

Screening for *in vitro* amoebicidal activity of plant essential oils against *Acanthamoeba* sp.

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Objective To screen for *in vitro* amoebicidal activity of essential oils extracted from 10 plant species from three botanical families against *Acanthamoeba* sp.

Methods Essential oils were extracted from dry material of 10 plant species using steam distillation; the percentage yields and physical characteristics of the derived essential oils were recorded. The cyst and trophozoite stages of *Acanthamoeba* sp. were harvested separately from monoxenic agar and axenic peptone yeast glucose (PYG) cultures, respectively. Amoebicidal activities of each essential oil were determined *in vitro* at a concentration of 50 mg/mL. After 24 h of incubation, morphological changes, cell viability, and percentage mortality of amoebae were investigated using a light microscope.

Results Essential oils were successfully extracted from all ten plant species with average yields on a dry weight basis ranging from 0.3 to 6.7% (v/w). Five out of the ten essential oils tested, including the oils of *Piper retrofractum* Vahl., *Piper nigrum* Linn., *Citrus hystrix* DC., *Curcuma longa* Linn., and *Kaempferia pandurata* Roxb., effectively eradicated *Acanthamoeba* trophozoites, with mortality up to 100%. Furthermore, both *P. retrofractum* and *C. hystrix* oils also exerted cysticidal activity against *Acanthamoeba* sp., with 3.35% and 43.62% mortality, respectively.

Conclusions This is the first report on the amoebicidal activities of these essential oils, of which the most effective were the *P. retrofractum* and *C. hystrix* oils which affected both the trophozoite and the cyst stages of *Acanthamoeba*. The results of this study provide basic knowledge for future studies of the amoebicidal potential of these plant essential oils which could potentially be developed and applied for the treatment of acanthamoebiasis for which no effective drug is currently available.
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Keywords: *Acanthamoeba* sp., amoebicidal activities, essential oils, *Piper retrofractum*, *Citrus hystrix*

Introduction

Acanthamoeba, a genus of free-living amoeba, is ubiquitous in the environment and sometimes plays a role as a human pathogen (1,2). Some *Acanthamoeba* spp. cause a life-

threatening disease, Granulomatous Amoebic Encephalitis (GAE), and a sight-threatening disease, *Acanthamoeba* keratitis (AK) (3). The prevalence of these diseases is lower than

similar illnesses contracted from other common pathogens; as a result these diseases tend to be misdiagnosed (4). Difficulties in curing these diseases are the result of many factors, e.g., lack of expertise with the amoeba leading to delayed diagnosis, use of improper drug doses, or lack of awareness of potential adverse effects due to drug toxicity. Moreover, the cyst stage, which possesses a tough, double-layered wall, is much more resistant to drugs than the trophozoite stage. Because of that, when therapeutic treatment is halted, the dormant cysts can transform into active trophozoites which can then result in recurrence of the infection (5).

So far, no effective drugs have been successfully used to treat human acanthamoebiasis. For example, GAE treatment involves several drug combinations, e.g., amphotericin B, rifampin, trimethoprim-sulfamethoxazole, ketoconazole, fluconazole, sulfadiazine, miltefosine, and albendazole, whereas the drug regimen for AK normally includes chlorhexidine in combination with diamidines and neomycin (6). Unfortunately, some drugs which have been shown to be effective such as propamidine and hexamidine are unavailable in some countries due to their high cost and specific usage.

Many plants used in traditional folk medicine have been extensively investigated in an effort to find new alternative compounds that can substitute for chemical drugs. This also brings up the idea of plant products that possess amoebicidal activities. To our knowledge, little is known about the acanthamoebicidal properties of plants from Thailand (7,8). One study of methanolic extract of *Pouzolzia indica* Benn, a member of the Urticaceae family, with the Thai name “kob-chanang-dang,” was found to show cysticidal efficacy against *Acanthamoeba* spp. isolated from a keratitis case. The aim of this study was to screen for potentially potent acanthamoebicidal activities of essential oils obtained from other Thai medicinal plants. A total of ten plants belonging to three families, all of which are used as folk remedies (9), were tested. This preliminary study of the amoebicidal activities of those plants may pro-

vide a useful perspective for future development of alternative compounds for treatment of *Acanthamoeba*.

