

***In vitro* activity of Triazolo[1,5-a] pyrimidine carboxylic acid extracted from microalgae *Hapalosiphon welweschii* against *Trichomonas vaginalis***

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### **Abstract :**

The present study targets the protozoan parasite *Trichomonas vaginalis* that causes a healthy problems among women and rarely among men , by the application of natural product or secondary metabolites extracted from the microalgae *Hapalosiphon welweschii* for the first time in Iraq . methanol extract was explained high activity in three concentration recording 100% of parasite death at 200 µg/ml of methanol extract in about two days while 150 and 100 µg/ml of extract reports activity against the parasite after three and four days post treatment respectively . GC-Mass spectrum of the methanol extract has explain presence of the compound Triazolo[1,5-a] pyrimidine carboxylic acid in about 13.28 % from the total composition of methanol extract of microalgae *H. welweschii* .

**Key words :** STD, T. vaginalis, methanol extract .

### **المستخلص :**

استهدفت الدراسة الحاليه طفيلي المشعره المهبليه الذي يسبب مشاكل صحيه للنساء ونادرا للرجال ، من خلال استعمال المركبات الطبيعىة او مركبات الايض الثانوي المعزوله من من الطحلب الاخضر المزرق الدقيق *H. welweschii* ولاول مره في العراق . فقد لوحظ هناك فعاليه عاليه للمستخلص الميثانولي ويثلاث تراكيز مسجلة 100% نسبة فعاليه للتركيز 200 مايكروغرام ا مللتر لهذا المستخلص وخلال يومين فقط في حين بين كل من التركيزين 150 و 100 مايكرواملتر الفعاليه نفسها ولكن بعد مرور ثلاث واربعه ايام على التوالي . وبين التحليل الكيمائي لمستخلص الميثانول لهذا الطحلب باستعمال تقنيه كروموتوكرافيا الغاز طيف الكتله احتواءه على نسبه عاليه من المركب Triazolo[1,5-a] pyrimidine carboxylic acid حوالي 13.28 % من المستخلص .

**الكلمات المفتاحية :** الامراض المنقله جنسياً ، طفيلي المشعره المهبليه ، المستخلص الميثانولي

### **Introduction :**

Trichomoniasis is a sexually transmitted disease (STD) with important health ramification ; it has been associated with vaginitis , Urethritis , and pelvic inflammatory disease (PID) . Trichomoniasis also impacts upon birth outcomes and is co – factor in human immunodeficiency virus (HIV) transmission and acquisition (Swygard *etal.*, 2004) . Symptoms in women with Trichomoniasis include vaginal discharge , dysuria , and pruritus . in men symptoms include the urethral discharge , urethral pruritus , and dysuria (Schwebke and Burgess 2004) . Approximately 180 million women worldwide may be infected with *T. vaginalis* . Prevalence estimates vary between population studies , but ranging from 5-74 % in women and 5 – 59 % in men , with the highest rate reported in either sex from sexually transmitted infection (STI) clinic and in other high risk population (Karyakarte and Damle 2003). The undesirable side effects associated with this classical drug, as well as the development of resistance, are encouraging research into alternative synthetic or natural compounds effective for the treatment of hydatid disease . In this regard, most studies have been focused on activity of natural products from algae chlorophyta and cyanophyta

, mainly due to their accessibility and use in traditional medicine . A range of pharmacological activities have also been observed with extracts of chlorophyta and cyanophyta as antibacterial , antifungal , anticancer ,and anti-parasitic compounds (Takeaki etal., 2003 and Abass, 2010).

