Antiparasite a ctivity of the microalgae Cyanobacteria *Hapalosiphon aureus* against the protoscolices of hydatid cyst , compared with albendazole drug .

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Abstract

Antiparasite a ctivity of the microalgae *Hapalosiphon aureus* from Basrah river in southern Iraq was studied. Water samples were collected from Shatt-Alarab river in the southern of Basrah , the algae cultured in BG-11 medium. Supernatants , alkaloidic and hexane extracts from biomass are isolated and screened against the Hydatidosis and compered with albendazole drug. The present study has resulted that 2- Methyl - 1-pyrroine and Ethylhexyl phthalate compounds have activity against the protoscolices of hydatid cyst similar to the activity of albendazole from the mean of weight of mice , mean of hydatid cyst number , mean of dimeter and weight of hydatid cyst.

.**Key words :** Algae , bioactive chemical compounds , antiparasite , isolation and identification .

فعالية الطحلب الأخضر المزرق (السيانوبكتريا) *Hapalosiphon aureus* ضد مرض الاكياس العدرية مقارنة بدواء الالبندازول

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المستخلص :

اختبرت فعالية الطحلب الاخضر – المزرق *Hopalosiphon aures* المعزول من انهار البصرة جنوب العراق . جمعيت عينات المياه الحاوية على الطحلب من مياه شط العرب . زرع في الوسط الزرعي BG-11 بعد عزله وتنقيته . اختبرت فعالية المستخلص القلويدي والزيتي للطحلب ضد الطفيلي المسبب لداء العدريات .استنتجت الدراسة الحالية ان المركب القلويدي 2-Methyl 3- pyrroine و المركب الزيتي Ethylhexyl phthalate امتلكا فعالية ضد مرض الاكياس العدرية مشابهة لفعالية دواء الالبندازول وقد لوحظ انخفاض في وزن الفئران المختبرية المعاملة بالمستخلصات الطحلبية وفي عدد الاكياس بالاضافة الى معدل اقطار واوزان الاكياس العدرية .

Introduction :

Microalgae are adiverse group of photosynthetic microorganisms found in the soil and fresh water environments (Metting and pyne , 1986) . They are able to produce a range of biochemical active compounds as antibacterial , antifungal , antivirale , enzyme inhibiting , immunostimulant , cytotoxic , ntiplasmodial activities (G hasemi *etal* ., 2oo4). and Antitrypanosomal (Lorena *etal* ., 2009) . Most of the isolated substances belong to groups of alkaloids , peptides , Tannins , Saponins , Triterpenes and phonols (Molera and semesi ,1996 as wellas carbohychates (Athbi 2010) . Hydatid disease, hydatidosis, cystic echinococcosis , Unilocular hydatid disease and *Echinococcus granulosus* Echinococcosis, all describe infections which are caused by cestodes of genus Echinococcus usually *Echinococcus granulosus* (Dar *etal.* , 1977 ; Akhan *etal* . , 2002 ; Georgopoulos *etal* . , 2007).

Hydatid cyst remains a significant public health problem in endemic areas such as Turkey, the Middle East, South America, New Zealand , Mediterranean region, Africa , China, Northern Kenya, Australia, and other sheep-raising areas (Morar and Feldman , 2003 ; McMnus *etal.*, 2003) . As an endemic disease, it causes social and economic losses for countries. WHO reports state that approximately 100,000 people in the world are infected with this disease every year (Roming,2003) which is common in rural population of underdeveloped countries because of their close association with domestic and wild animals(Parija and sheeladevi ,1999) . Until recently, surgery was the only option for treatment of echinococcal cysts, however, chemotherapy with benzimidazole compounds and, more recently, cyst puncture, and percutaneous aspiration, injection of chemicals, and reaspiration (PAIR) are increasingly seen to supplement or even replace surgery as the preferred treatment (Morar and Feldman , 2003)

The screening of microalgae and macroalgae for antibiotics and pharmacologically active compounds received ever increasing interest. A range of pharmacological activities have also been observed with extracts of algae and cyanobacteria as antibacterial , antifungal ,anticancer ,and anti-parasitic compounds (3,4,5,6). 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 Chloralin exhibited negative bacteria .Although very little research has focused the extracts of algae as asource of anti-parasitic compounds, recent studies have shown promising antimalarial activity in alga *Laurencia sp* . (Topcu *etal* ., 2003) in addition to their trypanocidal and leishmanicidal activity in *Fucus evanescens , pelvetia babingtonil* , *Ulva lactuca* and *Sargassum natans* (Nara *etal* ., 2000 ; and Orhan etal ., 2006) . The present study were design to examine the *in vivo* activity of bioactive chemical compounds (alkaloids and ethylaacetale) extracted from Cyanobacteria *(Hapalosiphon aureus)* against the protoscolices of hydatid cyst of *Echinococcus granulosus* and compared with albendazole drug.

Materials and methods

Isolation of microalgae

The microalga *Hapalosiphon aureus* is isolated from Shatt-Alarab River in Basra city, southern Iraq from January to April 2012. Primary culturing is done in BG-11 medium. After colonization, pure culture of the living specimens are prepared by using subculturing with agar plate method in Chu – 10 medium (Stein, 1975). Preserved specimens are prepared and the living specimens are incubated in 100 ml – conical flasks. Constant illumination is used at 60 $\mu E\ m^{-2}\ Sec^{-1}$ intensity with white fluorescent lamps. Temperature is 25 ±2°C. The resulted culture is identified based on morphology following taxonomy schemes of Prescott (1975) and Sant 'Anna (2004).

