



Antiparasitic activity of *Loranthus europaeus* Jacq. against *Acanthamoeba* sp.: An *in vitro* study

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Abstract

Acanthamoeba keratitis (AK), caused by species of the genus *Acanthamoeba*, has become more frequently reported in recent years, particularly among contact lens users and immunocompromised individuals. Although the disease is rare, it can cause blindness; treatment resistance and limited access to care are growing concerns. This study evaluated the antiparasitic activity of *Loranthus europaeus* Jacq. hexane extract against trophozoite and cyst stages of *Acanthamoeba* sp. and identify its chemical constituents by gas chromatography-mass spectrometry (GC-MS). Serial extract concentrations (1.25, 2.5, 5 and 10 mg/mL) were applied to trophozoite and cyst suspensions for 2, 4 and 6 min and viability was assessed microscopically using trypan blue exclusion. The extract demonstrated a clear concentration and time-dependent reduction in viability (two-way analysis of variance (ANOVA), $p < 0.001$), reaching 0.33 ± 0.58 % at the highest concentration and longest exposure for both stages. IC_{50} values ranged from 0.93–3.03 mg/mL across exposure times. The negative control showed no effect, whereas polyhexamethylene biguanide (PHMB, 0.02 %) reduced viability to a residual 30.67 ± 1.15 % at 6 min for both stages. The GC-MS analysis revealed a triterpenoid-rich profile 47.72 % dominated by Lup-20(29)-en-3 β -ol, acetate 42.06 % followed by sesquiterpene longipinane, germacrene D and caryophyllene. The GC-MS identifications further confirmed by retention index matching to published values. These findings point to preliminary, *in vitro* antiparasitic activity of *L. europaeus* extract against *Acanthamoeba* sp. an effect that may be linked to the terpenoid compounds detected in the extract. Before any translational application can be considered, further work is needed to purify the active compounds, clarify how they act and confirm the extract's safety for corneal cells.

Keywords: *Acanthamoeba*; antiparasitic; extract; *Loranthus europaeus*; trophozoites

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1. Introduction

Loranthus europaeus Jacq. is a hemiparasitic plant that grows on the branches of trees primarily parasitising oak and chestnut species in sunny places (1, 2). This species belongs to the family Loranthaceae and it is distributed in Anatolia, Europe, Iran, Iraq and Russia (3). The synonym of *L. europaeus* is *Viscum guernum* Rchb, while it is commonly known as Hib el-debgh and Fulful Hawa in Iraq and yellow mistletoe in Europe due to yellow colour of its berry fruits (2–5). The fruits have traditionally been used in Iraqi folk medicine for treating boils. People traditionally chew the dried fruit, which remains viscous even after drying, to form a pad with mouth moisture (4, 5).

Pharmacological studies have demonstrated several biological activities of *L. europaeus* extract, including antioxidant, cytotoxic activity (against AMJ13 breast cancer cells), enzyme-inhibitory (α -amylase, tyrosinase, cholinesterases), antifungal and antibacterial effects (6–8).

The genus *Acanthamoeba* is an opportunistic free-living amoeba with trophozoite and cyst life stages. Both stages serve as natural hosts for bacteria and other microorganisms to remain viable and even promote their virulence and infectiousness in humans (9).

Four species of *Acanthamoeba* can cause ocular keratitis *Acanthamoeba* keratitis (AK) and granulomatous amoebic encephalitis (GAE) especially in immunodeficient patients (10). The AK is increasingly reported among contact lens wearers and, although rare, can lead to corneal inflammation, scleritis and necrosis or blindness if not promptly treated (9, 11).

Although polyhexamethylene biguanide (PHMB) at 0.02 % is the prescribed treatment against both stages of *Acanthamoeba* (11), its use has been associated with adverse effects, including necrosis, death and loss of integrity of the plasma membrane of epithelial cells (12). Additionally, toxic keratopathy of the anterior and posterior corneal layers may occur at any stage of treatment and may require a major modification to the treatment strategy (13, 14). Finally, due to the robust cyst-stage walls, effective treatment needs several

months and infected tissues may develop resistance to the treatment, eventually requiring corneal scraping or keratoplasty (14, 15).

Anti-*Acanthamoeba* activity has been demonstrated by several plant-derived compounds and extracts in recent studies (16, 17). For example, *Iris setosa* extract showed amoebicidal effect against *Acanthamoeba castellanii* and *Acanthamoeba polyphaga*, alongside low cytotoxicity to human corneal cells (18). *Phragmites australis* extract has also been shown to induce amoebicidal, apoptosis-like cell death in *A. castellanii* trophozoites (19). *Trigonella foenum-graecum* L. seed extract exhibited anti-*Acanthamoeba* activity with favorable safety toward corneal-cells (20). These findings support plant-derived agents as a source of novel anti-*Acanthamoeba* therapeutics. Given the significant adverse effects associated with PHMB, the current mainstay of AK treatment, this study was designed to evaluate, *in vitro*, the antiparasitic effect of *L. europaeus* against the trophozoite and cyst stages of *Acanthamoeba* sp.

2. Materials and Methods

Plant sample collection

The plant materials were purchased from local markets in central Basra Governorate in January 2026 as a single batch. The material was authenticated by a botany specialist Eman M. A. Al-Rubaie, who identified it as *L. europaeus* (Fig. 1). A voucher specimen was prepared and deposited in the Herbarium of BASRAH (College of Science, University of Basrah) under the accession number 105 (2026) BAS.



Fig. 1. Dried *Lanthanum europaeus* fruits.

Extraction procedure

Fifteen grams of dried *L. europaeus* fruit powder was placed into a thimble, which was loaded inside the Soxhlet extractor and extracted with hexane at standard reflux temperature (about 69 °C) for 20 hr. Hexane was selected as a standard solvent for preliminary phytochemical screening, expected to selectively recover non-polar constituents, several classes of which have documented antiparasitic activity. The solvent was removed using a rotary evaporator and the resulting residue was dried to yield 3.25 g of extract (21.6 % w/w). The dried extract was stored at 4 °C until use.

For testing, the extract was first reconstituted in 20 % dimethyl sulfoxide (DMSO), producing a stock concentration of 20 mg/mL. Working concentrations (2.5, 5, 10 mg/mL) were prepared by serial dilution of this stock using the same 20 % DMSO vehicle, ensuring a constant final DMSO concentration across all tested conditions. Complete dissolution was achieved through vortex-mixing and sonication, followed by visual inspection to verify the absence of undissolved particles. The extract was then sterilised by 0.22 µm membrane filtration and showed no precipitation or colour change during short-term storage, indicating acceptable short-term stability.

Isolation and culture of *Acanthamoeba* sp.

Samples were collected prospectively from consecutive AK-suspected patients presenting to Basrah Teaching Hospital during the study period (12 January–30 March 2026). Sampling was performed under local anesthesia by an ophthalmologist using a sterile cotton swab, which was rolled gently across the affected corneal lesion from one side to the other. No corneal scrapings or conjunctival swabs were used in this study (21). Ethical approval for this study was waived by the Research and Ethical Review and Approval Committee, Department of Biology, College of Science, University of Basrah (Ref. Bio 26.35). Written informed consent was obtained from all adult participants prior to sampling.

