



## In Vitro Antitrichomonal Activity of Some Species of *Allium*

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### Abstract

**Background:** *Trichomonas vaginalis* infectious disease is an important worldwide health problem. Although, several drugs especially metronidazole and tinidazole have been used in the treatment, their-resistant strains have been developed and unpleasant adverse effects exist. Garlic and other *Allium* species are old medicinal plants, which were used for infectious diseases, cardiovascular disorders and hair and skin cosmetic diseases.

**Objectives:** We aimed to compare the anti-trichomoniasis activities of three species of *Allium*.

**Methods:** The *Trichomonas vaginalis* strain was isolated from vaginal discharge of women with vaginitis symptoms and cultured in a modified TYM medium. After confirmation of herbarium of the plants, their extracts were prepared. For testing, 24 sterile plates were used. In all 24 homes, 200  $\mu$ L of TYM were poured out. The tube containing the parasite, counting the number of angels with the aid of Neubauer slide and set to 500,000 per mL, was added to the amount of 100  $\mu$ L to all wells. In order to investigate the anti-trichomoniasis of the plant, concentrations of 37.5, 75, 150, 300, 600, 1200 and 2400  $\mu$ g/mL of extract have been tested. For positive control, 50 mL of metronidazole, 50  $\mu$ g/mL, was added to culture medium. In addition to, no herbal extract and metronidazole was added to the well of negative control.

**Results:** The leaf extract of bell circus with a concentration of 2000  $\mu$ g/mL after 48 hours and boiled cabbage bulb onion at a concentration of 24  $\mu$ g/mL and leaf extract of turmericum at a concentration of 1500  $\mu$ g/mL in 48 hours and boiled horseradish onions at 48  $\mu$ g/mL concentration in 24 hours could achieve 100% GI and significantly inhibit parasite growth. Leaf extract, boiled onions and essential oils inhibited the growth of *Trichomonas* parasites, were not as high as 100%.

**Conclusions:** According to the results obtained in our study, these *Allium* plants have inhibitory activity on growth of *Trichomonas vaginalis* parasite.

**Keywords:** *Allium*, *Trichomonas vaginalis*, Gastro-Intestinal Tract, Infectious Disease, Plant

### 1. Background

*Trichomonas vaginalis* (TV) is a mucosal flagellate pathogen, which causes one of the sexually transmitted diseases (STD). This pathogen can parasitize the human vagina, prostate gland, and urethra.

Moreover, the number of new adult patients of trichomoniasis in 2008 have been estimated 276.4 million cases between the ages of 15 and 49 years. In addition, the global prevalence of trichomoniasis of women and men has been estimated about 8.1 % and 1.0 %. Moreover, prevalence of trichomoniasis was estimated at 3.2 % based on culturing method in Tehran, Iran. Trichomoniasis may induce preterm delivery and low birth weight in female patients. The problems of this infection that can bother the

patient are vaginal discharge, itching, dysuria, abdominal pain and vulvar irritation (1, 2).

Despite the impact of these infections, there are just two drugs available to the therapist, metronidazole and tinidazole, which are 5-nitroimidazole drugs. The common disadvantages of these drugs are treatment failure due to resistance of *T. vaginalis* and side effects like potential carcinogenic, teratogenic and embryogenic effects and common effects such as nausea, dry mouth, bitter metallic taste and vomiting (3-5).

Natural products as the important sources for new therapeutic agents have been used for treatment of diseases and secondary metabolites of them have been studied for use as approved drugs (6).

*Allium* species as the medicinal plants have been used many years ago. Moreover, several biological activities such as antimicrobial, hypolipidemic and hypotensive have been reported (7, 8).

The *Allium* genus comprises biannual bulbous plants with solitary or clustered bulbs that are strongly aromatic. In addition, these plants have allicin and other sulfur containing organic compounds.

## 2. Objectives

We aimed to compare the anti-trichomoniasis effects of three species of *Allium*.

## 3. Methods

### 3.1. Plant Extraction

*Allium ampeloprasum* L., *Allium paradoxum* (M. Bib.) G. Don and *Allium ursinum* L. were collected from one of the villages of Mazandaran province, north of Iran. Herbariums were prepared and a systematic specialist confirmed their scientific names.

