Turkiye Parazitol Derg 2025;49(4):146-51
DOI: 10.4274/tpd.galenos.2025.52533

Investigation of the Antiparasitic Potential of Luteolin: *in vitro* Activity and Comparison with Standard Therapeutics

Luteolin'in Antiparaziter Potansiyelinin Araştırılması: in vitro Aktivitesi ve Standart Terapötiklerle Karşılaştırılması

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Cite this article as: Tileklioğlu E, Aydın E. Investigation of the antiparasitic potential of luteolin: *in vitro* activity and comparison with standard therapeutics. Turkiye Parazitol Derg. 2025;49(4):146-51.

ABSTRACT

Objective: Due to the limitations of current therapeutic approaches in treating parasitic diseases, there is a growing need for new and effective products, prompting interest in alternative approaches such as medicinal plants. Flavonoids, including luteolin, have shown promise in the treatment of many diseases due to their natural properties and pharmacological effects. This study aimed to investigate the *in vitro* activity of luteolin against *Acanthamoeba castellanii (A. castellanii), Entamoeba histolytica (E. histolytica),* and *Leishmania tropica (L. tropica).*

Methods: The reference parasite strains were tested for antiparasitic activity using luteolin concentrations ranging from 200 to 1.5 µg/mL. Positive controls included chlorhexidine, metronidazole, and glucantime, while dimethyl sulfoxide and parasite specific culture medium served as negative controls. Parasite mortality was assessed XTT [2,3-bis (2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide] and trypan blue dye exclusion assays. Minimum inhibitory concentration (MIC) and median lethal dose (LD_{50}) values were determined via non-linear regression analysis.

Results: Luteolin exhibited significant activity, with MIC values of 100 µg/mL for *A. castellanii* and *E. histolytica*, and 12.5 µg/mL for *L. tropica*. LD_{50} analysis revealed effective concentrations of 3.125 µg/mL for *E. histolytica* and 1.5 µg/mL for *A. castellanii*, while *L. tropica* displayed an LD_{50} below 1.5 µg/mL, indicating the highest sensitivity.

Conclusion: Luteolin demonstrated potent antiprotozoal effects *in vitro*, with *L. tropica* being the most susceptible, followed by *A. castellanii* and *E. histolytica*. Notably, luteolin's anti-leishmanial activity was comparable to glucantime. In conclusion, luteolin demonstrates significant potential as a broad-spectrum antiparasitic agent, and comprehensive *in vivo* studies are recommended to further evaluate its therapeutic efficacy.

Keywords: Luteolin, antiprotozoal activity, *A. castellanii*, *E. histolytica*, *L. tropica*

ÖZ

Amaç: Paraziter hastalıkların tedavisinde kullanılan mevcut tedavi ajanların sınırlılıkları nedeniyle yeni ve etkili ürünlere ihtiyaç duyulmaktadır. Bu durum, tıbbi bitkiler gibi alternatif yaklaşımlara olan ilgiyi artırmaktadır. Flavonoid grubu içerisinde olan luteolin, doğal özellikleri ve farmakolojik etkileri sayesinde birçok hastalığın tedavisinde umut verici potansiyeli olduğu belirtilmiştir. Bu çalışmada, luteolinin *Acanthamoeba castellanii (A. castellanii), Entamoeba histolytica (E. histolytica),* ve *Leishmania tropica'ya (L. tropica)* karşı *in vitro* aktivitesinin araştırılması amaçlanmıştır.

Yöntemler: Çalışmada kullanılan referans parazit suşlarına, 200 µg/mL ile 1,5 µg/mL arasında değişen luteolin konsantrasyonları uygulanarak antiparazitik aktivite test edildi. Pozitif kontroller olarak klorheksidin, metronidazol ve glukantim, negatif kontroller olarak dimetil sülfoksit ve parazite özgü kültür ortamları kullanıldı. Parazit mortalitesi, XTT[2,3-bis (2-metoksi-4-nitro-5-sülfenil)-(2H)-tetrazolium-5-karboksanilid] ve trypan blue boya testleri ile değerlendirildi. Minimum inhibitör konsantrasyon (MIC) ve medyan ölüm dozu (LD_{50}) değerleri, doğrusal olmayan regresyon analizi ile belirlendi.

Bulgular: Luteolinin, *A. castellanii* ve *E. histolytica* için 100 µg/mL, *L. tropica* için 12,5 µg/mL MIC değerleri ile önemli bir etkinlik göstermiştir. LD_{50} analizi, *E. histolytica* için 3,125 µg/mL ve *A. castellanii* için 1,5 µg/mL etkin konsantrasyonları ortaya koyarken, *L. tropica* 1,5 µg/mL'nin altında bir LD_{50} değeri göstererek en yüksek duyarlılığı sergilemiştir.