Diagnostic criteria, inclusion and exclusion criteria and patient characteristics

Patients were included only if they presented with clear clinical features strongly suggestive of AK, including severe ocular pain, photophobia, tearing and a characteristic ring-shaped stromal infiltrate (11). Inclusion criteria consisted of adults presenting with acute keratitis symptoms and visible corneal epithelial defects consistent with AK who consented to sampling.

Patients were excluded if they had confirmed bacterial, fungal, or viral keratitis; a history of recent ocular trauma or surgery or declined sampling. Bacterial keratitis was excluded by gram staining and culture on blood and chocolate agar; while fungal keratitis was excluded via potassium hydroxide (KOH) wet mount and culture on Sabouraud dextrose agar. Exclusion of viral keratitis was based on clinical findings, specifically the absence of dendritic or geographic lesions (22). All sampled individuals were symptomatic AK-suspected patients presenting to ophthalmology clinics; no asymptomatic individuals or healthy controls were included.

Prior treatment

None of the patients had received anti-*Acanthamoeba* therapy prior to sampling. Some had received empirical topical antibiotics, which do not eliminate *Acanthamoeba* and therefore do not interfere with microscopic detection.

Diagnostic confirmation and exclusion of other infections

Corneal swabs were inoculated onto non-nutrient agar (NNA) plates pre-seeded with heat-killed *Escherichia coli*. Definitive diagnosis was established by identifying active trophozoites and mature double-walled cysts under light microscopy, which are diagnostic for *Acanthamoeba* infection. Bacterial and fungal keratitis were excluded as described above. The direct isolation of viable *Acanthamoeba* trophozoites and cysts from symptomatic corneal lesions served as the primary etiological confirmation, even in the presence of incidental bacterial flora.

Culture preparation for bioassays

A total of 18 clinical samples tested positive for *Acanthamoeba* sp. based on microscopic criteria. To establish culture strains for the *in vitro* bioassays, all 18 clinical isolates were repeatedly subcultured and propagated on non-nutrient agar (NNA) plates pre-seeded with a lawn of heat-killed *E. coli* at 28 °C under monoxenic conditions. Organisms harvested from these active subcultures were subsequently utilised in the viability and membrane integrity assays.

All 18 microscopy-confirmed clinical isolates supplied the organisms used in the bioassays. Each isolate was cultured and maintained independently under monoxenic conditions and no pooling of isolates was performed. All 18 isolates were tested separately to capture biological variability among patient-derived strains. Although the specific isolate contributing to each replicate was not recorded, each replicate represented an independent biological sample drawn from the pool of 18 isolates. No reference strain was used in this study.

Morphological examination and classification of *Acanthamoeba* sp.

The wet-mount method was used to examine and classify the morphology of *Acanthamoeba* sp. A suspension of 100 µL was mixed with one drop of methylene blue and spread onto a glass slide, then observed under light microscope after covering the slide with a coverslip (23).

The identification of isolated *Acanthamoeba* sp. was performed based on detailed morphological characterisation of mature cysts using standard taxonomic keys (24, 25). Under light microscopy the isolated cysts displayed the defining diagnostic features of Group II a distinct double-walled structure consisting of a heavily wrinkled undulated outer ectocyst (EC) and a sharply polygonal, stellate inner endocyst (EN) with 4–6 prominent angular rays contacting the outer layer (Fig. 2). This group II morphology was consistently observed across all 18 clinical isolates examined in this study, with no morphological variation noted between isolates.

Cyst suspensions used in the bioassays reflected a natural clinical isolate population and were not restricted to fully mature cysts exclusively; light microscopic examination confirmed that > 90 % of the suspension consisted of fully mature cysts, identified by a complete, well-defined double wall.

Counting *Acanthamoeba* sp.

To collect the protozoa from the culture medium, cells were washed twice with sterile phosphate-buffered saline (PBS) (17.8 g of Na₂HPO₄, 2.4 g of KH₂PO₄, 80 g of NaCl and 2 g of potassium chloride (KCl) in 1 L of distilled water (DW)) (26). Following centrifugation at 2000 rpm for 5 min, the suspension was enumerated using a Neubauer haemocytometer under a microscope. The average count was multiplied by 10⁴/mL.

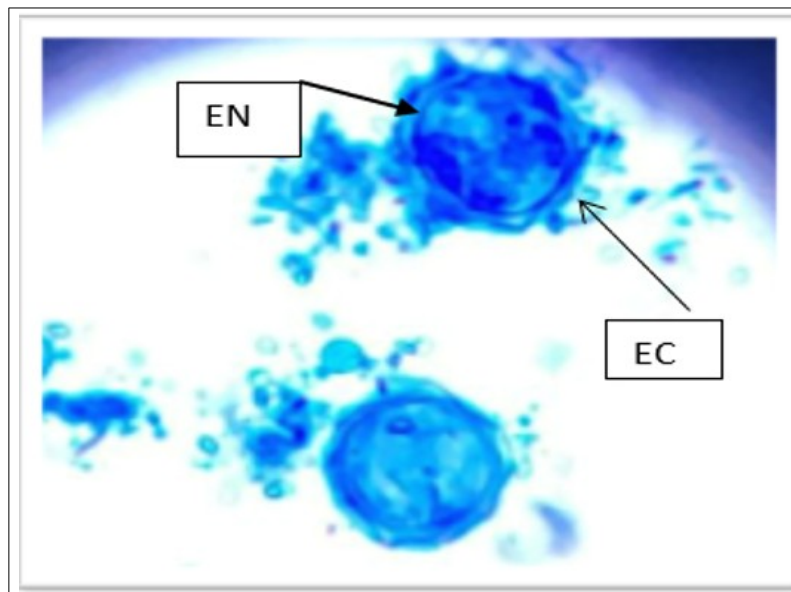


Fig. 2. Methylene blue staining of *Acanthamoeba* sp. cyst. The walls of the cysts, ectocyst (Ec) and endocyst (En) (× 100), were lightly stained with methylene blue.

Viability assessment

A mixture of 1 : 1 ratio of trypan blue dye and the parasite suspension was used to assess cell vitality (27). Following exposure to *L. europaeus* extract or PHMB for 2, 4 and 6 min, viability was assessed using haemocytometer (28). These short exposure times were selected to assess the immediate inhibitory response of *Acanthamoeba* trophozoites under rapid-contact conditions. For each condition, 100 organisms were counted per replicate across four haemocytometer quadrants. Non-viable cysts appeared stained, whereas viable cysts remained unstained; counts were recorded and expressed as percentages. A representative haemocytometer field is provided in Supplementary Fig. 1.

The study included 18 independent clinical isolates, each representing a distinct biological source. Biological replication was therefore derived from the diversity of the 18 isolates, while technical repeats consisted of multiple counts taken from the same biological sample.

