In vitro anti-*Trichomonas vaginalis* activity of *Pistacia lentiscus* mastic and *Ocimum basilicum* essential oil

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Abstract *Trichomonas vaginalis* is a protozoan parasite that causes trichomoniasis; a cosmopolitan sexually transmitted disease. Metronidazole is the drug of choice for *T. vaginalis* infections. The increase in metronidazole resistant parasites and undesirable side effects of this drug makes the search for an alternative a priority for the management of trichomoniasis. *Pistacia lentiscus* mastic and *Ocimum basilicum* oil are known for their antibacterial, antifungal, antiviral and antiprotozoal effects. The present study was carried out to investigate the in vitro effects of *P. lentiscus* mastic and *O. basilicum* oil on *T. vaginalis* trophozoites. The effects of different concentrations of *P. lentiscus* mastic (15, 10 and 5 mg/ml) and different concentrations of *O. basilicum* oil (30, 20 and 10 µg/ml) on multiplication of trophozoites at different time points (after 24, 48, 72 and 96 h) were determined, also morphological changes were reported by transmission electron microscopy (TEM). The results showed that both plants caused an inhibition of growth of *T. vaginalis* trophozoites. The minimal lethal concentration of *P. lentiscus* mastic was 15 mg/ml after 24 h incubation, 10 mg/ml after 48 h and 5 mg/ml after 96 h. The minimal lethal concentration of *O. basilicum* oil was 30 µg/ml after 24 h incubation, 20 µg/ml after 48 h and 10 µg/ml after 96 h. TEM study of trophozoites treated by *P. lentiscus* mastic or by *O. basilicum* oil showed considerable damage of the membrane system of the trophozoites, and extensive vacuolization of the cytoplasm. These results highly suggest that *P. lentiscus* mastic and *O. basilicum* oil may be promising phytotherapeutic agents for trichomoniasis treatment.

Keywords *Trichomonas vaginalis* · Trichomoniasis · *Pistacia lentiscus* mastic · *Ocimum basilicum* oil

Introduction

Trichomonas vaginalis (*T. vaginalis*) is the causative agent of trichomoniasis; the most common nonviral sexually transmitted disease, with 174 million new infections each year (WHO 2011). Infection with this protozoan may have serious consequences, such as adverse pregnancy outcomes (Cotch et al. 1997), predisposition to cervical cancer (Viikki et al. 2000) and pelvic inflammatory disease (Cherpes et al. 2006). Trichomoniasis is also a cofactor in human immunodeficiency virus transmission and acquisition (Van Der Pol et al. 2008). Moreover, Sutcliffe et al. (2006) reported a relationship between trichomoniasis and prostate cancer.

Although metronidazole is the drug of choice recommended by food and drug administration (FDA, USA) for the treatment of human trichomoniasis, carcinogenic and teratogenic effects as well as clinical or laboratory generated drug resistant isolates of *T. vaginalis* have been reported (Upcroft et al. 2006). According to some reports, metronidazole resistance is a major problem in clinical isolates of *T. vaginalis* in the USA and worldwide which has limited the therapeutic effectiveness of metronidazole (Rasolson et al. 2002). In addition, some side effects such as headache, dry mouth, metallic taste, glossitis and urticaria have been described (Upcroft et al. 2006).

In this context, there is an urgent need for new trichomonocidal agents with high effectiveness and low toxicity. The study of plants used by traditional medicine is a mean for finding alternative treatment, and several anti parasitic properties of many new natural product groups have been identified with their surprising efficacy (Kayser et al. 2003).

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Pistacia lentiscus mastic is a natural resin derived from the stem and leaves of the mastic tree, *P. lentiscus* Linn, native to Mediterranean areas (Takahashi et al. 2003). Traditional healers used it for the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Koo et al. 2000). Substantial evidence has also suggested that *P. lentiscus* mastic gum exhibits hepatoprotective, cardioprotective, anti-inflammatory and antioxidant properties (Giaginis and Theocharis 2011). Numerous studies showed that *P. lentiscus* mastic has impressive antibacterial and antimicrobial properties (Takahashi et al. 2003).

