ACTIVITY OF PLANT ESSENTIAL OILS AGAINST GIARDIA DUODENALIS

Supaluk Popruk¹, Kanthinich Thima¹, Ruenruetai Udonsom¹, Rachatawan Chiabchalard¹, Aongart Mahittikorn¹, Kaewmala Palukul² and Apanchanid Thepouypom³

¹Department of Protozoology, ²Department of Entomology, ³Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

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 IC₅₀ = 6.96 ± 0.13 µg/ml, followed by L. cubeba with IC₅₀ = 60.67 ± 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 Caccio, 2013). This suggests other animals may act as reservoirs for G. duodenalis and may be linked to zoonotic transmission.
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: po-y-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.

Essential oil densities were calculated
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 (2,300g, 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 *G. duodenalis* trophozoites can reduce MTT to a purple color with an absorbance at 550 nm.

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

\[
\% \text{ trophozoite survival} = \left( \frac{\text{sample absorbance} - \text{culture medium control absorbance}}{\text{non-treated control absorbance} - \text{culture medium control absorbance}} \right) \times 100.
\]

\[
\% \text{ inhibition} = 100 - \% \text{ trophozoite that survived}
\]

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).

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 aurantiifolia* (Christm.) Swing, a lime grown in subtropical and tropical regions, contains limonene, γ-terpinene, β-pinene and sabi

Lima et al. (2002) reported the essential oil of *Citrus aurantiifolia* (Christm.) Swing, a lime grown in subtropical and tropical regions, contains limonene, γ-terpinene, β-pinene and sabi

Lota et al. (2002) reported the essential oil of *Citrus aurantiifolia* (Christm.) Swing, a lime grown in subtropical and tropical regions, contains limonene, γ-terpinene, β-pinene and sabi

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 medic

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 medic

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 medic

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 medic

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

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

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

In conclusion, the essential oils of *C. × aurantiifolia* 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.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, for their support. This research project was supported by Mahidol University.

REFERENCES


Chueca B, Pagán R, García-Gonzalo D. Oxygen-
ated monoterpenes citral and carvacrol cause oxidative damage in *Escherichia coli* without the involvement of tricarboxylic acid cycle and Fenton reaction. *Int J Food Microbiol* 2014; 189: 126-31.


Li WR, Shi QS, Liang Q, Xie XB, Huang XM, Chen YB. Antibacterial activity and kinetics of *Litsea cubeba* oil on *Escherichia coli*. *PLOS One* 2014; 9: e110983.


