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Original papers

In-vitro antigiardial activity and GC-MS analysis of *Eucalyptus globulus* and *Zingiber officinalis* essential oils against *Giardia lamblia* cysts in simulated condition to human's body

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ABSTRACT. In order to finding of potent natural medication/agent which can kill giardial cysts in the interval between lysing of outer membrane of cysts due to exposure by acidic condition of stomach and their encystation in proximal small intestine, *In-vitro* antigiardial activity and GC-MS analysis of *Eucalyptus globulus* and *Zingiber officinalis* against *Giardia lamblia* were studied in simulated condition to human's body singly and in combination. Essential oils were extracted and their chemical components were identified via GC-MS method. After purification, cysts were exposure to acidic condition and were challenged by different concentrations of essential oils in simulated condition to human's body. Percentages of inactivated (killed) cysts as efficacy of antigiardial activity were calculated and analyzed statistically. Presence of 1,8-eucalyptol, α-pinene, α-terpineol acetate, etc. in essential oil of *E. globulus* and presence of geraniol, α-zingiberene, (E,E)-α-farnesene, etc. in *Z. officinalis* essential oil were identified. Highest antigiardial activity (73.55%) was observed for eucalyptus essential oil in time 480 minutes after exposure. Efficacies of ginger and combined essential oils were different in different times. This study shows considerable antigiardial activity for both of essential oils singly and in combination together against giardial cysts. In-vivo study of protective effect of these essential oils against giardiasis can be considered as a subject for next studies.

Key words: *Giardia lamblia*, antigiardial activity, eucalyptus, ginger, GC-MS analysis

Introduction

Giardia lamblia also known as *Giardia intestinalis* or *Giardia duodenalis*; common cause of water-borne [1], and food-borne diarrhea known as giardiosis [2], is a worldwide flagellated unicellular eukaryotic parasite [3]. Sometimes, it is a self-limiting infection without symptoms but severe gastrointestinal symptoms such as diarrhea, nausea, bloating, or mal absorption can also occur. *G. lamblia* is considered to be the most common parasite of the human gastrointestinal tract [4]. *Giardia* species have two major stages in the life cycle; infection of a host is initiated when the cyst is ingested with contaminated water or, less commonly, food or through direct fecal-oral contact. The cyst is relatively inert, allowing prolonged survival in a variety of environmental conditions. After exposure to the acidic environment of the stomach (approximately 1–2 hours), the outer membranes of cysts were lysed in stomach and then cysts encyst into trophozoites in the proximal small

intestine (about 6–10 hours after gulping). The trophozoite is the vegetative form and replicates in the small intestine, where it causes symptoms of diarrhea and mal-absorption. After exposure to biliary fluid, some of the trophozoites form cysts in the jejunum and are passed in the feces, allowing completion of the transmission cycle by infecting a new host [5].

As no prophylaxis against *Giardia* infections is available, countermeasures are limited to chemotherapy of established infections. Large selection of drugs is available for the treatment of giardiosis, the most commonly prescribed drugs are 5-nitroimidazoles metronidazole and tinidazole and the benzimidazole albendazole. Other drugs used are quinacrine, nitazoxanide, furazolidone, mebendazole, paromomycin, and bacitracin zinc [4]. Sometimes recurrent recalcitrant giardiosis occurs due to drug resistance [6]. Resistant *Giardia* strains have been repeatedly isolated from patients with refractory giardiosis from many years ago [7]. In addition, resistance to metronidazole and other 5-nitroimidazoles is relatively easy to induce in the laboratory by exposing *Giardia* to incremental doses of drug or to UV light [8]. Different resistance values of *G. lamblia* to Metronidazole and other 5-nitroimidazoles, Nitazoxanide, Albendazole, Furazolidone, and Quinacrine were reviewed by Leitsch [4]. Since prevention is always better than treatment, searching for new natural protective food additive/medications against resistant strains of *G. lamblia* is always necessary.

