

Original article

Evaluation of the Leishmanicidal potential of ethanolic extracts derived from *Cymbopogon citratus* leaves and *Ganoderma lucidum* fruiting bodies on *Leishmania martiniquensis* promastigotes

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Objective To evaluate the leishmanicidal potential of ethanolic extracts derived from *Cymbopogon citratus* leaves and *Ganoderma lucidum* fruiting bodies on *Leishmania martiniquensis* promastigotes.

Methods The chemical composition of ethanolic extracts from *C. citratus* leaves and *G. lucidum* fruiting bodies were analyzed using gas chromatography-mass spectrometry (GC-MS) and their leishmanicidal potential was evaluated *in vitro* with *L. martiniquensis* promastigotes. The half maximal inhibitory concentrations (IC₅₀) of the extracts was determined using alamarBlue™ Cell Viability Reagent. Cytotoxicity was tested on the human monocytic THP-1 cell line (THP-1 cells) and the selectivity indices (SI) of both extracts were calculated.

Results GC-MS analysis identified 17 compounds in the ethanolic extracts of *C. citratus* and 25 in the extracts of *G. lucidum* representing 76.87 and 95.45%, respectively, of all the components in each of those extracts. D:A-friedoursan-3-one (17.49%) and ergosterin (36.90%) were the main constituents of the *C. citratus* and the *G. lucidum* extracts, respectively. The *C. citratus* extract demonstrated moderate leishmanicidal activity with an IC₅₀ value of 20.26±2.8 µg/mL whereas the *G. lucidum* extract showed weak leishmanicidal activity (54.69±13.67 µg/mL). SI values of the extracts of *C. citratus* and *G. lucidum* were 5.38 and 3.54 µg/mL, respectively, showing that they were less toxic to THP-1 cells than to *Leishmania* parasites.

Conclusion The ethanolic extract of *C. citratus* with an SI value<10 has greater leishmanicidal activity than *G. lucidum*. Further investigations are needed to separate, purify, and identify the active components of these two plant sources as the extracts are complex mixtures of substances and pertinent active compounds may be present in low concentrations. **Chiang Mai Medical Journal 2019;58(4):181-9.**

Keywords: Ethanolic extracts, *Cymbopogon citratus*, *Ganoderma lucidum*, *Leishmania martiniquensis*, Promastigotes

Introduction

Leishmaniasis is a protozoan disease caused by *Leishmania* parasites. It is transmitted by the

bite of *Phlebotomus papatasi*, small insects known as sand flies. Leishmaniasis has been declared a

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newly emerging disease in Thailand (1). Many immunocompromised hosts infected with the parasite can develop severe conditions including disseminated cutaneous leishmaniasis and visceral leishmaniasis. The causative species in Thailand was first believed to be *Leishmania siamensis*. However, in 2014, comparison of DNA sequences carried out by Pothirat et al. revealed that there are two species, *Leishmania martiniquensis*, which is the cause of the majority of the leishmanial cases, and *Leishmania orientalis* (2,3). Amphotericin B is a therapeutic drug used to treat leishmaniasis in Thailand; however, the drug can cause serious side effects, the most concerning being renal toxicity (1). Although liposomal amphotericin B, a formulation that results in a significantly improved toxicity profile compared with the conventional amphotericin B (4), is available in other countries, the cost of prolonged use of liposomal amphotericin B is unaffordable for many Thai patients. Alternative therapeutic agents or combination therapies for the treatment of leishmaniasis in Thailand are needed.

Cymbopogon citratus (lemon grass or oil grass) and *Ganoderma lucidum* (lingzhi or reishi mushroom)

are both widely used as herbal medicines in many tropical countries. Antileishmanial properties of essential oils from *C. citratus* have been reported for several *Leishmania* species, e.g., *Leishmania chagasi*, *Leishmania amazonensis*, *Leishmania infantum*, *Leishmania tropica*, and *Leishmania major* (5-7). Furthermore, *C. citratus* has been shown to possess activities such as an antiproliferative effect against *Trypanosoma cruzi* (8) and an antimalarial effect against *Plasmodium falciparum* (9). *G. lucidum* has been used to promote health and longevity and as an alternative medicine in cancer patients in China and other countries (10,11). *G. lucidum* has been found to produce antimalarial activity against *P. falciparum* (12), however, its effect on *Leishmania* parasites has never been assessed. This study represents the first investigation of the leishmanicidal potential of ethanolic extracts of both plants on *L. martiniquensis* promastigotes.

