

In vitro assessment of anti-*Trichomonas* effects of *Zingiber officinale* and *Lavandula angustifolia* alcoholic extracts on *Trichomonas gallinae*

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Article Info	Abstract
Article history: Received: 25 January 2019 Accepted: 07 May 2019 Available online: 15 March 2021	<i>Trichomonas gallinae</i> is a parasite that acts as a canker-causing agent and leads to significant loss and mortality, especially in young birds. Metronidazole is the approved drug used for the treatment of trichomoniasis. A non-chemical alternatives such as medical plant extracts are also used to treat this disease due to drug resistance. This study aimed to assess <i>in vitro</i> antitrichomonal effects of <i>Lavandula angustifolia</i> and <i>Zingiber officinale</i> extracts on <i>T. gallinae</i> compared with metronidazole. The <i>T. gallinae</i> samples were obtained from infected pigeons. Multi-well plates filled with different concentrations (5.00, 10.00, 25.00, 50.00, and 100 µg mL ⁻¹) were used to perform <i>in vitro</i> analysis. The <i>Z. officinale</i> extract's minimum inhibitory concentration (MIC) in the 24-hr period was 25.00 µg mL ⁻¹ , while it was 50.00 µg mL ⁻¹ for metronidazole. The MIC value obtained for <i>L. angustifolia</i> extract in 24-hr was 50.00 µg mL ⁻¹ . The results indicated that the extracts of <i>Z. officinale</i> and <i>L. angustifolia</i> could act as potential natural agents against trichomoniasis. Furthermore, this study delineated the equal efficiency of <i>L. angustifolia</i> and <i>Z. officinale</i> with that of metronidazole in inhibiting the growth of <i>Trichomonas gallinae</i> trophozoites in culture media.
Keywords: <i>In vitro</i> Ginger Lavender <i>Trichomonas gallinae</i>	

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Introduction

As a flagellated protozoan, *Trichomonas gallinae* - belongs to the order referred to as Trichomonadida, and engenders trichomoniasis in birds. This parasite is found in various birds such as Columbiformes (doves and pigeons), domestic pigeon, *Columba livia* and occupies the upper gastrointestinal tract.¹⁻³ The disease is commonly seen as a caseous ulcer in the anterior region of the digestive tract. The severity of lesions varies, and in some cases, they might be able to kill the birds by obstructing the lumen of the esophagus.⁴

Various drugs are used for treating pigeons suffering from trichomoniasis. Metronidazole (MTZ) is highly suggested for curing the disease.^{5,6} Resistance to MTZ is well documented, and the mechanisms involved in its activities have been accurately defined.⁷ There are some side effects related to MTZ intakes, like transient neutropenia, nausea, cancer, and peripheral neuropathy.^{8,9} The side effects of chemical drugs have led scientists to look for non-chemical and natural equivalents to eradicate parasites.^{6,10-12}

The plant extracts taken from the genus *Lavandula* have been utilized for therapeutic purposes for several centuries.¹³ Lavender is a plant found in many countries and belongs to Labiatae (Lamiaceae) family.¹⁴ *Lavandula angustifolia* is known for its anti-fungal, antimicrobial, and anti-protozoan qualities.^{13,15-20} Moon *et al.* studied the anti-protozoan qualities of *L. angustifolia in vitro* and found that it acts against *Hexamita inflata*, *Trichomonas vaginalis*, and *Giardia duodenalis*.²¹

Ginger, *Zingiber officinale* Roscoe, Family: Zingiberaceae, as one of the widely used spices, has been investigated by scientists and results led to using it as a therapeutic herb due to its rhizomes.²² The antioxidant constituents of ginger include volatile oil, oleoresin (gingerols and shogaols), and phenolic derivatives (zingerone).²³ As an active component of ginger, gingerol is a pungent compound and can be altered to shogaols, zingerone, and paradol²⁴ to benefit from its antiparasitic,²⁵ hepatoprotective,²⁶ and antilarval,²⁷ antimicrobial²⁸ qualities. Another study conducted by Lin *et al.* investigated the larvicidal effects of hexahydrocurcumin,

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which was isolated from ginger.²⁹ They pointed out that hexahydrocurcumin might be used as a larvicidal agent against *Angiostrongylus cantonensis*. Arbabi *et al.* also showed that ethanol extract of ginger induced programmed death in *T. vaginalis*. Therefore, it is believed that ginger can be an alternative therapeutic plant to be used instead of MTZ, and it lacks the teratogenic effect of MTZ.³⁰

As far as our research is concerned, the anti-trichomonal qualities of *L. angustifolia* and *Z. officinale* have gone unnoticed by researchers. Thus, this study was conducted to analyze *in vitro* antitrichomonal features of *L. angustifolia* and *Z. officinale* in comparison with MTZ against *T. gallinae*.