Preparation extracts :

Preparation of extracts is done according to (Reichelt and Borowitazka, 1984) . In N-hexane extract 1 gm of *Hapalosiphon aureus* biomass are extracted by soxhlet continuously with 100 ml of ethyl acetate as solvent for 24 hour and then the extracts are concentrated at room temperature.The alkaloidal extract preparation for take 0.5 gm of dried culture extracted with acidic ethanol (ethanol absolute with 2% acetic acid) for 24 hour in a continuous extraction (soxhlet) apparatus . The extraction are filtered , and ethanol is evaporated on a rotary evaporator under vacuum at a temperature of 45C to a small volume (about a quarter) . Then a small amount of

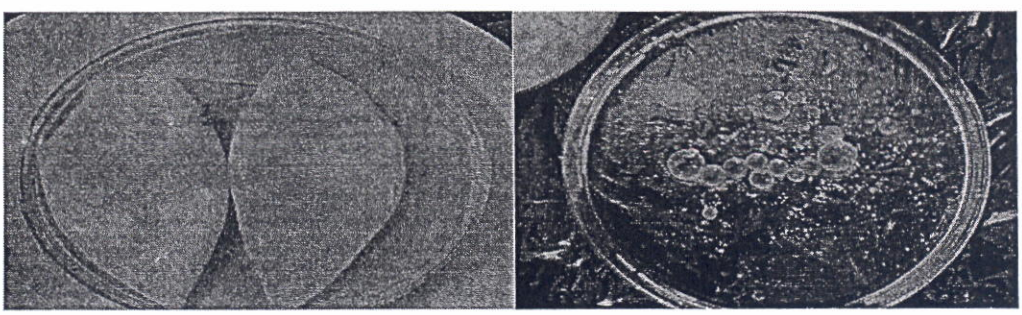
NH_3 (25%) is added to make pH of 9 . Subsequently , 100 ml of chloroform was added and slowly shacked for 10 mintes until alkaloid is separated for water and enter to the chloroform phase . This is repeated from three times and then total chloroform phase was evaporated , yielding a total alkaloid extract are dried under reduced pressure and stored in -20 C for further studes .

Identification of the Biochemical Active Compounds

Ultra – violet (UV) spectrum (LKB – Sweeden UV), Infra-red spectrum (IR) (Pye- Unicam Sp3- 3005 UK), gas Chromatography Mass (GC) (Agilent Technologies GC – mass 7890 AGC System) methods are applied for the identification and determination of the molecular weights and chemical formula and structure of the purified biochemical active compound.

Parasite materials :

Fresh hydatid cysts were obtained by surgery from human infected with hydatid disease from Al- Sadir Teaching Hospital in Basrah city . They were wrapped carefully in clean plastic bags, placed in an ice box, and transported to the Department of Biology, College of Education , Basrah University, where protoscolices were extracted (Smyth , 1964). . *E. granulosus* hydatid cysts containing protoscolices were removed under aseptic conditions from liver and lungs of naturally infected sheep and human. The outer surfaces of the cysts were sterilized with 70% ethanol before being dissected. Protoscolices were extracted according to (Smyth , 1964) (fig. 1).



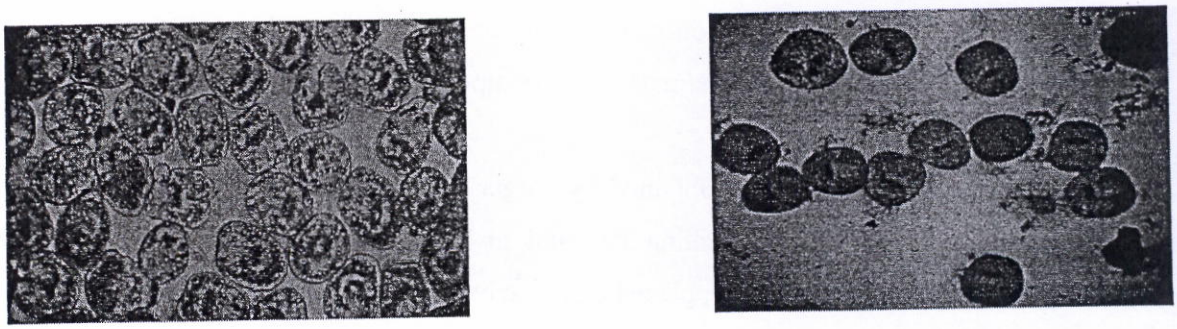
Hydatid cyst removed from human liver

Hydatid sand containing the daughter cyst , brood capsule and protoscolex after aspired from the hydatid cyst

Figure 1 . Hydatid cyst of *Echinococus granulosus*

Estimation of protoscolices viability :

200µl of hydatid fluid and 200µl of 0.1% eosin staining solution were combined in a microtube. After 20 minites incubation, the viability of protoscoleces are assessed by microscopic observation. Stained protoscoleces were considered as nonviable and the protoacoleces,which have not been stained with eosin, were considered as viable according to conventional.(Taran *et al* ., 2009) (fig. 2).



Viable protoscolices Non viable protoscolices

Figure 2 . Viable and non viable protoscolices

The counting of viable protoscolices

Protoscolices were counted according to the method cited by Al- Humairy (2010). After estimating the viability of protoscolices , 10 µl of the hydatid fluid was taken by a micropipette. The count was done under dissecting microscope (type Wild No.3) and repeated three times. The viable protoscolices were counted in 1ml based on the formula Viability in 1 ml = number of protoscolices in (10 µl) × 100 .

Injection of mice with protoscolices

A number of male *Mus musculus* mice Balb\C strain were injected with 0.2 ml 480/ (2400/5ml rate of viability) of protoscolices intraperitoneally (I.P.) by 1ml syringe syringe volume). The region of injection was sterilized with 70 % of ethyl alcohol . Al- Humairy (2010). .