ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

Natural Products: Alternative Therapeutic Compounds Against *Acanthamoeba* spp.

Chin Ei Wei[1], Chong Yoke Yeng[1], Tooba Mahboob[2], Lim Chooi Ling[1], Chandramathi Samudi Raju[2], Sahapat Barusrux[3,4], Veeranoot Nissapatorn[3,4,5]

[1]*School of Health Sciences, International Medical University, Malaysia.*
[2]*Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*
[3]*School of Allied Health Sciences,* [4]*Research Excellence Center for Innovation and Health Products (RECIHP), and* [5]*World Union for Herbal Drug Discovery (WUHeDD), Walailak University, Nakhon Si Thammarat, Thailand.*

[*]For correspondence: nissapat@gmail.com OR veeranoot.ni@wu.ac.th
Veeranoot Nissapatorn

Abstract: *Acanthamoeba*, *Balamuthia*, *Naegleria* and *Sappinia* are infective free-living amoeba (FLA), pathogenic to both humans and animals. *Acanthamoeba* spp. and *Balamuthia mandrillaris* have similar life cycles as they only exist in 2 forms, cyst and trophozoites. Meanwhile, *Naegleria fowleri* exists in 3 forms: cysts, trophozoites and flagellated forms. Opportunistic *Acanthamoeba* spp. gain entry into body *via* the eyes, nasal passages and through ulcerated skin, which then leads to epithelial keratitis, ocular keratitis and granulomatous amebic encephalitis (GAE) especially among immune-compromised individuals. Despite available anti-*Acanthamoeba* medications, there have been recurrent reports of toxicity with cases of untreatable GAE. Hence, there is a current demand for safe and effective drugs. Research on plant extracts against *Acanthamoeba* found that they possess amoebicidal and cytocidal effects, with no or negligible toxicity towards normal human cell lines. Therefore, natural product research should explore further on the discovery of effective drugs for potential use in treating *Acanthamoeba* and other FLA infections.

Keywords: Acanthamoeba, amoebicidal, cytocidal, natural products, future treatment

Acanthamoeba is single-celled eukaryote with a total of 17 different identified genotypes (T1-T17). *Acanthamoeba* are often easily retrieved from soil, fresh water, swimming pools, tap water and even heating or air conditioning units. It was reported that 20% of hot springs in Iran were contaminated with thermo-tolerant *Acanthamoeba* of pathogenic T3 and T4 genotypes, with the ability to grow at 37°C, 42°C and even at high temperatures of up to 70°C (1). This finding can be further explained by the capability of the *Acanthamoeba* spp. trophozoites to transform into double-walled cyst form upon exposure to harsh conditions such as food deprivation, desiccation and extreme chemical conditions (2,3). Despite its ubiquity in open environments, it is rare to be

ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

infected by *Acanthamoeba* spp. as there been evidence supporting the presence of anti-*Acanthamoeba* antibodies amongst the healthy population. Hence, *Acanthamoeba* spp. infections have a low occurrence rate but can lead to severe implications and even death, especially among immune-compromised individuals. *Acanthamoeba* exist in 2 forms, vegetative trophozoites and dormant cysts with minimal metabolic activity. Both infective trophozoites and cysts could gain entry into the human body through the oculus, causing severe keratitis and even visual loss. Many studies reported that *Acanthamoeba* keratitis (AK) is highly associated with contact lens wear, along with its primary causative genotype, T4 (87%) and T3 (13%) (4,5). *Acanthamoeba* trophozoites are known to exhibit a strong adherence to contact lens surfaces due to the presence of protein and lipid deposits (6,7). It was reported that scratches present on the lenses' surface and inconsistent washing of contact lenses are contributing factors in hastening *Acanthamoeba* colonization (8).

Furthermore, *Acanthamoeba* can lead to granulomatous amoebic encephalitis (GAE) by invading the central nervous system *via* entry through the respiratory system or through haematogenous dissemination *via* open wounds. Occurrence of GAE is more common among immuno-compromised hosts, with it is almost 100% fatality rate. However, it was reported that a 64-year old woman who underwent a deceased donor kidney transplant was diagnosed with GAE, which turned out to be most likely acquired through environmental exposure and not a donor-derived infection (9). Among the various diagnostic tests available, loop-mediated isothermal amplification assay (LAMP) is the most recommended in terms of its high sensitivity and specificity and less time-consuming when compared to the PCR method (10,11). Other detection methods include non-nutrient agar culture and fluorescent microscopy. Practicing good lens care and personal hygiene play a major role in preventing AK. Unfortunately, there is currently no promising treatment for both AK and GAE, and the only follow-up therapy is to receive early treatment for AK in order to reduce long-term visual sequelae. Even though numerous therapeutic studies reported strong amoebicidal effects on trophozoites, the compounds ultimately failed to eradicate the cyst form of *Acanthamoeba.* The current major concern is to develop an efficient treatment of GAE, whereas AK is of less concern due to wide range of commercially available topical medications in the market. For example, miltefosine (molecular weight 407.57g/moL) has shown a strong response against *Acanthamoeba hatchetti* clinical isolates infecting Syrian hamsters (12). After 7 days of miltefosine, 45% of the AK-infected Syrian hamsters were observed with great corneal opacity improvement, from visible corneal opacity gradually reverting to complete translucency. However, no observable improvement was detected in 15% of experimented hamsters with both visible corneal opacity and obscure iris vessels. Zubeyde *et al.* (year) assessed the efficiency of combination treatment with propamidine isetionate (0.1%) with polyhexamide (0.02%). However, the results were not as promising as miltefosine due to its slow improvement in corneal clarity, which was only observed after 21 days. Despite miltefosine's effect in AK which is suggestive of a potential treatment for GAE, a case was reported in Texas whereby an 11-year-old child diagnosed with GAE was prescribed with 50 mg miltefosine (2 times daily) per nasogastric tube for only 26.5 days before termination of treatment (13). Discontinuation of miltefosine was due to progression of neurologic deterioration and development of multiple organ dysfunction, which then lead to the suspicion of drug toxicity. The boy was pronounced dead after 40 days. In addition, Debnath *et al.* (2014) conducted ultrastructural analysis using transmission electron microscopy in order to illustrate distortion of trophozoite cell wall after 200 μM corifungin incubation, which then lead to cyst lysis (14). The ideal treatment for GAE would be a drug with the ability to cross the blood-brain barrier (BBB) with its lipophilic properties, along with a low molecular weight of not more than 600 Da. Despite this, Acea Biotech Inc. (USA) announced their preparation for a human clinical trial following their accomplishment in animal studies on assessing efficacy and toxicity on mice infected with *Candida albicans*, *Leishmania donocani* and *Naegleria fowleri,* also a type of brain-eating amoeba. Biguanides are known for its antimicrobial and amoebicidal action against *Acanthamoeba* spp. However, Cecilia *et al*. (year) identified that biguanides, especially chlorhexidine (CLX), are less effective against clinical isolates of

ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

Acanthamoeba cysts compared to polyhexamethylene biguanide (PHMB) (15). Furthermore, susceptibility of *Acanthamoeba* cysts to these drugs were only statistically significant when both clinical and ATCC (American Type Culture Collection) isolates were incubated for 48 hours with a combination of 0.04% PHMB with 0.04% CLX. In terms of biguanides' cytotoxicity in Human Umbilical Vein Endothelial Cells (HUVECs), a concentration of 0.04% of both PHMB and CLX induced cell death by apoptosis and necrosis respectively. However, the cytotoxic effect of combined drugs (0.04% PHMB+CLX) seemed to be decreased compared to that of a single instillation of biguanides. After 12 hours of incubation with these 2 biguanides drugs and their association, a low viability of human corneal epithelial cells (HCECs) was observed at concentrations of 0.02% PHMB and 0.02% PHMB + 0.02% CLX. Hence, this suggests that an alternative therapy may consist of 0.02% PHMB + 0.02% CLX even though it may not guarantee a strong cytocidal effect against *Acanthamoeba* cysts. Nevertheless, 7 out of 15 commercialized multipurpose disinfecting solutions (MPDS) available in Korea failed to kill *Acanthamoeba castellanii* even after 24 hours of exposure. Although the remaining showed positive amoebicidal effects and 2 brands have cytocidal effects, the cytotoxicity tested on human corneal epithelial cells were reported to range from 56% to 62% within 4 hours of exposure (16).

One of the new formulations for MPDS is by combining multipurpose disinfecting solution (MPDSs) with 100 μM chloroquine (CQ), and this mixture exhibited amoebicidal effects on immature cysts by inhibiting the formation of mature cysts (17). Due to the unpredictable consequences of these topical therapies and undesired cytotoxic effects, finding alternative methods or cures using natural products has been a popular research theme due to its purported minimal or lack of adverse effects. (Table 1). This systematic review was conducted by using electronic literature resources include original research papers as well as other information obtained from the NCBI database, PubMed, publishers such as Elsevier, and other databases as mentioned in the Reference list. Soil amoebae from the *Acanthamoeba* family, such as *A. castellanii,* were reported to have an oxidative detoxification mechanism against low concentrations of hydrogen sulphide (H_2S), of which *A. castellanii* will absorb H_2S and release N_2 as its by-product in the gas stream of mass spectrometry (18). However, when *A. castellanii* was exposed to increasing partial pressure of H_2S (>17 Pa), it experienced complete respiratory inhibition, resulting in the accumulation of H_2S in solution. It was also reported that mitochondrial respiration of *A. castellanii* was arrested at 38 μmoL^{-1} H_2S (19). In 2010, Carlos *et al.* conducted an *in vitro* evaluation of *Allium sativum* (Common name: garlic) extract as a potential therapeutic drug for *Acanthamoeba* keratitis due to its high sulphur-containing compounds (20). Significant data reported that the amoebicidal effect of *Allium sativum* extract on both cyst and trophozoite stages of *A. castellanii* are both dose and time-dependent. However, the effective dose against *A. castellanii* cysts, 62.5 mg/mL, showed marked cytotoxicity on New Zealand white rabbit corneal cells, while 15.62 and 7.81 mg/mL exert mild cytotoxicity. The most ideal dose is 3.9 mg/mL as it was non-toxic on corneal cells but required a long exposure time (>72 hours) to achieve 100% destruction of *A. castellanii* cysts. The extraction procedure adapted by Akin Polat *et al.* (2008) is complicated, time-consuming (6 hours of extraction in Soxhlet with methanol) and the final extracted product is in solid form, which may affect the absorption rate in subjects.

