



## Comparing effect of some herbal medicines extract with metronidazole for treatment of trichomoniasis in birds

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### ABSTRACT

*Trichomonas gallinae* is the flagellated protozoan parasite belongs to the category of zoomastigophorea, and order of trichomonadida. This parasite is located in the upper section of the gastrointestinal tract of numerous birds such as pigeons, chickens, eagles. Nitroimidazole compounds are carcinogenic, therefore is prohibited their use in veterinary medicine. . The aim of this study was to examine the effect of 6 herbals medicine extract on *Trichomonas gallinae* in vitro. The effect of different concentrations (1.25, 2.5 and 5 mg/ml) of extracts of *Quercus Persica*, *Allium sativum*, *Artemisia annua*, *Myrtus commuis*, *Zataria multiflora*, *Rosmarinus officinalis* was compared with metronidazole in 2, 4, 6, 18, 24, 48 and 72 hours after treating using trypan blue and eosin staining as vital stain. Sensitive of *T. gallinae* to the oak extract 2 mg/mL in 2 hours was more than the others and was close to the effect of metronidazole. In contrast to garlic extract did not show any sensitivity. The effect of the other extracts was between two mentioned extract. According to the results, the most effective extract on the trichomonas parasite was the oak fruit extract and then the rosemary extract. It is suggested, the study is also examined in vivo and if it was effective, used for the treatment of trichomoniasis in birds after the complementary studies.

**Key words:** *Trichomonas gallinae*, metronidazole, herbal medicine, extract

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### INTRODUCTION

*Trichomonas gallinae* is the flagellated protozoan parasite belongs to the category of zoomastigophorea, and order of trichomonadida, which lives in the trophozoite form (active form) [1,2]. This parasite is located in the upper area of the gastrointestinal tract (mouth, pharynx, esophagus and crop) of numerous birds such as pigeons, chickens, eagles [3,4] and depending on the strain of parasite and bird species causes various pathological symptoms, which is called trichomoniasis [5,6]. The symptoms including lethargy, depression, yellow and pasty diarrhea [7] and even in the pathogenic strains, due to the fibronectin wounds in the mouth, pharynx and digestive tract cause obstruction, and because of the severe starvations or secondary bacterial infections cause death [4]. The most sensitive birds to the disease, are the pigeons, especially young pigeons, with an intensity of mortality which sometimes reaches to 80-90% [8]. *Trichomonas gallinae* is only existed in the trophozoite form and disappeared in a short time outside of the body [9]. It is transmitted directly through feeding the chickens by the infected mothers, the use of shared watering place, the food and water, and hunting the infected birds by the prey birds [10]. The prevalence in family Columbidae as the main host is from 5.6% to 95% . The prevalence depends on the lifestyle, season or even the type of bird, for example, it is more common among the cage birds than wild birds [13], or it is more spread in the spring

and summer [14,15]. In the birds of prey, the prevalence of parasite is reported 19.6% [16]. The commercial drugs used in the treatment and prevention of this group of parasites are nitroimidazole compounds such as metronidazole, dimetridazole etc. [17]. First, in 1990, some reports based on the resistance against them were reported, which might be due to the excessive consumption of the preventive doses [18], second, since they are carcinogenic, the European Council in 1995 and the United States of America in 1997, declared its contraindication [19], which has become an introduction for the use of alternative compounds. The aim of this study was to examine the effect of 6 medicinal plants (*Quercus Persica*, *Allium sativum*, *Artemisia annua*, *Myrtus commuis*, *Zataria multiflora*, *Rosmarinus officinalis*) extract on *Trichomonas gallinae* in vitro.

## MATERIALS AND METHODS

This study was designed and performed after determining the prevalence of this parasite in the previous study [20].

### *Extraction of plant*

With cooperation of Agricultural Jihad Research Center, 6 plants including 1. *Quercus Persica* 2. *Allium sativum* 3. *Artemisia annua* 4. *Myrtus commuis* 5. *Zataria multiflora* 6. *Rosmarinus officinalis*, were collected and extracted. In order to provide the extract, the maceration method was used. Hence, after crushing the plant leaves, 50g of each sample was soaked and kept in 80% methanol for 48 hours. Then, the extract was condensed with normal filter paper and rotary evaporator device and after passing through the microbial filter with 0.45 micrometer pores, was dried at 40-50 ° C for 2 days [21].

### *Parasite culture*

Fresh stool samples of chickens, roosters and chicks were collected and transmitted to parasitology laboratory in appropriate conditions. First, the samples were studied with direct method and using physiology serum, and the moving and flagellated parasite was detected. Then, the positive samples were mixed with sterilized physiological serum and passed through a layer of moistened gauze. The filtered suspension was centrifuged at 5000 rpm for 4 minutes and the supernatant was discarded. The sediment was mixed, with ratio of 1 to 5, with RPMI<sub>1640</sub> culture medium strengthened with 10% inactive horse serum, starch with no antibiotics, and was incubated for 24 hours at 38 ° C, until the number of parasites reach to  $1 \times 10^6$  ml.