Materials and Methods

Preparation of plant extract. The plants were purchased from the local market, and the species of plants were identified and confirmed by Natural Resource Center (Herbarium No. 5489 and 6362). Some modifications were applied to the method used by Baqer *et al.*, and the alcoholic extract of plants was prepared.³¹ All the dry plant materials, rhizoms of ginger, and the dried flowering branch of lavender, were put in an electrical blender (Moulinex, Paris, France) and turned into powder. The powdered plant (100 g) was added to 500 mL of ethanol 70.00% in a magnetic stirrer and mixed for two hr. The obtained solution was left intact at room temperature for 24 hr, and was filtered after stirring again. A rotating evaporator separated the solvent. The remaining semisolid constituents were freeze-dried at 4.00 °C for futures applications.

Gas chromatography-mass spectrometry (GC-MS) analysis. The chemical composition of the extract was analyzed using GC-MS (Thermo Scientific™, Paris, France). The carrier gas was helium, and the split ratio was 0.50 mL⁻¹ per min. The GS conditions were as follows: Oven temperature program, initially 40.00 °C rising to 250 °C, 80.00 °C per min, for 3 min, injector and detector temperature of 250 °C. The identification of individual compounds was based on comparing their relative retention times with those of authentic samples on a capillary column and matching their mass spectra of peaks with those obtained from authentic samples and published data.³²

Parasites. Twenty-five pigeons were obtained from a local breeder in Urmia (West Azerbaijan, Iran). The pigeons were almost up to six weeks old. The wet mount method was used to recover *T. gallinae*.³³ Samples of membranous lesions in the oropharyngeal area of the birds were obtained by microbiology swabs, which were moistened with warm saline solution. Wet smears were prepared by rubbing the swabs on the glass slide. The slides were put under the light microscope (100× and

400× magnification), and Samour and Naldo method was applied to confirm *T. gallinae*.³³ The flagellated and pear-shaped structure of trophozoites was depicted under the microscope. According to Seddiek *et al.*, the average dimension of *T. gallinae* was $11.12 \pm 0.03 \times 8.14 \pm 0.01$ μm. *T. gallinae*'s trophozoites were isolated from the gastrointestinal tract through the mouth, and liver.⁶ Oral swabs were put in tryptone/yeast extract/maltose (TYM) medium (Oxoid Ltd., Basingstoke, Hampshire, England) to prepare the parasite cultures.³⁵ Fetal calf serum (10%; Sigma Chemical Co., St Louis, USA) was added to the culture, and it was incubated at 37.00 °C. Five days elapsed, and cultures were analyzed to monitor *T. gallinae*'s growth. In more than 95.00% mobility, the isolates were sub-cultured every 48 hr in TYM medium.⁶ Antibiotics, penicillin, and streptomycin per 120 IU were added in the initial phases to the subcultures; however, antibiotics use was halted after attaining the axenic culture.³³