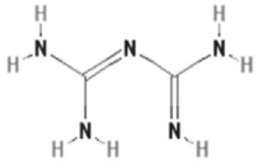
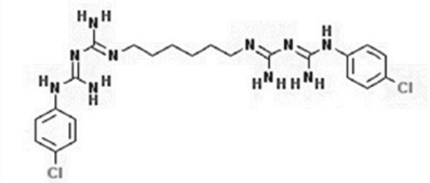
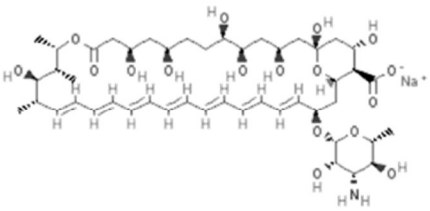
On the other hand, in the technique used by Satyal *et al* (2017), garlic extracts were obtained through hydro-distillation using the Clevenger apparatus for 3 hours. The pale yellow essential oil (liquid form) produced was further purified by decantation and dehydration with sodium chloride (21). Furthermore, Satyal *et al.* found that extracts of *Allium sativum* is composed of almost 99.3% sulphur-containing compounds, measured using Gas Chromatography-Mass Spectrometry (GCMS-QP2010 Ultra). In 2007, there was a study on the effectiveness of the methanolic extract of four *Allium* species against *Acanthamoeba castellanii* and its cytotoxicity on corneal cells *in vitro* (22). The *Allium* species used in this research are *A. sivasicum, A. dictyoprosum,*

ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

A. scrodoprosum subsp. rotundum, and *A. atroviolaceum*. As a result, only *A. scrodoprosum* subsp. *rotundum* showed amoebicidal effect towards *Acanthamoeba* trophozoites among the four plants and it failed to exert any cytotoxicity at the concentration of 32 mg/mL after one hour of treatment. Meanwhile, other *Allium* species did not show amoebidal effect towards *Acanthamoeba castellani* (22). Based on current literature, the bulb of *A. scrodoprosum* subsp. *Rotundum* has been found to have antibacterial, antifungal, antiviral and antioxidant properties (23) as well as amoebicidal effect towards *Acanthamoeba* (22). This plant also shows pharmacological properties such as antiseptic, hypotensive and diuretic effects (23).

Table 1 – Chemical drugs and their adverse effects with long-term use.

Drugs	Structural formula	Adverse effect
Polyhexamethylene Biguanide (PHMB) - Molecular formula: $C_8H_{19}N_5$ * - Molecular weight: 185.275 g/mol *		• Exhibit great cytotoxicity effect against HUVECs and HCECs. (15) • Insignificant ameobicidal effect at low percentage concentration.
Chlorhexidine (CLX) - Molecular formula: $C_{22}H_{30}Cl_2N_{10}$ * - Molecular weight: 505.452 g/mol *		• Can lead to chondrolysis of articular cartilage, dyspnea, urticarial and acute respiratory distress syndrome (a) • Ocular irritation was observed among chlorhexidine acetate-treated rabbits. (a)
Miltefosine - Molecular formula: $C_{21}H_{46}NO_4P$ * - Molecular weight: 407.576 g/mol *		• Teratogenicity, Stevensons-Johnson syndrome has been reported. (b) • Preclinical studies shown miltefosine can lead to both female and male infertility. (b)
Corifungin - Molecular formula: $C_{47}H_{72}NNaO_{17}$ * - Molecular weight: 946.073 g/mol *		• High molecular weight does not meet with the criteria for diffusing through BBB. • Hence, might not be effective in treating GAE.

*Source: National Center for Biotechnology Information. PubChem Compound Database; https://pubchem.ncbi.nlm.nih.gov
a. Source: HSBD. Record Name: Chlorhexidine. Available on: https://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+@rel+55-56-1
b. Source: DrugBank. Record Name: Miltefosine. Acessable from: https://www.drugbank.ca/drugs/DB09031
15. Mafra P, Carrijo-carvalho LC, Chudzinski-tavassi AM, Marques F, Taguchi DC, Foronda AS, et al. Antimicrobial Action of Biguanides on the Viability of Acanthamoeba Cysts and Assessment of Cell Toxicity. 2018.

The phenolic compounds found in this plant include gallic acid, chlorogenic acid and quercetin, which may bring about these medicinal properties (24). The *Lippia alba* plant of the Verbenaceae family and named after a French botanist Augustus Lippia, was found to be reactive against *Acanthamoeba* spp. Four flowering plants from the *Lippia* family (*L. alba, L. sidoides, L. gracilis, and L. pedunculosa*) was studied in 2015 to investigate their chemical components as well as their amoebicidal activity against *Acanthamoeba polyphaga* trophozoites, along with their cytotoxicity against the NCI-H292 human cell line (from pulmonary mucoepidermoid carcinoma). Among these, *L. gracilis and L. sidoides* exhibited greater inhibitory effects on *A.*

ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

polyphaga trophozoites at the concentration of 40 µg/mL, while the remaining two species, *L. alba* and *L. pedunculosa* failed to eliminate 100% of the trophozoites even at the highest concentration, 100 µg/mL (25).

These *Lippia* spp. extracts were analyzed using GC-MS and 4 major components were identified: carvacrol, carvone, rotundifolone and gamma-terpinene (depending on the species). Even though extracts of *Lippia* spp. exerted growth inhibition at 50 µg/mL, toxicity assays were not performed to assess its safety against normal human cell lines. Natural or unprocessed honey exhibited greater amoebistatic and amoebicidal effects compared to commercial honey (26). The concentration of natural honey used, 30% v/v, significantly induced reduction of amoebae numbers from control concentrations of $1x10^5$ to $5.8x10^3$ (94.2% decrease) after 24 hours of incubation. According to Khan *et al.* (2016), high phenolic and flavonoid content in natural honey is believed to play a role in its amoebicidal effect after observing no viable amoebae within 24 hours of incubation with peptone glucose yeast (PYG) medium, in contrast with glycerol and commercial honey-treated *A. castellanii*. Even though the effects of natural honey are promising, its phenolic and flavonoid content varies as according to the honeybee species and source of plant nectar, which are yet to be identified. Other concerns of using natural honey include contaminants coupled with its exposure to persistent organic pollutants (POPs) such as pesticides, insecticides, fungicides, herbicides and possible antibiotics used in bee farming (27-29).

