

Research Article

Testing the activity of Alkaloids extracted from *Clorella vulgaris* on the viability of *Trichomonas vaginalis* In vitro

AMAL KHUDAIR KHALAF

Dept. of microbiology \ College of medicine / University of Thi-Qar/ Iraq

Email ID: amalkhudair111@yahoo.com

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ABSTRACT

Three concentration were target the viable trophozoites of parasite in this study return to the alkaloid extract of the green algae or chlorophyte *Clorella vulgaris* in vitro , the results for the study were explain a high activity for the extract against the parasite where it is destroyed totally by 300 µg/ml in about four hours post treatment , further the decrease or the damage of parasite were started from the first 30 minutes from the treatment and in three concentrations that uses that included 150,200,300 µg/ml . the activity of alkaloid extract against *T. vaginalis* were reported a total damage for parasite after the third day in the case of 150 µg/ml , one day at 200 µg/ml , and 4 hours at 300 µg/ml post treatment .

Keywords: Natural products , *Clorella vulgaris*, *T. vaginalis*, alkaloids

INTRODUCTION

The traditional drugs have side effect on the organisms when used as well as the resistance that appear by the microbes against the use of antibiotic recently. So , there were a new trends to the use of natural products or secondary metabolites of of plant or algae as antimicrobial. Chlorophyta and cyanophyta are used highly for this matter as a pharmaceutical products acts as antibacterial, antifungal or antiprotozoal. (1,2).

Trichomoniasis is a sexually transmitted infection between male and female but it is rarely transmitted through the contaminated towels , this parasite is widely associated with Urethritis and vaginitis as well as the disease of pelvic or PID . *T. vaginalis* were also as co factor of virus of HIV transmitted (3) . the abnormal vaginal discharge and pruritis is the common Symptoms among female that infect with this parasite but symptoms in men are include urethral discharge and pruritis or itching (4) . the rate of infection is different between male and female but it is highly among female in comparison with male and about 180 million of female may be infected with parasite worldwide .the Prevalence or incidence is vary among studies but it is range between 5-74 % in female while 5 – 59 % in male. (5).

MATERIAL AND METHODS**Preparation of the alkaloid extract from *Clorella vulgaris* :**

Chu – 10 medium was used in this study for culturing of the microalgae *Clorella vulgaris* by using of one liter flasks that filled with 500ml of the liquid medium and inoculated with desired *Clorella* algae. All the inoculated flasks were transported to the growth chamber at 12-25 °C. white fluorescent loup at 60 µE/m²/cm intensity was used as Constant illumination .then after *C. vulgaris* or the microalgae was harvested by using GFA pre weighed filter paper and centrifuge methods at stationary phase of growth . the harvested algae were dried by the use of Freezing drier system to reach a dried microalgae at fixed weight . Finally , The alkaloid extracts was prepared , briefly, the dry weight were extracted in soxhlet apparatus by using the solution of acidic alcohol (ethanol absolute with 2% acetic acid) for 24 hours ,then pH of 9 were obtained by 25% of NH₃ .finally ,the solution was mixed with 100 ml of chloroform and shaken for 10 minutes and repeated for three times for evaporation of chloroform to yield alkaloid extract .

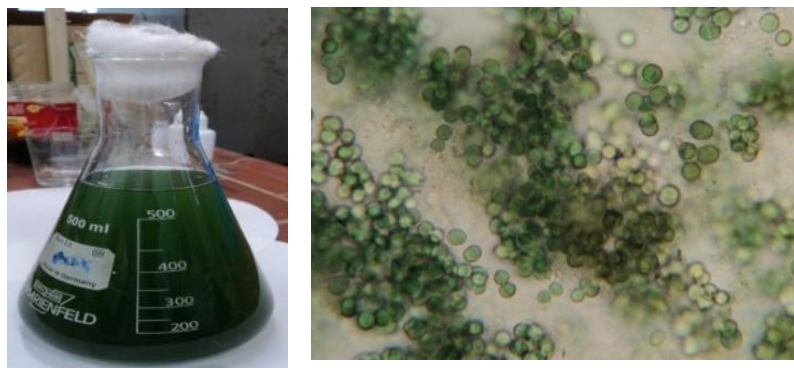


Fig.1: Culture of *Clorella vulgaris*

Parasite collection and culture:

T. vaginalis were collected from women with abnormal vaginal discharge by using High vaginal swab that was inoculated into Kupferberg broth media (Ph:6) and incubated at 37C° (3) and left for seven days , then after , three tube of parasite culture were treated with the extract in

three concentration only (150 , 200 , 300 µg/ml) ,lethal concentrations were chose from LD₅₀ , 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.