In vitro assessment of anti-parasitic activity

A suspension of 100 μL of the calibrated cyst/trophozoite ($4 \times 10^3/\text{mL}$) was prepared and transferred to a micro-centrifuge tube. Each tube was mixed with equal volume 100 μL of extract to obtain final test concentration of 1.25, 2.5, 5, 10 mg/mL, each concentration was performed in triplicate. Tubes were incubated for 2, 4 and 6 min at 26 °C. A vehicle-only control containing 10 % DMSO-the same final solvent concentration used in the extract-treated tubes- was included. The PHMB (0.02 %) served positive control as this concentration is established as first-line clinical therapy for AK (29). Equal volume (25 μL) of 0.4 % trypan blue and each extract concentration or control were mixed together and viability was assessed using a haemocytometer. Any tubes showing precipitated extract particles were excluded to prevent interference with trypan-blue staining and microscopic counting.

Statistical analysis

Two-way analysis of variance (ANOVA) was applied to analyse viability percentages of *Acanthamoeba* trophozoites and cysts, using extract concentration (1.25, 2.5, 5.0, 10.0 mg/mL) and exposure time (2, 4, 6 min) as fixed factors. To evaluate whether concentration effects depended on exposure duration, a concentration $\times$ time interaction term was included in the analysis. Because percentage data are bounded between 0 % and 100 %, values were subjected to an arcsine square-root transformation prior to analysis to stabilise variance. The ANOVA assumptions were evaluated using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. When significant main or interaction effects were detected, Tukey's HSD post-hoc test was applied for pairwise comparisons with control of family-wise error rate. The experimental unit was each independent replicate well ($n = 3$). Statistical analyses were performed using SPSS version 18.0 and *F* statistics, degrees of freedom and exact *p* values are

reported in the results. The differences at the level of $p \leq 0.05$ were considered statistically significant. Raw replicate-level values and organism counts are provided in the supplementary file.

Gas chromatography-mass spectrometry analyses

At Nahran Bin Omar, Basrah Oil Company, gas chromatography-mass spectrometry (GC-MS) spectrometry analysis was carried out utilising an Agilent Technologies 7890B GC system with an Agilent Technologies 5977A MSD and EI ion source that ran via an HP-5MS 5 % phenyl methyl siloxane column that measured 30 m $\times$ 0.25 mm $\times$ 0.25 μm . The oven temperature was set at 40 °C, held for 5 min then raised to 1 °C/min to 280 °C for 1 min; the temperature was then held at 280 °C for the remaining 20min. Helium was used as the carrier gas at constant flow mode of 1 mL/min and purge flow of 3 mL/min. A splitless injection mode was used with injection temperature of 290 °C and the injection sample volume was 1 μL . The mass spectrometer used Ion Source Temp, 230 °C, with scan speed of 1562 (N₂) and electron ionisation (70 eV) was obtained over the mass range 35–650 m/z. A hexane solvent blank was run to confirm the absence of carryover or contamination prior to sample analysis. The extract was analysed by a single GC-MS injection. Data were run through the NIST 2014 library database. Retention indices (RI) were calculated for all identified peaks using the Van den Dool and Kratz (1963) equation (30), based on a C7–C40 n-alkane standard (Sigma-Aldrich) run under identical GC conditions used for sample analysis. A quality threshold of ≥ 80 (NIST20 library match score) was applied to distinguish confidently identified compounds from tentative identifications. Full library search results for all peaks are provided in Supplementary Table 1 and retention times for the C7–C40 n-alkane standard are provided in Supplementary Table 2.

3. Results

Patients sampling

Out of 44 patient samples, 18 (41 %) were found to be infected, while the remaining 26 (59 %) were negative. All infected patients received a specialist diagnosis, based on initial clinical features including red-coloured eyes, inflamed conjunctiva and severe pain (Fig. 3a and b).

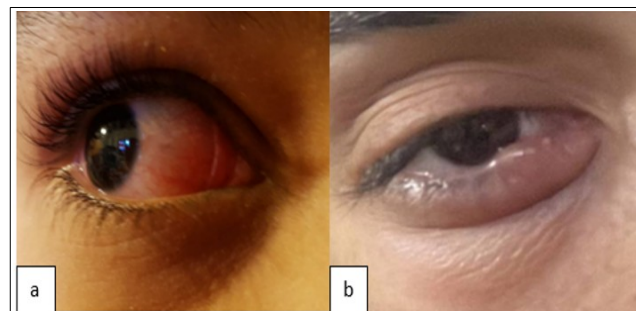


Fig. 3. Symptoms of infected eyes.

Morphological classification of *Acanthamoeba* sp.

After 24 hr, the isolated protozoan was classified to the genus level by inducing cyst development. The amoebae have changed into single-walled, spherical pre-cysts. After 48 hr of induction, the pre-cyst form was identified. A double-walled cyst stage with five sides was observed after 96 hr of cyst induction (Fig. 2). Based on the characteristics of the cyst stage, this isolate was confirmed as belonging to the genus of *Acanthamoeba*.

In vitro inhibitory activity of the *Loranthus europaeus* extract on *Acanthamoeba* trophozoite stage

Trophozoite viability decreased in a clear concentration- and time-dependent manner across all four tested concentrations (1.25–10 mg/mL), with each concentration differing significantly from control and from one another at most timepoints ($p < 0.001$; Table 1). The greatest reduction occurred at 10 mg/mL/6 min (0.33 ± 0.58 %), while the increase in effect with time was more pronounced at lower concentrations and plateaued at 5–10 mg/mL. The untreated control remained near 100 % viability throughout. The PHMB (0.02 %) showed a comparable time-dependent reduction, reaching 30.67 ± 1.15 % residual viability at 6 min (Table 1). Raw replicate-level counts for trophozoites are provided in Supplementary Table 3.

Effect of *Loranthus europaeus* extract on *Acanthamoeba* sp. cyst viability

Because cyst-stage residuals deviated from normality even after arcsine-square-root transformation, results were confirmed using the non-parametric Scheirer-Ray-Hare test. While both approaches confirmed significant main effects of concentration and time, the concentration $\times$ time interaction was significant only in the parametric model; given the normality violation, we consider the non-parametric result (no significant interaction) the more reliable conclusion for this specific term.

Cyst viability showed a similarly consistent concentration- and time-dependent decline across all tested concentrations (Table 2). The largest reduction occurred at the lowest concentration and longest exposure combination proportionally, with 1.25 mg/mL viability falling from 84.33 ± 0.58 % (2 min) to 31.33 ± 1.15 % (6 min; $p < 0.01$ vs. control at each timepoint). Higher concentrations (2.5–5 mg/mL) showed smaller relative decreases with increasing time, while the highest concentration (10 mg/mL) produced the greatest overall effect, reaching 0.33 ± 0.58 % viability at 6 min ($p < 0.001$ vs. all other concentrations and PHMB). The untreated control remained near 100 % viability throughout, while PHMB (0.02 %) showed a comparable time-dependent reduction, reaching 30.67 ± 1.15 % residual viability at 6 min. Raw replicate-level counts for cysts are provided in Supplementary Table 4.