The *Artemisia annua* plant, which is also commonly known as sweet wormwood or 'qinghao' in Mandarin has been grown commercially ever since its derivative, artemisinin had been found to be a potent antimalarial drug by Nobel Prize winner scientist Tu Youyou in 2015. Comparing natural honey with leave extracts of *Artemisia annua,* their phenolic contents are very similar (100 mg/g - 200 mg/g) but flavonoid concentration is much higher in extracts of *A. annua* leaves (600 mg/100 g) compared to that in natural honey (0.6 mg/g - 0.7 mg/g) (26,30). Derda *et al.* (2016) further discussed on extracts of *A. annua* leaves, and suggested artemisinin as potential therapy for acanthamoebiasis by examining the extracts on pathogenic *A. castellanii* strains (Ac32 & 309). Other than anticancer and antioxidant properties, artemisinin also tested positive in promoting amoebistatic and encystation, but its amoebicidal effect is yet to be determined. Furthermore, toxicity tests were carried out on both infected and non-infected mice, and results revealed that not only was artemisinin not toxic to non-infected mice, it even lengthened the survival period of infected mice by 3 to 4 times compared to non-treated infected mice (31). Therefore, natural product research groups should exploit the advantages of this plant extract as a possible suppressive drug toward GAE. In 2017, the oil derived from the Australian tea tree *Melaleuca alternifolia* leaves has been proven to exhibit amoebicidal and cytocidal effects on *Acanthamoeba castellanii* (24). Furthermore, tea tree oil was discovered to possess high content of 1-terpinen-4-ol, attributing to as much as 44.9% as reported by Instrumental Laboratories of Fritzsche Brothers, Inc.; New York (33). With its desirable antiseptic, anti-inflammatory, anti-fungal and antimicrobial properties, tea tree (*Melaleuca*) oil (TTO) has been greatly exploited in cosmetic and topical products (34-37). Greay *et al.* (2010) also demonstrated that TTO (42.4% of 1-terpinen-4-ol) exhibited anticancer effects on murine tumor cells lines (AE17 mesothelioma & B16 melanoma) in a dose-dependent manner. The reported IC_{50} values of TTO in fibroblast cells (L929 & HF32) after 24 hours is 0.1% and 0.08% respectively (38). In correlation with that, *Melaleuca alternifolia* TTO may be the most prospective natural remedy for cutaneous *Acanthamoeba* infections due to its ability to reduce cyst viability by 70-85% on the first day of exposure to 25 µL of TTO as previously reported (32). However, Bischoff *et al.* (1998) observed toxic effects in *Melaleuca* oil-treated cats after 24 hours including hypothermia, ataxia, dehydration, incoordination coupled with a two-fold increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, suggesting that *Melaleuca* oil may be hepatotoxic (39). Therefore, toxicity studies should be performed before endorsing its use as an amoebicidal, as there

ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

had been cases reported in regard to toxicosis after TTO ingestion. A 23-month-old and a 4-year old child experienced ataxia which progressed to unresponsiveness within 30 minutes after *Melaleuca* oil ingestion, although adverse effects were reversed by rehydration and consumption of activated charcoal, with removal of excess ingested TTO (40,41).