Balan et al. (2007) suggested that *P. lentiscus* mastic gum might be developed into a chemotherapeutic agent for the treatment of human cancer colon and other cancers. Several studies further evaluated the potential antiproliferative properties of mastic gum against several types of neoplasia (Giaginis and Theocharis 2011). *Pistacia khinjuk* mastic, another species of the genus *Pistacia*, showed an anti-helminthic effect against protoscoleces of *Echinococcus granulosus* (Taran et al. 2009). Markovics et al. (2012) strongly suggested that feeding goats to *P. lentiscus* foliage alleviates coccidiosis. Furthermore, Taran et al. (2010) reported that topical rubbing of *Pistacia atlantica* mastic decreased skin lesion size in the BALB/c mice infected with *Leishmania major* compared with that in the control, and it was concluded that *P. atlantica* mastic can be used for controlling cutaneous leishmaniasis caused by *L. major* and inhibiting development of cutaneous leishmaniasis lesions.

Ocimum basilicum Linn, a member of the Lamiaceae family, is an annual herb which grows in several regions around the world (Issazadeh et al. 2012). It is well known as a plant of a folk medicinal value. It is active against several species of bacteria and act as a natural powerful antifungal ingredient (Koba et al. 2009). *O. basilicum* oil contains rosmarinic acid, one of the most abundant caffeic acid esters; it has been reported to have antioxidant, anti-HIV and anti-inflammatory effects (Keita et al. 2000). Almeida et al. (2007) investigated the effects of *O. basilicum* essential oil on *Giardia lamblia* trophozoites, and they reported high anti-giardial potential. In this context, the present in vitro study aimed to evaluate the anti-*T. vaginalis* activity of *P. lentiscus* mastic and *O. basilicum* oil. As far as we know, this is the first report demonstrating the potential anti-*T. vaginalis* activity of these natural products.

Materials and methods

In vitro culture

Trichomonas vaginalis trophozoites were isolated from female patients attending the outpatient clinic, Gynecology and Obstetrics Hospital, Ain Shams University. The

trophozoites were cultured axenically in vitro at 37 °C in trypticase–yeast extract–maltose (TYM) medium (Diamond 1957), pH 6.0, supplemented with 1 ml heat-inactivated horse serum, crystalline penicillin (1,000,000 IU/ml), and streptomycin sulfate (100,000 µg/ml). Isolates were subcultured every 48 h in TYM medium and maintained in Parasitology Diagnostic and Research Unit, Faculty of Medicine, Ain Shams University.

Plant materials

Pistacia lentiscus mastic was obtained from Chios Gum Mastic Growers Association (Chios, Greece) and *O. basilicum* oil was obtained from Sigma-Aldrich (Germany), and each was dissolved in 5 % acetone (Duarte et al. 2009). *T. vaginalis* trophozoites were incubated in 5 ml TYM medium containing *P. lentiscus* mastic in different concentrations (5, 10, 15 mg/ml) for 24, 48, 72 and 96 h at 37 °C. Also, *T. vaginalis* trophozoites were incubated in 5 ml TYM medium containing *O. basilicum* oil in different concentrations (10, 20, 30 µg/ml) for 24, 48, 72 and 96 h at 37 °C.

Drug

The anti-*T. vaginalis* activity of the tested plant materials was compared with the drug metronidazole which was supplied as 250-mg tablets (Flagyl, Sanofi-Aventis, Egypt). Metronidazole tablets were dissolved in distilled water. *T. vaginalis* trophozoites were incubated in 5 ml TYM medium containing metronidazole in different concentrations (20 and 40 µg/ml) for 24, 48, 72 and 96 h at 37 °C.

Parasite control containing *T. vaginalis* trophozoites incubated in 5 ml TYM medium and solvent control containing *T. vaginalis* trophozoites incubated in 5 ml TYM medium with added solvent, were included in the study.