Plants have been used for their medicinal activities from ancient time [9]. They are known as great source of food additive and medications and are used to prevention or treatment of several infections, diseases and ailments. The use of plant food additives and medicines is widely accepted in all over the world [10]. Genus *Eucalyptus* knows by over 700 species distributed throughout the world [11]. This genus provides variety of components extracted from its essential oil known as acaricide and repellent agent [12]. Lethal and repellent activity of eucalyptus components were reviewed by Batish et al. [13]. *Eucalyptus* essential oil is a mixture of monoterpenes and sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones; however, the exact composition and proportion of which varies with species [11]. Essential oil of *Eucalyptus* has been placed under Generally Regarded As Safe (GRAS) category by Food and Drug Administration (FDA) of USA and classified as nontoxic [14].

Also ginger (rhizome of *Zingiber officinalis* Roscoe) is one of the most widely used herbal food additive and medications in oriental medicine for prevention and treatment of different pains, inflammations, stomach problems, nausea, vomiting, epilepsy, sore throat, cough, common cold, bruises, wounds, liver complaints, rheumatism, muscular pains, atherosclerosis, migraine headaches, high cholesterol, ulcers, and etc. [15]. Ginger essential oil can produce from fresh rhizomes and it has many efficient effects like antibacterial, antiviral, antifungal and other properties [16,17]. The essential oil of ginger rhizome is pale yellow to light-amber, contains both aromatic and pungent compounds and can be extracted with yields ranging from 1.5–3.0%, depending on the quality of the crop [18]. In the recent review, different chemical components and efficient effects of ginger were reviewed by Sharifi-Rad et al. [19].

To the best of authors knowledge and current date there is not any study on protective and/or lethal activity of *E. globulus* and *Z. officinalis* against *G. lamblia*. In order to find a natural and safe replacement for antigiardial medications and/or protective agents, in this novel research for the first time antigiardial effects of mentioned essential oils in an in-vitro condition like inside the human's stomach (acidity, temperature, humidity, etc.) were studied to evaluate their probable therapeutic potencies (protective/lethal effect) against *G. lamblia*. The main hypothesis of current study is finding of potent medication/agent which can kill giardial cysts in the interval between lysing of outer membrane of cysts after exposure to acidic condition of stomach and their encystation in proximal small intestine. Since there is a considerable interval between exposures of giardial cysts with acidic condition in stomach and their encystation in proximal small intestine and also with this fact in mind that trophozoites are more sensitive to medications than cysts, it is probable that if an agent has considerable effect against cyst after exposure to acidic condition of stomach and stop its life cycle, it may also kill trophozoite form in small intestine too.

Materials and Methods

Extraction of essential oils. Fresh leafs of *E. globulus* and rhizomes of *Z. officinalis* were used to essential oil extraction. Essential oils extractions were done separately for each sample via conventional

hydrodistillation method. 150 gram of each sample were crushed and added to 1000 ml distilled water in a round bottom flask. The flask was heated and the Clevenger apparatus was attached. The mixture was boiled at 100°C and then the temperature was reduced to 60°C and kept for 3 h, the recovered mixture was allowed to settle and finally essential oil was withdrawn for each sample separately [20].

GC-MS analysis and identification. GC-MS analysis and identification of essential oils were done according to the method described by Song et al. [21] for *E. globulus* and Singh et al. [22] for *Z. officinalis*. A Hewlett-Packard gas chromatograph (Model 6890) coupled with a quadruple mass spectrometer (Model HP 5973) and a Perkin Elmer Elite-5MS capillary column (5% phenylmethylsiloxane; 30 m, 0.25 mm I.D., 0.25 mm film thickness) were used in this study. Gas chromatograph and column were attached to a Mass spectrometer operated in negative chemical ionization mode. The initial oven temperature was 60°C, this was held for 4 min then rose at the rate of 4°C/min to 220°C, and then held for 15 min. Other operating conditions were as follows: carrier gas, Helium (99.999%), with a flow rate of 1 ml/min; injector temperature, 280°C; split ratio, 10:1. Mass spectra were recorded at 70 eV and the mass range was from *m/z* 33 to 500 amu. The components were identified on the basis of comparison of their retention indices and mass spectra with published data [22–25] and computer matching was done with the Wiley 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC–MS systems [21,22].

