Atimicrobial Bioactive Compound Isolated From Cyanobacterium *Nostoc linkia*

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الخلاصة

تم استخلاص وتنقية وتوصيف مركب كيميائي فعال بايلوجيا من مزرعة الطحلب *Nostoc linkia* والمعزول من انهار محافظة البصرة – جنوب العراق .

ان توصيف المركب اعتمد على قياسات الكتلة الطيفية والكروموتغرافيا الغاز فضلا عن تحديد ذوبانية وسمية ونقاوة والوزن الجزيئي للمركب المستخلص .

وتم اختبار الفعالية الضد مايكروبية لهذا المركب اتجاه بعض السلالات البكتيرية المرضية مثل المكورات العنقودية الذهبية واشريشيا القولون وخميرة المبيضات البيضاء باستخدام تقنية الانتشار بالاقراص وتحديد التركيز المثبط الادنى،اظهرت النتائج وجود فعالية ضد مايكروبية عالية اتجاه العزلات المختبرة.

Abstract

Bioactive chemical compound A_1 was extracted, purified and identified from the algal culture of ***Nostoc linkia*** isolated from Basra Rivers in southern Iraq. The identification of the compound by using GC-mass was confronted. Solubility, toxicity, purity and the chemical formula and molecular weight of A_1 compound were determined. The antimicrobial bioactivity of the purified compound against the bacterial strains *Escherichia coli* and *Staphylococcus aureus* and the dermatophytic fungus *Candida albicans* was tested by using a disk diffusion agar method. The Minimal Inhibitory Concentration (MIC) was also performed. The compound A_1 has shown a broader activity spectrum against all the test organisms.

Key words: Alga, bioactive compound, Candiasis, isolation and purification.

Introduction

Algae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients. Some important algae aspects, such as natural character, easy cultivation, their rapid growing (for many of the species) and the possibility of controlling the production of some bioactive compounds by manipulating the cultivation conditions, in this way algae can be considered as genuine natural photo bioreactors being (Plaza *et al*., 2008).

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They are able to produce a wide range of active substances with antibacterial, antifungal, antiviral, antiplasmodia, enzyme inhibiting, imunostimulatant and cytotoxic activities (Ghasemi *et al*., 2004). As well as antiprotoscolices (Khalaf, 2011). Part *et al.* (1944) were the first to isolate an antibacterial substance from *Chlorella which is* mixture of fatty acids, named Chlorellin exhibited negative bacteria.

Among the algal substances which have this kind of activity amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkanes, cyclic polysulphides, fatty acids an acrylic acid can be counted (Mtolera and Semesi, 1996). In this report an attempt was made to examine the algal culture metabolites particularly bioactive antimicrobial compounds and to be tested against a selected isolates of bacteria and dermatophytic fungus.

Materials and Methods

Isolation of microalga

The micro alga was isolated from Garmat – Ali River in Basra city southern Iraq from January to April 2013. Primary culturing was done in Chu – 10 medium. After colonization, pure culture of the living specimens were prepared by using sub culturing with agar plate method in Chu – 10 medium (Stein, 1975). Preserved specimens were prepared and the living specimens were incubated in 100 ml – conical flasks. Constant illumination was used at 60 µE m^{-2} Sec^{-1} intensity with white fluorescent lamps. Temperature was 25 ±2ºC. The resulted culture was identified based on morphology following taxonomy schemes of Prescott (1975) and Sant 'Anna (2004).

Extraction, Isolation and Purification of Bioactive compound

The algal culture was harvested after 18 days by centrifugation at 5000 rpm for 10 min. algal pellet was extracted with 100 ml of ethyl acetate by soxhelt apparatus for 24hrs. The extracted was dried under reduced pressure and stored in - 20ºC for further studies. Thin layer chromatography (TLC) was applied for the isolation of extracted metabolite and Rf value was measured. Purification of the extracted was made on silica gel column chromatography (GF243 Merck, Germany). A further purification of fraction compounds was made by a column chromatography method as shown in fig. 1.