Each experiment was performed in duplicates

Evaluation of the *P. lentiscus* mastic and *O. basilicum* oil anti-*T. vaginalis* efficacy was done by:

1. Detecting the inhibition of growth of *T. vaginalis* trophozoites by the tested plants materials. This was done by:
  - (a) Counting the number of trophozoites from culture tubes at time points of study using the improved Neubauer cell counting chamber and testing the viability of trophozoites using 0.3 % Trypan blue stain (Ali 2007).
  - (b) Calculation of motility percent of the trophozoites as: the ratio of motile number to total number

of parasites counted per 10 high power field (Ali 2007).

- (c) Calculation of percent of growth inhibition according to the equation: Percent of growth inhibition = $a - b/a \times 100$ where a = mean number of trophozoites in parasite control tubes and b = mean number of trophozoites in tested tubes (Palmas et al. 1984).
2. Detecting the minimal lethal concentration of each of *P. lentiscus* mastic and *O. basilicum* oil according to Meri et al. (2000).
3. Transmission electron microscopy (TEM). The ultra-structure changes of *T. vaginalis* trophozoites were studied using TEM, after 24 h incubation period using *P. lentiscus* mastic (10 mg/ml) and *O. basilicum* oil (20 µg/ml). The trophozoites were fixed with 2.5 % (v/v) cold glutaraldehyde and processed for examination with JEOL–JEM-1010 transmission electron microscope (Cedillo-Rivera et al. 2002).

Statistical analysis

Data were analyzed using SPSS version 19 for Windows (SPSS Inc., Chicago, IL, USA). Data were presented as the mean $\pm$ SD of duplicates, percent of growth inhibition, percent of viability and percent of motility. The mean numbers were compared at the same time interval using student's *t* test, the difference was considered significant when the *p* value was <0.05 .

Ethical consideration

An informed consent was taken from all the patients after explaining the aim of the study to them. The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University.

Results

The results of the present study are shown in Tables 1, 2, 3 and 4 and Figs. 1 and 2.

Growth inhibition and minimal lethal concentration

As regards *P. lentiscus* mastic treated culture, it showed 100 % inhibition of the parasitic growth with concentration of 15 mg/ml after 24 h incubation. 90 % inhibition of the parasitic growth was observed with concentration of 10 mg/ml after 24 h and complete inhibition of growth (100 %) after 48 h. 44 % inhibition of the parasitic growth was observed with concentration of 5 mg/ml after 24 h,

85.6 % after 48 h and 96.4 % after 72 h till complete inhibition of growth (100 %) after 96 h (Table 1).

Each concentration showed statistical significant difference ($p < 0.05$) when compared to parasite control and no statistical significant difference ($p > 0.05$) when compared to metronidazole 20 µg/ml except for the concentration of 15 mg/ml after 24 h which showed significant difference ($p < 0.05$) when compared to metronidazole 20 µg/ml. Minimal lethal concentration of *P. lentiscus* mastic was 15 mg/ml after 24 h, 10 mg/ml after 48 h and 5 mg/ml after 96 h (Table 1).

Concerning the viability and motility of *P. lentiscus* mastic treated culture, the higher the concentration and the longer the time of exposure the less the viability and motility percentage. Concentration of 15 mg/ml showed 0 % viable and motile trophozoites after 24 h incubation. Concentration of 10 mg/ml showed 10 % viable and 8.7 % motile trophozoites after 24 h decreased to 0 % for viability and motility after 48 h. Concentration of 5 mg/ml showed 56 % viable and 41 % motile trophozoites after 24 h, 14.4 % viable and 10.8 % motile trophozoites after 48 h, 5.4 % viable and 0.9 % motile trophozoites after 72 h, and decreased to 0 % for viability and motility after 96 h (Table 2).