Isolation of giardial cysts. Cysts were purified from positive feces samples of patients via floating method. Isolated cysts were identified by identification keys; they are usually 11–14 µm in length and 7–10 µm in width and they are either round or oval in shape and contain 4 nuclei, axonemes and median bodies. A halo effect outside the cyst wall may be observed because of the shrinkage which is caused by dehydrating agents, especially in the permanent staining [26,27]. Ten gram of positive stools was broken up in 100 ml tap water and was filtered through a 300 urn filter. 3 ml of feces suspension were added to 3 ml of 0.85 M sucrose and centrifuged at 2000–3000 rpm for 10 minutes. The cysts at the sucrose-water interface were aspirated with a Pasteur pipette and washed 3 times with water. Washed cysts were carefully added to the top of a discontinuous density gradient, consisting of 3 ml lavers of 0.85 M sucrose again. After centrifugation at 2000–3000 rpm for 10 min, cysts concentrated at the 0.85 M sucrose interface were collected and washed again [28]. In order to reaching of maximum number of cysts, 3 ml normal saline were added to washed cysts and then were centrifuged at 2000–3000 rpm for 5 min, so cysts precipitated in the bottom of tubes, upper liquids were removed and purified cysts were achieved.

Exposure of giardial cysts to acidic condition and their preparation. Since the normal PH and temperature of human's stomach are approximately 1–2.5 and 37–37.5°C, respectively [29], after isolation, in order to preparation of an *in-vitro* condition like the human's stomach, purified/concentrated cysts were added to the mixture of normal saline and hydrochloric acid. Initial PH of mentioned medium was about 1 and its final PH (after addition of purified cysts) was about 1.75. Cysts were incubated in this medium about 1.5 hours in 37°C, in a dark condition away from sunlight and other environmental factors (just like what is happening in normal condition of human's stomach). After 1.5 hours incubation, in order to deletion of acidic condition, cysts were washed and centrifuged 5 times by normal saline (10 ml, 2000–3000 rpm for 10 min) and for the last time purified cysts were concentrated as mentioned before. This suspension as a stock of cysts was stored at 4°C for a maximum of 3 days prior to use. In order to evaluation of purified cysts health after exposure to acidic condition, the concentrated cysts in 10 µl of concentrated cysts stock were observed via optic microscope (40× and 100×), healthy cysts had regular shape, clear color and continuous and fit membrane. 100% of examined cysts were health in this observation. Also in order to find estimated number of cysts per each ml of suspension, stock was shacked carefully then number of cysts in 10 µl of suspension was counted for three times, mean number was calculated and finally number of cysts per each ml of suspension were determined via multiplication, it was approximately about 5×10^4 cyst/ml suspension.

Examination groups. Different concentrations; 125 µl/ml, 250 µl/ml, 500 µl/ml, 750 µl/ml and 1 (pure) were prepared for essential oil of *E. globulus* and *Z. officinalis* separately via combination by different amount of normal saline, also mixed concentrations of 625 nl/ml *E. globulus* plus 625 nl/ml *Z. officinalis*, 125 µl/ml *E. globulus* plus 125 µl/ml *Z. officinalis*, 250 µl/ml *E. globulus* plus 250

Table 1. Identification of present components in essential oils of *Eucalyptus globulus* and *Zingiber officinalis*. Note that amounts below 1% are presented as trace.

Eucalyptus globulus	
Components	Amount (%)
1,8-eucalyptol	69.27
α-pinene	11.13
α-terpineol acetate	5.42
α-terpineol	3.99
alloaromadendrene	2.07
β-pinene	Trace
Pinocarveol	Trace
Camphene	Trace
β-sabinene	Trace
Limonene	Trace
γ-eudesmol	Trace
Ledol	Trace
Isoledene	Trace
Linalool	Trace
Alloocimene	Trace
Andaromadendrene	Trace
Terpinolene	Trace
Pinocarveol	Trace
Geranyl acetate	Trace
β-panasinsene	Trace
α-guaiene	Trace
Cubenol	Trace
Epiglobulol	Trace
β-gurjunene	Trace
Isopulegol acetate	Trace
etc.	Trace