Methods

Plant materials

Ten species of plants from six genera belonging to three botanical families, Piperaceae, Rutaceae, and Zingiberaceae (Table 1), were selected based primarily on their previously reported biological effects against *Acanthamoeba* spp. (10,11). The plant materials were either purchased from traditional herb suppliers or collected from their natural habitats in Chiang Mai province in northern Thailand. Taxonomic identification of the plant samples was carried out by Mr. James Franklin Maxwell, a botanist at the Chiang Mai University (CMU) Herbarium, Department of Biology, Faculty of Science, CMU, Chiang Mai, Thailand, and Ms. Wannaree Charoensup, a scientist at the Department of Pharmaceutical Science, Faculty of Pharmacy, CMU, Chiang Mai, Thailand. Voucher specimens of these plants were deposited for future reference at the Department of Parasitology, Faculty of Medicine, CMU.

Essential oil extraction

The identified plant material was air dried in the shade at ambient temperature for about one week then each type was separately ground using an electrical blender. The coarsely ground dried material from each plant was subjected to steam distillation for at least 3 h or until distillation was complete. Following the distillation process, the essential oil of each plant species was collected and separated using a separator funnel. The essential oil was then dehydrated using anhydrous sodium sulfate (Na_2SO_4) to eliminate any trace of moisture and was subsequently kept in amber-colored bottles at 4 °C until used for *in vitro* amoebicidal screening tests. The yields of the essential oils was averaged over triplicate extractions and calculated based on the dry weight of each type of plant material. The physical characteristics of the essential oils were also observed and recorded.

Culturing of *Acanthamoeba* sp.

The *Acanthamoeba* sp. used in this study was a clinical pathogen isolated from a keratitis patient, and was generously provided by Dr. Kosol Roongruangchai of the Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand (7). The amoeba isolate was maintained without shaking in a PYG defined medium (12) by sub-passage culture at room temperature.

To prepare the trophozoite stage, 50 mL of 3- to 5-day-old amoeba cultured in PYG medium was centrifuged at 600×g for 5 min. The trophozoite sediment was then washed twice with sterile Page's amoeba saline (PAS) (12) at the same speed. Viability of the trophozoite was determined using the trypan blue exclusion test (13). The viable and the nonviable trophozoites were separately enumerated using a hemocytometer (3). The living trophozoites were adjusted to a final concentration of 1×10^6 cells/mL and were used continuously for trophozoiticidal screening within the same day (14,15).

For the cyst stage preparation, 10 µL of the PYG medium, consisting of *Acanthamoeba* trophozoites in exponential growth, was centrally inoculated on a 1.5% non-nutrient agar plate coated with heat-inactivated *Escherichia coli* (NNE plate). The plate was then incubated at 30 °C for 14-21 days to insure complete encystment (10). To harvest the entire mature cysts from the plate, the agar surface was flooded with 5 mL of sterile PAS and gently scraped using a sterile spatula. The harvested cyst suspension was centrifuged at 900×g for 5 min, followed by washing of the cyst sediment twice with sterile PAS at the same speed. Cyst viability was determined and counted as mentioned in the trophozoite preparation method. Viable cysts were then adjusted to a final concentration of 1×10^6 cells/mL and were used for the cysticidal screening within the same day (14,15).

Screening for amoebicidal activity of plant essential oils

A concentration of 100 mg/mL of each essential oil was prepared by diluting the pure essential oil with suitable solvents that not only dissolved the oils but were also harmless to the tested amoeba. A volume of 100 µL of the cyst or trophozoite suspension (1×10^6 cells/mL) was pipetted into each well of a sterile 96-well plate. An equal volume of 100 mg/mL of each essential oil was added in order to yield a final concentration of 50 mg/mL (10,15,16). The same process was carried out for the positive and the negative control groups, which were exposed either to the drug (0.01% chlorhexidine) or to the solvent only, respectively (10). The cell suspension was mixed thoroughly via pipetting up and down. The plates were sealed and either incubated at 30 °C or at ambient temperature. After 24 h of incubation, the trypan blue exclusion test for cell viability was performed, and the percentage mortality was recorded. All of the experiments were repeated twice. The validity of the tests was accepted when the mortality in the negative solvent control group was less than 5%. In cases in which 100% mortality was de-

tected in a well, a cell suspension of nonviable cells was cultured on an NNE agar plate to confirm the results. In addition to recording cell viability and mortality rates, morphological changes in both the trophozoite and cystic stages of the essential oil-treated amoebae were also observed under a light microscope and compared with the positive and negative controls.