Table 1. Effect of *Loranthus europaeus* hexane extract and polyhexamethylene biguanide PHMB (0.02 %) on trophozoite-stage viability (%) of *Acanthamoeba* sp. at 2, 4 and 6 min exposure, determined by trypan blue exclusion

Concentration (mg/mL)	mean $\pm$ standard deviation of trophozoite viability (%)		
	2 min	4 min	6 min
1.25	60.33 ± 0.58^a	56.33 ± 5.03^a	52.67 ± 4.51^a
2.5	44.67 ± 4.51^a	42.33 ± 0.58^a	39.67 ± 6.35^a
5.0	37.67 ± 0.58^a	29.33 ± 0.58^a	24.67 ± 2.52^a
10.0	15.67 ± 1.53^a	0.67 ± 0.58^a	0.33 ± 0.58^a
Control	100.00 ± 0.00	99.67 ± 0.58	99.67 ± 0.58
PHMB 0.02 %	66.00 ± 4.00^a	43.67 ± 2.08^a	30.67 ± 1.15^a

Analysed by two-way ANOVA (concentration $\times$ time) on arcsine square-root transformed data, with Tukey's HSD post-hoc test.

^aSignificantly different from Control at the same timepoint (Tukey HSD, $p < 0.0001$). Control: untreated suspension in vehicle(DMSO)-matched medium; PHMB: polyhexamethylene biguanide, positive control.

Table 2. Effect of *Loranthus europaeus* hexane extract and polyhexamethylene biguanide (PHMB) (0.02 %) on Cyst-stage viability (%) of *Acanthamoeba* sp. at 2, 4 and 6 min exposure, determined by trypan blue exclusion

Concentration (mg/mL)	mean $\pm$ standard deviation of cyst viability (%)		
	2 min	4 min	6 min
1.25	84.33 ± 0.58^a	56.67 ± 0.58^a	31.33 ± 1.15^a
2.5	67.00 ± 4.36^a	62.33 ± 0.58^a	52.00 ± 1.73^a
5.0	36.33 ± 0.58^a	33.33 ± 2.08^a	24.67 ± 2.08^a
10.0	23.33 ± 0.58^a	16.33 ± 0.58^a	0.33 ± 0.58^a
Control	100.00 ± 0.00	99.33 ± 0.58	99.00 ± 1.00
PHMB 0.02 %	66.00 ± 4.36^a	43.67 ± 2.08^a	30.67 ± 1.15^a

Analysed by two-way ANOVA (concentration $\times$ time) on arcsine square-root transformed data, with Tukey's HSD post-hoc test.

^aSignificantly different from Control at the same timepoint (Tukey HSD, $p < 0.0001$). Control: untreated suspension in vehicle (DMSO)-matched medium; PHMB: polyhexamethylene biguanide, positive control. Cyst-stage residuals did not meet normality assumptions after transformation (Shapiro-Wilk $p = 0.001$); results were confirmed by the non-parametric Scheirer-Ray-Hare test.



To estimate the extract concentration producing 50 % viability (IC_{50}), dose-response curves were fitted using a four-parameter logistic model (Table 3). Trophozoites, IC_{50} values decreased as exposure time increased from 2.10 mg/mL (2 min) to 1.78 mg/mL (4 min) and 1.56 mg/mL (6 min). Cyst IC_{50} values followed a similar pattern 3.03, 2.60 and 0.93 mg/mL at 2, 4 and 6 min respectively, indicating that cysts were more susceptible with prolonged exposure.

Gas chromatography-mass spectrometry analysis of *Loranthus europaeus* extract

The GC-MS analysis of hexane extract of *L. europaeus* revealed 39 constituents, accounting for 100 % of the total chromatographic peak area, dominated by terpenoid compounds (triterpenoids 47.72 % and sesquiterpene 27.87 %), in addition to ester, ketone and long-chain hydrocarbons (Table 4; Fig. 4). The most abundant compound was triterpenoid compounds Lup-20(29)-en-3 β -ol, acetate (lupeol acetate) accounting for a combined 42.06 % of total area across

three peaks sharing an identical mass spectrum (individual peaks: 33.61, 5.38, 3.08 %); this identification relies on library spectral matching alone (Quality 99), as a reliable published retention index for lupeol acetate could not be located for independent confirmation. This was followed by sesquiterpene tentatively identified as longipinane (8.62 %, Quality 45 -a low-confidence match warranting cautious interpretation), germacrene D (4.53 %) and caryophyllene (3.30 %). Calculated retention indices for germacrene D (RI 1476) and caryophyllene (RI 1413) showed close agreement (within 10 RI units) with published literature values (1482 and 1419, respectively), supporting these two identifications independently of library matching. Full RI values, library match quality scores and identification confidence for all 39 peaks are presented in Table 4. For the majority of peaks, no published literature RI could be identified for comparison; this reflects limited RI reporting for these compound classes rather than a mismatch and these identifications rely on library spectral matching.

Table 3. Half-maximal inhibitory concentration (IC_{50}) of *Loranthus europaeus* hexane extract against *Acanthamoeba* sp. trophozoite and cyst stages at each exposure time, estimated by four-parameter logistic regression

Stage	Exposure time (min)	IC_{50} (mg/mL) $\pm$ SE	R^2
Trophozoite	2	2.10 $\pm$ 1.03	0.981
Trophozoite	4	1.78 $\pm$ 0.52	0.963
Trophozoite	6	1.56 $\pm$ 0.39	0.967
Cyst	2	3.03 $\pm$ 0.20	0.994
Cyst	4	2.60 $\pm$ 2.31	0.934
Cyst	6	0.93 $\pm$ 1.28	0.860

Table 4. GC-MS profile of *Loranthus europaeus* hexane extract with NIST20 library match quality, calculated retention index (RI, Van den Dool and Kratz, C7–C40 n-alkane standard) and identification confidence

Pk#	RT (min)	Area (%)	Compound	Qual	RI (calc.)	RI (lit.) / Note
Triterpenoids						
1	28.697	3.01	Squalene	99	2818	No literature RI available for comparison
2	31.993	0.85	γ -Sitosterol	98	3321	No literature RI available for comparison
3	32.634	0.93	Lupeol	94	3405	No literature RI available for comparison
4	32.926	0.87	A'-Neo-21 α H-gammacer-22(29)-en-3 β -ol, acetate	55	3438	Tentative (Qual < 80); no literature RI available
5	33.218	5.38	Lup-20(29)-en-3 β -ol, acetate (Lupeol acetate)	55	3471	Tentative (Qual < 80); no literature RI available
6	33.710	33.61	Lup-20(29)-en-3 β -ol, acetate (Lupeol acetate)	99	3522	No literature RI available for comparison
7	36.496	3.08	Lup-20(29)-en-3 β -ol, acetate (Lupeol acetate)	95	3751	No literature RI available for comparison
Sesquiterpenes						
8	15.308	1.05	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro...	98	1383	No literature RI available for comparison
9	15.709	3.31	Caryophyllene	99	1413	1419 (Δ 6)

