ACTIVITY OF PLANT ESSENTIAL OILS AGAINST *GIARDIA DUODENALIS*

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Abstract. *Giardia duodenalis* (synonyms: *Giardia lamblia* and *Giardia intestinalis*) is a common flagellate, zoonotic protozoan causing the diarrheal disease giardiasis. There is little information about the essential oils of plants found in Thailand against this parasite. We aimed to determine the efficacy of essential oils of the following plants against *G. duodenalis*: *Syzygium aromaticum*, *Zingiber officinale* Roscoe, *Alpinia galanga*, *Litsea cubeba*, *Illicium verum*, *Zanthoxylum rhetsa*, *Citrus* × *aurantifolia*, *Citrus hystrix*, *Citrus reticulata*, *Ocimum basilicum* and *Ocimum africanum*, using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay. It was found that essential oils of *C.* × *aurantifolia* was the most effective against *G. duodenalis* with $IC_{50} = 6.96 \pm 0.13$ μg/ml, followed by *L. cubeba* with $IC_{50} = 60.67 \pm 0.82$ μg/ml in dose-dependent fashion. The other essential oils had no efficacy against *G. duodenalis*, suggesting they may contain fewer antigiardial constituents. Future studies are needed to elucidate major active constituents of these essential oils and prove their efficacy and safety for treatment of *G. duodenalis*.

Keywords: *Giardia duodenalis*, essential oils, dose-dependent fashion

INTRODUCTION

Giardia duodenalis (synonyms: *Giardia lamblia* and *Giardia intestinalis*) is the most common flagellate protozoan infecting humans worldwide (Ramírez *et al*, 2015; Soares and Tasca 2016). Most infections occur from fecal-oral transmission through ingestion of contaminated water or food (Soares and Tasca 2016). There is a high prevalence of giardiasis among many children in developing countries (Kotloff *et al*, 2013). Giardiasis can cause greasy stools, flatulence, diarrhea, abdominal cramps, epigastric tenderness and malabsorption (Ankarklev *et al*, 2010; Bartelt and Sartor, 2015). The incidence of giardiasis depends on age, sanitation and personal hygiene (Stuart *et al*, 2003). *G. duodenalis* consists of eight assemblages (A-H). Human giardiasis is caused by assemblages A and B. Assemblages A and B have also been identified in other animals (Ryan and Cacciò, 2013). This suggests other animals may act as reservoirs for *G. duodenalis* and may be linked to zoonotic transmission.

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Metronidazole is the drug of choice to treat giardiasis; metronidazole is also effective against some anaerobic bacteria as well (Ankarklev *et al*, 2010; Martínez-Espinosa *et al*, 2015). Other drugs used to treat giardiasis include tinidazole, albendazole and furazolidone (Watkins and Eckmann, 2014). Common side effects reported for metronidazole include nausea, headaches, dizziness and a metallic taste (Alizadeh *et al*, 2006). Giardiasis treatment failures due to metronidazole have been reported (Solaymani-Mohammadi *et al*, 2010; Galeh *et al*, 2016).

Plant products have been widely used for medicinal purposes in human (Perumal Samy and Gopalakrishnakone, 2010). Natural plant-based products are a potential source for managing treatment failures. Little information is available regarding the use of plant products to treat giardiasis. We aimed to determine the efficacy of the essential oils of 11 different plants native to Thailand against *G. duodenalis*.

MATERIALS AND METHODS

Selected medicinal plants

The plants used in the study were *Syzygium aromaticum* (L.) Merr & L. M. Perry (local name: *kan-plu*), *Zingiber officinale* Roscoe (local name: *khing*), *Alpinia galanga* (L.) Wild. (local name: *kha*), *Litsea cubeba* (Lour.) Pers. (local name: *ta-krai-phu-kao*), *Illicium verum* Hook. f. (local name: *poy-kuck*), *Zanthoxylum rhetsa* (Roxb.) DC. (local name: *ma-khan*), *Citrus* × *aurantifolia* (Christm.) Swingle (local name: *ma-now*), *Citrus hystrix* DC. (local name: *ma-krood*), *Citrus reticulata* Blanco. (local name: *soom*), *Ocimum basilicum* L. (local name: *ho-ra-pha*) and *Ocimum africanum* Lour. (local name: *mang-luk*). Specific parts of each medicinal plant (Table 1) were processed through hydrodistillation. The selected plants were collected from various locations in Thailand. The plant samples were identified by the Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. Voucher specimens (015825-015832 and 015834–015836) were housed at the Herbarium of the Department of Botany, Faculty of Science, Chulalongkorn University.

