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ORIGINAL ARTICLE

Screening of Norharmane from Seven Cyanobacteria by High-performance Liquid Chromatography

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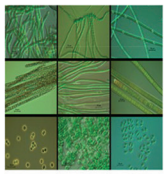
ABSTRACT

Background: Cyanobacteria, including pharmaceutically and medicinally valuable compounds attract the great attention lately. Norharmane (9H-pyrido (3,4-b) indole found in some cyanobacteria revealed a great number of biological effects. **Objective:** Seven cyanobacteria were isolated and identified from Yesilirmak River and Gaziosmanpasa University Campus to determine the norharmane content. **Materials and Methods:** Cyanobacteria collected from Tokat, Turkey were isolated and identified by morphologically. Norharmane (9H-pyrido [3,4-b] indole) quantities were presented for seven cyanobacteria, *Chroococcus minutus* (Kütz.) Nägeli, *Geitlerinema carotinosum* (Geitler) Anagnostidis, *Nostoc linckia* Bornet ex Bornet and Flahault, *Anabaena oryzae* F. E. Fritsch, *Oscillatoria limnetica* Lemmermann, *Phormidium sp.* Kützing ex Gomont, and *Cylindrospermum sp.* Kutzing ex E. Bornet and C. Flahault by high-performance liquid chromatography. **Results:** The norharmane amount indicated for cyanobacterial culture media altered in a species-dependent kind in the range of 0.81–10.87 µg/g. *C. minutus* produced the most norharmane among the investigated cyanobacteria as 10.87 µg/g. **Conclusion:** Cyanobacteria could be an important source of norharmane as well as pharmaceutically valuable compounds.

Key words: Cultivation, cyanobacteria, high-performance liquid chromatography, norharmane

SUMMARY

- Seven cyanobacteria were isolated and identified from Yesilirmak River
- Quantitative analysis of norharmane was executed on isolated cyanobacteria
- Four cyanobecteria species included the norharmane
- *Chroococcus minutus* contained the most norharmane (10.87 µg/g).



Cyanobacteria	Norharmane (µg/g)
Anabaena oryzae	2.25
Nostoc linckia	0.81
Geitlerinema carotinosum	1.92
Oscillatoria limnetica	-
Phormidium sp.	-
Cylindrospermum sp.	-
Chroococcus minutus	10.87

Abbreviations used: HPLC: High performance liquid chromatograph.

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INTRODUCTION

Secondary metabolites are natural products that have an important property in industrial and biomedical applications. Primer metabolites mediate synthesis of the required macromolecules, whereas the secondary metabolites are synthesized from metabolic intermediates that result from primary metabolism or from their final products.[1] Cyanobacterial metabolites may chemically be in the structure of peptides, alkaloids, indole alkaloids, polyketides, and terpenes, and many of these compounds exhibit a large variety of pharmaceutical properties.[2] Norharmane named as (9H-pyrido (3,4-b) indole, β-carboline class, is an alkaloid that has a heterocyclic amine structure derived from tryptophan amino acid. More than 140 different types of β-carbolines have been reported from plants and animals up to now.[3] Norharmane was also isolated from *Streptomyces* and marine dinoflagellates.[3] It is proto-toxic, including degeneration of nigrostriatal nerves in Parkinson's disease.[3] Several pharmacological effects of norharmane such as inhibition of monoamine oxidase, indoleamine 2,3 dioxygenase, nitric oxide synthase, and acetylcholinesterase enzymes were reported.[4-6] Norharmane has several pharmacological properties that affect the induction of apoptotic cell death in human neuroblastoma SH-SY5Y cells and the increase in insulin secretion from human Langerhans islets.[7]

In this study, the quantitative analysis of norharmane was investigated in *Chroococcus minutus*, *Geitlerinema carotinosum*, *Nostoc linckia*, *Anabaena oryzae*, *Oscillatoria limnetica, Phormidium sp.* and *Cylindrospermum sp.* which were collected and isolated from different location of Yesilirmak River and Gaziosmanpasa University Campus. Experimental results showed that *C. minutus* included the most norharmane (10.87 µg/g) among the investigated cyanobacteria.

