Progress in Microbes and Molecular Biology

HH PUBLISHER

Review Article

Recent Advances in Natural and Nanoparticle-Based Therapies for *Naegleria fowleri* Infections

Alexia Chloe Meunier[1], Reetashini Nair[2], Lee Yeong Zher[1], Natasha Sura Anak Lubau[1], Niwasini Krishna Kumar[3], Devandran Apparasamy[3]*, Usman Ahmed[2], Ayaz Anwar[2], Yuan Seng Wu[4,5], Subash CB Gopinath[6,7,8], Kavitha Rajendran[3]*

Article History

Received: 27 March 2025;

Received in Revised Form: 06 May 2025;

Accepted: 09 May 2025;

Available Online: 20 June 2025

[1]Jeffrey Cheah School of Medicine, Faculty of Health and Medical Science, Monash University, Malaysia; alexia.c.meunier@gmail.com (ACM); Yeong.Lee@monash.edu (YZ); natashasura.anaklubau@monash.edu (NSAL)

[2]Department of Biomedical Sciences, School of Medical and Life Sciences, Sunway University, Subang Jaya, 47500, Selangor, Malaysia; reetashininair@gmail.com (RN), 19094465@imail.sunway.edu.my (UA), ayazanwarkk@yahoo.com (AA)

[3]School of American Education (SAE), Sunway University, Subang Jaya, 47500, Selangor, Malaysia; niwasini17@hotmail.com (NKK)

[4]Department of Biomedical Sciences, Sir Jeffrey Cheah Sunway Medical School, Faculty of Medical and Life Sciences, Sunway University, Sunway City, Malaysia; yuansengw@sunway.edu.my (YSW)

[5]Sunway Microbiome Centre, Faculty of Medical and Life Sciences, Sunway University, Sunway City, Malaysia

[6]Center for Global Health Research, Saveetha Medical College & Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai – 602 105, Tamil Nadu, India; subash@unimap.edu.my (SCBG)

[7]Faculty of Chemical Engineering & Technology, 02600 Arau, and Institute of Nano Electronic Engineering, Universiti Malaysia Perlis (UniMAP), 01000 Kangar, Perlis, Malaysia

[8]Department of Technical Sciences, Western Caspian University, Baku AZ 1075, Azerbaijan

*Corresponding author: Kavitha Rajendran and Devandran Apparasamy; School of American Education (SAE), Sunway University, Subang Jaya, 47500, Selangor, Malaysia; kavithar@sunway.edu.my (KR) and devana@sunway.edu.my (DA)

Abstract: Primary amoebic meningoencephalitis (PAM), caused by *Naegleria fowleri*, remains an aggressively fatal infection with often ineffectual treatment avenues. This study reviews the antiamoebic efficacy of natural-derived compounds, including plant-based, marine-sourced, and nano-conjugated metabolites, bringing light to their potential as next-generation therapeutics.

Notably, plant-derived kaempferol and diosgenin were found to induce programmed cell death (PCD) in *N. fowleri* via mitochondrial signalling and production of reactive oxidative species (ROS), without the added toxicity to normal human cell lines. Terpenes such as thymol and forskolin effectively targeted both trophozoites and cysts by disrupting intracellular signalling and cell membrane integrity. The ability to cross the blood-brain barrier (BBB) was exhibited by marine-derived oxasqualenoids and sesquiterpenes a key requirement for effective PAM therapy, with the added ability to induce apoptosis in trophozoites. These compounds interfere with critical pathogenic mechanisms, including phagocytosis, adhesion, and protease activity highlighting their potential for multi-targeted therapy. Compared to current PAM treatments, which are limited by toxicity and poor central nervous (CNS) penetration, these natural compounds offer a safer and potentially more effective alternative. Additionally, nanoconjugation significantly enhanced the compound bioavailability, brain-targeting, and retention, further amplifying their therapeutic potential. This comprehensive review aims to bring to the apogee, the scientific importance of these findings and lays the foundation for future drug development strategies that harness the structural diversity and mechanistic specificity of natural products for treating PAM.

Keywords: *Naegleria fowleri*, primary amoebic meningoencephalitis, nanoparticles, anti-amoebic, natural compounds; SDG 3 Good health and well-being

1. Introduction

Naegleria fowleri, also known as the brain-eating amoeba, is a eukaryotic, free-living, pathogenic amoeba that causes primary amoebic meningoencephalitis (PAM), a rare but highly fatal infection. *Naegleria* spp. have been around for over a century, but a specific strain, *N. fowleri*, has gained growing attention for the past 50 years, as very few infection cases have been successfully treated and the currently available treatments have unpleasant side effects [1,2].