As regards *O. basilicum* oil treated culture, it showed 100 % inhibition of the parasitic growth with concentration of 30 µg/ml after 24 h incubation. Decrease of the trophozoites growth by 73.1 % was observed with concentration of 20 µg/ml after 24 h and complete inhibition (100 %) of growth after 48 h. Decrease of the trophozoites growth by 23.8 % was observed with concentrations of 10 µg/ml after 24 h, 78.4 % after 48 h and 95.6 % after 72 h till complete inhibition (100 %) of growth after 96 h (Table 3).

Each concentration showed statistical significant difference ($p < 0.05$) when compared to parasite control and no statistical significant difference ($p > 0.05$) when compared to metronidazole 20 µg/ml except for the concentration of 30 µg/ml after 24 h and 10 µg/ml after 72 h which showed significant difference ($p < 0.05$) when compared to metronidazole 20 µg/ml at the same time interval (Table 3).

Concerning the viability and motility of *O. basilicum* oil treated culture, the higher the concentration and the longer the time of exposure the less the viability and motility percentage, concentration of 30 µg/ml showed 0 % viable and motile trophozoites after 24 h incubation. 20 µg/ml showed 26.9 % viable and 19 % motile trophozoites after 24 h, and decreased to 0 % for viability and motility after 48 h. 10 µg/ml showed 76.2 % viable and 70 % motile trophozoites after 24 h, 21.6 % viable and 15 % motile trophozoites after 48 h, 4.4 % viable and 0.9 % motile trophozoites after 72 h, and decreased to 0 % for viability and motility after 96 h (Table 4).

Table 1 Effect of *P. lentiscus mastic* on the in vitro growth *T. vaginalis* trophozoites (10⁶) at different incubation periods

Dosage of treatment	After 24 h		After 48 h		After 72 h		After 96 h	
	(Trophozoites number) Mean ± SD	% of growth inhibition	(Trophozoites number) Mean ± SD	% of growth inhibition	(Trophozoites number) Mean ± SD	% of growth inhibition	(Trophozoites number) Mean ± SD	% of growth inhibition
Parasite control	(2.10–2.36) 2.23 ± 0.13	0	(3.96–4.36) 4.16 ± 0.20	0	(11.28–11.52) 11.4 ± 0.12	0	(4.94–5.16) 5.05 ± 0.11	0
Metronidazole, 20 µg	(0.78–0.82) 0.8 ± 0.02*	64.1	(0.07–0.73) 0.4 ± 0.33*	90.4	(0.03–0.17) 0.1 ± 0.07*	99.1	0.00*	100
Metronidazole, 40 µg	0.00*	100	0.00*	100	0.00*	100	0.00*	100
Solvent control	(2.05–2.35) 2.2 ± 0.15	1.3	(4.00–4.22) 4.11 ± 0.11	1.2	(11.2–11.4) 11.3 ± 0.1	0.9	(4.91–5.09) 5.0 ± 0.09	1
<i>Pistacia lentiscus</i> mastic, 5 mg/ml	(1.18–1.32) 1.25 ± 0.07*•	44	(0.58–0.62) 0.6 ± 0.02*•	85.6	(0.25–0.55) 0.4 ± 0.15*•	96.4	0.00*	100
<i>Pistacia lentiscus</i> mastic, 10 mg/ml	(0.1–0.35) 0.225 ± 0.125*•	90	0.00*•	100	0.00*•	100	0.00*	100
<i>Pistacia lentiscus</i> mastic, 15 mg/ml	0.00***	100	0.00*•	100	0.00*•	100	0.00*	100

SD standard deviation

* $p < 0.05$, statistically significant difference in comparison to parasite control in the same time interval; ** $p < 0.05$, statistically significant difference in comparison to metronidazole 20 µg in the same time interval; • no statistically significant difference ($p > 0.05$) in comparison to metronidazole 20 µg

Table 2 Percentage of viability and motility of *T. vaginalis* trophozoites in culture after exposure to *P. lentiscus* mastic at different incubation periods