Results

Essential oils derived by extraction

Extraction from each of the 10 types of plant materials provided essential oils with varying yields and different physical characteristics (Table 1). Most of the essential oils obtained appeared clear to light yellow, with the exception of *Piper sarmentosum* which was brown. With the exception of the oils of *P. sarmentosum* (1.06 g/mL) and *Murraya paniculata* (1.08 g/mL), the remaining essential oils possessed densities ranging from 0.56 g/mL to 0.94 g/mL, which are less than the density of water. The average percentage yield of derived oils on a dry weight basis ranged from 0.3% to 6.7% (v/w). The highest percentage yield was achieved from *Citrus reticulata* (6.7%), followed by *Citrus hystrix* (3.36%); yields from the other eight were all less than 1%.

Amoebicidal activity of essential oils against *Acanthamoeba* sp.

Dimethyl sulfoxide (DMSO) and 1% Tween 20 were used as solvents for essential oil dilution in cysticidal and for trophozoiticidal activity tests, respectively. These solvents did not cause any morphological changes in the amoebae. The *in vitro* amoebicidal activities of the essential oils tested are summarized in Table 2. After a 24 h exposure, the five essential oils extracted from *Piper retrofractum*, *Piper nigrum*, *C. hystrix*, *Curcuma longa*, and *Kaempferia pandurata* had effectively eradicated *Acanthamoeba* trophozoites, with a mean mortality of up to 100%. Complete cell death in a well was subsequently confirmed by agar plate culture which showed no cell growth. Interestingly, the *P. retrofractum* and *C. hystrix* oils also showed cysticidal activities against *Acanthamoeba* sp., with 3.35% and 43.62% mortality, respectively. However, the

Table 1. Physical characteristics and percentage yields (% yield) of essential oils isolated from selected herbs

Family/Species	Local name	Voucher number	Part used	Physical characteristics			
				Color	Odor	Density	% Yield
Piperaceae							
<i>Piper retrofractum</i>	Long pepper	PARA-PI-001-Fr/3	Fruit	Pale yellow	Pepper-like	0.86	0.64
<i>Piper nigrum</i>	Black pepper	PARA-PI-004-Fr/1	Fruit	Colorless	Pepper-like	0.94	0.39
<i>Piper sarmentosum</i>	Wild betel	PARA-PI-003-St-Le/1	Stem & Leaf	Brown	Pepper-like	1.06	0.31
Rutaceae							
<i>Citrus hystrix</i>	Kaffir lime	PARA-CI-001-Pe/2	Peel	Pale yellow	Intense citrus	0.86	3.36
<i>Citrus reticulata</i>	Mandarin orange	PARA-CI-004-Pe/2	Peel	Pale yellow	Intense citrus	0.84	6.70
<i>Murraya paniculata</i>	Orange Jessamine	PARA-MU-001-Le/1	Leaf	Colorless	Cool fragrance	1.08	0.30
Zingiberaceae							
<i>Amomum uliginosum</i>	Bustard	PARA-AM-002-Fr/4	Fruit	Pale yellow	Camphor-like	0.94	0.95
<i>Curcuma longa</i>	Turmeric	PARA-CU-005-Rh/1	Rhizome	Pale yellow	Ginger-like	0.56	0.56
<i>Curcuma zedoaria</i>	Zedoary	PARA-CU-004-Rh/6	Rhizome	Deep yellow	Cineolic-like	0.93	0.66
<i>Kaempferia pandurata</i>	Fingerroot	PARA-KA-001-Rh/2	Rhizome	Pale yellow	Ginger-like	0.94	0.67

strongest amoebicidal activity for the cyst and the trophozoite stages, with 63.26% and 100% mortality, respectively, were recorded in the 0.01% chlorhexidine-treated group. Regarding the solvent control groups, mortality of 0% trophozoite and 3.14% cysts were observed in 1% Tween 20- and DMSO-exposure groups, respectively.