Methods

Plant materials

Fresh leaves from *C. citratus* (Figure 1A) were collected from a natural habitat in Doi Saket

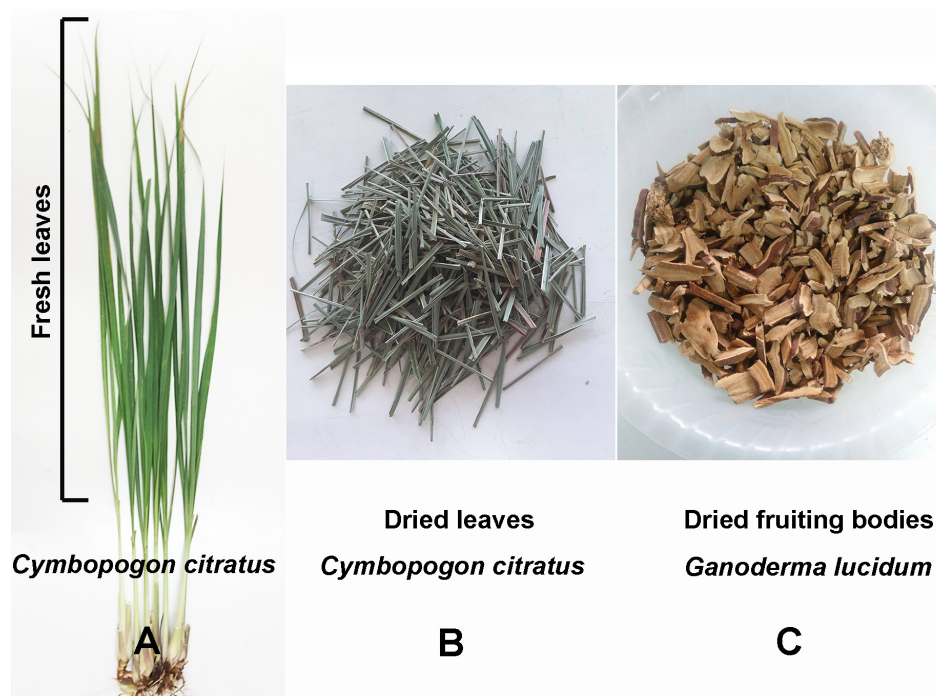


Figure 1. (A) Fresh and (B) dried leaves of *Cymbopogon citratus*. (C) Dried fruiting bodies of *Ganoderma lucidum*

district, Chiang Mai province and dried fruiting bodies of *G. lucidum* (Figure 1C) were purchased from a Chinese herbalist shop in the local market in Muang district, Chiang Mai province. Plant materials were identified and authenticated by an expert on herbal plants at the Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University. Reference specimens were kept and their voucher numbers (023244 for *C. citratus* and 023245 for *G. lucidum*) were deposited at the Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University.

Extraction and chemical analysis

Fresh leaves of *C. citratus* were shade-dried separately for 3-5 days in an open area with active ventilation and an ambient temperature of about 30 ± 5 °C. Dried leaves of *C. citratus* (Figure 1B) and dried fruiting bodies of *G. lucidum* were pulverized to a coarse/fine powder before extraction of the ethanolic extracts. One kilogram of finely powdered material from each plant was extracted exhaustively at room temperature by maceration with 1 L of 95% ethanol for 3 days. The extracted mixtures were filtered through a fine cotton cloth followed by filtering with Whatman No.1 filter paper and a vacuum Bucher funnel. They were then concentrated in a rotary evaporator and finally dried in a lyophilizer to completely remove the solvent and to obtain the dry ethanolic extracts.