Pk#	RT (min)	Area (%)	Compound	Qual	RI (calc.)	RI (lit.) / Note
10	16.235	2.32	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	99	1455	No literature RI available for comparison
11	16.498	4.54	Germacrene D	98	1476	~1482 ($\Delta 6$)
12	16.676	1.05	epi-(E)- β -Caryophyllene	99	1490	No literature RI available for comparison
13	17.677	2.82	1H-Cycloprop[e]azulen-7-ol, decahydro...	99	1575	No literature RI available for comparison
14	17.740	1.34	Caryophyllene oxide	93	1580	No literature RI available for comparison
15	18.055	0.44	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	70	1607	Tentative (Qual < 80); no literature RI available
16	18.907	1.73	Germacra-4(15),5,10(14)-trien-1 α -ol	99	1683	No literature RI available for comparison
17	27.954	0.67	9-Methyltetracyclo[7.3.1.0(2,7).1(7,11)]tetradecane	30	2712	Tentative (Qual < 80); no literature RI available
18	35.381	8.63	Longipinane, (E)-	45	3670	Tentative (Qual < 80); no literature RI available
Other compounds						
19	7.927	1.04	Benzene, 1-ethyl-2-methyl-	95	950	No literature RI available for comparison
20	8.676	1.75	Mesitylene	97	980	No literature RI available for comparison
21	9.311	0.36	Mesitylene	97	1008	No literature RI available for comparison
22	11.039	0.81	6-Methyl-3,5-heptadiene-2-one	97	1097	No literature RI available for comparison
23	15.113	0.94	Copaene	99	1368	1376 ($\Delta 8$)
24	16.985	0.37	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	98	1516	No literature RI available for comparison
25	17.557	0.43	3-Hexen-1-ol, benzoate, (Z)-	87	1564	No literature RI available for comparison
26	17.854	0.34	Salvia-4(14)-en-1-one	91	1590	No literature RI available for comparison
27	18.535	1.02	2-Phenylpropionic acid, 4'-(2-methyl-1-propenyl)-, methyl ester	30	1650	Tentative (Qual < 80); no literature RI available
28	19.731	1.2	Benzyl Benzoate	98	1759	No literature RI available for comparison
29	20.813	1.33	Benzoic acid, 2-hydroxy-, phenylmethyl ester	94	1864	No literature RI available for comparison
30	21.305	0.49	Hexadecanoic acid, methyl ester	99	1913	No literature RI available for comparison
31	22.901	0.34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	99	2082	No literature RI available for comparison
32	23.067	0.83	Phytol	99	2100	No literature RI available for comparison
33	23.536	1.22	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	95	2154	No literature RI available for comparison
34	24.417	1.18	Tributyl acetylcitrate	64	2255	Tentative (Qual<80); no literature RI available
35	25.218	0.61	9-Octadecenamide, (Z)-	99	2352	No literature RI available for comparison
36	27.759	1.12	Heptacosane	98	2684	No literature RI available for comparison
37	29.172	4.28	Nonacosane	99	2887	No literature RI available for comparison

Pk#	RT (min)	Area (%)	Compound	Qual	RI (calc.)	RI (lit.) / Note
38	32.440	0.44	2-(Pyridin-2-ylformamido)acetic acid, 2TMS	25	3380	Tentative (Qual < 80); no literature RI available
39	36.062	4.32	2-Pentadecylfuran	15	3722	Tentative (Qual < 80); no literature RI available

RI (calc.): experimental retention index calculated using the Van den Dool and Kratz (1963) equation against a C7–C40 n-alkane standard run under identical GC conditions. For most peaks, no published literature RI value could be identified for direct comparison; this reflects limited RI reporting in the literature for these compound classes, not a failed or mismatched comparison and identification for these peaks relies on library spectral matching (Quality score, NIST20) alone. Where literature RI values were available (copaene, caryophyllene, germacrene D), calculated values showed close agreement (within 10 RI units, shown as Δ), providing independent confirmation of these identifications beyond library matching. 'Tentative' indicates a low-confidence library match (Quality < 80); these identifications should be regarded as provisional regardless of RI availability. No authentic reference standards were co-injected in this study.

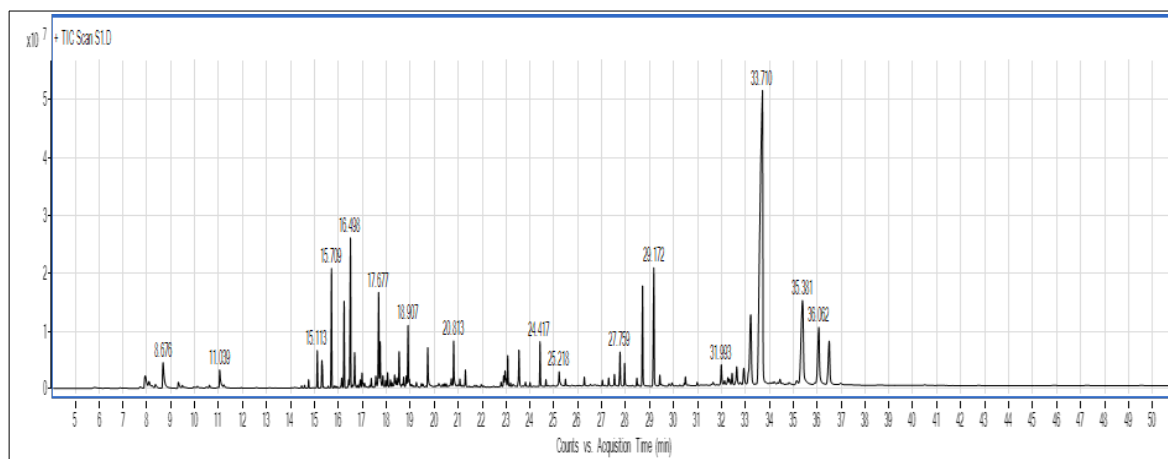


Fig. 4. Gas chromatogram of *Loranthus europaeus* hexane extract.

4. Discussion

In the current study, *Acanthamoeba* sp. was identified in eye samples based on the morphological characteristics described earlier (24, 25). *Loranthus europaeus* extracts have traditionally been used for treating leishmaniasis (4) and have reported antioxidant, antibacterial and antifungal activity (6–8). To our knowledge, the present work provides the first evidence of antiparasitic activity of *L. europaeus* against *Acanthamoeba* sp.

This finding should be considered alongside established methodology in the anti-*Acanthamoeba* literature. Previous plant-extract studies have tested activity directly against *Acanthamoeba* species, including *I. setosa* extract tested against *A. castellanii* and *A. polyphaga* (18). *Trigonella foenum-graecum* seed extract evaluated on a clinical T4 isolate and *P. australis* extract tested against *A. castellanii* (19, 20). These studies typically use much longer exposure periods (24–72 hr) and confirm amoebicidal (irreversible, non-recoverable) activity through excystation/outgrowth recovery assays, distinguishing it from amoebistatic (reversible growth-inhibitory) activity. Although direct quantitative comparison is limited by differing exposure durations and viability metrics, the concentration range at which *L. europaeus* extract reduced viability in this study (1.25–10 mg/mL over min) is broadly comparable to or lower than concentrations reported effective for

other plant extracts over much longer exposure windows. For example, *T. foenum-graecum* required up to 450–750 mg/mL over 24 hr (20), suggesting *L. europaeus* may warrant further investigation as a comparatively fast-acting candidate, pending confirmation of true amoebicidal activity.