Observations under light microscopy of cell viability and morphological alterations revealed differences in the structural appearance of the tested amoebae. Some morphological characteristics are shown in Figure 1. In the solvent control group, both the cyst and the trophozoite stages of viable amoebae presented unstained intact morphology with clear cytoplasm as they were able to exclude trypan blue (Figure 1A, 1D). Viable cysts were ovoid, spherical, or polygonal in shape with double layer walls: outer (ectocyst), and inner (endocyst) cyst walls (Figure 1A). The ectocyst appeared wrinkled, with a clear space separating it from the smooth endocyst. The live trophozoites also had specific characteristics such as acanthopodia and contractile vacuoles (Figure 1D). In contrast, dead amoebae stained with trypan blue, a result of the loss of cell permeability, were observed in the presence of chlorhexidine (Figure 1B, 1E), and the most effective *C. hystrix* oil (Figure 1C, 1F). With exposure to chlorhexidine, nonviable cysts showed a broader space between the endocyst and the ectocyst (Figure 1B), while those treated with *C. hystrix* oil were shrunken (Figure 1C). Remarkable alterations in trophozoites in the presence of chlorhexidine and the oil groups were also observed (Figure 1E, 1F). Under both exposure conditions, trophozoites became rounder with a distorted cell membrane, and the organelles inside the cytoplasm became clumped.

Discussion

The preliminary trials for amoebicidal screening against the *Acanthamoeba* pathogenic isolate at a discriminating dosage of 50 mg/mL demonstrated the promising efficacy of essential oils derived from five of the ten plants tested, *P. retrofractum*, *P. nigrum*, *C. hystrix*,

Table 2. Amoebicidal activity of plant essential oils on *Acanthamoeba* sp., trophozoites and cysts

Family	% Mortality of trophozoites			% Mortality of cysts		
	Experiment I	Experiment II	Mean±SE	Experiment I	Experiment II	Mean±SE
Piperaceae						
<i>Piper retrofractum</i>	100	100	100±0	3.13	3.57	3.35±0.31
<i>Piper nigrum</i>	100	100	100±0	0	0	0
<i>Piper sarmentosum</i>	0	0	0	0	0	0
Rutaceae						
<i>Citrus hystrix</i>	100	100	100±0	42.42	44.83	43.62±1.70
<i>Citrus reticulata</i>	0	0	100±0	0	0	0
<i>Murraya paniculata</i>	0	0	0	0	0	0
Zingiberaceae						
<i>Amomum uliginosum</i>	0	0	0	0	0	0
<i>Curcuma longa</i>	100	100	100±0	0	0	0
<i>Curcuma zedoaria</i>	0	0	0	0	0	0
<i>Kaempferia pandurata</i>	100	100	100±0	0	0	0
Control						
1% Tween 20	0	0	0	-	-	-
DMSO	-	-	-	3.66	2.63	3.14±0.73
0.01% Chlorhexidine	100	100	100±0	64.71	61.82	63.26±2.04