Zingiber officinalis	
Components	Amount (%)
Geraniol	30.24
α-zingiberene	7.83
(E,E)-α-farnesene	6.55
Neral	6.37
ar-curcumene	4.68
Gingerols	4.11
Borneol	2.94
α-pinene	2.57
1,8-Cineole	2.46
Shogaols	2.22
Diarylheptanoids	1.89
Guaiol	1.34
Acorenone B	1.23
Criptone	1.18
Camphor	1.07
α-copaene	Trace
Geranyl acetate	Trace
Terpinen-4-ol	Trace
2-Nonanone	Trace
Limonene	Trace
Sabinene	Trace
Terpinolene	Trace
Elemol	Trace
Germacrene-B	Trace
Geranyl acetate	Trace
etc.	Trace

µl/ml *Z. officinalis*, 375 µl/ml *E. globulus* plus 375 µl/ml *Z. officinalis* and 500 µl/ml *E. globulus* plus 500 µl/ml *Z. officinalis* were prepared with the same method. In order to sure of tests validity, positive control groups with the concentration of 50 mg/ml were prepared via dilution of 1 tablet of Metromax® (Metronidazole Tablet, Oral 250 mg, Tehranchemie Company, Tehran, Iran) in 5 ml normal saline, in the normal condition metronidazole has small and weak effects against giardial cysts but after exposure to acidic condition of stomach it has more effect against cysts [30], also negative control groups were prepared from pure normal saline without any additive components.

Challenge of cysts with groups. In order to deletion of bias in results, purified cysts were studied at the date of preparation. 1 ml of each group were transferred to separate tubes, and then 200 µl of cyst's stock (approximately 5000 cysts) were added to each examination groups and also control groups. Triplicate tests were done for every group. Then tubes were incubated in 37°C and at the times of 30, 60, 120, 240 and 480 minutes after exposure.

Determination of efficacy. At the end of each incubation period, tubes were shaken carefully and then 100 µl of each tube was transferred to new tube, then 50–100 µl eosin stain 5–10% were added, after about 3–5 min, a little amount (about 50 µl) was smeared to glass slide and then was observed carefully via optic microscope (40× and 100×). For each slide 300 cysts were counted and number of dead cyst recorded, then percentage of dead cysts as an efficacy value was calculated for each replication of every examination group. Dead cysts were identified by their different shape than active cysts. Finally, mean number of efficacy for each concentration (group) was calculated.

Statistical analysis. The analyzed data were expressed as the Mean ± standard error of the mean (SEM) using Sigma stat (version 3.1) software. Groups were compared using one-way ANOVA for repeated measurements. A value of ($P \leq 0.05$) was considered significant.

Table 2. Efficacy or percentage of killed/inactive cysts for different concentrations of eucalyptus essential oil (Mean±SEM%) in different times

Concentrations	30 min	60 min	120 min	240 min	480 min
125 µl/ml	$3.42\pm0.71^{A,a*}$	$7.76\pm0.55^{A,a}$	$12.21\pm0.88^{A,b}$	$15.75\pm2.43^{A,b}$	$31.49\pm2.51^{A,c}$
250 µl/ml	$6.07\pm0.65^{A,a}$	$8.32\pm0.34^{A,a}$	$16.52\pm1.25^{A,b}$	$20.08\pm1.36^{A,b}$	$47.61\pm3.73^{B,c}$
500 µl/ml	$10.03\pm0.27^{B,a}$	$13.28\pm1.14^{B,a}$	$22.73\pm2.07^{A,b}$	$30.57\pm2.52^{B,c}$	$51.64\pm4.17^{B,d}$
750 µl/ml	$11.96\pm1.02^{B,a}$	$15.02\pm1.31^{B,b}$	$27.89\pm1.56^{B,c}$	$31.76\pm0.85^{B,c}$	$58.16\pm2.33^{B,d}$
1 (pure)	$38.43\pm1.67^{C,a}$	$47.27\pm0.39^{C,a}$	$59.09\pm2.23^{C,b}$	$65.98\pm4.27^{C,b}$	$73.55\pm3.91^{C,c}$
Negative Control	$0^{D,a}$	$0^{D,a}$	$0^{D,a}$	$0^{D,a}$	$0^{D,a}$
Positive Control	$93.21\pm0.22^{E,a}$	$100^{E,b}$	$100^{E,b}$	$100^{E,b}$	$100^{E,b}$

*Presence of different superscript uppercase letters ($^{A-E}$) show the significant differences ($P\leq0.05$) between different concentrations in each time (column) and presence of different superscript lowercase letters ($^{a-d}$) show the significant differences ($P\leq0.05$) between different times for each concentration (row).