N. fowleri has been identified in 35 countries and can be found in water sources on all continents except Antarctica [3]. Its natural habitats include rivers, freshwater lakes, hot springs, and ponds. This parasite has also been detected in urban zones, including hotel swimming pools, waterparks, recreational fountains, and tap water used for nasal rinsing. As a thermophilic organism, it thrives under warmer conditions.

According to the latest statistics, 488 cases of primary amoebic meningoencephalitis (PAM) have been reported worldwide according to latest statistics, with 164 cases occurring in the USA between 1962 and 2023 [4,5]. However, the number of global cases is widely

underestimated owing to the difficulty in the diagnosis of PAM and the rapid progression of the infection, leading to patient death within 48 hours of the first symptoms [6]. The clinical symptoms are highly similar to those of viral and bacterial meningitis [7]. Headaches, fever, stiff neck, nausea followed by seizures, neurological impairments, cerebral hemorrhage, and death are the reported symptoms and prognosis of infected patients [7,8].

N. fowleri is a thermophilic amoeba that exists in three forms: the cyst form, the trophozoite form, and the motile flagellate form. Although all forms are capable of causing central nervous system infection, the trophozoite is the main proliferative and tissue-invasive stage associated with disease progression. During hostile conditions, the amoeba transforms into a metabolically inactive cyst, measuring 8–12 μm in diameter, characterized by a thick double wall with pores [9]. In this stage, the organism remains dormant and highly resistant to various physical and chemical stresses [2,7]. Although cysts can be transported by dust particles and enter the nasal mucosa, no cysts have been identified in brain tissue [10]. Moreover, the trophozoite and flagellate forms are typically inhaled during activities such as swimming, diving, or contact with contaminated water. These forms are the only ones found in the affected tissues and cerebrospinal fluid of infected individuals [11]. Trophozoites attach to the nasal mucosa, penetrate the olfactory epithelium, and traverse the cribriform plate to invade the brain [9]. Once in the central nervous system, trophozoites trigger intense inflammation, leading to neuronal damage and extensive tissue destruction [9]. Currently, no drugs are specifically designed for PAM [12]. The current treatment for PAM includes a combination of antifungal agents (amphotericin B), anti-cancer drugs (miltefosine), and antibiotics (rifampin) [10]. However, the use of these medications is often associated with severe adverse effects. The blood-brain barrier further complicates drug delivery to the central nervous system during infection. Consequently, higher doses are typically required to achieve minimal inhibitory effects, thereby increasing the risk of toxicity [13]. Amphotericin B, for instance, is associated with unpleasant side effects such as nausea and, in severe cases, nephrotoxicity [14]. Furthermore, rifampin monotherapy is discouraged due to the rapid emergence of drug resistance [11]. Therefore, there is an urgent need to develop safer and more effective therapeutic strategies [2].

In the search for improved therapies, natural products offer a promising alternative. Composed of multiple bioactive compounds, natural substances exhibit a broad range of biological activities, including antiparasitic, antifungal, antibacterial, anticancer, and antiviral effects. For example, several natural compounds have showed activity against disease-causing organisms such as *Acanthamoeba*, fungi, bacteria, and *Leishmania*. Beyond their biomedical applications, natural compounds also possess electrochemical and photochemical properties, making them attractive for renewable technologies such as solar panels [15,16]. Natural products

have historically played a central role in drug discovery. According to the World Health Organization, approximately 80% of the global population relies on medicinal plants for disease management [17], and it is estimated that up to 50% of currently marketed drugs have natural origins [18]. In addition, microorganisms have proven to be rich reservoirs of therapeutic agents, exemplified by the discovery of penicillin from *Penicillium* fungi. This breakthrough ushered in a new era of natural-product-derived medicines, soon followed by landmark discoveries such as streptomycin, cyclosporine, and erythromycin. Despite ongoing efforts to explore natural chemical diversity, only a small fraction of newly discovered bioactive molecules advances through rigorous clinical evaluation [19]. Given the limitations of current treatments for infections such as PAM, natural products remain a largely untapped but highly promising source for novel drug development [20]. Moreover, notable examples such as amphotericin B (AmB) and ivermectin both isolated from *Streptomyces* species underscore the therapeutic potential of microbial-derived compounds [21]. Several studies have evaluated the effects of naturally derived compounds on *N. fowleri*, primarily targeting the trophozoite stage due to its active role in infection. Although cyst and flagellate forms are also infectious, they are less frequently studied because of the difficulty in establishing consistent experimental models. Nevertheless, promising inhibitory agents have been identified; however, their mechanisms of action remain poorly understood, limiting their translation into clinical applications.