### *Comparing effect of plant extracts and metronidazole*

For each different concentrations of plant extract and metronidazole (1.25, 2.5 and 5 mg/ml), 1.5 ml of RPMI<sub>1640</sub> culture medium, 0.5 ml of parasites cultured ( $5 \times 10^5$  parasites), and the extract and metronidazole were separately added. The drug-free culture medium containing parasite, and the extract were provided as positive control. The samples were incubated at the temperature of 38 ° C. Then, 2, 4, 6, 18, 24, 48 and 72 hours after the incubation, with the use of trypan blue and eosin staining, the percentage of live parasites was calculated compared to the positive control. All the tests were performed in three series.

## RESULTS

In this study *Trichomonas* showed sensitivity to metronidazole, so that with minimum concentration (1.25 mg/ml) and the minimum time (2 hours) caused 100% death of the parasites (Table 1). Plant extracts based on proximity to the effect of metronidazole, were divided into four groups as follows:

First group: The oak fruit extract at the same time and concentration of metronidazole (1.25 mg/ml, 2 hours) was able to eliminate 60% of the parasites, but in the concentration of 2.5 mg/ml, and within 2 hours, all the parasites were eliminated (Table 1).

Second group: The *Rosmarinus* and *Artemisia* extracts at the highest concentration (5 mg/ml), were able to eliminate 100% of parasites, respectively, within 3 and 4 hours. The difference was in the effectiveness time (Table 1).

Third group: The *Myrtus* and *Zataria* extracts at the highest concentration (5 mg/ml) respectively, within 18 and 24 hours, eliminated 100% of parasites (Table 1).

Fourth group: The garlic extract that was not effective at none of the concentrations and times on the parasite, and was exactly against the metronidazole (Table 1).

According to the results, the most effective extract on the trichomonas parasite was the oak fruit extract and then the rosemary extract.

**Table 1: The percentage of live *Trichomonas gallina* after effecting the extracts**

Time Concentration (mg/mL) Control	2 h			4 h			6 h			18 h			24 h			48 h			72 h		
	1.2	2.5	5	1.2	2.5	5	1.2	2.5	5	1.2	2.5	5	1.2	2.5	5	1.2	2.5	5	1.2	2.5	5
Control	100	10	10	100	10	10	100	10	10	100	10	10	100	10	10	100	10	10	100	10	10
Metronidazole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosmarinus officinalis	50	3	0	40	0	0	30	0	0	15	0	0	10	0	0	0	0	0	0	0	0
Quercus Persica	40	0	0	30	0	0	20	0	0	10	0	0	5	0	0	0	0	0	0	0	0
Myrtus communis	100	10	90	100	10	85	100	10	85	90	40	30	60	10	0	50	40	0	10	0	0
Artemisia annua.l	100	10	5	100	85	0	100	82	0	100	80	0	100	75	0	85	70	0	60	40	0
Zataria multiflora	100	10	90	100	10	80	100	90	75	100	70	0	90	70	0	0	0	0	0	0	0
Allium sativum	100	10	10	100	10	10	100	10	10	100	10	10	100	10	10	100	10	10	100	10	10

## DISCUSSION

Among the studied extract of the plants, the oak fruit extract, which is obtained from the local trees and also can be found plentifully, had the greatest impact on the parasite death. So that with concentration of 2.5 mg/ml within 2 hours, eliminated 100% of the parasites in vitro, but metronidazole with higher concentration (5 mg/ml) had the same impact. By comparing the results of oak extract and metronidazole, it seems that the oak extract was appropriate and can be used as an inexpensive and effective local medicine against the trichomoniasis in birds. In particular, no report has been provided on the resistance and the adverse effects caused by its consumption and most people also tend to use plant medicines [22].

Although, it is expected that any combination such as herbals medicine extracts which are digested with the foods, changed along the path which are differentiated with vitro conditions. But the studies have shown that the effect of them and also nitroimidazole compounds such as metronidazole, tinidazole on *Trichomonas gallinae* in vitro are similar to their effect on body [23], hence, the use of oak extract in the treatment of trichomoniasis is also promising.

The important point of this study, which has caused the difference between the effect of extracts to each other, and groups treated with metronidazole and the control group, was the impact of extracts and their effectiveness time, which have been evaluated as the effective factors.

Since the foods and herbal extracts have passed through the digestive system within a time range, the lower are the two factors, dose and effectiveness time, they will be more useful. For example, with effectiveness time of 18 and 24 hours, respectively, such as thyme and myrtle extracts, they might be discarded with no effect but for the rosemary and artemisia extracts, within 2 and 4 hours, they have eliminated 100% of parasites, and considered effective compounds in comparison with other studies with maximum elimination of 80% [23]. However, they are weaker compared to oak extract and metronidazole with longer duration and higher effective minimum dose. It is suggested, the study is also examined in vivo and if it was effective, used for the treatment of trichomoniasis in birds after the complementary studies.

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