The flower extract of *Lonicera japonica* Thunb showed remarkable amoebicidal activity against *Acanthamoeba triangularis* trophozoites even at a low dosage of 0.5 mg/mL (42). However, the effect of *L. japonica* Thunb butanol fraction at 1.5 mg/mL dosage seemed to have nullified the effect against both trophozoites and cysts after 72 hours of incubation. Furthermore, the ethyl acetate fraction of *L. japonica* at the concentration of 1.5 mg/mL stimulated cyst growth at 8.2%, while inhibiting trophozoite growth by 48.9%. In comparison with *Pericampylus glaucus* (Lam.) Merr. extracts, betulinic acid (triterpene) and periglaucine A. (alkaloid) at the concentration of 100 µg/mL were found to exhibit acanthamoebicidal activity at a level similar to that of 4 µg/mL CLX (43). There is also another study showing the chemotherapeutic properties of the plants towards *Acanthamoeba*. These plants, namely *Rubus chamaemorus*, *Pueraria lobata*, *Solidago virgaurea* and *Solidago graminifolia*, showed chemotherapeutic properties which may be used in a combined treatment with antibiotics to treat *Acanthamoeba* infections. In this study, the experimentally-infected animals showed longer survival periods compared to the control group. However, these plant extracts didn't show amoebicidal, rather only amoebistatic properties which extended the animal's survival period. Besides, the plant extracts did not show any toxic effect towards the animals (44). In the study, *Rubus chamaemorus* leaf extract was found to contain ellagic acid as the main phenolic compound (44). Based on available literature, *Rubus chamaemorus* fruit and leaf extracts have been shown to have antimicrobial activity against many different bacterial strains including *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis* (45,46). *R. chamaemorus* is known as cloudberry and is commonly found in Northern countries of Europe, Asia and America (46). Meanwhile *Pueraria lobate* is often used in traditional Chinese medicine, as the root can be used to treat diseases like fever, diabetes and cardiovascular diseases. Besides, flower of *P. lobate* can be used to treat alcohol intoxication, dysentery and contain antioxidant, antidiabetic, anti-stroke and hypoglycemic properties (46). The beneficial chemical constituents include isoflavones, puerarin and triterpenoids (46,47). Another medicinal plant, *Solidago virgaurea*, also known as Goldenrod, is traditionally used to treat urinary tract inflammation. Studies have shown that this plant possess antimicrobial, antibacterial, antifungal, antitumor and anti-inflammatory properties. It contains phenolic constituents such as lipophilic flavonoids (44), polyphenolic acids, gallic acid and clerodane diterpenes (48). In 2011, a study was done to evaluate the *in vitro* amoebicidal activity of the methanolic extracts using *Teucrium polium* and *T. chamaedrys* (49) The results revealed that the plant extracts exerted amoebidal effects on the trophozoites and cysts (49). To be specific, *T. chamaedrys* plant extracts showed the amoebicidal effect on the trophozoites as there is no trophozoites observed within 48 hours with extract concentrations of 16 mg/mL or above. At the concentration of 16 g/mL, the extracts killed 18% of the total cysts at the end of the experiment. Meanwhile *T. polium* was found to show toxicity towards *Acanthamoeba* trophozoites at the concentration of 32 mg/ml within 48 hours. It also showed amoebicidal effect towards the cysts with the same concentration (49). The *Teucrium* genus has about 300 species distributed all around the world (50). Some *Teuricum* species are used traditionally as diuretics and in the treatment of gout, stomach pain and asthma. For instance, *T. polium* has been found to show anti-tumor, antimicrobial, antioxidant and hypoglycaemic properties (50-53). Phenolic compounds isolated from this plant include glycosides, flavonoids and poliumosides (50,53). Meanwhile, *T. chamaedrys* possesses strong antioxidant and antimicrobial properties which has been used to treat abscesses, cellulite and digestive disorders (54,55). Flavonoids are the phenolic component that can be found in most plants and it exerts a strong antioxidant effect which can inhibit the oxidation of molecules (54).

ASIAN JOURNAL OF PHARMACOGNOSY Review
Asian J. Pharmacogn 3(2):29-38

eISSN-0128-1119

In 2012, extracts of four *Peucedanum* species (*P. caucasicum, P. palimbioides, P. chryseum, and P. longibracteolatum*) were used to evaluate the amoebicidal effects towards *Acanthamoeba castellanii* cysts and trophozoites. As a result, *P. longibracteolatum* possess the strongest effect among the four extracts. There were no trophozoite or cyst found in the 32 mg/mL extract group within 72 hours (56). The genus *Peucedanum* has more than 120 species distributed in Asia, North America, Africa and Europe (57). It is used as folk medicine to treat gastrointestinal disorders, headaches, epilepsy and sore throat. The *Peucedanum* species has been found to contain bioactive substances including flavonoids, phenolic acids, diterpenes and praeruptorins (57,58). Praeruptorins was found to have anti-inflammatory, hepatoprotective, antiasthma and anti-tumor properties (58,59). According to an existing literature, the *Peucedanum* species has also been found to exert an amoebicidal effect on the trophozoites of *Entamoeba histolytica* (60) (Table 2). Active components of natural products which have been studied to be effective against *Acanthamoeba* spp. are phenolic compounds, terpenes (terpinen-4-ol, gamma-terpinene), flavonoids, carvacrol, carvone, rotundifolone and high sulphur-content natural products. More natural product extracts should be evaluated for their possible potential as future alternative treatments against free-living *Acanthamoeba spp.* Alternative drug delivery methods should be introduced to offer superior bioavailability and minimal toxicity in comparison to oral and intravenous routes. For instance, utilizing nanotechnology in drug delivery, especially for high-sulphur content natural products such as garlic, minimizes its cytotoxic effects while enhancing its penetrative power into both trophozoite and cystic stages of *Acanthamoeba*.

Table 2 – Natural products with amoebicidal effect against the cyst and trophozoite stages of *Acanthamoeba* spp.

Natural Products	**Major composition**	**Research findings/Drawbacks**
Allium sativum	Sulphur-containing compounds (99.3%)	Amoebicidal effect against cyst and trophozoites of *A. castellanii*. Drawbacks: garlic extract shown to have cytotoxicity effect towards rabbit corneal cells. (20)
Allium scrodoprosum subsp. Rotundum	Gallic acid, Chlorogenic acid and quercetin	Amoebicidal effect against trophozoites of *A. castellanii.* (22)
Lippia alba	Carvacrol, carvone, rotundifolone and gamma-terpinene (depending on specific species)	Minimum inhibitory concentration (MIC) = 40µg/mL Drawbacks: 1. High extract concentration of *L. alba* and *L. pedunculosa* does not inhibit trophozoites growth. (25) 2. None toxicity assay was done to access *Lippia spp.* extracts against human cell line.
Natural honey	High phenolic and flavonoid content	Induces growth inhibition by 94.2% after incubation period of 24hours. (26) Drawbacks: Phenolic and flavonoid content may vary; depending on its environment.
Artemisia annua	Artemisinin	Promote amoebistatic and encystation. Lengthen survival period of *Acanthamoeba* infected mice while no adverse effect observed in healthy mice. (31) Drawback: No amoebicidal effect yet to be observed.
Melaleuca alternifolia	1-terpinen-4-ol; 44.9% (v)	Portrait cysticidal effect by 70-85% on first 24hours of exposure with 25µL of tea tree oil. Drawback: Mild adverse effect was observed, such as dehydration, hypothermic and also elevated serum ALT and AST. (39)