Sonuç: Bu çalışma ile luteolinin *in vitro* ortamda güçlü antiprotozoal etkisi belirilenmiştir. Çalışmada en duyarlı parazit *L. tropica*, ardından *A. castellanii* ve *E. histolytica* olmuştur. Özellikle luteolinin anti-leishmanial aktivitesi, glukantim ile karşılaştırılabilir

Received/Geliş Tarihi: 23.09.2024 **Accepted/Kabul Tarihi:** 26.08.2025 **Epub:** 10.09.2025 **Publication Date/Yayınlanma Tarihi:** 01.12.2025

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düzeydedir. Sonuç olarak luteolinin, geniş spektrumlu bir antiparazitik ajan olarak önemli potansiyel göstermektedir. Terapötik etkinliğinin değerlendirilmesi için kapsamlı *in vivo* çalışmaların yapılması önerilmektedir.
Anahtar Kelimeler: Luteolin, antiprotozoal aktivite, *A. castellanii*, *E. histolytica*, *L.tropica*

INTRODUCTION

Millions of individuals, predominantly in low- and middle-income countries, are affected by parasitic infections, particularly those caused by protozoans such as *Entamoeba* spp., *Acanthamoeba* spp., and *Leishmania* spp., which contribute to significant morbidity and mortality globally (1-3).

Amoebiasis, caused by *Entamoeba histolytica* (*E. histolytica*), is a notable parasitic disease, leading to an estimated 55,500 deaths and 2.237 million disability-adjusted life years annually, as reported by the Global Burden of Disease 2010 Study (4). The prevalence of *E. histolytica* infection can reach up to 50% in some developing regions, including parts of South and Central America, Africa, and Asia. In Türkiye, the incidence varies regionally, with an average rate of 2.27% (1). Although the disease is often asymptomatic, it can present with symptoms ranging from diarrhea to dysentery, and in severe cases, it may disseminate to other organs such as the liver, lungs, and brain, leading to abscess formation. The standard pharmacological treatment includes nitroimidazoles like metronidazole (MTZ), but these drugs have limitations, including adverse effects such as neurotoxicity with prolonged use and the emergence of drug-resistant strains (5).

Acanthamoeba castellanii (A. castellani) is an opportunistic, free-living amoeba found in soil and water, associated with approximately 2.9 cases per million globally. It causes two primary clinical conditions: *Acanthamoeba* keratitis (AK) and granulomatous *Acanthamoeba* encephalitis. AK begins with the attachment of trophozoites to the corneal epithelium, potentially progressing to stromal invasion and vision loss or blindness (2). Current treatments for *Acanthamoeba* infections include 0.02% chlorhexidine and 0.02% polyhexamethylene biguanide, though prolonged use of these topical agents can result in corneal toxicity (6).

Leishmaniasis, caused by over 20 pathogenic *Leishmania* species, is transmitted by various species of female phlebotomine sandflies (3). The disease is endemic in more than 102 countries/regions bordering the Mediterranean and the Black Sea, including Türkiye, with thousands of new cases reported annually (7). Clinical manifestations vary based on the *Leishmania* species and the host's immune response. Treatment options include antimony (first line), amphotericin B (second line), imidazole's, miltefosine, paromomycin, and liposomal amphotericin B. However, these drugs are expensive, exhibit toxic side effects, and are often rendered ineffective by drug-resistant strains (8).

Considering the challenges associated with current treatments for these parasitic diseases, there is growing interest in exploring medicinal plants for alternative therapies. Flavonoids, a diverse group of polyphenolic compounds found in various plants, are gaining attention due to their pharmacological properties (9). With over 9,000 types identified, flavonoids are characterized by their C6-C3-C6 structural backbone and include several subgroups based on structural variations (10). Luteolin, a prominent flavonoid found in numerous plants used in traditional medicine, has demonstrated a range of biological effects, including anti-diabetic, anti-allergic, and anti-cancer activities. Despite extensive research on its antibacterial and antiviral properties, its antiparasitic activity remains underexplored (11-13). This study aims to evaluate the *in vitro* activity of luteolin against *A. castellanii*, *E. histolytica*, and *Leishmania tropica (L. tropica),* contributing to the search for novel therapeutic agents.

METHODS

Ethical Approval

No clinical material or data were used in this study. Therefore, ethics committee approval is not required.

Preparation and Storage of Luteolin Stock Solution

Luteolin was purchased from Sigma-Aldrich (St. Louis, MO). A stock solution of one hundred millimolars in dimethyl sulfoxide (DMSO) was ready and held on at -20 °C.