Both extracts were sent to the Science and Technology Service Center, Faculty of Science, Chiang Mai University, for analysis by gas chromatography-mass spectrometry (GC-MS). Analytical GC was carried out with a Hewlett-Packard 7890A (Wilmington, CA, USA) gas chromatograph equipped with a single quadrupole mass selector (Agilent Technologies, Wilmington, CA, USA) and an MSD 5975C (EI) gas chromatograph/mass. Selective Detector (Agilent Technologies, Wilmington, CA, USA). The column was a DB-5MS (30 m $\times$ 0.25 mm ID $\times$ 0.25 μ m film thickness). The total GC-MS running time was 20 min. The analytical conditions were injector and transfer line temperatures of 250 and 280 °C.

The oven temperature was programmed to increase from 50 to 250 °C at a rate of 10 °C/min. The carrier gas was helium at 1.0 mL/min, the injection was 0.2 μ L (1/10% v/v, in CH_2Cl_2), and the split ratio was 100:1. An electron ionization system with an ionization energy of 70 eV was used for GC-MS detection. Data were acquired over a range of 50-550 atomic mass units (amu) with a scan rate of 2.91 scans/s. The relative percentages of the constituents is expressed as a percentage by peak area normalization. Chemical components of the ethanolic extract were identified based on direct comparison of their spectra with those of the GC-MS (NIST 2008 and Wiley 8NO8) spectral libraries.

Cytotoxic activity of *C. citratus* and *G. lucidum* extracts

The extracts of *C. citratus* and *G. lucidum* were evaluated for cytotoxicity against the human monocytic THP-1 cell line using alamarBlue™ Cell Viability Reagent (Thermo Fisher Scientific, Waltham, MA, USA) using the method described by the manufacturer. THP-1 cells were incubated in an RPMI-1640 medium (Hyclone, Logan, UT, USA) containing 25 ng/mL of phorbol myristate acetate (PMA) and plated on 96-well flat-bottom plates at 2.5×10^5 cells/mL for 24 h at 37 °C and 5% CO_2 in a humidified atmosphere. Then the cultures were washed and replaced with fresh medium. THP-1 cells were incubated for 48 h before addition of a two-fold diluted solution of the extracts with final concentrations ranging from 800 to 0.0977 μ g/mL. Cells were treated with different concentrations of extracts for 72 h. AlamarBlue™ Cell Viability Reagent was then added to all wells at a final concentration of 10% and incubated for 6 h before determination of absorbance at 570 nm and 600 nm using a plate reader. The percentage survival of THP-1 cells was calculated and analyzed. The 50% cytotoxic concentration (CC_{50}) values of both extracts, defined as the concentration of extract required to induce 50% cell death, were determined. Three independent experiments were performed in triplicate.

Parasite culture

A strain of *L. martiniquensis* from northern Thailand, originally isolated from a patient with visceral leishmaniasis in 2012 (MHOM/TH/2012/LSCM1) (2), was used. The parasites had been cryopreserved in liquid nitrogen. The parasites were cultured at 26 °C in Schneider's insect medium pH 7.0 (SIM) (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and subpassaged to fresh medium every four days to maintain the growth and viability of the parasites.

Leishmanicidal activity assays on promastigotes

Each of the extracts of *C. citratus* and *G. lucidum* was initially dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution at a concentration of 25 mg/mL. The final DMSO concentration never exceeded 0.4%, a level which has been proven to be non-toxic to the protozoa (5). Both solutions were stored at -20 °C. The viability of the log phase promastigotes was evaluated by using trypan blue under a light microscope before being treated with the extract solution. The viability percentage was determined by counting a total of at least 200 cells. Log phase promastigotes with 98-99% viability were used in all assays. The stock solution was diluted two-fold and added into the log phase promastigotes (90 µL of 10⁶ parasites/mL) in SIM supplemented with 10% FBS at pH 7.0 to achieve a final range of concentrations from 100 to 0.0977 µg/mL. The parasites were incubated at 26 °C in the medium either in the absence or presence of the different concentrations of extracts. Amphotericin B deoxycholate (AmpB deoxycholate) was used as a reference drug. As a control, blank wells containing only culture media (no cells) were included on each plate. Leishmanicidal activity assays on promastigotes were conducted using alamarBlue™ Cell Viability Reagent. After a 48 h incubation, 10 µL of alamarBlue™ Cell Viability Reagent was added to each well. Following an additional 24 h incubation period, absorbance of the resulting mixtures was measured at wavelengths of 570 nm and 600 nm using a plate reader. The color produced

by resorufin in the alamarBlue™ Cell Viability Reagent is proportional to the number of viable parasites. The concentration that inhibited culture growth by 50% (IC₅₀) after a 72 h incubation was calculated. Three independent experiments were run in triplicate.