eISSN-0128-1119

Lonicera japonica Thunb	Chlorogenic acid ($C_{16}H_{18}O_9$)	MIC against *A. triangularis* = 0.5mg/mL Drawback: Long incubation period of *L. japonica* extract exert low growth inhibition. (42)
Pericampylus glaucus (Lam.) Merr.	Betulinic acid (triterpene), periglaucine A. (alkaloid)	It exhibits acanthamoebicidal power similar with 4 µg/mL CLX. (43)
Rubus chamaemorus	Ellagic acid	It shows amoebistatic properties by extended survivor period of animals while no toxic effect was observed. (44)
Pueraria lobata	Isoflavones, puerarin and triterpenoids	It possesses amoebistatic effect by lengthen animal's survival period while no adverse effect was observed. (44)
Solidago virgaurea	Flavonoids, polyphenolic acids and gallic acid	It shows amoebistatic properties by extended survivor period of animals while no toxic effect was observed. (44)
Teucrium polium	Glycosides, flavonoids and poliumoside	It shows amoebicidal properties towards trophozoites of *A. castellanii* in the concentration of 32mg/mL within 48 hours. It shows amoebicidal effect towards cysts in the concentration of 32 mg/mL at the end of the experimental process. (49)
Teucrium chamaedrys	Flavonoids	It possesses amoebicidal effect towards trophozoites of *A. castellanii* in the concentration of 16mg/mL within 48 hours. It shows amoebicidal effect towards cysts in the concentration of 16 mg/mL at the end of the experimental process. (49)
Peucedanum species	Flavonoids, phenolic acids, diterpenes and praeruptorins	It shows amoebicidal action on *A. castellanii* trophozoites and cysts. (56)

ACKNOWLEDGMENT

The authors would like to extend their gratitude to Assoc. Prof. Dr. Veeranoot Nissapatorn and Dr. Tooba Mahboob for their guidance and support during the preparation of the manuscript. We also credit Walailak University, Thailand and International Medical University (IMU), Malaysia for their hospitality and usage of their electronic databases.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Solgi R, Niyyati M, Haghighi A, Taghipour N, Tabaei SJS, Eftekhar M, et al. Thermotolerant *Acanthamoeba* spp. Isolated from therapeutic hot springs in northwestern Iran. J Water Health 2012; 10(4): 650-6.
2. Sriram R, Shoff M, Booton G, Fuerst P, Visvesvara GS. Survival of *Acanthamoeba* cysts after desiccation for more than 20 years. J Clin Microbiol 2008; 46(12): 4045–8.
3. Sawyer TK, Lewis EJ, Galassa M, Lear DW, O'Malley ML, Adams WN, et al. Pathogenic amoebae in ocean sediments near wastewater sludge disposal sites. J Water Pollut Control Fed 1982; 54(9): 1218–323.
4. Booton GC, Joslin CE, Shoff M, Tu EY, Kelly DJ, Fuerst PA. Genotypic identification of *Acanthamoeba* sp. isolates associated with an outbreak of *Acanthamoeba* keratitis (AK). Cornea 2009; 28(6): 673-6.
5. Cope JR, Collier SA, Rao MM, Chalmers R, Mitchell GL, Richdale K, et al. Contact lens wearer demographics and risk behaviors for contact lens-related eye infections--United States, 2014. MMWR Morb Mortal Wkly Rep 2015; 64(32): 865–70.
6. Jones L, Senchyna M, Glasier M-A, Schickler J, Forbes I, Louie D, et al. Lysozyme and lipid deposition on