***In vitro* assay.** After applying a few modifications to the method described by Tabari *et al.*, we conducted the *in vitro* analysis.³⁵ Metronidazole (Alborz-Daru, Tehran, Iran) was chosen as the standard anti-trichomonal agent. The reaction of *T. gallinae* to MTZ and extracts of *Z. officinale* and *L. angustifolia* was analyzed by incubating the trophozoites with *Z. officinale* and *L. angustifolia* extracts and MTZ in multi-well plates. Each well received 100 μL of culture medium. The culture contained 1.00×10^4 parasites and pre-diluted MTZ, and extracts of *Z. officinale* and *L. angustifolia* were added to the wells to obtain the highest concentrations of 5.00, 10.00, 25.00, 50.00, and 100 μg mL⁻¹. The control was the plates with no treatment. To provide an anaerobic situation, wells were sealed by rubbing Vaseline (50.00 μL) on top of the wells. After that, the plates were incubated for 72 hr at the temperature of 37.00 °C.³³ Hemocytometer was used to count the trophozoites of the culture. To distinguish between living and dead trophozoites, an equal amount of trypan blue (Sigma Chemical Co.) 0.40% was supplemented to the samples.³³ The results were gained by a minimum of three replications of each experiment. The minimum inhibitory concentration (MIC) was defined as the minimum concentration of a drug at which the parasites were no longer motile.⁶ The following equation was used to obtain the growth inhibition percentage:

$$\text{Growth inhibition (\%)} = (A-B) / A \times 100$$

where, *A* is the average number of the control group's trophozoites, and *B* is the average number of trophozoites in the test group.⁶

Statistical Analysis. Statistical analysis was performed by SPSS (version 19.0; IBM Corp., Armonk, USA). The analysis of variance (ANOVA) investigated the difference between control and test groups. *p* values less than 0.05 were considered significant.

Results

Gas chromatography-mass spectrometry analysis.

Major chemical compounds of GC-MS analysis *Z. officinale* and *L. angustifolia* alcoholic extracts are presented in Table 1.

In vitro results. Table 2 summarizes the efficiency results of various concentrations of *Z. officinale* and *L. angustifolia* extracts as antitrichomonal agents. It could be observed that the extracts of *Z. officinale* and *L. angustifolia* had a higher rate of anti-trichomonal action on *T. gallinae*. The minimum inhibitory concentration (MIC) of *Z. officinale* extract equaled 25.00 $\mu\text{g mL}^{-1}$ in 24 hr, while it was 50.00 $\mu\text{g mL}^{-1}$ for MTZ. These values were 25.00 and 10.00 $\mu\text{g mL}^{-1}$ for *Z. officinale* in 48 and 72 hr, respectively. It is interesting to note that MIC for 48 and 72 hr was obtained as 25.00 and 10.00 $\mu\text{g mL}^{-1}$ for MTZ. The 24 hr MIC value for *L. angustifolia* extract was 50.00 $\mu\text{g mL}^{-1}$. The study showed that *L. angustifolia* with the MIC of 50.00 $\mu\text{g mL}^{-1}$ was able to eradicate all living *T. gallinae* in 48 hr, while the period for the MIC of 10.00 $\mu\text{g mL}^{-1}$ was 72 hr (Table 2). The control samples did not exhibit any reduction in the number of cells.

Figure 1 depicts the results of growth inhibition percentage (GI, %) for the groups treated with the extracts of *Z. officinale* and *L. angustifolia*, and MTZ in 24, 48, and 72 hr period. Comparing the results with that of the control group, we noticed that there was a significant difference in the growth inhibition percentage of the groups treated with the extracts of *Z. officinale* and *L. angustifolia* and MTZ with the control group. Therefore, the study showed that *Z. officinale* and *L. angustifolia* extracts had higher antitrichomonal acidity *in vitro*.

Table 2. Major chemical compounds in ethanol extracts of *Lavandula angustifolia* and *Zingiber officinale* identified by GC-MS.

Ethanol extracts	Major compounds	Percent
<i>L. angustifolia</i>	Ocimene	7.82
	Camphor	4.55
	Linalyl acetate	5.60
	Borneol	22.70
	1,8-cineol	11.50
	α -pinene	14.30
<i>Z. officinale</i>	linalool	26.20
	Gingerol	19.67
	α -Curumene	27.51
	Methyl 2,5-octadecadiyanoate	4.50
	Cis-6-shagole	5.67
	α -gigeberene	22.56
	cyclohexane	11.45
	α -fernesene	8.76
Cineole	2.08	

Discussion

There is a recent surge of interest in using natural extracts with antiparasitic qualities in treating various diseases. Some herbal extract plays a vital role as replacements of chemical drugs. Various studies prove that some herbal extracts are effective in eradicating protozoa, such as *Plasmodium falciparum*,³⁶ *Trichomonas vaginalis*,³⁷⁻³⁹ *Trypanosoma brucei*,⁴⁰ *Cryptosporidium* sp.,⁴¹ *Entamoeba histolytica*,^{37,38} and *Giardia* sp.^{37,38} This study investigated the ant-trichomonas action of *Z. officinale* and *L. angustifolia* extract, *in vitro*. As far as our knowledge is concerned, this was the first study on the antitrichomonal effects of *Z. officinale* and *L. angustifolia* on *T. gallinae*.