Data analysis

The CC₅₀ and IC₅₀ were calculated using the computer software program GraphPad Prism v6.0 (GraphPad Software, USA) in conjunction with a nonlinear regression model of variable slope with results expressed as mean±SD. Selectivity indices (SI) of the extracts were also calculated. SI for promastigotes of *L. martiniquensis* was calculated as the ratio between the CC₅₀ for THP-1 cells and the IC₅₀ for the parasite. Extracts with SI values >10 were considered low cytotoxic agents and defined for the selection of an antiparasitic hit agent (13). According to the classification of antiparasitic activity by Bero et al. (14), extracts that have a measurable effect against parasites with an IC₅₀ value <2 µg/mL (or µM for pure compounds) were considered to be strongly active, and those with an IC₅₀ value <21 µg/mL (or µM for pure compounds) were considered to be moderately active, while those with a higher IC₅₀ value (≥21 µg/mL or µM for pure compounds) were considered to be less active and not crucial to the study outcome.

Results

Chemical composition of plant ethanolic extracts

GC-MS studies of the chemical constituents of ethanolic extracts of *C. citratus* and *G. lucidum* revealed the presence of 17 and 25 compounds, constituting 76.87 and 95.45% of the total composition of the two plant sources, respectively (Tables 1 and 2). The compounds in the ethanolic extract of *C. citratus* were comprised mostly of D:A-friedoursan-3-one (17.49%), followed by 4,6-bis (4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-1-carbaldehyde (14.59%), and mono (2-ethylhexyl) phthalate (MEHP) (9.73%). The major chemical constituents of the *G. lucidum* ethanolic extracts were ergosterin (36.90%), followed by 7,22-ergostadienol (18.68%), and mono (2-ethylhexyl) phthalate (MEHP) (7.44%).

Table 1. Chemical composition of the ethanolic extract of *C. citratus*

No.	Chemical constituent	RT ^a (min)	Percentage composition
1	trans-Geraniol	14.650	6.107
2	Geranic acid	17.488	1.937
3	Geranyl acetate	18.026	2.765
4	Neophytadiene	28.663	1.051
5	Palmitic acid	31.323	1.841
6	Ethyl palmitate	31.804	1.554
7	4,6-bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-1-carbaldehyde	33.886	14.586
8	Ethyl linolenate	34.980	1.360
9	1H-Benz[F]indole-2-carboxylic acid,9-methoxy,1-methyl-, ethyl ester	35.563	0.920
10	Mono (2-ethylhexyl) phthalate (Mehp)	41.017	9.734
11	Cyclooctacosane	49.073	0.735
12	Cholesterol	49.130	0.254
13	α-Tocopherol	49.308	6.460
14	Ergost-14-en-3-ol	50.498	1.172
15	β-Stigmasterol	50.847	2.114
16	γ-Sitosterol	51.728	6.793
17	D:A-friedoursan-3-one	53.656	17.490
	Total identified		76.873

^aRetention time (min)

Cytotoxic and leishmanicidal activities of *C. citratus* and *G. lucidum* extracts

In regard to the cytotoxic activity on THP-1 cells, the ethanolic extracts of *C. citratus* and *G. lucidum* exhibited CC₅₀ values of 109±3.23 and 193.5±4.67 µg/mL, respectively, higher than that of AmpB deoxycholate (57.53±1.9 µg/mL). The *C. citratus* extract exhibited leishmanicidal activity with an IC₅₀ value of 20.26±2.8 µg/mL, whereas the *G. lucidum* extract had an IC₅₀ value of 54.69±13.67 µg/mL. SI values of the ethanolic extracts of *C. citratus* and *G. lucidum* were 5.38 and 3.54 µg/mL, respectively, indicating that they were less toxic to THP-1 cells than to *Leishmania* parasites (Table 3).

Discussion

The use of available drugs for the treatment of leishmaniasis is limited due to their side effects and to increasing drug-resistance in the parasites. The development of new treatments is an urgent need. Natural products obtained from plants offer the possibility of the discovery of promising new

active compounds for the treatment of leishmaniasis (15).