Dosage of treatment	After 24 h (%)		After 48 h (%)		After 72 h (%)		After 96 h (%)	
	Viability (%)	Motility (%)	Viability (%)	Motility (%)	Viability (%)	Motility (%)	Viability (%)	Motility (%)
Parasite control	100	98	100	76.2	100	21	100	9.6
Metronidazole, 20 µg	35.9	33.1	9.6	7.1	0.9	0.2	0	0
Metronidazole, 40 µg	0	0	0	0	0	0	0	0
Solvent control	98.7	97	98.8	76	99.1	20	99	9.5
<i>Pistacia lentiscus</i> mastic, 5 mg/ml	56	41	14.4	10.8	5.4	0.9	0	0
<i>Pistacia lentiscus</i> mastic, 10 mg/ml	10	8.7	0	0	0	0	0	0
<i>Pistacia lentiscus</i> mastic, 15 mg/ml	0	0	0	0	0	0	0	0

Transmission electron microscopy study (TEM)

As regards *P. lentiscus* mastic treated culture, TEM showed that the membrane system of *T. vaginalis* trophozoites was considerably damaged (Fig. 1b, c), with abnormal vacuolization of the cytoplasm (Figure 1a–c), and extensive cytoplasmic destruction (Fig. 1b, c).

As regards *O. basilicum* oil treated culture, TEM showed that the membrane system of the trophozoites was considerably damaged (Fig. 2b, c), with abnormal vacuolization of the cytoplasm (Fig. 2a–c), and extensive cytoplasmic destruction (Fig. 2b, c).

Discussion

The present in vitro study was carried out to evaluate the effects of *P. lentiscus* mastic and *O. basilicum* oil on *T. vaginalis*. Taking into account that some clinical cases of trichomoniasis are caused by *T. vaginalis* isolates resistant to metronidazole and its side effects specially with higher doses, the treatment constitutes a major challenge, and the options are limited (Cudmore et al. 2004). In the present study, different concentrations of *P. lentiscus* mastic and *O. basilicum* oil were tested, on the axenic culture of *T. vaginalis* in comparison to parasite control and metronidazole

Table 3 Effect of *O. basilicum* oil on the in vitro growth *T. vaginalis* trophozoites (10^6) at different incubation periods

Dosage of treatment	After 24 h		After 48 h		After 72 h		After 96 h	
	(Trophozoites number) Mean $\pm$ SD	% of growth inhibition	(Trophozoites number) Mean $\pm$ SD	% of growth inhibition	(Trophozoites number) Mean $\pm$ SD	% of growth inhibition	(Trophozoites number) Mean $\pm$ SD	% of growth inhibition
Parasite control	(2.10–2.36) 2.23 $\pm$ 0.13	0	(3.96–4.36) 4.16 $\pm$ 0.20	0	(11.28–11.52) 11.4 $\pm$ 0.12	0	(4.94–5.16) 5.05 $\pm$ 0.11	0
Metronidazole, 20 μ g	(0.78–0.82) 0.8 $\pm$ 0.02*	64.1	(0.07–0.73) 0.4 $\pm$ 0.33*	90.4	(0.03–0.17) 0.1 $\pm$ 0.07*	99.1	0.00*	100
Metronidazole, 40 μ g	0.00*	100	0.00*	100	0.00*	100	0.00*	100
Solvent control	(2.05–2.35) 2.2 $\pm$ 0.15	1.3	(4.00–4.22) 4.11 $\pm$ 0.11	1.2	(11.2–11.4) 11.3 $\pm$ 0.1	0.9	(4.91–5.09) 5.0 $\pm$ 0.09	1
<i>Ocimum basilicum</i> oil, 10 μ g/ml	(1.6–1.8) 1.7 $\pm$ 0.1**•	23.8	(0.88–0.92) 0.9 $\pm$ 0.02**•	78.4	(0.45–0.55) 0.5 $\pm$ 0.05***	95.6	0.00*	100
<i>Ocimum basilicum</i> oil, 20 μ g/ml	(0.5–0.7) 0.6 $\pm$ 0.1**•	73.1	0.00**•	100	0.00**•	100	0.00*	100
<i>Ocimum basilicum</i> oil, 30 μ g/ml	0.00***	100	0.00**•	100	0.00**•	100	0.00*	100