Table 1
Parts of selected plants used in essential oil extractions.

Plant	Part
Syzygium aromaticum	Flower
Zingiber officinale	Rhizome
Alpinia galanga	Mature fleshy fruit
Litsea cubeba	Mature fleshy fruit
Illicium verum	Mature flower
Zanthoxylum rhetsa	Mature flower
Citrus × *aurantifolia*	Peel
Citrus hystrix	Peel
Citrus reticulata	Peel
Ocimum basilicum	Mature leaf
Ocimum africanum	Mature leaf

Table 2
Concentrations of selected plant essential oils.

Plant	Density (g/ml)
Syzygium aromaticum	1.07
Zingiber officinale	0.95
Alpinia galanga	0.86
Litsea cubeba	0.89
Illicium verum	0.95
Zanthoxylum rhetsa	0.80
Citrus × *aurantifolia*	0.88
Citrus hystrix	0.88
Citrus reticulata	0.80
Ocimum basilicum	0.95
Ocimum africanum	0.95

Essential oil densities were calculated

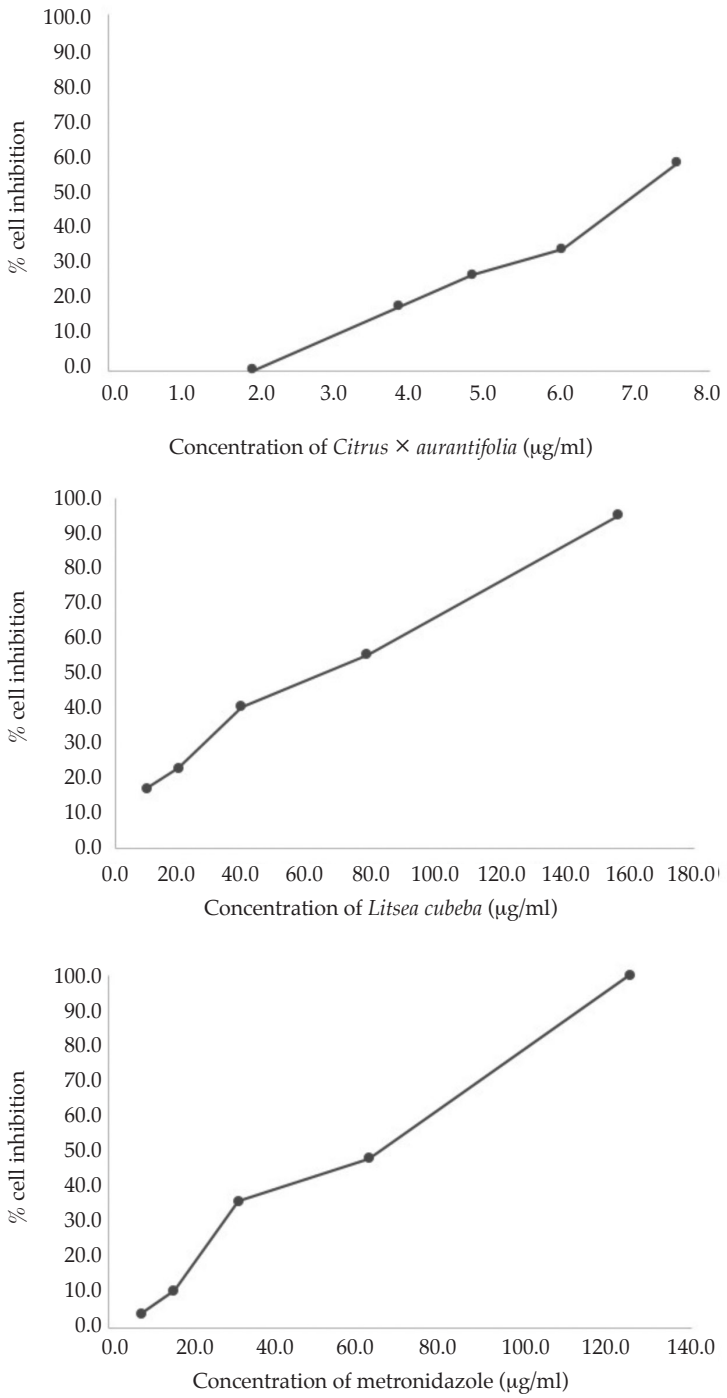

Fig 1–Mean inhibitory effect of *Citrus* × *aurantifolia*, *Litsea cubeba* and metronidazole against *G. duodenalis* trophozoites.

using the following formula: D = M/V, where D represents density (g/ml), M represents mass (g) and V represents volume (ml). Essential oils were dissolved in dimethyl sulfoxide (DMSO) (Table2) prior to the testing process.

G. duodenalis culture

G. duodenalis trophozoites were grown anaerobically in borosilicate glass screw-cap culture tubes at pH 6.85 in modified TYI-S-33 medium (Keister, 1983). The medium was supplemented with 10% heat-inactivated bovine serum, bovine bile and 3% NCTC-135 (Gibco, Paisley, Scotland) (Keister, 1983). The trophozoites were examined during the log phase of growth. Subculturing was performed three times a week. Gentamicin (Sigma-Aldrich, St Louis, MO) was added during routine culturing. Log-phase cultures (2 to 3 days) were harvested by cooling (4°C/7 minutes) and centrifuging (2,300*g*, at 4°C for 7 minutes). The trophozoites were counted in a hemocytometer. The *G. duodenalis* trophozoites were then used for the study.