In vitro Cultures of *A. castellanii, E. histolytica* and *L. tropica*

Axenic cultures of *A. castellanii* trophozoites (ATCC 30010) were propagated in protease peptone-yeast extract-glucose medium. This medium contained 0.75% (w/v) protease peptone, 0.75% (w/v) yeast extract, and 1.5% (w/v) glucose, supplemented with penicillin G (500 U/mL) and streptomycin (50 µg/mL) to maintain sterility and promote optimal axenic growth. Cultures were maintained in 25 mL cell culture flasks (Sigma), refreshed weekly, and incubated at 30 °C. For cell harvesting, the culture medium was removed by centrifugation at 1500 rpm for 5 minutes, followed by three washes in phosphate-buffered saline to eliminate any remaining medium components. To detach the trophozoites adhering to the flask walls, the flasks were gently agitated on ice for 30 minutes.

The *E. histolytica* strain (ATCC 30459) was generously provided by Dr. Charles Graham Clark from the London School of Hygiene and Tropical Medicine. *E. histolytica* trophozoites were axenically grown in LYI (liver digest, yeast extract, iron) medium, which included 880.0 mL of LYI broth, 20.0 mL of a vitamin mixture, and 100.0 mL of heat-inactivated adult bovine serum. The medium was further supplemented with penicillin G (500 U/mL) and streptomycin (50 µg/mL). To ensure continuous growth and viability, trophozoites were routinely subculture into screw-capped test tubes containing 7 mL of LYI medium.

L. tropica promastigotes (ATCC 50129) were cultured at 26 °C in RPMI-1640 medium (Sigma), supplemented with 10% heat-inactivated fetal bovine serum sourced from Cegrogen, Stadtallendorf, Germany. This enriched medium provided the necessary nutrients for promastigote growth and development. The cultures were maintained in 25 mL flasks, ensuring optimal conditions to support the promastigotes' stationary phase proliferation.

In vitro Antiparasitic Test of Luteolin

A. castellanii, *E. histolytica*, and *L. tropica* were seeded into 96-well microtiter plates (Greiner, Germany), with luteolin applied in serial concentrations ranging from 200 µg/mL to 1.5 µg/mL. For

this, the trophozoite densities of *A. castellanii* and *E. histolytica* were adjusted to 5×10^4 cells/mL and 1×10^6 cells/mL, respectively, and *L. tropica* promastigotes were standardized to 1×10^5 cells/mL. The trophozoites of *A. castellanii* and *E. histolytica* were given 20 minutes to adhere to the wells, a process that was confirmed under a Leica inverted microscope (Leica, Wetzlar, Germany). The plates were then incubated at 30 °C for 24 hours for *A. castellanii*, at 26 °C for 72 hours for *E. histolytica*, and at 37 °C for 48 hours for *L. tropica*.

Two distinct assays were employed to assess the antiprotozoal activity of luteolin *in vitro*. The anti-leishmanial effect was evaluated using the XTT cell proliferation kit from Roche Molecular Biochemicals (Mannheim, Germany), following the manufacturer's instructions (14). A viability assay was conducted for *A. castellanii* and *E. histolytica*, which involved staining the cells with 0.1% trypan blue [(TB) 0.4%] at a 1:1 ratio. Live cells remained unstained, while alive cells were stained, and both were counted using a hemocytometer (15). The percentage of parasite mortality was calculated using the formula: % mortality = (negative control-test sample) × 100/negative control.

Parasite mortality was determined as 100% when no motile parasites were observed. Minimum inhibitory concentration (MIC) and LD_{50} values were determined, with MIC representing the lowest concentration that fully inhibited parasite growth and LD_{50} representing the dose required to kill 50% of the parasites. To verify trophozoite and promastigote viability, the samples were reinoculated into fresh media and monitored over 24, 48, and 72 hours for regrowth. LD_{50} values were calculated by fitting a non-linear sigmoidal dose-response curve (four-parameter logistic regression) to the mortality data using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). The 95% confidence intervals (CI) were determined from the regression model. Analyses were performed separately for each protozoan species.

Each experiment included appropriate controls. Negative controls consisted of DMSO (final concentration <1%), which was used as the solvent for luteolin, and parasite specific culture medium appropriate for each organism without luteolin. For *A. castellanii*, the negative control medium was PYG, for *E. histolytica*, LYI medium; and for *L. tropica*, RPMI-1640. These media served as parasite maintenance controls to ensure that any observed mortality was due solely to the treatment and not to culture conditions. The positive controls consisted of MTZ (Specia Rhone Poulenc Rorer, Paris, France) for *E. histolytica*, chlorhexidine (Sigma) for *A. castellanii*, and N-methyl meglumine (Glucantime™, Rhone Poulenc, France) for *L. tropica*. All assays were conducted three times in triplicate to ensure reliability and reproducibility of the results.