Despite the discovery of several natural compounds with inhibitory effects against *N. fowleri*, challenges such as poor solubility, low bioavailability, and inefficient delivery to target tissues have hindered their clinical application. Therefore, innovative strategies, particularly advanced drug delivery systems, are critical to unlocking the therapeutic potential of these natural agents. One promising approach is nano-conjugation, which has gained significant attention in drug discovery studies. Nano-carriers can enhance the pharmacokinetics and pharmacodynamics of cargo drugs, improving their solubility, stability, and targeted delivery [1]. In recent years, scientists have successfully conjugated a wide range of compounds including natural products and existing drugs with nanoparticles. Among these, gold, silver, and various metal oxides are the most extensively researched carriers for nanoparticle-based drug delivery systems [13]. For example, a recent study investigated the activity of amphotericin B conjugated with silver nanoparticles and found that the nanoconjugated AmB exhibited enhanced activity against *Naegleria* [13]. In this review, we present a comprehensive summary of natural compounds identified over the past decade that demonstrate inhibitory activity against *N. fowleri* trophozoites. By consolidating these findings, we aim to support ongoing research toward the development of safer and more effective therapeutic strategies against this devastating and often fatal infection.

2. Methods

A comprehensive literature search was conducted using Google Scholar, PubMed, and Scopus to collect information on reported cases of *N. fowleri* inhibition published between 2014 and 2024. Keywords such as "*Naegleria fowleri*," "natural compounds," "natural extracts," "natural metabolites," "*Naegleria fowleri* inhibition," "nanoconjugated compound," and related terms were used to ensure an inclusive and focused search. Titles and abstracts were screened for relevance, and studies focusing solely on synthetic compounds were excluded.

For each selected publication, detailed information was extracted, including the origin of the tested compound, amoebicidal potential, cytotoxic activity, details of any nano-conjugation, and proposed mechanisms of action. Additional data, such as compound concentrations, experimental models (*in vivo* or *in vitro*), and statistical measures (e.g., IC_{50} values when available), were also recorded.

Multiple reviewers independently assessed the publications to enhance the objectivity of the review process. Disagreements were resolved through consensus and discussion to minimize potential bias. This systematic approach facilitated the development of a robust dataset, providing valuable insights into the potential of natural compounds and nanoconjugates in inhibiting *N. fowleri* viability and supporting future therapeutic advancements.

3. Results and discussion

3.1. Potential of Plant-Derived Compounds as Anti-microbial Agents against N. fowleri

The emergence of multidrug-resistant microorganisms requires the development of new anti-microbial agents. Plant-derived compounds have emerged as promising candidates because they possess diverse chemical structures and biological activities. A broad range of plant secondary metabolites, including alkaloids, flavonoids, terpenes, and phenolic compounds, have been studied for their anti-microbial properties [22–26]. They have shown a range of pharmacological effects, including disruption of bacterial cell membranes, interference with RNA synthesis, modulation of bacterial efflux pump expression, and inhibition of quorum sensing mechanisms [22–26]. Because of their broad-spectrum activity, these phytochemicals are now being studied for their potential against free-living amoeba, such as *N. fowleri* and *Acanthamoeba spp.* as shown in Table 1.

One promising source of anti-microbial agents is *Momordica charantia* (bitter melon), a medicinal plant traditionally used in Asian, South American, and Caribbean medicine [27]. Previous studies have shown that diosgenin, a steroidal saponin extracted from *M. charantia*,

exhibits broad pharmacological activities, including antiviral, anti-fungal, and antihelminthic effects [28–30]. In a study on the amoebicidal activity of diosgenin against *N. fowleri* trophozoites, diosgenin showed 98% inhibition at 100 μg/mL of the extract after 6 h of exposure and 100% inhibition after 24 hours. Diosgenin was shown to disrupt trophozoite membrane integrity and inhibit cysteine protease activity of *N. fowleri* through mechanistic studies, with Diosgenin as a potential therapeutic agent [31].