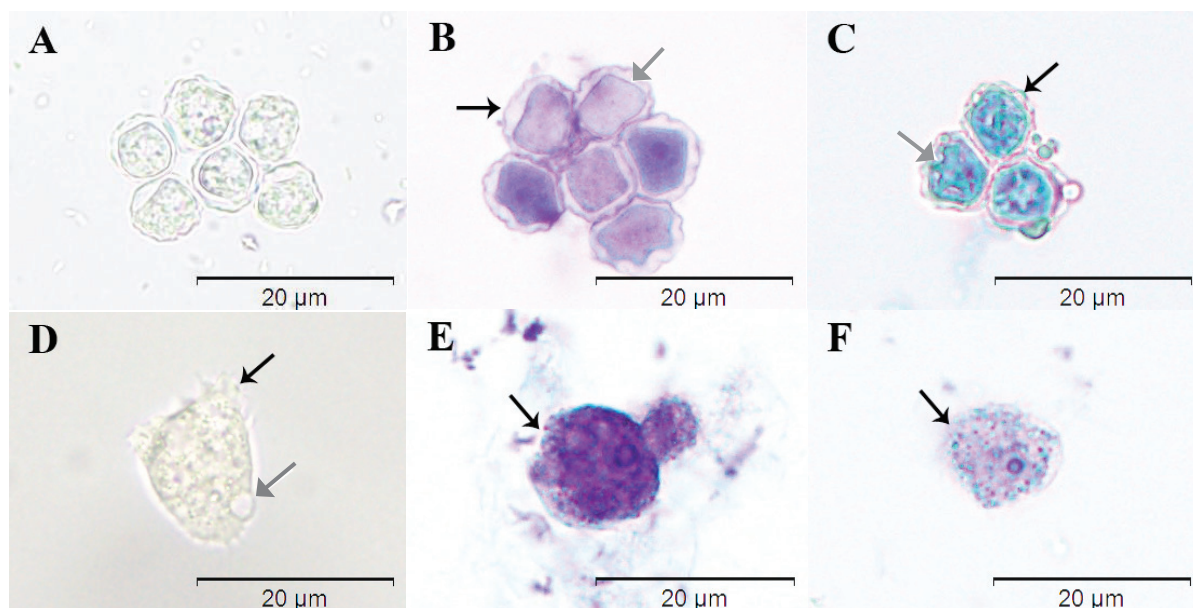


Figure 1. Morphological observation of *Acanthamoeba* sp., cysts (A–C) and trophozoites (D–F) after the trypan blue exclusion test (magnification x400). Under normal conditions: (A) Unstained living cysts in DMSO. (D) An unstained living trophozoite in 1% Tween 20, showing acanthopodia and contractile vacuole (black and gray arrows, respectively). Under 0.01% chlorhexidine conditions: (B) Stained cysts showing broader space between the ectocyst (black arrow) and the endocyst (gray arrow). (E) Stained trophozoites with distorted cell membrane (arrow) and cytoplasmic clumping. Under *C. hystrix* oil conditions: (C) Stained cysts presenting the shrunken ectocyst (black arrow) and the endocyst (gray arrow). (F) Stained trophozoites with distorted cell membrane (arrow) and cytoplasmic clumping.

Cu. longa, and *K. pandurata*. The essential oils of *P. retrofractum* and *C. hystrix* showed greater amoebicidal action than the others, eradicating both the cyst and the trophozoite stages of *Acanthamoeba* sp., while *P. nigrum*, *Cu. longa*, and *K. pandurata* oils could kill only trophozoites. These effective oils were isolated from edible plants that are regularly used as food ingredients and in traditional herbal medicines. In traditional or folk medicine, fruits of both *P. retrofractum* and *P. nigrum* are used for their anti-flatulent, expectorant, antitussive, antifungal, and appetite-enhancing properties (17) as well as for pain relief, rheumatism, flu, muscular aches, colds, exhaustion, and fevers (18-20). *C. hystrix* is widely used in traditional medicine for treatment of flu, fever, hypertension and abdominal pains as well as for diarrhea in infants (21). The fruit of *C. hystrix* is used as a digestive stimulant and blood purifier, and to reduce high blood pressure (22,23), while the peel is used as a carminative and as a stomachache alleviator (24). A herbal ingredient derived from the dried roots of *Cu. longa* has traditionally been utilized for relieving stagnation, stasis, and pain, as well as for treating amenorrhea and wounds (25,26). In Thailand, a folk drug derived from *K. pandurata* rhizome is used as an aphrodisiac and for colic disorders (27); additionally, this rhizome is used in combination with other local herbs to treat many illnesses such as coughs, aphtha, stomach distension, uterus inflammation, vaginal infection, and as a diuretic; it is also used for helminthic diseases in Indonesian traditional medicine (28,29).

In addition to folk medical uses, these plant species have been evaluated for bioactivities against various human pathogenic organisms. Toxic action against bacteria (30), fungi (31), and protozoa such as *Leishmania* (32) and *Trichomonas* (33) have been discovered in some *Piper* species. Solvent extracts of pepper plants, including *P. retrofractum*, *P. nigrum*, *P. longum*, and *Piper cubeba*, have been found to exhibit antibacterial activity against both Gram positive bacteria, e.g., as *Staphylococcus albus* and *Bacillus megaterium*, and Gram negative bacteria, e.g., *Escherichia coli*,