Results

Phytochemistry

Results of current study show the presence of 1,8-eucalyptol, α-pinene, α-terpineol acetate, α-terpineol, alloaromadendrene, β-pinene, pinocarveol, etc. in essential oil of *E. globulus* and presence of geraniol, α-zingiberene, (E,E)-α-farnesene, neral, ar-curcumene, etc. in *Z. officinalis* essential oil. Table 1 shows the different identified components for each essential oil in details.

Efficacy of *Eucalyptus* essential oil

Results of current study show different amounts of efficacy (lethal effect) for each concentration of *Eucalyptus* essential oil versus time. In time 30 minutes; great acts for every concentration were seen, at this time efficacy of metronidazole 50 mg/ml (positive control) was not perfect and its full performance was seen in time 60 minutes. Concentration 1 (pure) of essential oil had the greatest activity in every period. The most increasing of efficacy in treatments were occurred during the incubation period between 240 minutes and 480 minutes. The best performance was observed for concentration 1 (pure) of essential oil in time 480 minutes. In time 480 minutes, whole the cysts were survived and died in negative control and

Table 3. Efficacy or percentage of killed/inactive cysts for different concentrations of ginger essential oil (Mean±SEM%) in different times

Concentrations	30 min	60 min	120 min	240 min	480 min
125 µl/ml	$1.23\pm0.05^{A,a*}$	$5.51\pm1.14^{A,b}$	$9.77\pm1.53^{A,c}$	$14.18\pm0.67^{A,d}$	$29.56\pm3.77^{A,e}$
250 µl/ml	$4.55\pm0.76^{A,a}$	$7.63\pm0.85^{A,a}$	$13.26\pm2.37^{A,b}$	$22.34\pm2.89^{B,c}$	$33.75\pm2.11^{A,d}$
500 µl/ml	$12.56\pm3.64^{B,a}$	$16.73\pm2.23^{B,a}$	$20.59\pm3.52^{B,b}$	$28.72\pm3.37^{B,c}$	$40.43\pm3.92^{B,d}$
750 µl/ml	$16.29\pm2.91^{B,a}$	$17.78\pm2.65^{B,a}$	$25.99\pm3.07^{B,b}$	$34.01\pm1.76^{C,c}$	$46.37\pm3.25^{B,d}$
1 (pure)	$27.96\pm3.52^{C,a}$	$33.88\pm3.13^{C,b}$	$46.15\pm1.39^{C,c}$	$53.19\pm2.94^{D,d}$	$61.15\pm4.73^{C,d}$
Negative Control	$0^{D,a}$	$0^{D,a}$	$0^{D,a}$	$0^{E,a}$	$0^{D,a}$
Positive Control	$88.99\pm4.36^{E,a}$	$100^{E,b}$	$100^{E,b}$	$100^{F,b}$	$100^{E,b}$

*Presence of different superscript uppercase letters ($^{A-F}$) show the significant differences ($P\leq0.05$) between different concentrations in each time (column) and presence of different superscript lowercase letters ($^{a-e}$) show the significant differences ($P\leq0.05$) between different times for each concentration (row).

positive control respectively. Detailed results for efficacy of eucalyptus essential oil are shown in Table 2.

Efficacy of ginger essential oil

Efficacy of different concentrations of ginger essential oil in time 30 minutes was less than other treatments. The best performance in time 30 minutes was seen for concentration 1 (pure), at this time the performance of metronidazole 50 mg/ml (positive control) was not perfect again and its full performance was seen in time 60 minutes. An increasing in efficacy for every concentration was occurred gradually and the highest activity for all groups was seen in time 480 minutes. The highest activity of concentrations was seen for concentration 1 (pure) of essential oil and in time 480 minutes. Comparison of results for ginger essential oil is shown in Table 3.