MATERIALS AND METHODS

Cyanobacteria collection and isolation

Algae samples, taken from the pelagic regions of Yesilirmak River and Gaziosmanpasa University Campus between October and November 2014 in Tokat province and surrounding areas were collected and kept in 1 l plastic containers and tromped by filter paper (GF/C filter paper, Whatman). The samples were incubated for 2 weeks at 12/12 h (light/dark) in an enrichment medium (F2) at an average of 2465 lux at 26°C.[8] Cyanobacteria were identified and subjected

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to mechanical isolation under an inverted microscope through a micropipette and microinjector. *C. minutus* (40° 17 40.19" N, 36° 19 28.81" E), *G. carotinosum* (49° 19' 49.12" N, 36° 34' 2.06" E), *N. linckia* (40° 19' 45.655" N, 36° 33' 45.06"E), *A. oryzae* (40° 19' 43.77" N, 36°28' 22.26" E), *O. limnetica* (40° 19' 51.27" N, 36° 23' 4.69" E), *Phormidium sp.*(40°19' 50.85" N, 36° 28' 37.46" E) and *Cylindrospermum sp.*(40° 20' 4.39" N, 36° 28' 37.46" E) were isolated. After mechanical isolation, they were streaked by 1.5% agarized medium.[9]

Cultivation

Bristol, Blue Green Algae (BG-11) and BG-11° (without $NaNO_3$) which were the suitable media for cyanobacteria growth were used for cultivation of isolated cyanobacteria. An appropriate nutrient media (235 ml) and 10% of each cyanobacterium was inoculated into 250 ml flasks at 26°C, 2465 lux and kept to develop for about 2 weeks.[10]

Extraction

The cultures were centrifuged at 4000 rpm for 15 min. The pellets were washed with distillated water for twice then lyophilized and were weighed on a precision scale. Each sample (0.50 mg) was placed in 1 ml glass tube and dissolved in methanol-chloroform (1/1) then vortexed for 1 min, and placed in an ultrasonic bath for 10 min.[11] After that, it was vortexed for another 1 min and filtered with a polytetrafluoroethylene syringe (Chrom Tech, 0.45 µm 13 mm).

High-performance liquid chromatography Analyses

The each extract sample (20 µl) was injected into the column. Quantitative analysis was executed by high-performance liquid chromatography (HPLC) (Shimadzo, Japan) with C18 120A (Thermo, 4.6 mm × 150 mm, 3 µm particle size) reverse phase column. Norharmane metabolite was detected at 247 nm wavelength with photodiode array detector. The flow rate was adjusted to 1 ml/min using a gradient system of A, water with 0.1% formic acid and B, acetonitrile. The gradient program was fixed as follows: 0–14 min, 100% A; 14–29 min, 80% A, 29–32 min, 60% A, 32–34 min, 0% A.[12] The amount of norharmane was calculated by the calibration curve using the Gauss method.

RESULTS AND DISCUSSION

Cyanobacteria and systematic

The needs for the life of cyanobacteria are mainly water, light, carbon dioxide, and simple inorganic compounds.[13] However, cyanobacteria can grow rapidly under certain environmental conditions.[14] Cyanobacteria were artificially developed in cultural media. While the *C. minutus*, *G. carotinosum* were cultivated in Bristol medium, *O. limnetica*, *Phormidium* sp. MBIC10025 were developed in BG11 medium and other cyanobacteria, *Nostoc* sp., *A. oryzae* and *Cylindrospermum* sp. CENA33 were cultivated in BG-11° [Table 1].

Quantitative analysis of norharmane

It was found out that four purified cyanobacteria, *C. minutus*, *N. linckia*, *A. oryzae* and *G. carotinosum* included norharmane, whereas the other three, *O. limnetica*, *Phormidium sp*., *Cylindrospermum* sp. did not. The retention time of norharmane was observed at 10th min in HPLC chromatogram [Figure 1]. Interestingly, the cyanobacteria species excreted norharmane belonged to the Nostocales order and Nostocaceae family [Table 1].

Norharmane quantity was calculated according to the Gauss method by plotting the calibration curve over the absorbance value of the standard at 247 nm wavelength [Table 2].

Table 1: Systematics of isolated cyanobacteria and cultivation media

Cyanobacteria	Order	Family	Medium
Chroococcus minutus	*Chroococcales*	*Chroococcaceae*	Bristol
Geitlerinema carotinosum	*Oscillatoriales*	*Coleofasciculaceae*	BG-11
Oscillatoria limnetica	*Oscillatoriales*	*Oscillatoriaceae*	BG-11
Phormidium spp.	*Oscillatoriales*	*Oscillatoriaceae*	BG-11
Cylindrospermum spp.	*Nostocales*	*Nostocaceae*	BG-11°
Nostoc linckia	*Nostocales*	*Nostocaceae*	BG-11°
Anabaena oryzae	*Nostocales*	*Nostocaceae*	BG-11°