eISSN-0128-1119

silicone hydrogel contact lens materials. Eye Contact Lens 2003; 29(1 Suppl): S75-9.
7. Simmons PA, Tomlinson A, Connor R, Hay J, Seal D V. Effect of patient wear and extent of protein deposition on adsorption of *Acanthamoeba* to five types of hydrogel contact lenses. Optom Vis Sci 1996; 73(6): 362–8.
8. Sehgal R, Saini J, Singh KD, Bhatti HS. *Acanthamoeba* adherence to soft contact lens and human corneal stroma. Indian J Pathol Microbiol 2002 ; 45(1): 63–7.
9. Salameh A, Bello N, Becker J, Zangeneh T. Fatal granulomatous amoebic encephalitis caused by *Acanthamoeba* in a patient with kidney transplant: A case report. OFID (Oxford University Press). 2015.
10. Ge Z, Qing Y, Zicheng S, Shiying S. Rapid and sensitive diagnosis of *Acanthamoeba* keratitis by loop-mediated isothermal amplification. Clin Microbiol Infect 2013;19(11): 1042–8.
11. Mewara A, Khurana S, Yoonus S, Megha K, Tanwar P, Gupta A, et al. Evaluation of loop-mediated isothermal amplification assay for rapid diagnosis of *Acanthamoeba* keratitis. Indian J Med Microbiol 2017; 35(1): 90–4.
12. Ak Z, Obwaller A, Vural A, Walochnik J. Efficacy of miltefosine for topical treatment of *Acanthamoeba* keratitis in Syrian hamsters. Parasitol Res 2012;110(2): 515-20.
13. Roy SL, Atkins JT, Gennuso R, Kofos D, Sriram RR, Dorlo TPC, et al. Assessment of blood-brain barrier penetration of miltefosine used to treat a fatal case of granulomatous amebic encephalitis possibly caused by an unusual *Balamuthia mandrillaris* strain. Parasitol Res 2015 ;114(12): 4431-9.
14. Debnath A, Tunac JB, Silva-Olivares A, Galindo-Gómez S, Shibayama M, McKerrowa JH. *In vitro* efficacy of corifungin against *Acanthamoeba castellanii* trophozoites and cysts. Antimicrob Agents Chemother 2014; 58:1523–8.
15. Mafra P, Carrijo-carvalho LC, Chudzinski-tavassi AM, Marques F, Taguchi DC, Foronda AS, et al. Antimicrobial action of biguanides on the viability of *Acanthamoeba* cysts and assessment of cell toxicity. Invest Ophthalmol Vis Sci 2013; 54(9): 6363-72.
16. Moon E-K, Park H-R, Quan F-S, Kong H-H. Efficacy of Korean multipurpose contact lens disinfecting solutions against *Acanthamoeba castellanii*. Korean J Parasitol 2016; 54(6): 697-702.
17. Moon E-K, Lee S, Quan F, Kong H-H. Chloroquine as a possible disinfection adjunct of disinfection solutions against *Acanthamoeba*. Vol. 188, Exp Parasitol 2018; 188:102-106.
18. Lloyd D, Kristensen B, Degn H. Oxidative Detoxification of hydrogen sulphide detected by mass spectrometry in the soil amoeba *Acanthamoeba castellanii*. J Gen Microbiol 1981; (126): 167–70.
19. Vaquer-Sunyer R, Duarte CM. Sulfide exposure accelerates hypoxia-driven mortality. Limnol Oceanogr 2010; 55(3): 1075–82.
20. Polat ZA, Vural A, Ozan F, Tepe B, Özcelik S, Cetin A. *In vitro* evaluation of the amoebicidal activity of garlic (*Allium sativum*) extract on *Acanthamoeba castellanii* and its cytotoxic potential on corneal cells. J Ocul Pharmacol Ther 2008; 24(1): 8–14.
21. Satyal P, Craft JD, Dosoky NS, Setzer WN. The chemical compositions of the volatile oils of garlic (*Allium sativum*) and wild garlic (*Allium vineale*). Foods (Basel, Switzerland). 2017; 6(8). pii: E63.
22. Polat ZA, Vural A, Tepe B, Cetin A. *In vitro* amoebicidal activity of four *Allium* species on *Acanthamoeba castellanii* and their cytotoxic potentials on corneal cells. Parasitol Res 2007; 101(2):397-402.
23. Tasci B, Koca I. Use of *Allium scorodoprasum* L. subsp. *rotundum* as food. Acta Hortic 2016; (1143):153–8.
24. Mollica A, Zengin G, Locatelli M, Picot-Allain CMN, Mahomoodally MF. Multidirectional investigations on different parts of *Allium scorodoprasum* L. subsp. rotundum (L.) Stearn: Phenolic components, *in vitro* biological, and in silico propensities. Food Res Int 2018; 108:641-649.
25. Santos IG de A, Scher R, Rott MB, Menezes LR, Costa EV, Cavalcanti SC de H, et al. Amebicidal activity of the essential oils of *Lippia* spp. (Verbenaceae) against *Acanthamoeba polyphaga* trophozoites. Parasitol Res 2016;115(2): 535-40.
26. Khan NA. Antiacanthamoebic properties of natural and marketed honey in Pakistan. Asian Pac J Trop Biomed 2016; 6(11): 967–72.
27. Zawislak J (University of ASD of A. New FDA rules affect beekeeper access to antibiotics [Internet]. 2017 [cited 2018 Aug 24].
28. Roszko MŁ, Kamińska M, Szymczyk K, Jędrzejczak R. Levels of selected persistent organic pollutants (PCB, PBDE) and pesticides in honey bee pollen sampled in Poland. PLoS One. 2016; 11(12): 1-22.
29. Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ. Antibiotic, Pesticide, and microbial contaminants of honey: Human Health Hazards. Sci World J 2012; 2012(Table 1):1–9.
30. Iqbal S, Younas U, Chan KW, Zia-Ul-Haq M, Ismail M. Chemical composition of *Artemisia annua* L. leaves and antioxidant potential of extracts as a function of extraction solvents. Molecules 2012;17(5):6020–32.
31. Derda M, Hadaś E, Cholewiński M, Skrzypczak Ł, Grzondziel A, Wojtkowiak-Giera A. *Artemisia annua* L. as a plant with potential use in the treatment of acanthamoebiasis. Parasitol Res 2016;115(4):1635–9.
32. Hadaś E, Derda M, Cholewiński M. Evaluation of the effectiveness of tea tree oil in treatment of *Acanthamoeba* infection. Parasitol Res 2017;116(3): 997–1001.
33. Swords G, Hunter GLK. Composition of Australian tea tree oil (*Melaleuca alternifolia*). J Agric Food Chem 1978 1;26(3):734–7.
34. Carson CF, Hammer KA, Riley T V. *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 2006;19(1): 50–62.
35. Li W-R, Li H-L, Shi Q-S, Sun T-L, Xie X-B, Song B, et al. The dynamics and mechanism of the antimicrobial activity of tea tree oil against bacteria and fungi. Appl Microbiol Biotechnol. 2016 Oct;100(20):8865–75.