Their leaves were separated, dried, powdered and kept in dark containers. Extraction of leaves was performed with methanol solvent by maceration method. In this method, 100 g of all leaves were macerated in methanol for 72 hours and repeated triplet, separately. Then, all extracts were collected. Moreover, a rotary evaporator was used for evaporation of the solvent. In addition, fresh bulbs of them were crushed in a mixture machine and were boiled in water for ten minutes and sterilized by filters.

In addition, their essential oils were prepared by the enflurage method. In this method, crushed onions are placed in an oven container and added to olive oil. The essential oils are extracted into the oil. Then, the essential oil is separated from the oil-by-oil and decanting method and finally distilled (9, 10).

### 3.2. Identification and Separation of Onion Compounds

The filtered essential oils were subjected to GC-MS analyses. GC-MS was carried out on an HP 5890 GC system coupled to a Quadrupole Mass Detector. Helium was used as a carrier gas in a constant flow mode at 1 mL/min. The temperature of the column in initial procedure was 70°C, which was gradually increased by 10°C up to 280°C. The instrument was set to a 70°C as the initial temperature for 2 min. Then, the temperature was raised up to 280°C, at the increase rate of 5°C/min to 9 min. In addition, the ionization voltage was 70eV. Separation was achieved by RTS-volatile column about 30 m long. Quadrupole Mass Detector was employed to detect compounds when they were

vented from the column. The temperature of the detector was 300°C. Using data library such as NIST database (The National Institute of Standards and Technology), the mass spectra obtained through GC-MS, were analyzed, and the volatile compounds of the plant samples were identified (10, 11).

### 3.3. Culture

In this interventional study, the studied population was *Trichomonas vaginalis* isolated from urine sedimentation in women with vaginitis symptoms that after separation was cultured in a TYM medium (Trypticase, Yeast extract, Maltose) containing 10% sterile calf serum and by adding antibiotics ciprofloxacin with 50 µg/mL, ceftriaxone with 100 µg/mL and amphotericin B with 2.5 µg/mL concentration to this environment and during the four passages of a day in between, it was pure and duplicated (12-14).

### 3.4. In Vitro Exposure and Evaluation

For testing, 24 sterile plates were used. For further accuracy, each dilution of the extracts (leaf extracts and bulb essential oils) was performed in 2 wells and the test was repeated 3 times. According to protocol, in all 24 wells, 200 µL of TYM was dispensed. Then a parasite tube that counts the number of parasites with Neubar slide and sets it to 500,000 per mL were shaken completely to make it uniform and 100 µL was added to all wells. The herbal powder was treated with sterile distilled water and concentrated to 6 mg/mL. After filtering, it was poured (12-14).

In order to investigate the anti-trichomoniasis properties of the plant, concentrations of 37.5, 75, 150, 300, 600, 1200 and 2400 µg/mL of extract were tested. For positive control, instead of herbal extracts, 50 µL metronidazole with 500 µg/mL has been added to the positive control medium.

For negative controls, no extraction of herbal extracts and metronidazole was added. In the study, dilutions of herbal extracts and metronidazole with sterile PBS (phosphate buffer saline) with pH = 6.4 were prepared. In addition, plates were placed in a 37-degree incubator. To check the growth rate of the parasite after 24 and 48 hours, after ensuring that the wells are mixed with a pipette method, 10 microliters were removed from each well. The number of parasites was counted with Neubar slide. In order to achieve a minimum laboratory and human error rate, tests were performed using double blinded method and three times and the average parasite count was reported as the result three times. At 24 and 48 hours, the motility and survival of the parasites with the trypan blue color (depleted

parasites and blue, while the live parasites remain colorless) were investigated. The time of impact-effect concentration the number of parasites in each stage-was the survival and parasitic movement (12-14).

### 3.5. Statistical Analysis

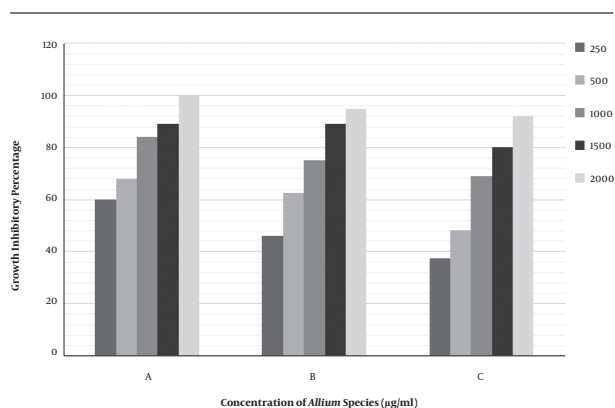
The results of parasite counting were calculated as growth inhibitory percent (percentage of growth inhibition GI%) using the following formula:  $GI\% = 100 \times (a - b)/a$