## **Material And Methods :**

### **1- Microalgae and methanol extract preparation :**

*Hapalosiphon welweschii* were cultured by using Chu – 10 medium , briefly , jars of 5 liters were filled with 3 liters of liquid medium , inoculated with desired algae, and then transferred to growth chamber at 12-25 °C. Constant illumination was used at 60  $\mu\text{E}/\text{m}^2/\text{cm}$  intensity with white fluorescent loup . Algae was harvested at the medium of stationary phase by using GFA pre weighed filter paper and centrifuge methods . Freeze – dried weighted again to reach a fixed weight of dried microalgae . The methanol extracts to be prepared; dry mass in ratio (1: 15 g/ml) was extracted using magnetic starrier through 24 hours. The precipitates were removed by filtration and left to dry until use, and then the filtrates were concentrated at room temperature .

### **- GC-Mass spectra analysis:**

Gas chromatography - mass spectra of fraction applied for the identification and determination of the molecular weight and chemical formula and structure of the purified chemical active compounds . it was done in Bruker company , Iran and Al- Elbait university in Jordin.

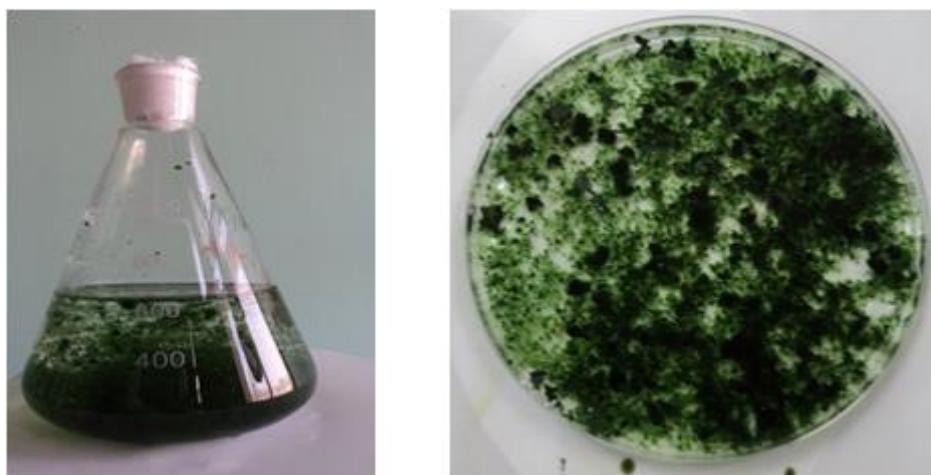


Figure (1) : Microalgae *Hapalosiphon welweschii* in culture

### **2- Collection of Parasite sample and culturing :**

High vaginal swab (HVS ) was collected from women infected with *T. vaginalis* after the insertion of speculum. the swab was inoculated into Kupferberg trichomonas broth media (Ph:6) and incubated at 37°C (Swygard *et al.*,2004) and left for seven days , there after , three tube of parasite culture were treated with the extract in three concentration only (100 , 150 , 200  $\mu\text{g}/\text{ml}$ ) ,lethal concentrations were chose from LD<sub>50</sub> , forth tube consider as control sample without treatment . the four tube were microscopically examined and counting of parasite for seven days started with one hour.



Figure (2) : *Trichomonas vaginalis* in vaginal discharge and culture

## Results

Methanol extract of *Hapalosiphon welweschii* recorded high activity at 200  $\mu\text{g/ml}$  after 2 – days post treatment , while 100  $\mu\text{g/ml}$  has explained activity after 4 days and 150  $\mu\text{g/ml}$  revealed activity after 3 days – post treatment. The previous results of extract activity reported after days for each concentration till use , but the activity of this product were observed after one hour as explain at the following table(1) :

Table (1): Viability of *Trichomonas vaginalis* treated with methanol extract of *Hapalosiphon welweschii*

Concentration $\mu\text{g/ml}$	time of treatment \ days								
	1 h	4 h	1 day	2 days	3 days	4 days	5 days	6 days	7days
100	70.0	61.4	42	31.66	20	0	0	0	0
150	66.21	50	38.66	26.33	0	0	0	0	0
200	57.33	42.6	23.66	0	0	0	0	0	0
Control	92.9	88.0	80.75	78.22	75.0	67.1	59.8	54.2	50.5
L.S.D.	0.854								
Significant differences , $P \leq 0.05$									