Similarly, *Larrea tridentata*, referred to as the creosote bush, endemic to the remote southwestern United States, is known for its varied pharmacological properties, including antioxidant and anti-bacterial properties [1]. Nine secondary metabolites from *L. tridentata* were evaluated against *N. fowleri* in an assay conducted by Bashyal et al. [32]. The amoebicidal activities of seven of these compounds against *N. folweri* were determined to be potent, with EC_{50} values between 37 and 235 μM. The primary mechanism of action was found to be inhibition of cysteine protease activity, a process required for trophozoite survival and pathogenicity [32].

Another medicinal plant, *Inula viscosa* has been studied for its antiamoebic properties. *Inula viscosa* crude extracts showed inhibitory activity against *N. fowleri* trophozoites, with an IC_{50} of 17.89 μg/mL. Subsequent purification led to the identification of two bioactive compounds, Inuloxin A and Sakuranetin. These compounds induced programmed cell death (PCD) in trophozoites, as previously described by Zeouk *et al*. [33]. PCD hallmarks, such as chromatin condensation, mitochondrial damage, cell membrane disruption, and increased production of ROS, were induced, demonstrating their potential as effective therapeutic agents against amoebic infections [33].

Polyphenolic compounds, called flavonoids, have shown considerable promise in fighting protozoal infections, including those arising from free-living amoebae. Lê *et al*. [28] recently evaluated the antiamoebic activity of 18 flavonoids against *N. fowleri*. Among these compounds, they identified the most potent compounds to be demethoxycurcumin and kaempferol, with IC_{50} values below 40 μM. Kaempferol has been extensively studied for its antimicrobial and antiparasitic properties and is widespread in many fruits and vegetables. This compound has shown efficacy against pathogenic bacteria, fungi, and protozoa [34]. Recently, kaempferol was tested against *N. fowleri* and was shown to induce PCD in trophozoites. The mechanisms of action include disruption of cellular processes such as chromatin condensation, mitochondrial dysfunction, increase of ROS production, culminating in apoptotic, ultimately leading to 'non-apoptotic,' cell death. These findings suggest that kaempferol is a promising candidate for new treatment strategies against *N. fowleri* infections [35].

Recent findings highlight the presence of many terpenes that exhibit strong antiamoebic activities with both *N. fowleri* trophozoites and cysts. Forskolin, thymol, borneol, and andrographolide showed potent inhibitory effects against parasites with low cytotoxicity to human cells. These terpenes have diverse mechanisms of action, including membrane disruption, inhibition of enzymatic activities, and modulation of cellular signaling pathways, which signify their potential as promising candidates for further development as antiamoebic agents [36].

Additionally, propolis is known to have broad-spectrum anti-microbial potential. Recent studies have demonstrated that propolis is effective against *N. fowleri*, exhibiting both amoebicidal and cysticidal activity. Treatment of *N. fowleri* trophozoites with propolis at a concentration of 5 μg/mL for 24 hours resulted in a 96% reduction in viability, indicating its potential as a therapeutic agent against this highly pathogenic amoeba. The potency of propolis is likely attributed to its complex chemical composition, rich in flavonoids, phenolic acids, and terpenes, which together may disrupt cellular functions and ultimately lead to cell death in amoebae [37].

Terpenes are a diverse class of naturally occurring organic compounds that exhibit anti-fungal, antibacterial, and antiparasitic activities. Our group has revealed that terpenes such as forskolin, thymol, borneol, and andrographolide possess the most potent activity against *N. fowleri.* Although these compounds inhibited trophozoites and cysts of *N. fowleri* as effectively as possible, they demonstrated low toxicity to human cells [36].

Forskolin, a diterpene extracted from *Coleus forskohlii*, has been reported to disrupt protozoan cellular signalling pathways, while thymol, a monoterpenoid phenol found in thyme oil, compromises the intergrity of microbial cell membranes [36,38]. Borneol's antibacterial activity improves its ability to penetrate microbial cells. Andrographolide, a diterpenoid lactone from *Andrographis paniculata*, shows strong anti-inflammatory and anti-parasitic activities and might account for its action against *N. fowleri*. Together, these terpenes suggest their potential use as part of a multi-targeted therapeutic intervention against amoebic infections [36].