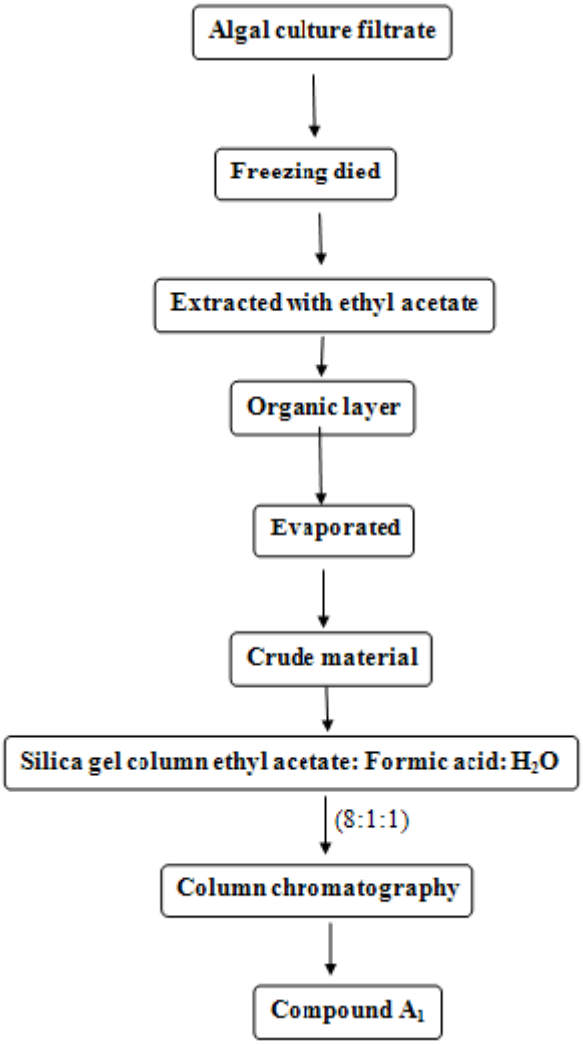

Figure- 1: Steps of extraction and purification of bioactive compound A_1 from the alga *N. linkia*

Antibacterial and antifungal bioassay

Discs diffusion agar method was used (Ghasemi *et al*., 2004). To examine the antimicrobial activity of the purified compound. Two strains of bacteria: *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for this purpose. Antifungal bioactivity of this compound was also tested against *Candida albicans* (Reference strain). Fungal cultures were obtained from the Basra general Hospital.

The Minimum Inhibitory Concentration (MIC) Test

The MIC values were determined by the standard serial dilution assay according to (Baron and Finegold, 1990).

The MIC values in this assay were indicated by the absence of bacterial or fungal growth at the minimal concentration of the compound Gmonas Sabourauds dextrose broth (ESDB) medium was used for this test.

Cytotoxic Test

Cytotoxicity of the purified compound was examined by using human RBC following a previously described method (Xian – guo and Ursula, 1994).

Solubility Test

The solubility of the biochemical active compound in various solvents (ethanol, methanol, chloroform, hexane, dimethyl sulphoxide, DMSO and water)was carried out.

Identification of the Biochemical Active Compound

Ifra-red spectrum (IR) (Pye- Unicam Sp3- 3005 UK), Gas Chromatography mass (GC) (Agilent Technologies GC – mass 7890 AGC System) methods were applied for the identification and determination of the molecular weights and chemical formula and structure of the purified biochemical active compound.

Results

Algal extract showed a single spot on TLC referred as A_1 compound with Rf value of 0.83 solubility test of A_1 indicated that this component is in soluble in hexane and ethylacetate but is soluble or partially soluble in the other examine solvents (table – 1)..

Table – 1: solubility test of purified compound A in different solvents

Purified	Ethyl acetate	Hexane	Methanol	Ethanol	Chloroform	DMSO	Water
component A	non-soluble	non-soluble	Soluble	Soluble	Soluble	Soluble	Partially Soluble

IR spectrum revealed that that A_1 composed of various functional molecules structure (table – 2). The purified A_1 compound exhibited a very strong spectra band at 2856 cm^{-1} representing a specific chemical functional group (fig.2).

Table - 2: Infrared (IR) data of component

Wave length (cm^{-1})	Assignment
3408	OH strech.
2924.09, 2854.65	CH aliphatic
1736	C=O stretch
1633	C - N strech.
1369	O-C bend
719	CH_2 bend

strech.: (stretching vibration); bend.:(bending).

