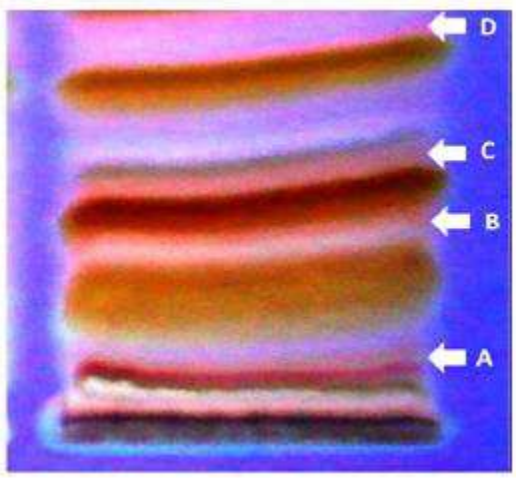

Table 1. Bioassay of TLC spots (25 µgml-1) of *L. aestuarii* against bacterial and fungal strains.

Designated Inhibition Zone (Mm)		
Spots in 2nd TLC	***E. aerogenes***	***C. albicans***
A	2.21	Not detected
B	Not detected	5.56±0.348
C	1.60	0.0
D	1.2	0.0
25% Methanol	Not detected	Not detected

DISCUSSION

Our research represents a significant contribution to the ongoing efforts aimed at discovering novel antimicrobial compounds from natural sources. Given the pressing issue of antibiotic resistance, the exploration of alternative reservoirs for potential antibiotics and antifungals is of paramount importance (11). In this study, we focused on the methanolic extracts of cyanobacterial species, *Lyngbya aestuarii*, as a potential sources of bioactive compounds. The exploration of natural sources for novel antimicrobial compounds has become increasingly vital in the face of rising antibiotic resistance (12). In this study, we investigated the methanolic extracts of cyanobacterial species, *Lyngbya aestuarii* for its potential to yield bioactive compounds with antimicrobial properties.

CONCLUSION

Our research unveils the exciting antimicrobial potential hidden within cyanobacteria, exemplified by the compounds. These discoveries highlight the vast diversity of bioactive compounds in these organisms, igniting hope for novel antibiotics and antifungals. By drawing connections with prior studies and employing robust structural identification techniques, we have bolstered the credibility of our findings. While our work represents a significant step forward, the path to combating antimicrobial resistance is long. Future research should delve into mechanism elucidation, toxicity assessment, and clinical trials. Our study serves as both a promising starting point and a reminder of nature's potential in this critical battle.

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