This study showed the efficiency of the *Z. officinale* and *L. angustifolia* extract against *T. gallinae*. Our study's results were in agreement with that of Arbabi *et al.*, in which he delineated that ginger led to apoptosis in *T.*

Table 1. Effect of different metronidazole concentrations, *Zingiber officinale*, and *Lavandula angustifolia* extracts on the *in vitro* growth of *Trichomonas gallinae* trophozoites (10^4). Data are presented as mean \pm SD.

Treatments	Concentration ($\mu\text{g mL}^{-1}$)	Trophozoites number		
		24 hr	48 hr	72 hr
Control	-	6.53 \pm 0.21 ^a	8.20 \pm .025 ^a	7.09 \pm 0.12 ^a
	5.00	4.75 \pm 0.32 ^b	1.87 \pm 0.07 ^b	0.98 \pm 0.05 ^b
	10.00	1.87 \pm 0.02 ^d	0.78 \pm 0.03 ^d	0.00 ^d
Metronidazole	25.00	0.59 \pm 0.09 ^c	0.00 ^d	0.00 ^d
	50.00	0.00 ^c	0.00 ^d	0.00 ^d
	100.00	0.00 ^c	0.00 ^d	0.00 ^d
	5.00	4.12 \pm 0.40 ^b	1.24 \pm 0.12 ^b	0.74 \pm 0.04 ^b
	10.00	1.54 \pm 0.12 ^d	0.49 \pm 0.02 ^d	0.00 ^d
<i>Lavandula angustifolia</i>	25.00	0.15 \pm 0.12 ^c	0.04 \pm 0.01 ^d	0.00 ^d
	50.00	0.00 ^c	0.00 ^d	0.00 ^d
	100.00	0.00 ^c	0.00 ^d	0.00 ^d
	5.00	5.40 \pm 0.31 ^b	1.72 \pm 0.11 ^b	0.49 \pm 0.09 ^b
	10.00	1.22 \pm 0.15 ^c	0.44 \pm 0.35 ^d	0.00 ^d
<i>Zingiber officinale</i>	25.00	0.00 ^c	0.00 ^d	0.00 ^d
	50.00	0.00 ^c	0.00 ^d	0.00 ^d
	100.00	0.00 ^c	0.00 ^d	0.00 ^d

Different superscript letters in a column indicate significant differences ($p < 0.05$).

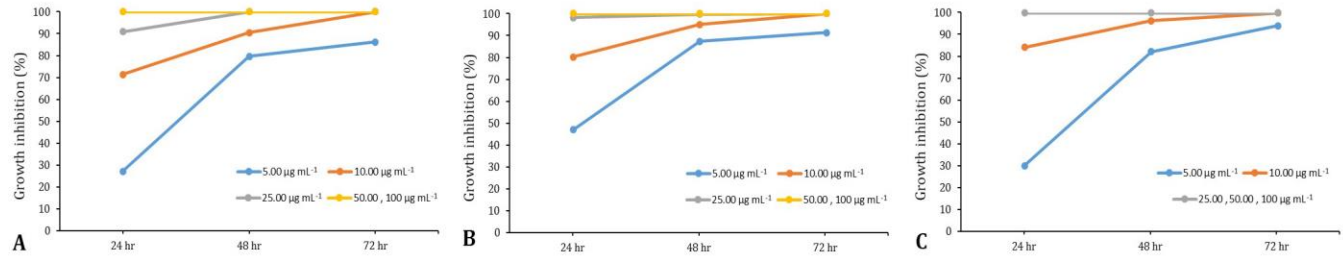


Fig. 1. Comparison of growth inhibition (%) of *Trichomonas gallinae* trophozoites in the presence of different concentrations of **A)** metronidazole, **B)** *Lavandula angustifolia* extract and **C)** *Zingiber officinale* extract.