BG: Blue Green

Table 2: Quantification of norharmane from cyanobacteria

Cyanobacteria	Norharmane (µg/g)
Chroococcus minutus	10.87
Geitlerinema carotinosum	1.92
Nostoc linckia	2.25
Anabaena oryzae	0.81
Oscillatoria limnetica	ND
Phormidium spp.	ND
Cylindrospermum spp.	ND

ND: Not detected

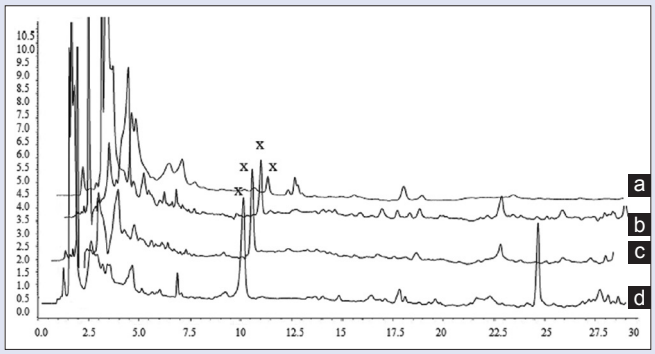

Figure 1: High-performance liquid chromatography chromatogram of norharmane (x) on *Anabaena oryzae* (a), *Geitlerinema carotinosum* (b), *Nostoc linckia* (c), *Chroococcus minutus* (d)

Cyanobacteria are the inevitable sources of natural compounds used in biotechnology for the benefit of human beings, and bioactive secondary metabolites are of great interest in medicine and agriculture. Some cyanobacterial metabolites have significant pharmaceutical potentials as antimicrobial, anticancer, antiviral, and enzyme inhibitory activities.[15,16] Indole alkaloids, bauerins A-C were isolated from terrestrial cyanobacteria (Dichotrix baueriana GO25-5) and were revealed the activity against herpes simplex virus 2.[17] Cytotoxic antiviral indolocarbazoles were isolated from *Nostoc sphaericum* EX-5-1.[18]

Norharmane is the simplest example of *β*-carboline exhibiting the various biological and pharmacological effects.[19] The investigation of norharmane in different cyanobacteria is rather scarce, but some researches on norharmane for *Nodularia harveyana*, *Nostoc insulare* and *Synechocystis* sp., *Anabaena cylindrica*, *Anabaena inaequalis*, *Anabaenopsis siamensis*, *C. minutus*, *Nostoc carneum*, *Nostoc commune, Nodularia harveyana*, and *Phormidium foveolarum* were mentioned.[7,20,21]

C. minutus showed high and rapid ability to remove nonylphenol including bioaccumulation and biodegradation. Hence, *C. minutus* can be used for removing of nonylphenol contaminated aqueous systems effectively by biodegradation.[22] In addition, *C. minutus* has the ability of decolorizing cyclic azo dyes as well.[23] Unsaturated,

hydroxy, n-saturated, branched, dioic fatty acids were determined from *C. minutus* and major fatty acids were found as hexadecanoic (16:0), 9-hexadecenoic (16:1), hexadecadienoic (16:2), octadecanoic (18:0), and 9-octadecenoic (18:1).[24]

N. linckia can be used for selenium nanoparticles synthesis. *N. linckia* biomass with selenium nanoparticles may be promising agent for medicinal, pharmaceutical, and technological objective.[25] *Nostoc sp.* methanol extract which included the phytol, n-hexadecanoic acid, 9,12-octadecadienoic acid, 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester as the major products revealed the antimicrobial activity.[26] Moreover, *Nostoc sp.* displayed the acetylcholinesterase inhibitory activity.[27]

In the present study, the presence of norharmane, the target pharmacological secondary metabolite in the cyanobacteria taxa was determined by HPLC. The amount of norharmane was detected in *C. minutus*, *G. carotinosum*, *N. linckia* and *A. oryzae* as 10.87, 1.92, 0.81, and 2.27 (μg/g dried algae), respectively. However, norharmane was not detected in *O. limnetica, Phormidium sp. and Cylindrospermum sp.* in this work.

CONCLUSION

Screening the norhamane from algae samples, four of those comprised of the norharmane compound. Due to the consisting of bioactive compounds, cyanobacteria have a potency to be used in drug discovery and development process. Moreover, *C. minutus*, *G. carotinosum*, *N. linckia* and *A. oryzae* as could be a source of norharmane production. The isolation of bioactive secondary metabolites including norharmane from these cyanobacteria could be effective in the pharmaceutical industry.

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Conflicts of interest

There are no conflicts of interest.

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