Statistical Analysis

The mean, degrees of freedom, and t-value (t) were calculated. Data on the antiprotozoal activity were analysed for statistical significance by using the two-tailed Student's t-test for unpaired samples. A p-value of 0.05 or less was considered indicative of a statistically significant difference. LD_{50} regression was conducted using GraphPad 10 (GraphPad Software, San Diego, CA, USA). LD_{50} values were determined by fitting the data to a non-linear regression model (four-parameter logistic, variable slope). The goodness-of-fit (R^2) and 95% CIs for each LD_{50} estimate were calculated to assess model reliability. Dose response curves were plotted for each parasite species to visualize differences in susceptibility. All experiments were performed in triplicate and repeated three independent times, and the results are presented as mean ± standard error.

RESULTS

According to the *Acanthamoeba* TB dye exclusion assays, mortality in the negative control group was minimal, with a maximum value of 8%, while significantly higher mortality rates were recorded in both the luteolin and chlorhexidine groups, reaching 100%. The highest mortality was determined at concentrations of 100 µg/mL and 200 µg/mL for both luteolin and chlorhexidine, with 100% mortality. At the lowest concentration (1.5 µg/mL), the mortality rate decreased to 30% for luteolin, while it remained 98% for chlorhexidine. Overall, an increase in mortality rates was observed as the concentration increased (Figure 1a). The *in vitro* effect of luteolin on *Acanthamoeba* was determined to be significant and dose dependent. A statistical relationship was not found between luteolin and the positive control ($p=0.0172$; $t=2.7026$; $df=14$).

The TB dye exclusion assays demonstrated that luteolin achieved complete effectiveness (100% mortality) against *E. histolytica* trophozoites at concentrations of 100 µg/mL and 200 µg/mL. At 50 µg/mL, luteolin induced 98% mortality, while at lower concentrations, the parasites were affected but their viability remained higher compared to the positive control. The number of motile trophozoites increased as the drug concentrations decreased (Figure 1b). No statistical relationship was detected between luteolin and the positive control (MTZ) ($p=0.0953$; $t=1.7886$; $df=14$).

According to XTT analysis, luteolin induced 100% mortality at concentrations ranging from 200 to 12.5 µg/mL against the promastigote form of *L. tropica*. Mortality rates between 95% and 98% were determined at the three lowest concentrations (6.25, 3.125, and 1.5 µg/mL). No significant differences were detected between luteolin and glucantime ($p=0.1108$; $t=1.8249$; $df=7$).

The MIC of luteolin was determined as 100 µg/mL for *A. castellanii* and *E. histolytica*, while a value of 12.5 µg/mL was noted for *L. tropica*. Histogram graphs showing the *in vitro* activity of luteolin against *A. castellanii*, *E. histolytica*, and *L. tropica* are presented in Figures 2-4, respectively.

LD_{50} regression analysis, performed using a non-linear dose-response model, revealed that luteolin exhibited the highest potency against *L. tropica*, with an LD_{50} value below 1.5 µg/mL (95% CI: X-Y) (Figure 5). For *A. castellanii* and *E. histolytica*, the LD_{50} values were calculated as 1.5 µg/mL (95% CI: X-Y) and 3.125 µg/mL (95% CI: X-Y), respectively (Figure 5a and 5b). The steep slope of the dose–response curve for *L. tropica* indicates a rapid decline in viability with small increases in concentration, suggesting greater susceptibility compared with the other protozoa. In contrast, *E. histolytica* demonstrated the least sensitivity to luteolin within the tested range, as reflected by the higher LD_{50} value.

DISCUSSION

The decreasing effectiveness of conventional therapeutic agents in treating parasitic diseases has led to delays and failures in treatment. Moreover, long-term use of these agents often

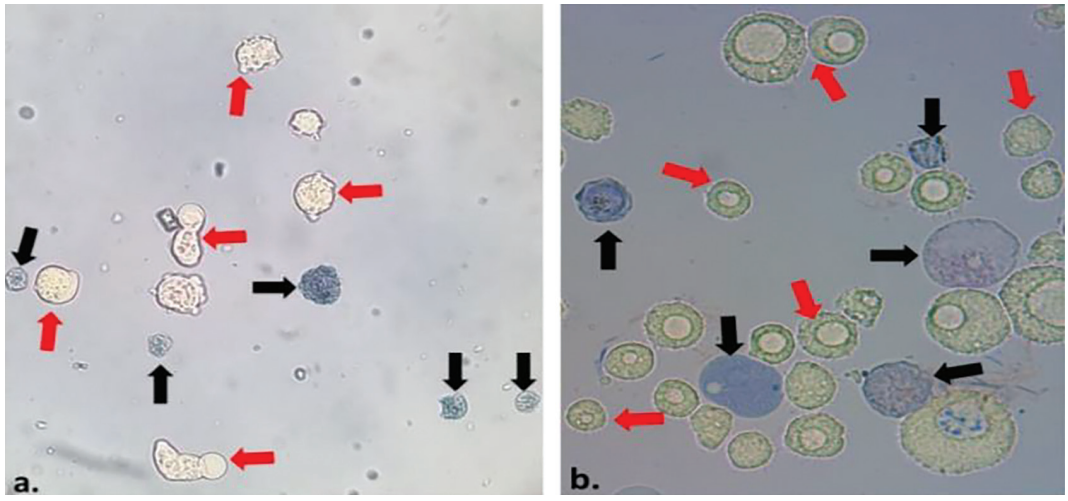

Figure 1. Trophozoites of *E. histolytica* **(a)** and *A. castellanii* **(b)** with trypan blue dye exclusion assays (40x). Alive (red arrow-unstained) and dead (black arrow-stained) cells