## GC- Mass spectrum of methanol Extract of microalgae :

The GC – Mass spectrum (Fig,1) of the methanol extract of *H. welweschii* revealed that are 22 peaks of different sizes. The results of spectrum showed that Triazolo[1,5-a] pyrimidine carboxylic acid consist 13.28 % of the total methanol extract followed by Diterpine ( 13.03 %) as illustrated below :

Table ( 2) :bioactive chemical compounds of methanol extract of *H. welweschii*

Peak	R.T.	% of total	Compounds	M.W.
1	24.628	13.28	- Triazolo[1,5-a] pyrimidine carboxylic acid	164.12
2	27.673	13.03	- Diterpine	286.45
3	28.951	4.36	- 3,7,11,15,tetramethyl- 2- hexadecan-1-ol	296
4	30.362	2.42	-18- Nonadecen- 1-amine	283

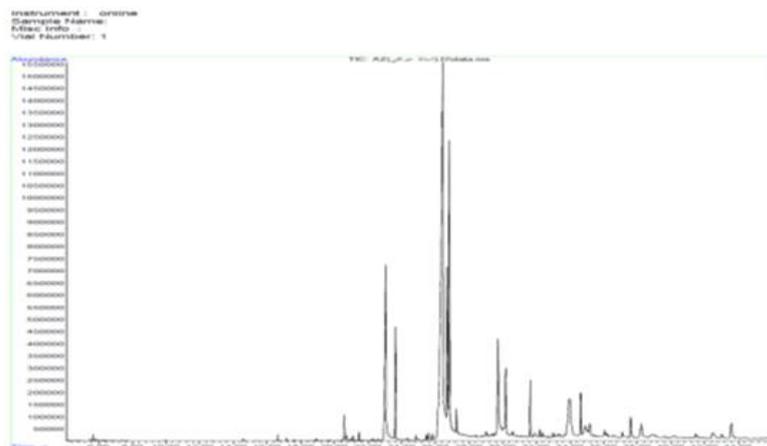


Figure (3) : GC- Mass spectrum of Methanol extract

### **Discussion :**

Natural products have been the source of therapies since the advent of traditional medicine and healing; it remains a dominant source to date. The World Health Organization (WHO) estimates that 80% of the world's inhabitants depend mainly on traditional medicine for their primary health care (Tuney *et al.*, 2006 ; Taran *et al.*, 2009 ). Three concentrations of the methanol extract of *H. welweschii* were used in the present study and it had an *in vitro* activity against *T. vaginalis* and the time plays an important role in the treatment since the decreased concentration leads to increase the time of treatment . The activity of the methanol extract could be explained by the presence of the compound Triazolo[1,5-a] pyrimidine carboxylic acid. It is difficult to speculate the mechanism by which these bioactive compounds act as parasitocidal agents. In this regard Sepulveda-Boza and Cassels (1996) suggested that many bioactive chemical compounds exhibited their parasitocidal activity by virtue of their interference with the redox balance of the parasites, acting either on the respiratory chain or the cellular defenses against oxidative stress. It is also known that some bioactive compounds act by binding with the DNA of the parasite. For example, dihydroorotate dehydrogenase (DHOD), the fourth enzyme in the *de novo* pyrimidine biosynthetic pathway, is essential to parasites, including the electron acceptor capacity and cellular localization (Morales-Landa *et al.*, 2007). In this way, it has been recently demonstrated that the methanol extracts of brown algae *Ishige okamurae*, *Fucus evanescens*, and *Pelvetia babingtonii* contain potent noncompetitive inhibitors against *Trypanosoma cruzi* DHOD (Takeaki *et al.*, 2003 and Morales-Landa *et al.*, 2007).

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