Salmonella typhi, and *Pseudomonas aeruginosa*. Antifungal activities against the fungus *Aspergillus niger* were also observed with these plant extracts (34). Bactericidal activity against three oral pathogens, including *Streptococcus mutans*, *Streptococcus sanguinis*, and *Porphyromonas gingivalis*, has been identified from the leaf and the fruit peel oils of *C. hystrix* (35). Hydrodistilled essential oils extracted from *C. hystrix* and *Citrus aurantiifolia* epicarps exhibited stronger antifungal activity against aflatoxin-producing strains of *Aspergillus flavus* and *Aspergillus parasiticus* as well as other common spoilage fungi than their ethyl acetate extracts (36). Similarly, antimicrobial activities of *Cu. longa* (37-39) and *K. pandurata* (40,41) have been reported against various bacteria, viruses, fungi, and parasites, e.g., *Trypanosoma*, *Leishmania*, and *Plasmodium*.

To the best of our knowledge, the plants chosen in this study, *P. retrofractum*, *P. nigrum*, *C. hystrix*, and *K. pandurata*, have never been evaluated for amoebicidal activity against *Acanthamoeba*. Therefore, this is the first study showing the acanthamoebicidal effect of these four plant oils. Interestingly, the oils extracted from *C. hystrix* and *P. retrofractum* exhibited the highest acanthamoebicidal activity not only to the cyst stage, but also to the trophozoite stage, although *in vitro* amoebicidal activity against *Acanthamoeba castellanii* of *Cu. longa* has been previously reported. Ethanol rhizome extract of *Cu. longa* has been shown to exhibit a significant inhibitory effect on the multiplication of *Acanthamoeba* cysts as compared to the drug (chlorhexidine) and non-treated controls (10). However, the present study noted no cysticidal activity for *Cu. longa* rhizome oil, although it could kill *Acanthamoeba* trophozoites. In an *in vitro* amoebicidal study which focused on 200 Southeast Asian plants, extract from *Kaempferia galangal*, which belongs to the same genus as *K. pandurata*, exhibited a lytic effect and encystment induction on three species of *Acanthamoeba* tested, *A. culbertsoni*, *A. castellanii*, and *A. polyphaga* (42). Combining the results from this study with data from previous

studies, it can be postulated that products of identical botanical species or plants from the same genus do not necessarily exhibit comparable activity against a given microorganism. Differences in the bioactivity of particular plant species could be attributed to qualitative and/or quantitative variability in activity level and to specific chemical properties. In addition, factors that can potentially contribute to differences in the chemistry, e.g., chemicals and techniques used in extraction as well as plant-related factors, including climatic and growth conditions, plant maturation, plant storage, and plant preparation, should be carefully considered and standardized when analyzing and utilizing plants and plant-derived products (43-45).

For treatment of acanthamoebiasis, a variety of drugs have been used clinically, but no agent has manifested effectiveness against all *Acanthamoeba* isolates (42). One issue related to the difficulty in the treatment of *Acanthamoeba* infections that is often mentioned is the reversible transformation between the cyst and the trophozoite stages. During drug administration, the trophozoite makes temporary biological changes to the dormant cyst in which elaborately reinforced double cyst walls are produced to protect it from the actions of the drug. Increased drug dosage is needed to overcome this barrier. There are compound drugs for *Acanthamoeba* treatment of both cysts and trophozoites; however, doses sufficiently high to be effective have toxicity and side effect issues (46,47). Active compounds, particularly those of plant origin, that are capable of inducing high susceptibility of amoebae at the low doses with less toxicity are, therefore, highly desirable. There has been increasingly frequent investigation of plants with medicinal properties with regard to their antimicrobial activity in an effort to discover more effective and safer products capable of replacing conventional pharmaceutical drugs (30-32,37). In the present study, *C. hystrix* and *P. retrofractum* oils, which have demonstrated amoebicidal potential against both cysts and trophozoites of *Acanthamoeba*, are promising potential new chemotherapeutic agents for

acanthamoebiasis. To that end, further studies on the dose-response amoebicidal property as well as chemical identification and isolation of these plants should be carried out. To increase understanding, investigation using purified plant products is necessary to identify the principles that account for the amoebicidal activities and to elucidate their mechanism of action both *in vitro* and *in vivo*. In addition, the minimum concentrations necessary for amoebae eradication should also be determined. The findings of the current study provide a basis for future research exploring the amoebicidal potential of natural essential oils that could potentially be beneficial in the treatment of acanthamoebiasis for which no drug of choice is currently available.