SD standard deviation

* $p < 0.05$, statistically significant difference in comparison to parasite control in the same time interval; ** $p < 0.05$, statistically significant difference in comparison to metronidazole 20 μ g in the same time interval; • no statistically significant difference ($p > 0.05$) in comparison to metronidazole 20 μ g

Table 4 Percentage of viability and motility of *T. vaginalis* trophozoites in culture after exposure to *O. basilicum* oil at different incubation periods

Dosage of treatment	After 24 h (%)		After 48 h (%)		After 72 h (%)		After 96 h (%)	
	Viability (%)	Motility (%)	Viability (%)	Motility (%)	Viability (%)	Motility (%)	Viability (%)	Motility (%)
Parasite control	100	98	100	76.2	100	21	100	9.6
Metronidazole, 20 μ g	35.9	33.1	9.6	7.1	0.9	0.2	0	0
Metronidazole, 40 μ g	0	0	0	0	0	0	0	0
Solvent control	98.7	97	98.8	76	99.1	20	99	9.5
<i>Ocimum basilicum</i> oil, 10 μ g/ml	76.2	70	21.6	15	4.4	0.9	0	0
<i>Ocimum basilicum</i> oil, 20 μ g/ml	26.9	19	0	0	0	0	0	0
<i>Ocimum basilicum</i> oil, 30 μ g/ml	0	0	0	0	0	0	0	0

control. *P. lentiscus* mastic and *O. basilicum* oil had lethal effects on growth of the trophozoites, with ultrastructure destructive changes.

In this study the effect of *P. lentiscus* mastic and *Ocimum basilicum* oil on the inhibition of trophozoites growth showed statistically significant difference when compared to parasite control, inhibition of trophozoites growth and trophozoites motility were in relation to concentration and incubation time.

The minimal inhibitory concentration at 24 h interval of *P. lentiscus* mastic was 15 mg/ml, and of *O. basilicum* oil was 30 μ g/ml, showing potency comparable to that of metronidazole 40 μ g/ml. *P. lentiscus* 15 mg/ml and *O. basilicum* oil 30 μ g/ml showed statistically significant

difference when compared to metronidazole 20 μ g/ml after 24 h incubation. These findings were in accordance with the results of Ali (2007) who reported similar inhibitory effect when using Propolis, and also those of Rocha et al. (2012) when using saponins from *Quillaja*, *Passiflora* and *Ilex* species on the axenic culture of *T. vaginalis*. The similarity between the effects of natural products in this study and other studies on *T. vaginalis* trophozoites can be attributed to the possibility that different plants may share the same active ingredients.

With lower concentrations of *P. lentiscus* mastic and *O. basilicum* oil longer incubation period was required to obtain complete loss of viability and motility. The study recorded that a 90 % inhibition of growth with

Fig. 1 TEM of *T. vaginalis* trophozoites from *P. lentiscus* mastic (10 mg/ml) treated culture at 24 h incubation period showing abnormal vacuolization (V) (a–c), defects in the cell membrane (CM) with extensive cytoplasmic destruction (Cyto) (b, c). N nucleus, Fl flagella, ER endoplasmic reticulum

concentration of 10 mg/ml *P. lentiscus* mastic after 24 h incubation period increased to 100 % inhibition after 48 h which represents the minimal inhibitory concentration at that point of time, while concentration of 5 mg/ml needed up to 96 h to completely inhibit the trophozoites growth. Also, a 73.1 % inhibition of growth with concentration of 20 µg/ml of *O. bacilicum* oil after 24 h incubation period reached 100 % inhibition after 48 h, while concentration of 10 µg/ml needed up to 96 h to completely inhibit the trophozoites growth. These results were comparable to that of metronidazole 20 µg/ml whose inhibitory effect progressed over time to reach complete inhibition after 96 h, however being natural products, they have the advantage over metronidazole of being more safe.