C. citratus is known to have a wide range of biologically active chemicals which have been shown to have therapeutic effects. It has been reported to exhibit immunomodulatory, anti-inflammatory, antiviral, anticarcinogenic, antihyperglycemic, antioxidant, antimalarial, antimutagenic, antimicrobial and antiglycation properties (16). Previously, the leishmanicidal activity of essential oils from *C. citratus* has been studied with several *Leishmania* species (5-7). *C. citratus* essential oil contains two major constituents, citral, representing 81.5% of the oil, and myrcene with 10.8% (17). In this study, the *in vitro* activity of ethanolic extracts of *C. citratus* against *L. martiniquensis* promastigotes was evaluated for the first time. The major compounds in the ethanolic extract of *C. citratus* were D:A-friedoursan-3-one (17.49%), 4,6-bis(4'-methylpent-3'-en-1'yl)-6-methylcyclohexa-1,3-diene-1-carbaldehyde (14.59%), and mono (2-ethylhexyl) phthalate (MEHP) (9.73%). D:A-friedoursan-3-one or

Table 2. Chemical composition of the ethanolic extract of *G. lucidum*

No.	Chemical constituent	RT ^a (min)	Percentage composition
1	Isopentylidene isopentylamine	8.842	0.195
2	N-Ethyl-2-carbethoxyazetidine	19.794	0.914
3	Pentadecanoic acid	29.246	0.387
4	11-Hexadecenoic acid	31.014	0.326
5	Palmitic acid	31.323	3.281
6	Ethyl palmitate	31.804	0.371
7	Linoleic acid	34.585	4.817
8	Petroselaidic acid	34.694	2.449
9	Oleic acid	34.739	0.111
10	Ethyl linolenate	34.980	1.119
11	Ethyl oleate	35.020	1.039
12	Mono (2-ethylhexyl) phthalate (Mehp)	41.017	7.441
13	α-Matriline	46.910	1.073
14	9-Phenanthrenol, 4B,5,6,7,8,8A,9,10-octahydro-1,3,4-trimethoxy-4B,8,8-trimethyl-2-(1-methylethyl)-, [4BS- (4Bα,8Aβ,9α)]-[1,1':4',1''-tercyclohexane]-4-carboxylic acid,4''-propyl-,propyl ester, [trans[trans(trans)]]-	47.059	2.795
15	Triacetylcybrodol	47.278	0.459
16	3,9-dimethoxy-1,11-dimethylbenzo(1,2-B:5,4-B) bisbenzofuran-6,12-dione	47.431	0.413
17	6-Oxo-11,12,14-trimethoxy-abieta-8,11,13-triene	47.643	2.461
18	(22E,24S)-24-Methylcholesta-5,7,9(II),22-tetraen-3B-ol	49.725	3.830
19	Ergosterin	50.298	36.897
20	7,22-Ergostadienol	50.521	18.681
21	δ7-campestenol	51.316	2.520
22	Anthraergostatetraenol	52.392	0.739
23	Ergone	52.655	0.419
24	Isovaleraldehyde 2,4-dinitrophenylhydrazone	53.141	1.581
25	Methyl-23,24-dinor-3-oxolean-1,4,12-trien-28-oate	57.780	1.131
Total identified			95.449

^aRetention time (min)**Table 3.** Cytotoxic activity, leishmanicidal activity, and selectivity index of ethanolic extracts and the reference drug AmpB deoxycholate

Ethanolic extracts or Drug	THP-1 cells	<i>L. martiniquensis</i>	
	CC ₅₀ ^a (μg/mL)	IC ₅₀ ^b (μg/mL)	SI ^c (μg/mL)
<i>C. citratus</i>	109±3.23	20.26±2.8	5.38
<i>G. lucidum</i>	193.5±4.67	54.69±13.67	3.54
AmpB deoxycholate	57.53±1.9	0.085±0.01	676.82

^a50% cytotoxic concentration, mean±SD, ^b50% inhibitory concentration, mean±SD^cSelectivity index=CC₅₀÷IC₅₀

cymbopogonol is a new triterpenoid which was reported by Hanson et al. (18), its biological function is still unknown. Many factors are in-

involved in the process of obtaining different constituents or fractions derived from plants. Not only the specific part of the plant, the age

or stage of maturity, the season of harvest, the geographical origin, and any genetic differences, but also the extraction methods, including the different solvents used, can influence the derived bioactive constituents and yield of *C. citratus* (19).