vaginalis, and this process largely depended on dose and time.³⁰ Another study conducted by Ezatpur *et al.* showed the antitrichomonas action of *L. angustifolia*. Their study depicted that essential oils with a concentration of 0.10 and 0.01 had a significant effect on *T. vaginalis*.⁴² Tabri *et al.* showed the anti-trichomonal action of *Peganum harmala* on *T. gallinae*. 24 hr MIC of *P. harmala* extract was measured as 15.00 µg mL⁻¹, while this amount was 50.00 µg mL⁻¹ for MTZ. Their study showed that the alkaloid extract of *P. harmala* was significantly useful in treating *T. gallinae* isolates, which were resistant to MTZ.³⁵

Our study was proof of the anti-trichomonas action of ginger extract *in vitro*. In this study, we identified gingerol (19.67%) in the ginger extract. The ginger extract was found to contain small amounts of other compounds. This study confirmed the results obtained by an earlier study on the anti-parasitic qualities of gingerol, hexahydrocurcumin, and shogaol.^{22,29,43} Our *in vitro* study showed that, in a period of 24 hr, there were no trichomonads after adding the ginger extract (25.00 µg mL⁻¹) to the culture medium. Other studies also showed that ginger extract acts efficiently against *Trichomonas vaginalis*,³⁰ *Giardia* spp.,⁴⁴ *Leishmania* spp.,⁴⁵ and *Cryptosporidium* spp.⁴⁶ *in vitro*. Ginger is also a biocidal plant that acts as an anthelmintic^{29,42} and antimicrobial agent.⁴⁷ As Arbabi *et al.* suggested, the adverse effect of *Z. officinale* against *T. gallinae* was related to its potential in inducing apoptosis. According to earlier studies, apoptosis induction of *Z. officinale* was a major superior compared to other widespread drugs like MTZ. The other advantage of *Z. officinale* is that it does not engender a severe immune response.³⁰ Khademvatan *et al.* and Arbabi *et al.* determined the apoptosis quality of at least nine species of a unicellular organism like *Leishmania* and *T. vaginalis*.^{30,48}

There have been several studies aimed at determining the pharmacological qualities of *linalool* and *L. angustifolia*. The results of these studies suggested that *L. angustifolia* extract and alkaloids were active in curbing grain weevils, clothes moths, mites, and aphids.^{13,15-20} Reports indicated *L. angustifolia* extract therapeutic effects on *Giardia duodenalis*, *Trichomonas vaginalis*, and *Hexamita inflata* infections. *L. angustifolia* oil is potentially powerful, and its low concentration is sufficient to eradicate *T. vaginalis*, *G. duodenalis*, and *H. inflata* in culture. It should also be

noted that *L. angustifolia* acts via lysis of the cells in order to eliminate the parasites, mentioned above.²¹

Metronidazole is a widely used drug for the treatment of trichomoniasis, but some isolates of *T. gallinae* are resistant to drugs, which can be hazardous to free-living birds.³⁴ These isolates are reported in various countries like Spain, Belgium, USA, and Iran.^{3,4,33,49} Our study showed that *Z. officinale* and *L. angustifolia* extracts were the best alternatives to be used for treating the isolates. A 24 hr MIC (15.60 µg mL⁻¹) was set as the cutoff to assess MTZ resistance in *T. gallinae* strains.³ Our study measured the 24 hr MIC of MTZ as 50.00 µg mL⁻¹, which significantly differed from that Rouffauer *et al.*³ Therefore, we could conclude that the obtained strains of *T. gallinae* in this study had a high level of resistance to MTZ. Our results agreed with that of Tabari *et al.*, which testified the existence of MTZ-resistant strains of *T. gallinae* in Iran.³⁵

It could be concluded that the extracts of *Z. officinale* and *L. angustifolia* had the potential to be used as antitrichomonas agents. Our results proved that *in vitro* activity of these extracts signified their activity in birds, as well. Further studies are required to analyze the possible adverse effects of *Z. officinale* and *L. angustifolia* and to prove their antitrichomonal qualities *in vivo*.

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Conflict of interest

The authors disclose no potential conflict of interest.

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