The GC-MS profile revealed several terpenoid constituents, including lupeol acetate. This compound appeared as three separate peaks, but all three shared an identical mass spectrum, most likely representing minor conformers or fragmentation-related forms of a single pentacyclic triterpenoid acetate, rather than three distinct compounds. It is also worth noting that the library peak-area percentages reported here are only semi-quantitative; they should not be interpreted as validated compositional values. Furthermore, because no literature retention index could be located for lupeol acetate specifically, its identification relies on library spectral matching alone, without independent confirmation.

These observations do not establish that any single compound is responsible for the antiparasitic activity observed. Lupeol acetate is one plausible candidate, as it has previously been shown to inhibit *Trypanosoma cruzi* cysteine synthase, without notable toxicity or structural interference and its lipophilic character is broadly consistent with the effect seen here (31). However, its individual contribution was never

isolated or tested directly in this study, so the link remains hypothesis-generating rather than mechanistically confirmed. It is also possible that the activity results from synergistic interactions among several constituents, from minor or unidentified compounds not captured by library matching, or from the hexane extraction method itself, which would have excluded more polar compounds, including the phenolics reported in other *L. europaeus* studies (7, 32–35). Additionally, because the *L. europaeus* fruits used in this study were obtained from local markets, the specific host tree could not be identified or verified, a further limitation when interpreting the phytochemical findings, given that phenolic and host-related composition can vary geographically (36).

Lupeol, also identified in this study, has previously been isolated from *Loranthus micranthus* Hook.f. (Eastern Nigeria mistletoe) (37) and has been reported to possess antiparasitic, anthelmintic and antifungal properties (38). However, the only anti-*Acanthamoeba*-specific evaluation found in the literature reported that lupeol lacked potent amoebicidal activity against *A. castellanii* (39). In contrast to related triterpenoids such as betulinic acid and betulin, which were tested in the same study. This suggests that lupeol alone is unlikely to account for antiparasitic effect observed in the present study and that activity may instead arise from other detected constituents (lupeol acetate, caryophyllene, germacrene D) or from a synergistic effect among the crude extract's components.

Additionally, caryophyllene and germacrene D, phytol and β -sitosterol were identified in the present study and have been reported previously for their antiparasitic properties against unrelated pathogens. Caryophyllene and germacrene D identifications were further supported by close agreement between calculated and published retention indices (within 10 RI units), providing independent confirmation of compound identity beyond library matching. These compounds should be regarded as supporting evidence for general antiparasitic bioactivity of this compound class rather than direct evidence of anti-*Acanthamoeba* mechanism, as no *Acanthamoeba*-specific mechanistic data exist for these compounds at present. Caryophyllene (3.30 %) has demonstrated activity against *T. cruzi* and *Leishmania brasiliensis* (40). It was also reported that activity of essential oils from leaves of *Xylopi frutescens* against *T. cruzi* Y strain due to sesquiterpenes compound E-caryophyllene (8.5 %) and germacrene D (27.0 %) (41).

β -sitosterol has been documented to possess anti-leishmanial properties against *Leishmania donovani* and *Leishmania amazonensis*. Molecular docking also displayed binding of β -sitosterol to targets trypanothione reductase TR and leishmanolysin GP63 receptors (42). Another study, confirmed the activity of β -Sitosterol significantly against *L. donovani* promastigotes. Redox balance is disturbed by β -sitosterol through producing intracellular reactive oxygen species (ROS) that initiate cellular programmed death through altered structure, promoted accumulation of lipid molecules, depolarised

mitochondrial membrane, depletion of non-protein thiol and translocation of phosphatidylserine (43).

Phytol has shown promising activity against adult *Schistosoma mansoni* (44) and β -caryophyllene oxide has shown antiparasitic activity against *T. cruzi* through the inhibition of ABC channels, as suggested *in silico* studies (45).

Limitations and future work

Acanthamoeba sp. identification relied on morphological criteria rather than molecular confirmation. Future work will include *Acanthamoeba*-specific polymerase chain reaction (PCR), 18S rRNA sequencing and genotype assignment with sequence submission for an accession number.

Viability was assessed by trypan blue exclusion at short exposure intervals (2–6 min, rapid-contact disinfectant-style testing), which measures membrane permeability rather than irreversible viability and cannot distinguish amoebicidal from amoebistatic effects, since cyst recovery/excystation was not assessed. Longer, more clinically representative exposure durations are also needed particularly for meaningful comparison with PHMB. Future studies will incorporate wash/recovery excystment assays and a complementary viability method, using independent cyst preparations.

Human corneal epithelial cytotoxicity was not assessed; a selectivity index could not be calculated and this is planned before any translational claims. Bioassay-guided fractionation to isolate and identify the active constituents is planned to establish causal attribution and enable a true potency comparison with PHMB.

The host tree of the market-sourced fruit could not be traced and findings should be interpreted as batch-specific; field-collected material with documented host identity is needed. The extract, from a single batch, was not standardised to a quantified marker compound; quantification remained semi-quantitative (GC-MS peak-area percentages) and formal standardisation against authentic standards is a necessary next step toward pharmacological development.

5. Conclusion

Loranthus europaeus exhibited significant antiparasitic effects on both trophozoite and cyst stages of *Acanthamoeba* sp. The effects observed at lower concentrations and shorter exposure times than reported for several other plant extracts tested against *Acanthamoeba*. Because the extract was crude, unfractionated and tested at higher mass concentrations than the standardised polyhexamethylene biguanide (PHMB), these results represent a proof-of-concept for antiparasitic activity rather than a direct potency comparison. Isolation of the active constituents will be required to establish true relative potency. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a terpenoid-rich profile including lupeol acetate,



caryophyllene and germacrene D that offers a plausible, though still unconfirmed, explanation for the effect observed. Building on this, future work should focus on purifying and identifying the active constituents, testing alternative solvents and plant parts and confirming activity against *Acanthamoeba* and other hard-to-treat pathogens.

If these preliminary findings hold up under further testing, the next steps toward translation would involve standardising the active compound, developing a stable ophthalmic formulation suited to the terpenoids' lipophilic nature and evaluating efficacy and safety in an established *in vivo* model of *Acanthamoeba* keratitis (AK) before any clinical work could even be considered. Given the preliminary, *in vitro* nature of this study, such translational development remains a distant and speculative prospect at this stage.

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Authors' contributions

AHSA carried out GC-MS analysis, writing of the original draft and visualisation. EMAA participated in plant extraction and funding acquisition. MAM performed statistical analysis and contributed to study design and project administration. SAA carried out the investigation, including antiparasitic activity and identification of parasite species. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

Ethical approval for this study was waived by the Research and Ethical Review and Approval Committee, Department of Biology, College of Science, University of Basrah (Ref. Bio 26.35) on 05.01.2026. The waiver was granted because corneal swabs were collected as part of routine diagnostic procedures and all samples were fully anonymised before laboratory analysis. No personal identifiers or clinical data were retained. The study complies with the ethical principles of the Declaration of Helsinki (1964) and its later amendments.