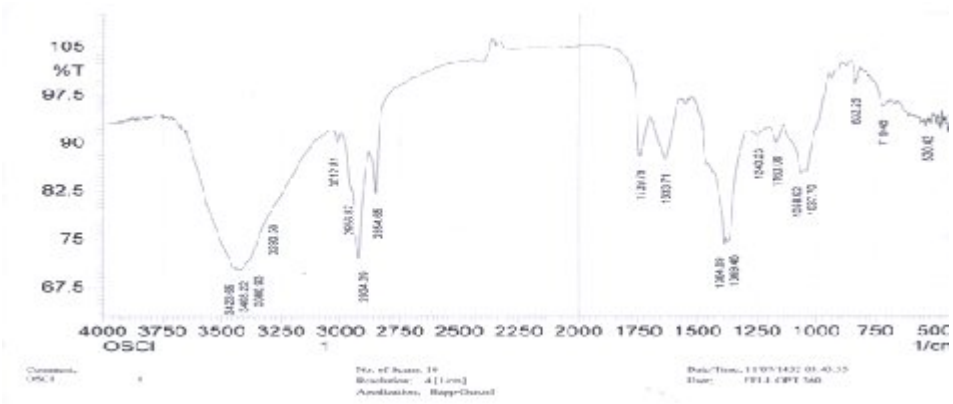

Figure - 2 : Infrared (IR) spectrum of component of *N. linkia*

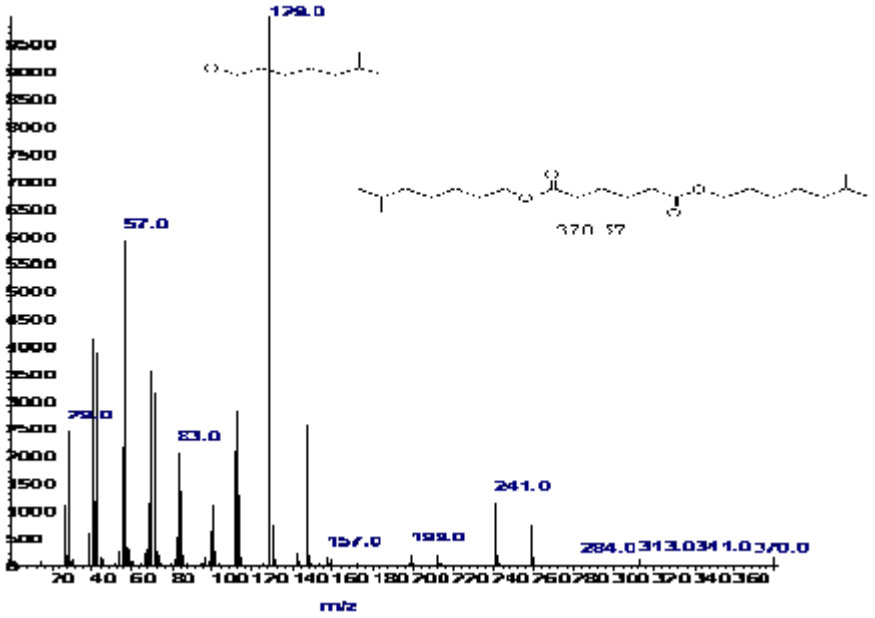

Figure - 3 : GC- Mass spectrum of Bis (6- methylheptyl) hexane dioate

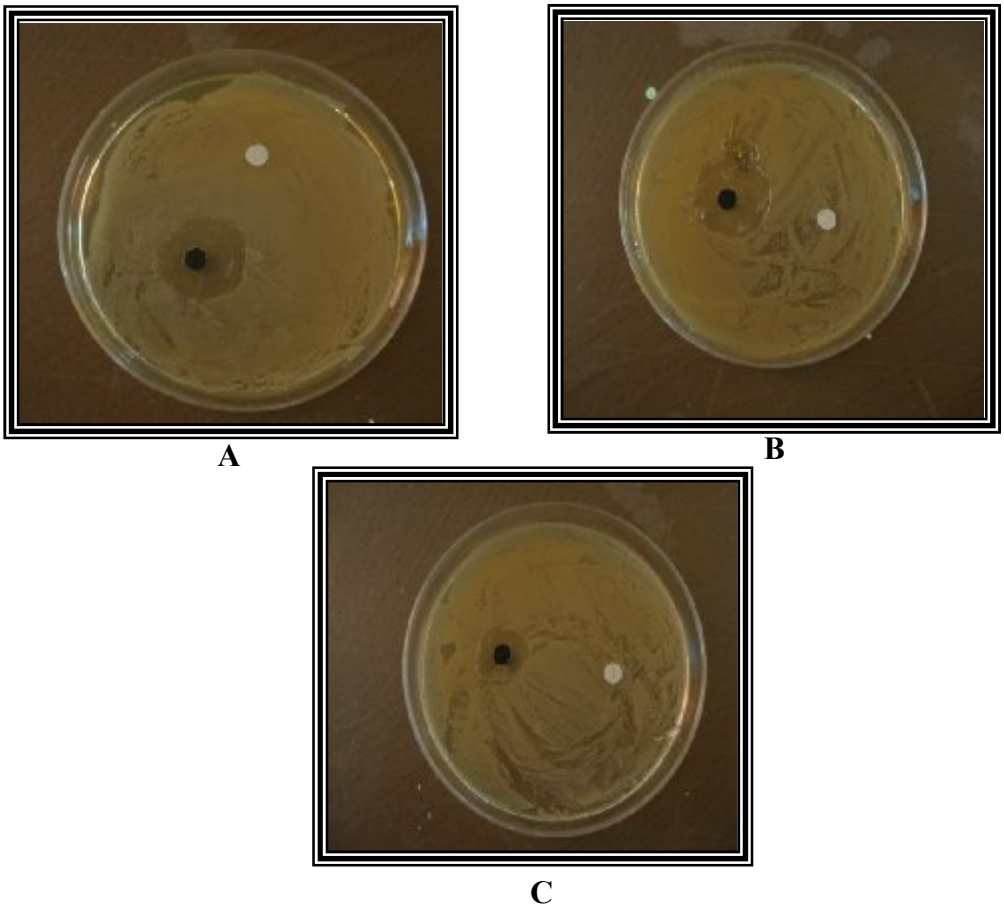

Plate 1: Inhibition zones exhibited by the purified A1 compound against (A) *E. coli* (B) *S. aureus* . and (C) *C. albicans*

Analysis spectrum on GC- mass method, it appeared that the molecular formula of A_1 compound is C22H42O4 (fiug.3), with a molecular weight 370.5 kd and it chemical structure is Bis (6methylheptyl) hexanedioate.

This indicated that A_1 compound is more related to ester group by comparing its spectroscopic data with available literature.

The MIC value of this bioactive compound is 7.32 µg/L for *E. coli* and *Staph. Aureus* 13.48 µg/L for the selected isolate of the dermatophytic fungus *Candida albicans*.

A clear zone inhibition of 18mm diameter was observed by using algal crude extract against both bacterial strains *E.* coli and *Staph. aureus* and 17 mm against *Candida albicans*. However, the inhibition zones diameters revealed by purified A compound were greater reaching to 22 mm and 21 mm for *E.* coli and *Staph. aureus* respectively and 18 mm for *Candida albicans*

(Plate, 1) The isolated A compound didn't show any toxicity against human RBC.

Discussion

Algal in general are a good source for antimicrobial agents (Ghasemi *et al*., 2004). The microalgae such as *Chlorella vulgaris*, *Chroococcus disperses*, *Chlamydomonas reinhardtii* (Ghasemi *et al*., 2007) *Chlorella vulgaris* (Abdel-Raouf, 2004), *Euglena rigidis* (Das *et al*., 2005), *Nostoc* Spp. (Jaki *et al*., 2000), *Cladophora crispata* (Khalaf, 2011; Athbi, 2010), *Chlorella* spp., *Scenedesmus* spp. (□rd□g *et al*., 2004), have been reported as the main groups of microalgae to produce antimicrobial substances.