Oliveira et al. (5) demonstrated that *C. citratus* essential oil inhibits *in vitro* *L. chagasi* promastigotes in a dose-dependent manner, with an $IC_{50}/72\text{ h}$ of $45\text{ }\mu\text{g/mL}$. The essential oil of *C. citratus* has shown inhibitory effects on promastigotes as well as causing remarkable morphological and ultrastructural alteration to occur in them. It has also been shown to have antiproliferative activity on *L. amazonensis* promastigotes at concentrations corresponding to $IC_{50}/72\text{ h}$ of $1.7\text{ }\mu\text{g/mL}$ (6). Machado and colleagues (7) demonstrated the inhibitory effect of the essential oil of *C. citratus* on *L. infantum*, *L. tropica* and *L. major* promastigote growth at IC_{50} concentrations ranging from 25 to $52\text{ }\mu\text{g/mL}$. In this study, according to the classification of antiparasitic activity for extracts and fractions derived from plants and natural products as defined by Bero et al. (14), the ethanolic extract of *C. citratus* showed moderate leishmanicidal activity on *L. martiniquensis* promastigotes, with IC_{50} values of $20.26\pm 2.8\text{ }\mu\text{g/mL}$. These results suggest that the extract of *C. citratus* has promising antileishmanial properties.

The leishmanicidal potential of the ethanolic extract of *G. lucidum* was also evaluated for the first time on *L. martiniquensis* promastigotes *in vitro*. The major constituent of the *G. lucidum* ethanolic extracts was ergosterin (36.90%). Ergosterin, also known as ergosterol, is a crystalline sterol which is a biochemically important natural substance. It is abundant in mushrooms and has shown chemopreventive activity (20). Although the extract of *G. lucidum* showed only weak leishmanicidal activity on promastigotes with an IC_{50} value of $54.69\pm 13.67\text{ }\mu\text{g/mL}$, its activity was higher than the extracts of other mushrooms, e.g., ethanolic extracts from *Pleurotus ostreatus* (oyster mushroom) on *L. major* ($IC_{50}=160\pm 2\text{ }\mu\text{g/mL}$) (21), ethanol extract from *Grifola frondosa* (maitake mushroom) against *L. donovani* promastigotes ($IC_{50}=93.9\text{ }\mu\text{g/mL}$) (22), and water extract

from *Agaricus blazei* Murill (known as 'Cogumelo do Sol' in Brazil, or 'Himematsutake' in Japan) on *L. amazonensis*, *L. chagasi*, and *L. major* ($IC_{50}=67.5$, 65.8 , and $56.8\text{ }\mu\text{g/mL}$, respectively) (23).

Based on the classification of cytotoxicity of extracts and fractions derived from plants and natural products as defined by Study Program and Disease Control (24) and the selection of an antiparasitic hit agent (13), the evaluated ethanolic extracts from both plants were classified as moderately toxic ($100 < CC_{50} \leq 1000\text{ }\mu\text{g/mL}$, $SI < 10$). However, the SI of both extracts on THP-1 cells were lower than for *Leishmania* parasites, indicating that the extracts displayed leishmanicidal activity to a greater extent than cytotoxicity. Although both extracts evaluated in this study were less active than AmpB deoxycholate, a drug used clinically, further investigation is needed to extract, fractionate and identify their active components. As the extracts used in this study were crude extracts consisting of complex substances, the effective components might be present only in low concentrations and other components in the extracts might have affected the results.

Conclusions

The ethanolic extract of *C. citratus* has a level of leishmanicidal activity greater than that of *G. lucidum* with an SI value < 10 . However, both ethanolic extracts are a potential natural source for production of new antileishmanial agents as these extracts are complex mixtures of substances and the active components may be present only in low concentrations. Further investigation is needed to separate, purify, and identify the active components. In addition, assessment of leishmanicidal activity in *in vitro* intracellular amastigotes and *in vivo* experimental models, assessment for toxicity in animals, and determination of their mode of action are necessary prior to developing the compounds for the treatment of leishmaniasis.