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Supplementary Table 1. Raw gas chromatography-mass spectrometry (GC-MS) library search results (top library match per peak) for the *Loranthus europaeus* hexane extract, obtained from NIST20 library search (MassHunter, Agilent Technologies)

Pk#	RT (min)	Area (%)	Top library match (NIST20)	Qual
1	7.927	1.04	Benzene, 1-ethyl-2-methyl-	95
2	8.676	1.75	Mesitylene	97
3	9.311	0.36	Mesitylene	97
4	11.039	0.81	6-Methyl-3,5-heptadiene-2-one	97
5	15.113	0.94	Copaene	99
6	15.308	1.05	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-	98
7	15.709	3.31	Caryophyllene	99
8	16.235	2.32	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	99
9	16.498	4.54	Germacrene D	98
10	16.676	1.05	epi-(E)-beta-Caryophyllene	99
11	16.985	0.37	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	98
12	17.557	0.43	3-Hexen-1-ol, benzoate, (Z)-	87
13	17.677	2.82	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-	99
14	17.740	1.34	Caryophyllene oxide	93
15	17.854	0.34	Salvia-4(14)-en-1-one	91
16	18.055	0.44	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	70
17	18.535	1.02	2-Phenylpropionic acid, 4'-(2-methyl-1-propenyl)-, methyl ester	30
18	18.907	1.73	Germacrene-4(15),5,10(14)-trien-1a-ol	99
19	19.731	1.2	Benzyl Benzoate	98
20	20.813	1.33	Benzoic acid, 2-hydroxy-, phenylmethyl ester	94
21	21.305	0.49	Hexadecanoic acid, methyl ester	99
22	22.901	0.34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	99
23	23.067	0.83	Phytol	99
24	23.536	1.22	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	95
25	24.417	1.18	Tributyl acetylacrylate	64
26	25.218	0.61	9-Octadecenamide, (Z)-	99
27	27.759	1.12	Heptacosane	98
28	27.954	0.67	9-Methyltetracyclo[7.3.1.0(2,7).1(7,11)]tetradecane	30
29	28.697	3.01	Squalene	99
30	29.172	4.28	Nonacosane	99
31	31.993	0.85	gamma.-Sitosterol	98
32	32.440	0.44	2-(Pyridin-2-ylformamido)acetic acid, 2TMS	25
33	32.634	0.93	Lupeol	94
34	32.926	0.87	A'-Neo-21aH-gammacer-22(29)-en-3b-ol, acetate	55
35	33.218	5.38	Lup-20(29)-en-3beta-ol, acetate (Lupeol acetate)	55
36	33.710	33.61	Lup-20(29)-en-3beta-ol, acetate (Lupeol acetate)	99
37	35.381	8.63	Longipinane, (E)-	45
38	36.062	4.32	2-Pentadecylfuran	15
39	36.496	3.08	Lup-20(29)-en-3beta-ol, acetate (Lupeol acetate)	95

Pk# = peak number as assigned by the GC-MS acquisition software; RT: retention time; Area (%): percentage of total integrated chromatographic peak area; Qual: NIST20 library match quality score (0–100, higher indicates greater spectral match confidence). Full compound identification, calculated retention indices and identification confidence levels are presented in Table 4.

Supplementary Table 2. Retention times of the C7-C40 n-alkane standard series (Sigma-Aldrich), run under identical GC conditions to the sample extract, used for calculation of retention indices (Van den Dool and Kratz, 1963) reported in Table 4

Carbon number (Cn)	Retention time (min)
C8	3.636
C9	6.694
C10	9.162
C11	11.097
C12	12.737
C13	14.194
C14	15.540
C15	16.798
C16	17.976
C17	19.099
C18	20.166
C19	21.177
C20	22.140
C21	23.064
C22	23.947
C23	24.799
C24	25.612
C25	26.392
C26	27.148
C27	27.873
C28	28.573
C29	29.258
C30	29.911
C31	30.548
C32	31.161
C33	31.830
C34	32.594
C35	33.478
C36	34.521
C37	35.747
C38	37.204
C39	38.972
C40	41.082

Data acquired 13 August 2026 (Agilent MassHunter GC-MS, method: MSD 5977A Split.M). C7 was not detected/integrated in this run; retention indices were calculated using the C8–C40 range shown.

Supplementary Table 3. Replicate-level trypan blue exclusion counts for *Acanthamoeba* sp. trophozoites

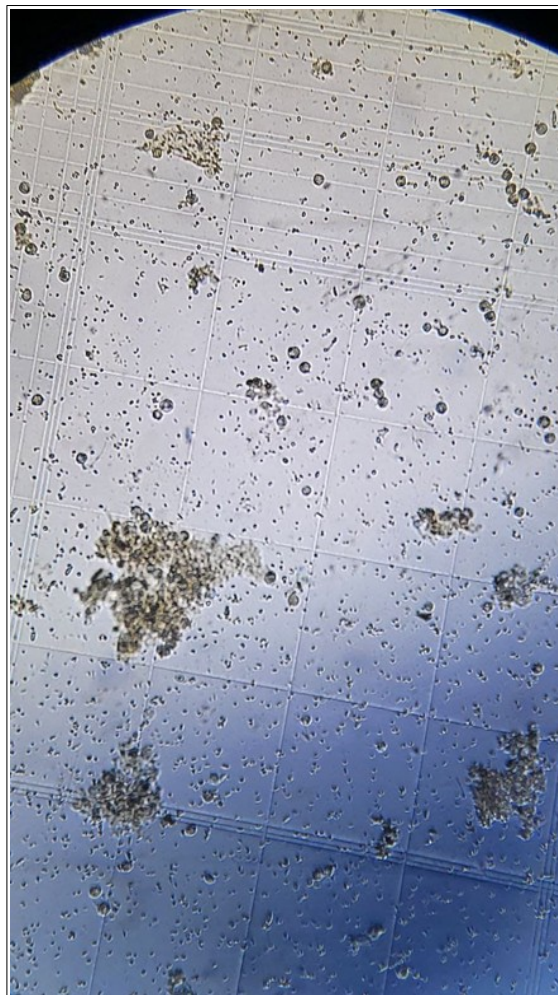
Concentration (mg/mL)	Exposure time (min)	Replicate	Total counted	Viable (unstained)	Non-viable (stained)	Viability % (calc)	
1.25	2	1	100	60	40	60.00	
1.25	2	2	100	60	40	60.00	
1.25	2	3	100	61	39	61.00	
		Mean $\pm$ SD				60.33	0.58 %
1.25	4	1	100	57	43	57.00	
1.25	4	2	100	51	49	51.00	
1.25	4	3	100	61	39	61.00	
		Mean $\pm$ SD				56.33	5.03 %
1.25	6	1	100	48	52	48.00	
1.25	6	2	100	53	47	53.00	
1.25	6	3	100	57	43	57.00	
		Mean $\pm$ SD				52.67	4.51 %
2.5	2	1	100	40	60	40.00	
2.5	2	2	100	49	51	49.00	
2.5	2	3	100	45	55	45.00	
		Mean $\pm$ SD				44.67	4.51 %
2.5	4	1	100	42	58	42.00	
2.5	4	2	100	42	58	42.00	
2.5	4	3	100	43	57	43.00	
		Mean $\pm$ SD				42.33	0.58%
2.5	6	1	100	47	53	47.00	
2.5	6	2	100	36	64	36.00	
2.5	6	3	100	36	64	36.00	
		Mean $\pm$ SD				39.67	6.35 %
5.0	2	1	100	37	63	37.00	
5.0	2	2	100	38	62	38.00	
5.0	2	3	100	38	62	38.00	
		Mean $\pm$ SD				37.67	0.58 %
5.0	4	1	100	29	71	29.00	
5.0	4	2	100	29	71	29.00	
5.0	4	3	100	30	70	30.00	
		Mean $\pm$ SD				29.33	0.58 %
5.0	6	1	100	27	73	27.00	
5.0	6	2	100	25	75	25.00	
5.0	6	3	100	22	78	22.00	