The ability to produce antimicrobial agents may be significant not only as a defensive instrument for the algal strains but also as a good source of the new bioactive compounds from pharmaceutical point of view. Different bioactive chemical compounds identified as antimicrobial and antifungal screening from different microalgae. Some of these compounds including chlorellin (Metting and Pyne, 1986) Nostocin A (Hirata *et al*., 1996), Nostocyclyne A (Ploutno and Carmeli, 2000), Ambigo A and B (Flach *et al*., 1997), parsiguine (Ghasemi *et al*., 2004).

Few reports on the bioactive secondary metabolites 2 –(N, N-Dimethyl hydrazine) Cyclohexane carbonitrite compound has been isolated from this algal species and found to be effective from parasite (Antiprotoscolices) (Khalaf *et al*., 2011)The present study revealed that the purified extract (A compound) of *C. glomerata* exhibited an inhibitory action against both bacterial strains *E. coli* and *Staph. aureus* and antifungal dermatophyte *Candida albicans*.

The production of metabolite substances by algae in general, is often affected by various growth conditional factors mainly the medium of algal culturing (Baron, 1990). The liquid medium (Chu – 10) which is used. The present study is efficient for mass production of metabolite by the selected alga. Meantime, the isolated A1 compound showed a good bioactivity against the dermatophyte *Candida albicans*. This dermatophytic species frequently isolated from patients with skin infections(Candidiasis) in southern Iraq (Muhsin *et al*., 1997). The purified A1 compound is more likely to be chemically related to ester group (□rd□g *et al*., 2004) based on their chemical structure verification by using GC-mass techniques.

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