In this formula, the number of live parasites in the microtube wells is negative and b is the number of live parasites in the well containing the extract. Also, by counting the number of live parasites in different concentrations of Neubar and tripan blue for 24 hours and 48 hours, IC<sub>50</sub> (inhibitory concentration) was calculated using Graph pad software. Moreover, the findings were analyzed using ANOVA in SPSS (Ver. 20) software ( $P \text{ Value} \leq 0.05$ ) (12, 13).

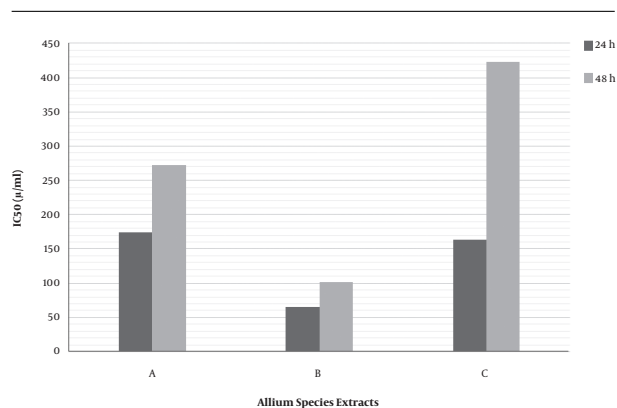
## 4. Results

Essential oils of *Allium* species bulbs have some compounds, including carvacrol, cyclohexasiloxane and cyclopentasiloxane in *Allium paradoxum*, camphor, nootkatone and dipropyl disulfide in *Allium ampeloprasum* and thymol, carvacrol and thymyl acetate in *Allium ursinum*, chiefly. These compounds are the important agents for biological activity of the plants. Table 1 shows the composition of essential oil with retention time (Rt) and percentage of each compound.

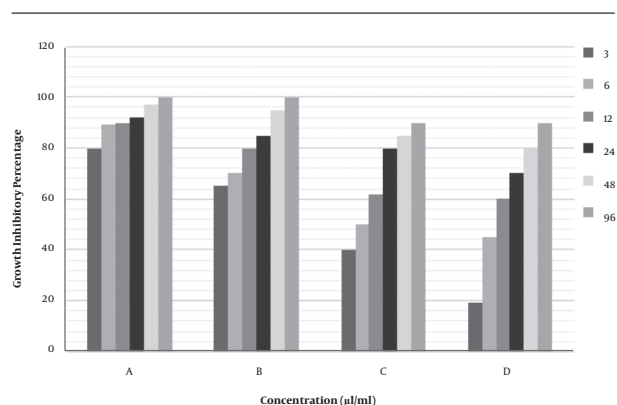
The growth inhibitory effect and IC<sub>50</sub> of leaf extracts and bulb essential oil of *Allium ursinum*, *Allium ampeloprasum* and *Allium paradoxum* are shown in Figures 1-4.



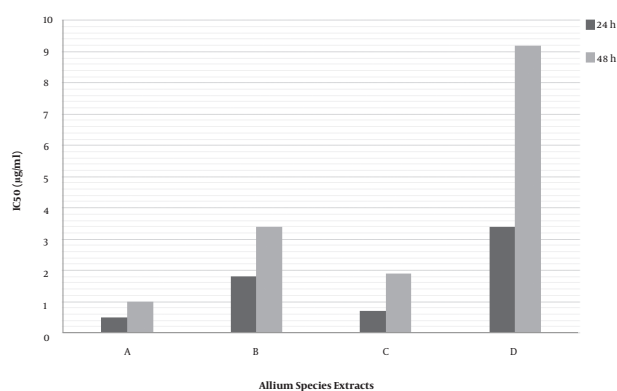
**Figure 1.** Growth inhibitory percentage of different concentrations of *Allium ursinum* (A), *Allium ampeloprasum* (B) and *Allium paradoxum* (C) leaves extraction (24 h).



**Figure 2.** IC<sub>50</sub> of leaf extraction of *Allium ursinum* (A), *Allium ampeloprasum* (B) and *Allium paradoxum* (C) After 24 and 48 hours.