The anti *T. vaginalis* effect of *P. lentiscus* mastic reported in this study can be attributed to the ability of *P. lentiscus* mastic to induce apoptosis (Li et al. 2011), and to a variety of phytochemicals constituents which are medically important such as α -pinene, β -pinene, β -myrcene, limonene, *trans*-caryophyllene, camphene, and to phenolic

compounds which revealed considerable antimicrobial activity and particular antifungal activity (Ansari and Siddiqui 2012). These activities are probably in relationship with the structure of the phenolic compound gallic acid, which is a component of *P. lentiscus* mastic. Gallic acid has been tested for its ability to inhibit the proliferation of *Trypanosoma cruzi* trypomastigotes in vitro (Koide et al. 1998). Also, it was suggested that the anti *Trichomonas tenax* and anti-*T. vaginalis* effect of pomegranate peel extract can be attributed to its gallic acid content (El-Sherbini and Shoukry 2012). It would be of interest to determine which of the constituents of *P. lentiscus* mastic, alone or in synergy with another constituent, have the ability to produce the anti *T. vaginalis* activities reported in this study.

The ability of the crude extracts of *O. bacilicum* oil to inhibit the growth of bacteria and fungi is an indication of its broad-spectrum antimicrobial potential, which may be employed for the management of microbial infections. Primary phytochemical screening of *O. bacilicum* oil,

Fig. 2 TEM of *T. vaginalis* trophozoites from *O. basilicum* oil (20 $\mu\text{g/ml}$) treated culture at 24 h incubation period showing abnormal vacuolization (V) (a–c), membranous defects (CM) with extensive cytoplasmic destruction (Cyto) (b, c). Fl flagella

revealed the presence of saponin, steroids, tannins, glycosides, alkaloids and flavonoids (Issazadeh et al. 2012). In general, isolated plant's substances such as flavonoids, alkaloids, coumarins, saponins and glycosides have demonstrated activity against different flagellates such as *G. lamblia* (Barbosa et al. 2007), *Trichomonas gallinae* (Adebajo et al. 2006), *T. vaginalis* (Arthan et al. 2008), and *Leishmania infantum* (González-Coloma et al. 2011). Anti-*T. vaginalis* activity of other plant's saponins was reported (Rocha et al. 2012). Thus, the anti *T. vaginalis* activity reported in this study can be attributed to the saponin content of *O. basilicum* oil as well as the biologically active flavonoids.

Almeida et al. (2007) reported high anti-giardial potential of *O. basilicum* essential oil by inhibition of proteolytic activity of cysteine proteinases of *G. lamblia* trophozoites. Cysteine proteinases are present in a wide range of parasitic protozoa, including *T. vaginalis* and appear to be

important in several aspects of the life cycle and of the parasite-host relationships (Gradoni and Ascenzi 2004). So we can suggest that the inhibitory effect of *O. basilicum* oil on the growth of *T. vaginalis* trophozoites is, also possibly, through its ability to inhibit cysteine proteinase's activity of *T. vaginalis*.

TEM of each of *P. lentiscus* mastic and *O. basilicum* treated culture showed considerable damage of the membrane system of the trophozoites, abnormal vacuolization and extensive destruction of the cytoplasm, after incubation period of 24 h with the concentration of 10 and 20 $\mu\text{g/ml}$, respectively. Those findings are in accordance with results of Oxberry et al. (1994) who reported loss of the cytoplasmic material, vacuolization and disruption of cytoplasmic membrane after usage of metronidazole. Once this occurs other degenerative changes associated with cell death and decline growth are also likely to occur ending up by complete loss and disintegration of trophozoites.

In conclusion, the results from this study support our perspective for the possibility of using *P. lentiscus* mastic and *O. basilicum* oil as anti-*T. vaginalis* agents.

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