Efficacy of combined essential oils

Efficacy of combined essential oils in time 30 minutes was strange. Its performance in concentration 625+625 nl/ml essential oils was medium and between the performance of individual essential oils and its performance for other concentrations was greater than individual essential oils, also the best performance was observed for concentration 500+500 µl/ml at this time. Performance of metronidazole 50 mg/ml (positive control) in first incubation period was not perfect again and its full performance was seen in time 60 minutes. The most increasing were seen between the intervals of times 60–120 min and 240–480 min, respectively. The highest activity was seen for concentration 500+500 µl/ml at time 480 minutes. Results for combined essential oils are shown in Table 4 in detail.

Compartment of groups versus time

In time 30 minutes, the highest activity was observed for concentration 1 (pure) eucalyptus essential oil and the performance of positive controls in this time were not perfect. In time 60 minutes, the full performance of positive control groups were seen. Comparison between results shows that efficacy of eucalyptus essential oil was more than others in whole concentrations and times. But efficacy of ginger and combined essential oils was different versus different times. Figure 1 shows the graphical comparison between treatments for each time.

Discussion

Natural products, such as essential oils which are produced by the secondary metabolism of herbs have uses in human consumption as functional food, food additives, medicines, nutritional supplements and the manufacture of cosmetics due to their properties [31]. In this study antigiardial activity of *E. globulus* and *Z. officinalis* against *G. lamblia* in the simulated *in vitro* condition to human's stomach were studied singly and in combination together.

Several results were reported about antigiardial activity of different essential oils: Calzada et al. [32]

Table 4. Efficacy or percentage of killed/inactive cysts for different concentrations of eucalyptus plus ginger essential oils (Mean±SEM%) in different times

Concentrations	30 min	60 min	120 min	240 min	480 min
625+625 nl/ml	$2.71\pm0.28^{A,a*}$	$8.23\pm2.83^{A,b}$	$15.51\pm2.17^{A,c}$	$18.36\pm1.52^{A,c}$	$27.62\pm1.26^{A,d}$
125+125 µl/ml	$10.36\pm1.21^{B,a}$	$13.51\pm2.05^{B,a}$	$18.88\pm1.62^{A,b}$	$24.55\pm3.73^{B,c}$	$36.32\pm1.51^{B,d}$
250+250 µl/ml	$15.32\pm1.18^{C,a}$	$19.68\pm1.37^{C,a}$	$27.16\pm2.08^{B,b}$	$31.14\pm1.66^{C,b}$	$43.09\pm2.87^{C,c}$
375+375 µl/ml	$26.58\pm3.23^{D,a}$	$32.63\pm3.57^{D,a}$	$42.38\pm1.28^{C,b}$	$44.36\pm3.81^{D,b}$	$50.72\pm4.08^{D,c}$
500+500 µl/ml	$34.04\pm4.36^{E,a}$	$38.53\pm2.96^{E,a}$	$51.73\pm3.61^{D,b}$	$57.28\pm3.53^{E,c}$	$59.67\pm2.45^{E,c}$
Negative Control	$0^{F,a}$	$0^{F,a}$	$0^{E,a}$	$0^{F,a}$	$0^{F,a}$
Positive Control	$96.35\pm1.08^{G,a}$	$100^{G,b}$	$100^{F,b}$	$100^{G,b}$	$100^{G,b}$

*Presence of different superscript uppercase letters ($^{A-G}$) show the significant differences ($P\leq0.05$) between different concentrations in each time (column) and presence of different superscript lowercase letters ($^{a-d}$) show the significant differences ($P\square0.05$) between different times for each concentration (row).