In vitro antigiardial assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was used in this study (Wright *et al*, 1992; Bénéré *et al*, 2007). Viable *Giardia* trophozoites can reduce MTT to a purple color with an absorbance at 550 nm.

Each essential oil tested and metronidazole (Sigma-Aldrich, St Louis, MO) as a positive control were prepared in 2-fold serial dilutions in 100% DMSO. A non-treated control (100% growth) and a culture medium control (0% growth) were included in each plate as well. Briefly, the various concentrations of essential oils and metronidazole, the non-treated control and the culture medium control were added to the wells of a 96-well microplate. Then, 10^5 trophozoites were added to each well to give a final volume of 100 µl. The concentration of the DMSO was < 0.5% and this did not have an effect on the test. The plates were sealed and incubated at 37°C under anaerobic conditions in 2.5-liter Pack-Rectangular Jars (Mitsubishi Gas Chemical, Tokyo, Japan). After 24 hours incubation, the growth medium was removed gently to avoid affecting the adherent phase of the trophozoites before adding the MTT. MTT at 50 mg/well was added to each well and the plates incubated for another 4 hours. After that, 100 µl of 100%DMSO was added to dissolve the crystals in each well prior to recording the absorbance at 550 nm.

The percentage trophozoite viability at various concentrations of the essential oils and metronidazole was determined using the following formula:

% trophozoite survival = [(sample absorbance - culture medium control absorbance)/ non-treated control absorbance - culture medium control absorbance] × 100.

% inhibition = 100 - % trophozoite that survived

All experiments were performed in triplicate. The inhibitory concentration of 50% (IC_{50}) was defined as the concentration of essential oil or metronidazole required to inhibit cell growth by 50%. The mean IC_{50} ± standard deviations (SD) values for the studied essential oils and metronidazole were determined using SPSS version 18.0 (IBM, Armonk, NY).