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Conflict of interest statement

There are no conflicts of interest.

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การคัดกรองฤทธิ์ฆ่าเชื้ออะมีบา *Acanthamoeba* sp. ในหลอดทดลองของน้ำมันหอมระเหยจากพืช

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วัตถุประสงค์ เพื่อคัดกรองฤทธิ์ฆ่าเชื้อแอมบาในหลอดทดลองของน้ำมันหอมระเหยที่สกัดได้จากพืช 10 ชนิด ใน 3 วงศ์พืช

วิธีการ ทำการสกัดน้ำมันหอมระเหยจากวัสดุแห้งของพืชที่วินิจฉัยชนิดแล้วโดยวิธีกลั่นด้วยไอน้ำ พร้อมบันทึกผลผลิตร้อยละและลักษณะทางกายภาพของน้ำมันหอมระเหยที่สกัดได้ เตรียมเชื้ออะแคนทามิบาระยะซิสต์และโทรโฟซอइटจากการแยกเพาะเลี้ยงเชื้อบนอาหารวุ้นและอาหารเหลวตามลำดับ แล้วทดสอบฤทธิ์ฆ่าอะแคนทามิบาในหลอดทดลองของน้ำมันหอมระเหยแต่ละชนิด โดยทำที่ความเข้มข้น 50 มิลลิกรัมต่อมิลลิลิตร เมื่อเวลาผ่านไป 24 ชั่วโมง สังเกตการเปลี่ยนแปลงทางสัณฐานวิทยาของเซลล์ พร้อมบันทึกการมีชีวิตรอดและร้อยละการตายของเซลล์ภายใต้กล้องจุลทรรศน์

ผลการทดลอง พืชทั้งหมดให้ผลิตผลของน้ำมันหอมระเหยอยู่ในช่วง 0.3-6.7% (v/w) จากน้ำหนักแห้ง ในการทดสอบฤทธิ์ฆ่าอะแคนทามิบาพบว่าน้ำมันหอมระเหย 5 ใน 10 ชนิด ได้แก่ น้ำมันหอมระเหยจากติป्ली (*Piper retrofractum* Vahl.), พริกไทย (*P. nigrum* Linn.), มะกรูด (*Citrus hystrix* DC.), ขมิ้นชัน (*Curcuma longa* Linn.) และกระชาย (*Kaempferia pandurata* Roxb.) มีฤทธิ์ฆ่าอะแคนทามิบาในระยะโทรโฟซอइटได้ โดยพบอัตราการตายเท่ากับร้อยละ 100 ยิ่งไปกว่านั้นน้ำมันหอมระเหยจากติป्लीและมะกรูดยังมีฤทธิ์ฆ่าอะแคนทามิบาในระยะซิสต์ได้ โดยพบอัตราการตายเท่ากับร้อยละ 3.35 และ 43.62 ตามลำดับ

สรุป เป็นการรายงานครั้งแรกเกี่ยวกับฤทธิ์ฆ่าอะแคนทามิบาของน้ำมันหอมระเหยที่ทดสอบในการศึกษานี้ โดยเฉพาะน้ำมันหอมระเหยจากติป्लीและมะกรูดที่มีประสิทธิภาพสูงสุด โดยมีผลต่อทั้งสองระยะของอะแคนทามิบา ผลการศึกษานี้ถือเป็นความรู้พื้นฐานสำหรับการศึกษาต่อไปเกี่ยวกับฤทธิ์ฆ่าอะแคนทามิบาของน้ำมันหอมระเหยจากพืชที่สามารถนำไปพัฒนาและประยุกต์ใช้ในการรักษาโรคที่เกิดจากเชื้ออะแคนทามิบาซึ่งในปัจจุบันยังไม่มียารักษาที่ได้ผล **เชียงใหม่เวชสาร 2561;57(2):89-98.**

คำสำคัญ: *Acanthamoeba* sp., ฤทธิ์ฆ่าอะแคนทามิบา น้ำมันหอมระเหย ติป्ली มะกรูด