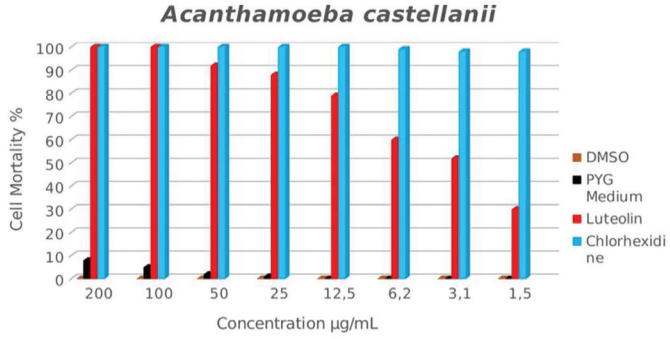

Figure 2. Mortality of luteolin against *A. castellanii* trophozoites

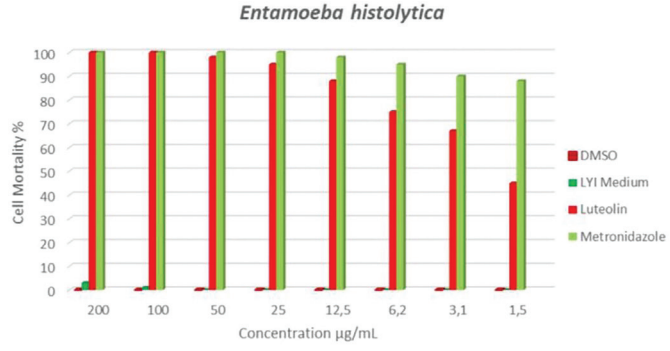

Figure 3. Mortality of luteolin aganist *E. histolytica* trophozoites

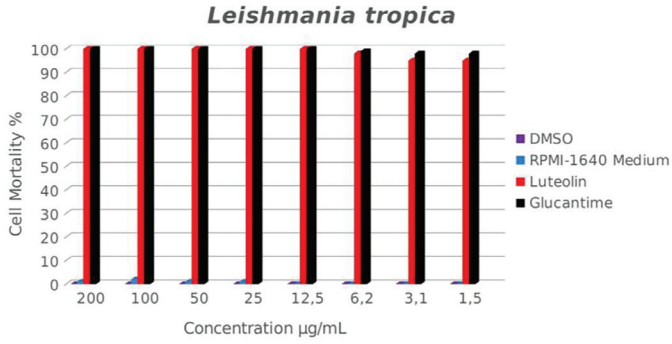

Figure 4. Mortality of luteolin aganist *L. tropica* promastigote

results in toxicity and adverse side effects (16). Accordingly, there has been a shift towards the discovery of natural medicinal compounds with antiparasitic properties. Medicinal plants, commonly used as raw materials across various sectors, including medicine and pharmacy, produce phytoalexins in response to microbial invasion. These compounds, derived from secondary metabolites, are mainly found in flavonoids (17). Many studies have demonstrated that flavonoids possess a wide array of biological activities, such as antioxidant, antimutagenic, antibacterial, antiangiogenic, anti-inflammatory, antiallergic, enzymatic regulatory, and anticancer effects (10,18,19).

Luteolin and its glycosides are significant flavonoids with demonstrated antimicrobial activity. Several studies have reported their effectiveness against bacteria, viruses, and fungi. However, studies on the antiparasitic activity of luteolin are limited. For instance, research has shown that flavonoids, when used alone or in combination, are effective against *Cryptosporidium parvum* and *Encephalitozoon intestinalis* (20). One study revealed that luteolin inhibits the transformation of *Plasmodium falciparum* rings into more advanced stages, without affecting the sensitivity to chloroquine or artemisinin (21). In addition, luteolin was shown to markedly inhibit the growth of *Babesia bovis*, *Babesia bigemina*, *Babesia caballi*, and *Theileria equi*, starting from an initial parasitemia of 1%. Furthermore, at a dosage of 5 mg/kg, luteolin resulted in a 77.5% reduction in the growth of *Babesia microti* in BALB/c mice, indicating its potential applicability in the treatment of babesiosis. (22).