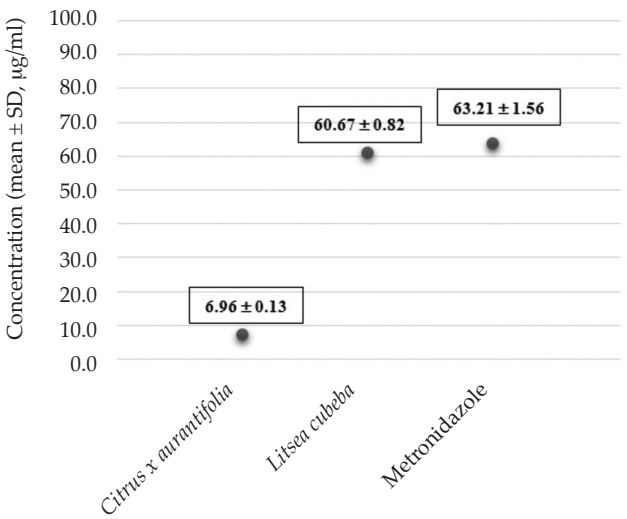

Fig 2–The mean inhibitory concentration (IC_{50}) ± SD of plant essential oils and metronidazole effective against *Giardia duodenalis*.

RESULTS

Of the essential studied, only *C.* × *aurantifolia* (IC_{50} ± SD = 6.96 ± 0.13 µg/ml), *L. cubeba* (IC_{50} ± SD = 60.67 ± 0.82 µg/ml) and metronidazole (IC_{50} ± SD = 63.21 ± 1.56 µg/ml) had efficacy against *G. duodenalis* in dose-dependent fashion (Fig 1, 2).

DISCUSSION

In our study, of the 11 plant essential oils studied, only *C.* × *aurantifolia* and *L. cubeba* inhibited the growth of *G. duodenalis*. Calzada *et al* (2006) reported plants have been used in traditional Mexican medicine to treat *G. duodenalis*

and *Entamoeba histolytica*. Lota *et al* (2002) reported the essential oil of *Citrus aurantifolia* (Christm.) Swing, a lime grown in subtropical and tropical regions, contains limonene, γ-terpinene, β-pinene and sabinene at varying concentrations. Limonene is a colorless liquid hydrocarbon of the monoterpene group, having a strong odor (Ruiz and Flotats, 2014; Subramenium *et al*, 2015).

Litsea cubeba is a quick growing plant commonly found in southern China and Southeast Asia that has been used for detoxification and to treat arthritis and diarrhea in traditional Chinese medicine (Kong *et al*, 2015; Lin *et al*, 2016). Its essential oil is pale-yellow and has a sour lemon-like smell (Li *et al*, 2014). The substances found in the essential oil are dependent on the plant parts used for the extraction (Wang and Liu, 2010). The environment and harvesting time affect the proportions of the essential oil's compounds. The major substances of *L. cubeba*'s essential oil are aldehydes (citral, citral isomer, citronellal and citronellal isomer) and the minor substances are alkenes (limonene, sabinene, 1R-α-pinene and 4-methyl-1,4-hepta-diene) and alcohols (linalool, terpineol and 2,7-dimethyl-2,7-octanediol), which are active ingredients in disinfectants (Li *et al*, 2014).

We hypothesize the ability of limonene, a component of the essential oil of *C.* × *aurantifolia*, to kill or inhibit the *Giardia* parasite, is promoted by other complementary substances, such as citral and carvacrol (Chueca *et al*, 2014).

The other essential oils, we extracted from *Z. rhetsa*, *I. verum*, *A. galangal*, *S. aromaticum*, *O. basilicum*, *O. africanum*, *C. hystrix*, *C. reticulata* and *Z. officinale* had no efficacy against *G. duodenalis*, suggesting they may contain fewer antigiardial substances, such as limonene, citral and carvacrol.

In conclusion, the essential oils of *C.* × *aurantifolia* and *L. cubeba* had antigiardial properties. Future studies are needed to determine which components of these plants in what proportions are the major active ingredients and have the potential to be used in future safety and efficacy studies.

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