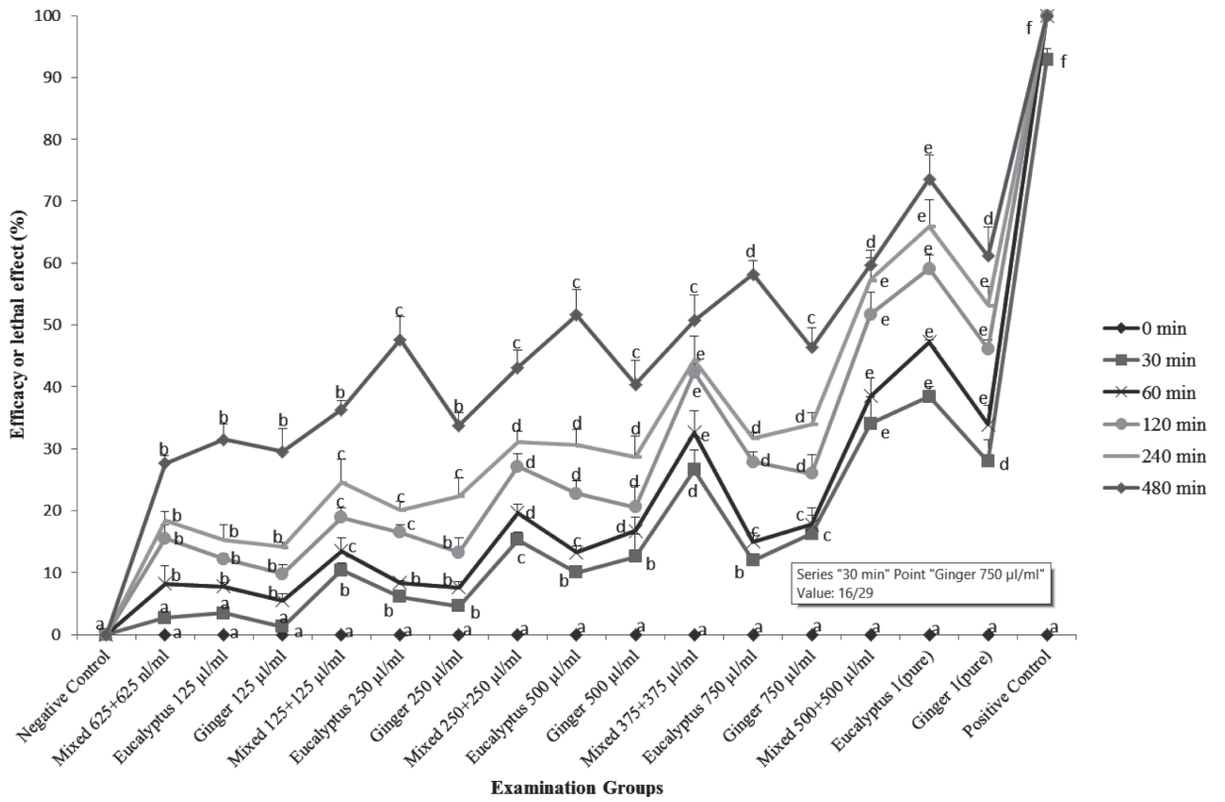

Fig. 1. Comparison of gained results for different treatments in different times. Presence of different lowercase letters (a-f) in each line (time) shows the significant differences (P≤0.05) between different treatments in each time (line).

reported that some plants are use in traditional Mexican medicine in order to giardiosis treatment. They reported that *Dorstenia contrajerva*, *Senna villosa* and *Ruta chalepensis* are the most active toward *G. lamblia*. In other study researcher reported that hydroalcoholic leaf extracts of *Anacardium occidentale* L., *Chenopodium ambrosioides* L., *Passiflora edulis* Sims, *Psidium guajava* L. and *Stachytarpheta cayennensis* have great antigiardial activity [33]. Results of current study are agree with them and show the considerable values of protective/antigiardial activity for essential oils of *E. globulus* and *Z. officinalis* against *G. lamblia* singly and in combination together in the in-vitro condition like human's stomach.