Although research on luteolin's effects against *Acanthamoeba* is scarce, it has been suggested that luteolin could play an important role in developing alternative treatments for *Acanthamoeba* infections. Luteolin has demonstrated cytotoxicity against mouse macrophages (J774A.1) and has been reported to induce

programmed cell death in *A.castellanii* (23). However, luteolin's effects appear to vary among species. For example, in a study evaluating the anti-amoebic activity of 18 flavonoids against *A. castellanii*, *A. polyphaga*, and *Naegleria fowleri* (*N. fowleri*), luteolin was highly effective against *N. fowleri* but less so against *A. castellanii*. Notably, *A. polyphaga* was found to be more sensitive to luteolin than *A. castellanii*, with the former exhibiting higher sensitivity index values (>11) and lower IC_{50} values (<30 µM) (24). In the present study, the highest mortality rate of luteolin on *A. castellanii* was 100% at concentrations of 200 µg/mL and 100 µg/mL. The MIC and LD_{50} values were determined to be 100 µg/mL and 3.125 µg/mL, respectively. However, luteolin's efficacy at lower concentrations was less pronounced compared to chlorhexidine, highlighting the need for further *in vitro* and *in vivo* studies to fully understand luteolin's effects on *Acanthamoeba* species.

Recent research has increasingly focused on the potential of medicinal plants as complementary or specific treatment strategies with amoebicidal properties against *Entamoeba* species, given the limitations of current therapeutic agents (25). Notably, natural compounds have been observed to induce morphological changes in amoebae, including chromatin condensation and cytoskeletal protein rearrangement (26). In one study, ten flavonoids and four iridoids were evaluated for their anti-amoebic activity and cytotoxicity against MT-4 cells. Among them, kaempferol (IC_{50}=10.3±2.3 µg/mL), apigenin (IC_{50}=12.7±4.3 µg/mL), and luteolin (IC_{50}=17.8±4.3 µg/mL) exhibited stronger activity than their glycoside counterparts (27). In the current study, luteolin demonstrated a potent effect against *E. histolytica* at higher concentrations, with 100% mortality at 200 and 100 µg/mL and 98% mortality at 50 µg/mL. The mortality effect decreased dose-dependently, with an MIC value of 100 µg/mL and an LD_{50} of 1.5 µg/mL. Although MTZ proved more effective at lower concentrations, these results indicate that luteolin effectively eliminates half of the trophozoites at the lowest concentration tested.

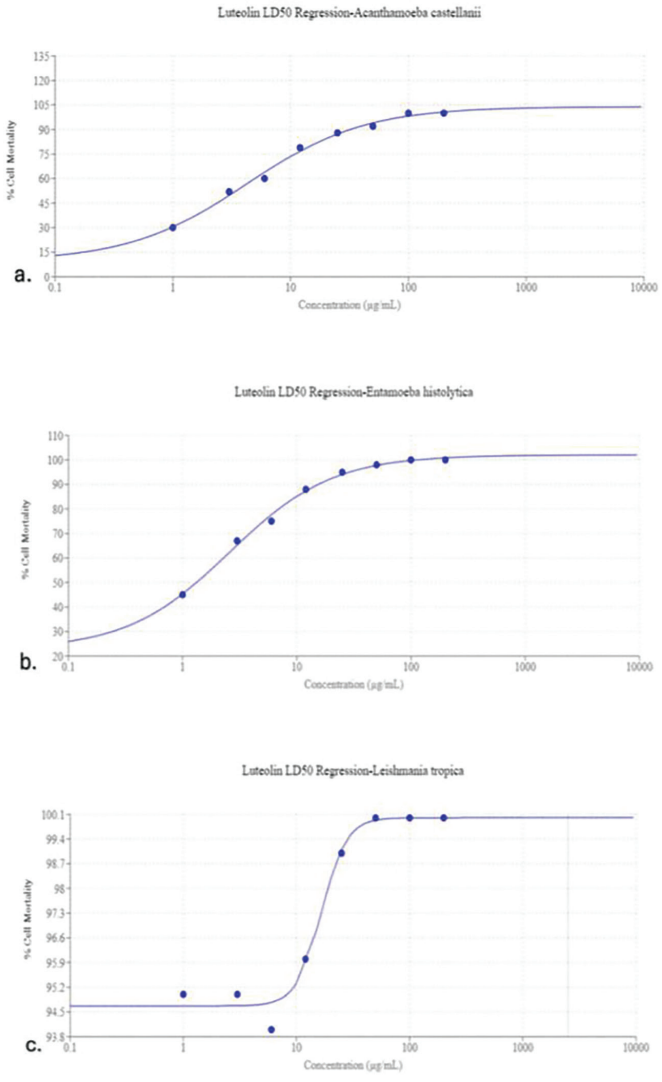

Figure 5. LD_{50} regression analysis of luteolin aganist *A. castellanii* **(a)**, *E. histolytica* **(b)** and *L. tropica* **(c)**