**Figure 3.** Growth inhibitory percentage of different concentrations of bulb essential oils of *Allium ursinum* (A), *Allium ampeloprasum* (B) *Allium paradoxum* (C) and *Allium paradoxum* (D) (24 h).



**Figure 4.** IC<sub>50</sub> of bulb essential oils of *Allium ursinum* (A), *Allium ampeloprasum* (B) and *Allium paradoxum* (C) and *Allium paradoxum* (D).

According to the results, *Allium ursinum* leaf extract has the most growth inhibitory effects and the *Allium am-*

**Table 1.** Composition of Essential Oil with Retention Time and Percentage of Each Compound

Compounds	<i>Allium</i> Species					
	<i>Allium paradoxum</i>		<i>Allium ampeloprasum</i>		<i>Allium ursinum</i>	
	Rt	%	Rt	%	Rt	%
Cyclopentasiloxane	14.8	4.46	-	-	-	-
Cyclohexasiloxane	20.66	5.35	-	-	-	-
Carvacrol	21.18	32	-	-	20.91	55.57
Dipropyl Disulfide	-	-	13.28	13.62	-	-
Camphor	-	-	14.53	6.31	-	-
Nootkatone	-	-	35.12	13.63	-	-
Thymol	-	-	-	-	20.64	17.78
Thymyl acetate	-	-	-	-	22.37	5.58

*peloprasum* leaf has the lowest IC50. In addition, the leaf extracts and bulb essential oil of *Allium ursinum* have the highest inhibitory activities and the lowest IC50.

## 5. Discussion

In this study, the effect of *Allium ursinum*, *Allium ampeloprasum* and *Allium paradoxum* leaf extracts and bulb essential oils on *T. vaginalis* growth has been evaluated in vitro. According to the results, the crude extract of leaves and bulbs, bulb essential oils of *Allium ursinum* showed a higher growth inhibitory effect compared to the controls with the lowest concentration in the 24 and 48 hours of incubation.

Humans usually have used the plants as therapeutic agents. In the recent years, studies have attempt to analyze, select the suitable medicinal plants, and extract the components for therapeutic outputs. With the explanations, biological effects of some medicinal plants on *Trichomonas vaginalis* have been documented in different investigations. Several studies have been done about the efficacies of natural compounds on trichomoniasis.

Yousofi Darani et al. analyzed the effects of *Chaerophyllum macropodum* extracts on *Trichomonas vaginalis* in vitro (15). According to the study, all extract concentrations showed some degrees of growth inhibition activity on *T. vaginalis* and crude extract was more efficient.

Gokmen et al. studied efficacy of *Haplophyllum myrtifolium* on trichomoniasis (16). In the study, the efficacy of the plant was evaluated. According to the results, ethanol extract caused a more effective lysis on *T. vaginalis* trophozoites compared with total alkaloid extract ( $P < 0.05$ ). In addition, no compounds except for fluoroquinolone alkaloid skimmianine prepared above 37.5  $\mu\text{g}/\text{mL}$  were found to have any inhibitory effect on *T. vaginalis* trophozoites.

Moreover, Mahmoudvand et al. evaluated efficacies of some Iranian medicinal plants on *Trichomonas vaginalis*

(17). According to the study, some plant species such as *Quercus infectoria* have been evaluated.

### 5.1. Suggestion

More studies about treatment of infectious diseases, especially life-threatening disorders by natural-based drugs were suggested.

### 5.2. Limitations

The low number of cases, the culture of parasites and experimental instruments were the limitation of the study.

### 5.3. Conclusions

The results of this study showed that the extracts of leaves and the essential oil of bulbs of *Allium ursinum*, *Allium ampeloprasum* and *Allium paradoxum* with different concentrations have anti-trichomonal activity.

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## Footnotes

**Authors' Contribution:** Atena Majidi, Hojat-Allah Arab, Zohreh Momeni and Ali Davoodi contributed to study design, data collection and carried out experiments. Mohammad Azadbakht and Hajar Ziaei Hezarjaribi were supervisors of the research project and contributed to original data, critical editing and reviewing the manuscript. All the authors read and approved the final manuscript.

**Conflict of Interests:** The authors declare no conflict of interest.

**Ethical Approval:** The study protocol was approved by Ethics Committee of Mazandaran University of Medical Sciences.

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