eISSN-0128-1119

36. Nenoff P, Haustein UF, Brandt W. Antifungal activity of the essential oil of *Melaleuca alternifolia* (tea tree oil) against pathogenic fungi *in vitro*. Skin Pharmacol. 1996;9(6):388–94.
37. May J, Chan CH, King A, Williams L, French GL. Time–kill studies of tea tree oils on clinical isolates. J Antimicrob Chemother. 2000;45(5):639–43. Available from: http://dx.doi.org/10.1093/jac/45.5.639
38. Greay SJ, Ireland DJ, Kissick HT, Levy A, Beilharz MW, Riley T V., et al. Induction of necrosis and cell cycle arrest in murine cancer cell lines by *Melaleuca alternifolia* (tea tree) oil and terpinen-4-ol. Cancer Chemother Pharmacol. 2010;65(5):877–88.
39. Bischoff K, Guale F. Australian tea tree (*Melaleuca alternifolia*) oil poisoning in three purebred cats. J Vet Diagnostic Investig 1998; 10(2): 208–10.
40. Jacobs MR, Hornfeldt CS. Melaleuca oil poisoning. J Toxicol Clin Toxicol 1994; 32(4): 461-4.
41. Morris MC, Donoghue A, Markowitz JA, Osterhoudt KC. Ingestion of tea tree oil (*Melaleuca* oil) by a 4-year-old boy. Pediatr Emerg Care 2003;19(3):169-71.
42. Mahboob T, Azlan A, Tan T, Samudi C, Sekaran SD, Nissapatorn V, et al. Anti-encystment and amoebicidal activity of *Lonicera japonica* Thunb. and its major constituent chlorogenic acid *in vitro*. Asian Pac J Trop Med 2016;9(9):866–71.
43. Mahboob T, Azlan A, Natalia F, Boonroumkaew P, Sya N, Azman N, et al. Acanthamoebicidal activity of periglaucine A and betulinic acid from *Pericampylus glaucus* (Lam.) Merr. *in vitro*. Exp Parasitol 2017; 183: 160–6.
44. Derda M, Hadaś E, Thiem B. Plant extracts as natural amoebicidal agents. Parasitol Res. 2009;
45. Thiem B, Goślińska O. Antimicrobial activity of *Rubus chamaemorus* leaves. Fitoterapia. 2004;75(1):93–5.
46. Rauha J-PP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavoniods and other phenolic compounds. Int J Food Microbiol . 2000;56(1):3–12. Available from: file://g/Library/Reference Manager/Papers/1564. Int J Food Microbiol 2000; 56, 3-12.
47. Wong KH, Li GQ, Li KM, Razmovski-Naumovski V, Chan K. Kudzu root: Traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases. J Ethnopharmacol 2011; 134(3): 584–607.
48. Starks CM, Williams RB, Goering MG, O'Neil-Johnson M, Norman VL, Hu JF, et al. Antibacterial clerodane diterpenes from Goldenrod (*Solidago virgaurea*). Phytochem 2010; 71(1): 104-9.
49. Tepe B, Malatyali E, Degerli S, Berk S. *In vitro* amoebicidal activities of T*eucrium polium* and *T. chamaedrys* on *Acanthamoeba castellanii* trophozoites and cysts. Parasitol Res. 2012; 110(5):1773-8.
50. De Marino S, Festa C, Zollo F, Incollingo F, Raimo G, Evangelista G, et al. Antioxidant activity of phenolic and phenylethanoid glycosides from *Teucrium polium* L. Food Chem 2012;133(1):21-8.
51. Esmaeili MA, Yazdanparast R. Hypoglycaemic effect of *Teucrium polium*: Studies with rat pancreatic islets. J Ethnopharmacol 2004; 95(1): 27–30.
52. Darabpour E, Motamedi H, Nejad SMS. Antimicrobial properties of *Teucrium polium* against some clinical pathogens. Asian Pac J Trop Med 2010; 3(2): 124–7.
53. Elmasri WA, Hegazy MEF, Mechref Y, Paré PW. Structure-antioxidant and anti-tumor activity of *Teucrium polium* phytochemicals. Phytochem Lett 2016; 15:81-7.
54. Kadifkova Panovska T, Kulevanova S, Stefova M. *In vitro* antioxidant activity of some *Teucrium* species (Lamiaceae). Acta Pharm. 2005;55:207-14.
55. Vlase L, Benedec D, Hanganu D, Damian G, Csillag I, Sevastre B, et al. Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. Molecules. 2014;19(5):5490–507.
56. Malatyali E, Tepe B, Degerli S, Berk S, Akpulat HA. *In vitro* amoebicidal activity of four *Peucedanum* species on *Acanthamoeba castellanii* cysts and trophozoites. Parasitol Res. 2012; 110(1):167-74.
57. Sarkhail P. Traditional uses, phytochemistry and pharmacological properties of the genus *Peucedanum*: A review. J Ethnopharmacol 2014;156:235–70.
58. Sarkhail P, Shafiee A, Sarkheil P. Biological activities and pharmacokinetics of praeruptorins from *Peucedanum* species: A systematic review. Biomed Res Int. 2013; 2013: 343808
59. Schillaci D, Venturella F, Venuti F, Plescia F. Antimicrobial and antiproliferative activity of *Peucedanum nebrodense* (Guss.) Strohl. J Ethnopharmacol 2003; 87(1): 99-101.
60. Degerli S, Tepe B. Phenolic acid composition and anti-parasitic effects of four *Peucedanum* species on *Entamoeba histolytica* trophozoites. Iran J Parasitol 2015;10(3): 420–31.