Studies have also suggested that flavonoids could serve as food supplements in the treatment of leishmaniasis due to their low IC_{50} values (28). In one study, it was noted that the side effects of quercetin and luteolin on cutaneous wounds caused by *Leishmania* species were less severe than those caused by meglumine (29). While quercetin exhibited non-specific effects on normal human T-cells, luteolin was found to be non-toxic and a strong candidate for anti-leishmanial drug development (30). Additionally, *in vivo* studies have demonstrated that luteolin has greater cytotoxicity against lymphocytes and is a more potent inhibitor of *L. tropica* amastigotes than luteolin-4'-O-β-D-glucopyranoside (12). Similarly, another study evaluated the cytotoxicity of 105 compounds on mammalian L6 cells and their antiparasitic activities, identifying fisetin, 3-hydroxyflavone, luteolin, and quercetin as the most potent anti-leishmanial agents, with IC_{50} values of 0.6, 0.7, 0.8, and 1.0 µg/mL, respectively (31). In the present study, luteolin exhibited strong anti-leishmanial activity against *L. tropica*, comparable to glucantime. According to the XTT analysis, luteolin induced 100% mortality at concentrations ranging from 200 to 12.5 µg/mL, with mortality rates of 95-98% at the lower concentrations. These findings are consistent with glucantime's effect, which induced 100% mortality at concentrations of 200-12.5 µg/mL and 98-99% at the lower concentrations. No meaningful distinction was found between luteolin and glucantime (p=0.1108; t=1.8249; df=7). The MIC value of luteolin was 12.5 µg/mL, and its LD_{50} value at even lower concentrations underscores its potential as an effective anti-leishmanial agent.

CONCLUSION

This study highlights the significant antiparasitic potential of luteolin against *E. histoytica*, *A. cestallanii* and *L. tropica*. Luteolin demonstrated comparable effectiveness to established treatments like MTZ, chlorhexidine, and glucantime under *in vitro* conditions. The lack of significant statistical differences between luteolin and these standard treatments suggests that luteolin may possess similar antiparasitic properties. Furthermore, its effective performance, particularly against *L. tropica*, supports the potential for luteolin to be explored further as an anti-leishmanial agent. Our results may form the basis for future studies to evaluate the anti-parasitic activity of luteolin in potential therapeutic applications.

*Ethics

Ethics Committee Approval: No clinical material or data were used in this study. Therefore, ethics committee approval is not required.