Also results of current study show the presence of 1,8-eucalyptol, α-pinene, α-terpineol acetate, α-terpineol, alloaromadendrene, β-pinene, pinocarveol, etc. in essential oil of *E. globulus* and geraniol, α-zingiberene, (E,E)-α-farnesene, neral, ar-curcumene, etc. in *Z. officinalis* essential oil. These results are agreed with results of Liu et al. [34] which show the presence of 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, andaromadendrene in *E. globulus* essential oil and results of Sivasothy et al. [35] that show the presence of gingerols, shogaols, diarylheptanoids, phenylbutenoids, flavanoids, diterpenoids and sesquiterpenoids in *Z. officinalis* essential oil. Presence of mentioned chemical agents explains that why these essential oils have great antigiardial activities, even against cyst form.

Amaral et al. [36] described that 153 plant species from 69 families have giardicidal activity. Antigiardial activity of phenolic-rich essential oils gained from some aromatic plants have been reported by Machado et al. [37], in their study; the tested essential oils inhibited the growth of *G. lamblia* and *Thymbra capitata* essential oil was the most active. The tested essential oils inhibited parasite adherence since the first hour of incubation and were able to kill almost 50% of the parasites population. Also, antigiardial activity of *Citrullus lanatus* fruit has been reported in other study; they reported that all crude extracts and isolated compounds were active against *G. lamblia* [38]. In current study, all the treatments had antigiardial

activity but the highest activity was observed for eucalyptus essential oil in concentration 1 (pure) and at the time of 480 minutes.

Antigiardial activity of 27 crude methanolic extracts derived from 26 plants used in Mexican traditional medicine for treatment of diarrhea and dysentery were reported by Brandelli et al [39]. In other study antigiardial activity of garlic (*Allium sativum*) studied and it was found to be efficient antigiardial agent [40]. Also antiprotozoal activities of 42 essential oils were reviewed in other study and reported that those from the Lamiaceae family were the most powerful agents [41]. Antigiardial activity of essential oil of *Ocimum basilicum* was studied in other experiment, it was reported that essential oil of *Ocimum basilicum* and its purified compounds, especially linalool, have a potent antigiardial and antimicrobial activity [42]. Results of current study are agree with mentioned studies and show that essential oils of *E. globulus* and *Z. officinalis* have potent activities against *G. lamblia* singly and in combination together.

Antigiardial activity of *Syzygium aromaticum* and its major compound eugenol also were studied; this essential oil inhibited trophozoites adherence since the first hour of incubation and was able to kill almost 50% of the parasites population in a time dependent manner [43]. Antiprotozoal activities of many plants were reviewed by Monzote et al. [44].

Result of current study is agree with whole of mentioned studies and shows also a great amount of protective/lethal activity for essential oil of *E. globulus* and *Z. officinalis* against *G. lamblia* cysts after exposure to acidic condition like stomach. Since there is a considerable interval between exposures of giardial cysts with acidic condition in stomach and their encystation in proximal small intestine and also with this fact in mind that trophozoites are more sensitive to medications than cysts, it is clear that potent natural medications like examined essential oils can be effective against different stage of *G. lamblia* life cycle.

In conclusion, this novel research for the first time shows antigiardial effects of essential oils of ginger and eucalyptus against *G. lamblia* cysts after exposure to acidic condition in an In-vitro condition like inside the human's stomach. Since there is a considerable interval between exposures of giardial cysts with acidic condition in stomach and their encystation in proximal small intestine and also with this fact in mind that trophozoites are more sensitive to medications than cysts, it is probable that these essential oils which have considerable effect against giardial cysts after exposure to acidic condition of stomach and stop their life cycle, then they may also kill trophozoite form in small intestine too, but more studies are needed. Presence of 1,8-eucalyptol, α-pinene, α-terpineol acetate, α-terpineol, alloaromadendrene, β-pinene, pinocarveol, etc. in essential oil of *E. globulus* and presence of geraniol, α-zingiberene, (E,E)-α-farnesene, neral, ar-curcumene, etc. in *Z. officinalis* essential oil were identified too. Antigiardial activity of eucalyptus essential oil was more than others in whole concentrations and times and its highest performance (about 74%) was observed in time 480 minutes after exposure. Efficacies of ginger and combined essential oils were different in time but their highest antigiardial activities were also observed in time 480 minutes after exposure too. *In vivo* study of protective effect of these essential oils against giardiosis can be considered as a subject for next studies.

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