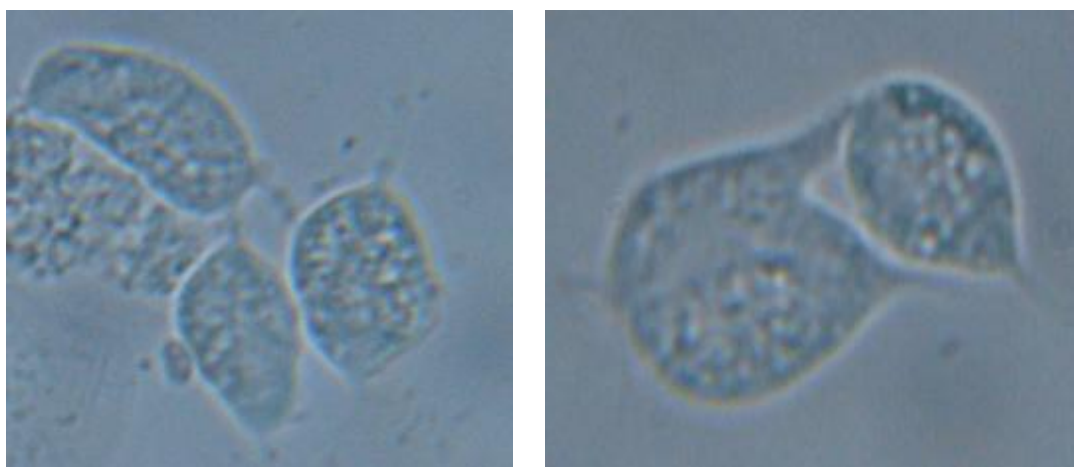


Fig.2: *Trichomonas vaginalis* trophozoites

GC- spectrum analysis

GC-spectrum or chromatogram of alkaloid was done in agriculture college at university of Basrah in Iraq.

STATISTICAL ANALYSIS

The statistical analysis was conducted by using the analysis T test. The L.S.D. test at 0.05 level was used to analyze differences in the mean of viability of the parasite that treated with bioactive chemical compounds in vitro (SPSS, 1998) .

RESULTS

High effect were recorded at the current study when testing the activity of alkaloid extract against the trophozoites of the pathogenic protozoan *T. vaginalis* , where the parasite was damaged at the third concentration (300 µg/ml) from the fourth hour through the study . the activity of alkaloid extract against *T. vaginalis* revealed that the parasite killing started from 30 min from treatment at 150,200,300 µg/ml concentration reporting a total damage for parasite after the third day in the case of 150 µg/ml , one day at 200 µg/ml , and 4 hours at 300 µg/ml post treatment as listed in the following table:

Table 1: efficacy of the alkaloid extract against *T. vaginalis*

Extract Conc. µg/ml	Treatment time per days								
	30min.	1 h	2 h	3 h	4 h	1 day	2days	3days	4 days
150	90.4	78.29	70.11	58.9	47.24	31.7	12	0	0
200	88.1	72.1	61.82	54.21	32.86	0	0	0	0
300	71.32	60.12	48.2	32.54	0	0	0	0	0
Control	97.1	95.0	92.75	90.22	87.0	82.1	79.8	75.2	70.5
L.S.D.	0.824								
Significant differences , $P \leq 0.05$									

GC –spectrum of alkaloid Extract:

The following GC –spectrum explains the chromatogram of the alkaloid extract as the showing diagram

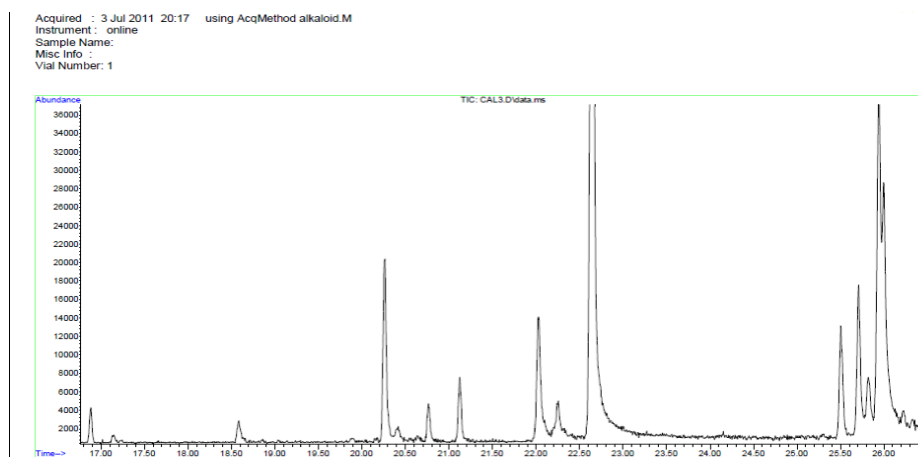


Fig.1: GC spectrum explains alkaloid extract of *C. vulgaris*

DISCUSSION

There are another source for therapy instead of the traditional pharmaceutical industries by the use of the natural products the bioactive materials that extracted from the organisms like algae and plants , till now this benefit for natural product were remain dormant or use as primary care for health (6,8).Secondary metabolites or the Natural products are the alternative compound that used in the pharmaceutical therapies because the complication or the side effects followed that the use of classical drugs. (6,7) . The medical action of this products or secondary metabolites was come from the inhibition of the action of enzymes through the interaction with group of thymol of the enzymes and its joining with DNA and RND causing the destruction of these DNA and RND , further , inhibition of the protein cell biosynthesis

and damage of the metabolism of lipids ,carbohydrates and sterols.

alkaloid extract at the three concentrations used in this study in vitro to inactivate *T. vaginalis* were revealed an activity against the parasite at different time in a adifferent concentration .the mechanism of action for these compound is difficult to be understand or how can act as parasitocidal inhibitors . (7) remembered that natural product may be in activate the parasite by their interaction the balance of rodex of the parasite, or their action against oxidative stress by the cellular defense, and may be act on the respiratory chain. Further action is known by the binding of these bioactive compound with DNA of parasites. For example, the forth enzyme or dihydroorotate dehydrogenase in the biosynthesis of pyrimidine pathway that is essential to protozoal parasites in the capacity of electron

acceptor capacity and localization of cell (7,8). This is similar to that inhibitors for dihydroorotate dehydrogenase or DHOD of *Trypanosoma cruzi* parasite that target or inactivated by the methanol extract from the algae *Fucus evanescens* and *Ishige okamurae* during the study that have been recently demonstrated (1 ,8).

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