		Mean ± SD				24.67	2.52 %
10.0	2	1	100	16	84	16.00	
10.0	2	2	100	17	83	17.00	
10.0	2	3	100	14	86	14.00	
		Mean ± SD				15.67	1.53 %
10.0	4	1	100	0	100	0.00	
10.0	4	2	100	1	99	1.00	
10.0	4	3	100	1	99	1.00	
		Mean ± SD				0.67	0.58 %
10.0	6	1	100	0	100	0.00	
10.0	6	2	100	0	100	0.00	
10.0	6	3	100	1	99	1.00	
		Mean ± SD				0.33	0.58 %
Control	2	1	100	100	0	100.00	
Control	2	2	100	100	0	100.00	
Control	2	3	100	100	0	100.00	
		Mean ± SD				100.00	0.00 %
Control	4	1	100	100	0	100.00	
Control	4	2	100	100	0	100.00	
Control	4	3	100	99	1	99.00	
		Mean ± SD				99.67	0.58 %
Control	6	1	100	100	0	100.00	
Control	6	2	100	99	1	99.00	
Control	6	3	100	100	0	100.00	
		Mean ± SD				99.67	0.58 %
PHMB 0.02 %	2	1	100	62	38	62.00	
PHMB 0.02 %	2	2	100	66	34	66.00	
PHMB 0.02 %	2	3	100	70	30	70.00	
		Mean ± SD				66.00	4.00 %
PHMB 0.02 %	4	1	100	46	54	46.00	
PHMB 0.02 %	4	2	100	42	58	42.00	
PHMB 0.02 %	4	3	100	43	57	43.00	
		Mean ± SD				43.67	2.08 %
PHMB 0.02 %	6	1	100	30	70	30.00	
PHMB 0.02 %	6	2	100	30	70	30.00	
PHMB 0.02 %	6	3	100	32	68	32.00	
		Mean ± SD				30.67	1.15 %

Supplementary Table 4. Replicate-level trypan blue exclusion counts for *Acanthamoeba* sp. cysts

Concentration (mg/mL)	Exposure time (min)	Replicate	Total counted	Viable (unstained)	Non-viable (stained)	Viability % (calc)	
1.25	2	1	100	84	16	84.00	
1.25	2	2	100	84	16	84.00	
1.25	2	3	100	85	15	85.00	
		Mean $\pm$ SD				84.33	0.58 %
1.25	4	1	100	57	43	57.00	
1.25	4	2	100	57	43	57.00	
1.25	4	3	100	56	44	56.00	
		Mean $\pm$ SD				56.67	0.58 %
1.25	6	1	100	32	68	32.00	
1.25	6	2	100	32	68	32.00	
1.25	6	3	100	30	70	30.00	
		Mean $\pm$ SD				31.33	1.15 %
2.5	2	1	100	65	35	65.00	
2.5	2	2	100	72	28	72.00	
2.5	2	3	100	64	36	64.00	
		Mean $\pm$ SD				67.00	4.36 %
2.5	4	1	100	62	38	62.00	
2.5	4	2	100	62	38	62.00	
2.5	4	3	100	63	37	63.00	
		Mean $\pm$ SD				62.33	0.58 %
2.5	6	1	100	50	50	50.00	
2.5	6	2	100	53	47	53.00	
2.5	6	3	100	53	47	53.00	
		Mean $\pm$ SD				52.00	1.73 %
5.0	2	1	100	36	64	36.00	
5.0	2	2	100	36	64	36.00	
5.0	2	3	100	37	63	37.00	
		Mean $\pm$ SD				36.33	0.58 %
5.0	4	1	100	35	65	35.00	
5.0	4	2	100	31	69	31.00	
5.0	4	3	100	34	66	34.00	
		Mean $\pm$ SD				33.33	2.08 %
5.0	6	1	100	24	76	24.00	
5.0	6	2	100	23	77	23.00	
5.0	6	3	100	27	73	27.00	

		Mean ± SD				24.67	2.08 %
10.0	2	1	100	23	77	23.00	
10.0	2	2	100	23	77	23.00	
10.0	2	3	100	24	76	24.00	
		Mean ± SD				23.33	0.58 %
10.0	4	1	100	17	83	17.00	
10.0	4	2	100	16	84	16.00	
10.0	4	3	100	16	84	16.00	
		Mean ± SD				16.33	0.58 %
10.0	6	1	100	0	100	0.00	
10.0	6	2	100	0	100	0.00	
10.0	6	3	100	1	99	1.00	
		Mean ± SD				0.33	0.58 %
Control	2	1	100	100	0	100.00	
Control	2	2	100	100	0	100.00	
Control	2	3	100	100	0	100.00	
		Mean ± SD				100.00	0.00 %
Control	4	1	100	100	0	100.00	
Control	4	2	100	99	1	99.00	
Control	4	3	100	99	1	99.00	
		Mean ± SD				99.33	0.58 %
Control	6	1	100	100	0	100.00	
Control	6	2	100	99	1	99.00	
Control	6	3	100	98	2	98.00	
		Mean ± SD				99.00	1.00 %
PHMB 0.02 %	2	1	100	71	29	71.00	
PHMB 0.02 %	2	2	100	64	36	64.00	
PHMB 0.02 %	2	3	100	63	37	63.00	
		Mean ± SD				66.00	4.36 %
PHMB 0.02 %	4	1	100	46	54	46.00	
PHMB 0.02 %	4	2	100	42	58	42.00	
PHMB 0.02 %	4	3	100	43	57	43.00	
		Mean ± SD				43.67	2.08 %
PHMB 0.02 %	6	1	100	30	70	30.00	
PHMB 0.02 %	6	2	100	30	70	30.00	
PHMB 0.02 %	6	3	100	32	68	32.00	
		Mean ± SD				30.67	1.15 %



Supplementary Fig. 1. Representative haemocytometer counting field used for enumeration of *Acanthamoeba* sp. trophozoites/cysts (light microscopy).