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In Vitro Activity of Methanol Extract of Microalgae *Hapalosiphon aureus* Against *Trichomonas vaginalis*

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Abstract **—The present study targets the protozoan parasite Trichomonas vaginalis that causes a healthy problem among women and rarely among men, by the application of natural product or secondary metabolites extracted from the microalgae Hapalosiphon aureus 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 fourand fivedays post treatment respectively. GC- Mass spectrum of the methanol extract has explain presence of the compound (2- deca - 3,d- dienyloxy) carbonyl benzeoic acid in about 13.28 % from the total composition of methanol extract of microalgae.**

Keywords— Sexually transmitted disease, T. vaginalis, methanol extract.

I. INTRODUCTION

Trichomoniasis is a sexually transmitted disease (STD) with important health ramification; it has been associated with vaginatis, 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 *et al.*, 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 studiesbut 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 2004). 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 *et al.*, 2003; Abass, 2010).

II. MATERIAL AND METHODS

- Microalgae and methanol extract preparation

Hapalosiphon aureus were cultured by using Chu – 10 mediums, 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 ^{0}C. Constant illumination was used at 60 µE\m\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.

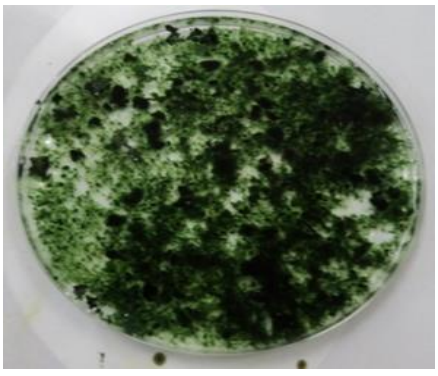

Fig. 1. Microalgae *Hapalosiphon aureus* in culture

- 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 37C°

(Swygard *et al.*,2004) and left for seven days, thereafter, three tube of parasite culture were treated with the extract in three concentration only (100 , 150 , 200 µg\ml) ,lethal concentrations were chose from LD_{50} , 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.

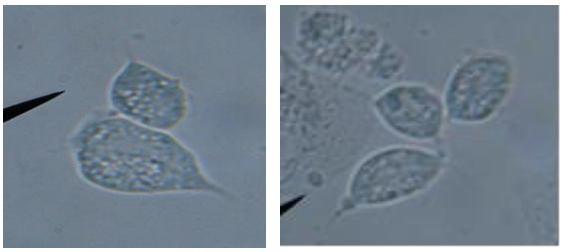

Fig. 2 . *Trichomonas vaginalis* in vaginal discharge and culture

MEASURMENTS

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.

III. Results

Methanol extract of Hapalosiphon aureus recorded high activity at 200 µg\ml after 2 – days post treatment , while 100 µg\ml has explained activity after 5 days and 150 µg\ml revealed activity after 4 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 *Hapalosiiphon aureus*

Concentration µg\ml	**Time of treatment\ days**								
	1 h	***4 h***	***1 days***	***2 days***	***3 days***	***4 days***	***5 days***	***6 days***	***7 day***
100	70.0	61.4	42	31.66	20	12.11	0	0	0
150	66.21	50	38.66	26.33	9.22	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 ≤ 0.05									

GC – Mass spectrum (Fig, 3) of the methanol methanol extract has recorded 22 peaks , (2- deca - 3,d- dienyloxy) carbonyl benzeoic acid consisted of 22.57% (R.T. 22.647) of the total extract followed by ethyl linoleolate (13.13% and 25.946 min of R.T.) , and other compounds .

TABLE 2 Bioactive chemical compounds of methanol extract of *microalgae*

Peak	**R.T.**	**% of total**	**Compound**	**M.W.**
1	20.264	3.26	- Phytol	298
2	22.647	22.57	- (2- deca - 3,d- dienyloxy) carbonyl benzeoic acid	302
3	25.946	13.13	- Ethyl linoleolate	293
4	32.411	1.65	- Diterpine	286.45

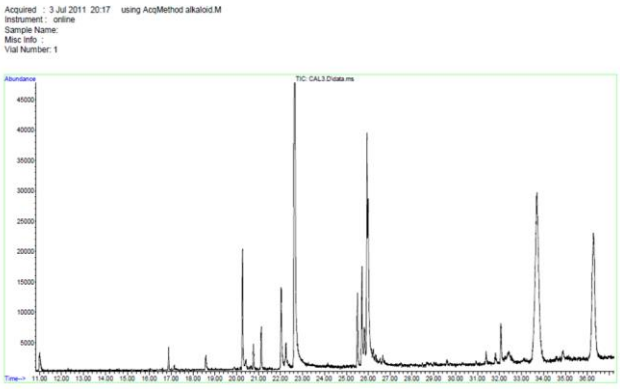

Fig. 3. GC spectrum of methanol extract of *Hapalosiphon aureus*

IV. IV. 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). The biochemical medicinal activity of natural products results from inhibition of enzymes action by interaction with thymol group (Sh) of enzymes and linking with DNA & RND, then destruction of these nucleic acids and finally inhibition the biosynthesis of cell proteins, metabolism of each of carbohydrates, and lipids.

Three concentrations of the methanol extract of H. aureus 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 (2- deca - 3,d- dienyloxy) carbonyl benzeoic acid.

It is difficult to speculate the mechanism by which these bioactive compounds act as parasitecidal agents. In this

regard Sepulveda-Boza and Cassels (1996) suggested that many bioactive chemical compounds exhibited their parasitecidal 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; Morales-Landa *et al.*, 2007).

V.**REFRENCE**

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