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Conflict of interests

The authors declare that they have no conflict of interests.

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การประเมินศักยภาพการฆ่าเชื้อ *Leishmania martiniquensis* ระยะโปรแมสตีโกต ของสารสกัดเอธานอลที่ได้จากใบตะไคร้แกง และดอกเห็ดหลินจือ

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ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

วัตถุประสงค์ เพื่อประเมินศักยภาพการฆ่าเชื้อ *Leishmania martiniquensis* ระยะโปรแมสตีโกต ของสารสกัดเอธานอลที่ได้จากใบตะไคร้แกง และดอกเห็ดหลินจือ

วิธีการ การวิเคราะห์องค์ประกอบทางเคมีของสารสกัดเอธานอลจากใบตะไคร้แกง และดอกเห็ดหลินจือ โดยวิธี Gas Chromatography-Mass Spectrometry (GC-MS) และ ประเมินศักยภาพการฆ่าเชื้อ *L. martiniquensis* ระยะโปรแมสตีโกต เป็นครั้งแรกในหลอดทดลอง ค่าความเข้มข้นการยับยั้งสูงสุดครั้งหนึ่ง (IC_{50}) วัดได้จากการใช้ alamarBlue™ Cell Viability Reagent ความเป็นพิษต่อเซลล์ ได้ทดสอบในเซลล์ไลน์ monocytic THP-1 (เซลล์ THP-1) และคำนวณหาดัชนีการเลือกใช้ (SI) ของสารสกัดทั้งสองด้วย

ผลการศึกษา จากการวิเคราะห์ด้วย GC-MS พบว่า มีสารประกอบ 17 และ 25 ตัว จากสารสกัดเอธานอลของใบตะไคร้แกง และดอกเห็ดหลินจือ คิดเป็นร้อยละ 76.87 และ 95.45 ขององค์ประกอบทั้งหมด ตามลำดับ D:A-friedoursan-3-one (ร้อยละ 17.49) และ ergosterin (ร้อยละ 36.90) เป็นองค์ประกอบหลักของสารสกัดเอธานอลของใบตะไคร้แกง และดอกเห็ดหลินจือ ตามลำดับ สารสกัดจากใบตะไคร้แกง มีฤทธิ์ฆ่าเชื้อลิสมาเนียปานกลาง โดยมีค่า IC_{50} เท่ากับ $20.26 \pm 2.8 \mu\text{g/mL}$ ในขณะที่สารสกัดจากดอกเห็ดหลินจือ มีฤทธิ์ฆ่าเชื้อลิสมาเนียต่ำ ($54.69 \pm 13.67 \mu\text{g/mL}$) ค่า SI ของสารสกัดจากใบตะไคร้แกงและดอกเห็ดหลินจือ เท่ากับ 5.38 และ 3.54 $\mu\text{g/mL}$ ตามลำดับ แสดงให้เห็นว่า สารสกัดทั้งสองมีความเป็นพิษต่อเซลล์ THP-1 น้อยกว่า เชื้อลิสมาเนีย

สรุป การศึกษาครั้งนี้แสดงให้เห็นว่า สารสกัดเอธานอลจากใบตะไคร้แกง มีฤทธิ์ฆ่าเชื้อลิสมาเนียดีกว่าสารสกัดเอธานอลของดอกเห็ดหลินจือ ด้วยค่า $SI < 10$ การตรวจสอบเพิ่มเติม ควรดำเนินการเพื่อแยกส่วน ทำให้บริสุทธิ์ และระบุส่วนประกอบที่ออกฤทธิ์ เนื่องจากสารสกัดมีส่วนผสมที่ซับซ้อน ของสารประกอบหลายชนิด และสารประกอบที่ออกฤทธิ์ อาจพบในความเข้มข้นที่ต่ำ **เชียงใหม่เวชสาร 2562;58(4):181-9.**

คำสำคัญ: สารสกัดเอธานอล ตะไคร้แกง เห็ดหลินจือ *Leishmania martiniquensis* โปรแมสตีโกต