Informed Consent: Not required.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: E.T., Concept: E.T., E.A., Design: E.T., E.A., Data Collection or Processing: E.T., E.A., Analysis or Interpretation: E.T., E.A., Literature Search: E.T., E.A., Writing: E.T., E.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Atabati H, Kassiri H, Shamloo E, Akbari M, Atamaleki A, Sahlabadi F, et al. The association between the lack of safe drinking water and sanitation facilities with intestinal *Entamoeba* spp infection risk: a systematic review and meta-analysis. PLoS One. 2020; 15: e0237102.
2. Zhang Y, Xu X, Wei Z, Cao K, Zhang Z, Liang Q. The global epidemiology and clinical diagnosis of *Acanthamoeba* keratitis. J Infect Public Health. 2023; 16: 841-52.
3. Haftom M, Petrucka P, Gemechu K, Nesro J, Amare E, Hailu T, et al. Prevalence and risk factors of human Leishmaniasis in ethiopia: a systematic review and meta-analysis. Infect Dis Ther. 2021; 10: 47-60.
4. Turkeltaub JA, McCarty TR 3rd, Hotez PJ. The intestinal protozoa: emerging impact on global health and development. Curr Opin Gastroenterol. 2015; 31: 38-44.
5. Morán P, Serrano-Vázquez A, Rojas-Velázquez L, González E, Pérez-Juárez H, Hernández EG, et al. Amoebiasis: advances in diagnosis, treatment, immunology features and the interaction with the intestinal ecosystem. Int J Mol Sci. 2023; 24: 11755.
6. Seal DV. *Acanthamoeba* keratitis update-incidence, molecular epidemiology and new drugs for treatment. Eye (Lond). 2003; 17: 893-905.
7. Özbel Y, Töz S, Muñoz C, Ortuño M, Jumakanova Z, Pérez-Cutillas P, et al. The current epidemiology of leishmaniasis in Turkey, Azerbaijan and Georgia and implications for disease emergence in European countries. Zoonoses Public Health.407-395 :69 ;2022.
8. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis. 2017; 11: e0006052.
9. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016; 5: e47. Erratum in: J Nutr Sci. 2025; 14: e11.
10. Rakha A, Umar N, Rabail R, Butt MS, Kieliszek M, Hassoun A, et al. Anti-inflammatory and anti-allergic potential of dietary flavonoids: a review. Biomed Pharmacother. 2022; 156: 113945.
11. López-Lázaro M. Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem. 2009; 9: 31-59.
12. Iqbal K, Jamal Q, Iqbal J, Afreen MS. Luteolin as a potent anti-leishmanial agent against intracellular *Leishmania tropica* parasite. Trop J Pharm Res. 2017; 16: 337-42.
13. Muruganathan N, Dhanapal AR, Baskar V, Muthuramalingam P, Selvaraj D, Aara H, et al. Recent updates on source, biosynthesis, and therapeutic potential of natural flavonoid luteolin: a review. Metabolites. 2022; 12: 1145.
14. Kuhn DM, Balkis M, Chandra J, Mukherjee PK, Ghannoum MA. Uses and limitations of the XTT assay in studies of Candida growth and metabolism. J Clin Microbiol. 2003; 41: 506-8.
15. Tennant JR. Evaluation of the trypan blue technique for determination of cell viability. Transplantation. 1964; 2: 685-94.
16. Dziduch K, Greniuk D, Wujec M. The current directions of searching for antiparasitic drugs. Molecules. 2022; 27: 1534.
17. Bizuneh GK. The chemical diversity and biological activities of phytoalexins. Adv Tradit Med. 2021; 21: 31-43.
18. Ammar RB, Kilani S, Bouhlel I, Ezzi L, Skandrani I, Boubaker J, et al. Antiproliferative, antioxidant, and antimutagenic activities of flavonoid-enriched extracts from (Tunisian) Rhamnus alaternus L.: combination with the phytochemical composition. Drug Chem Toxicol. 2008; 31: 61-80.
19. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutrients. 2020; 12: 457.
20. Mead J, McNair N. Antiparasitic activity of flavonoids and isoflavones against *Cryptosporidium parvum* and *Encephalitozoon intestinalis*. FEMS Microbiol Lett. 2006; 259: 153-7.
21. Lehane AM, Saliba KJ. Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. BMC Res Notes. 2008; 1: 26.
22. AbouLaila MR, Abd El-Aziz A, Yokoyama N, Igarashi I. Luteolin: target validation in *Babesia bovis* by reverse transcription polymerase chain reaction and *in vivo* inhibition of *Babesia microti*. J Clin Res Med Rep. 2019; 1: 103.
23. Sifaoui I, López-Arencibia A, Martín-Navarro CM, Reyes-Batlle M, Wagner C, Chiboub O, et al. Programmed cell death in *Acanthamoeba castellanii* Neff induced by several molecules present in olive leaf extracts. PLoS One. 2017; 12: e0183795.
24. Lê HG, Võ TC, Kang JM, Nguyễn TH, Hwang BS, Oh YT, et al. Antiamoebic activities of flavonoids against pathogenic free-living amoebae, *Naegleria fowleri* and *Acanthamoeba* species. Parasites Hosts Dis. 2023; 61: 449-54.
25. Nezaratizade S, Hashemi N, Ommi D, Orhan IE, Khamesipour F. A systematic review of anti-*Entamoeba histolytica* activity of medicinal plants published in the last 20 years. Parasitology. 2021; 148: 672-84.
26. Martínez-Castillo M, Pacheco-Yepez J, Flores-Huerta N, Guzmán-Téllez P, Jarillo-Luna RA, Cárdenas-Jaramillo LM, et al. Flavonoids as a natural treatment against *Entamoeba histolytica*. Front Cell Infect Microbiol. 2018; 8: 209.
27. Cimanga RK, Kambu K, Tona L, Hermans N, Apers S, Totté J, et al. Cytotoxicity and *in vitro* susceptibility of *Entamoeba histolytica* to Morinda morindoides leaf extracts and its isolated constituents. J Ethnopharmacol. 2006; 107: 83-90.
28. Manjolin LC, dos Reis MB, Maquiaveli Cdo C, Santos-Filho OA, da Silva ER. Dietary flavonoids fisetin, luteolin and their derived compounds inhibit arginase, a central enzyme in *Leishmania* (Leishmania) amazonensis infection. Food Chem. 2013; 141: 2253-62.
29. Hamidizadeh N, Ranjbar S, Asgari Q, Hatam G. The evaluation of quercetin and luteolin efficacy on cutaneous leishmaniasis in mice infected with *Leishmania major*. J Pharm Negat Results. 2017; 8: 43-8.
30. Mittra B, Saha A, Chowdhury AR, Pal C, Mandal S, Mukhopadhyay S, et al Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. Mol Med. 2000; 6: 527-41.
31. Tasdemir D, Kaiser M, Brun R, Yardley V, Schmidt TJ, Tosun F, et al. Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. Antimicrob Agents Chemother. 2006; 50: 1352-64.