More recently, *Pinus densiflora*, a member of the Pinaceae family, widely distributed in East Asia and used in traditional medicine was tested against *N. fowleri* [39]. The leaf extracts of *P. densiflora* showed promising amoebicidal activity with low toxicity against glial cell lines making it a good candidate for development of novel drug for PAM.

Table 1. Naturally derived compoinds with anti-amoebic activity against *N. fowleri*, including their key active compounds, their mechanisms of action, and inhibitory effects.

Compounds Source	Active Compounds	Activity on N. *fowleri* trophozoites	Mechanism of action	References
Plant Based				
Momordica charantia	Diosgenin	98% inhibition of trophozoites at 100 µg/ml	Disrupt membrane integrity, Inhibition of cysteine protease	[31]
Larrea tridentata	Multiple Secondary metabolites	EC_{50}: 37- 235 µM	Inhibition of cysteine protease activity	[32]
Inula viscosa	Inuloxin A and Sakuranetin	IC_{50} 17.89 µg/mL Inuloxin A at 21.27 µM Sakuranetin: 74.83 µM	Induction of programmed cell death (PCD)	[33]
Propolis	Propolis extracts, Flavonoids, and phenolic acids	96% inhibition of trophozoites after 24 hours	Disrupts cell membrane, induces cell death	[37]
Variety of plants	Kaempferol	Low IC_{50} values (< 40 µM). IC_{50} of 29.28 ± 0.63 µM	Induces apoptosis-like cell death	[28]
Medicinal Plants	Forskolin, thymol, andrographolide, borneol	IC_{50} (µM): Thymol 153.601 µM, Forskolin 189.170 µM, Percentage Inhibition: Borneol: 49% Andrographolide:46% Inhibits trophozoites and cysts, low human cell toxicity	Disrupts cellular processes, membrane integrity	[36]
Microbes				
Streptomyces sanyensis	Indolocarbazoles	IC_{50} ranging 0.08 - 11.51 uM	Induction of programme cell death	[40]
Fungal isolates	Bioactive extracts	> 33% inhibition	Not reported	[41]
Fungal	Lovastatin	IC_{50} 19.742 uM	Not reported	[42]

B*acillus spp.*	Tyrocidines - derived peptides	94% inhibition after 24h	Not reported	[43]
Marine Compounds				
Laurencia viridis	Oxasqualenoids	IC_{50} :16.25 ± 1.23 μM and 12.70 ± 2.64 μM	Not reported	[44]
Gongolaria abies-marina,	Meroterpenoids	IC_{50} ranging 19.86 - 58.91	Induction of programmed cell death	[45]
Laurencia dendroidea.	Sesquiterpenes	IC_{50} : 61.52 ± 12.97 μM	Not reported	[46]
Laurencia Johnstonii	Laurinterol Debromolaurinterol Isolauritenol	IC_{50}: μM Laurinterol: 13.42 Debromolaurinterol: 18.76 Isolauritenol: 28.18	Not reported	[20]

3.2. The Therapeutic Potential of Marine Bioactive Compounds

The uniqueness of the marine environment and the extremes of its habitats have emerged as a source of bioactive compounds with significant potential for drug discovery. Marine organisms, including algae, sponges, and corals, are prolific sources of structurally diverse metabolites that demonstrate a wide spectrum of bioactivities, notably antiviral, antibacterial, anticancer, and anti-inflammatory properties [47–49]. In recent years, efforts have focused on harnessing these marine-derived compounds to develop novel therapeutic agents against free-living amoebae, such as *N. fowleri*, the causative agent of PAM, a rare but often fatal infectious process.

Another promising avenue for research is the extraction of oxasqualenoids from the red algae *Laurencia viridis* [44]. These compounds also significantly inhibited *N. fowleri* trophozoites. Yucatone, most active oxasqualenoid identified, exhibits potent antiamoebic activity with an IC_{50} value of 28.53 μM. This efficacy may be attributed to the unique squalene-derived backbone of oxasqualenoids, which is thought to disrupt membrane integrity and induce cell death in parasitic amoebae. These findings position red algae-derived metabolites as a novel and promising class of antiamoebic agents [44].

Another group of marine-derived secondary metabolites, meroterpenoids, have also demonstrated potent antiamoebic activity [45]. Six bioactive compounds were extracted from the brown alga *Gongolaria abies-marina*, all of which inhibited *N. fowleri* trophozoites to a comparable degree. Of these, the most potent inducer of PCD in the parasite was identified as Gongolarone B. Several cellular events are involved in PCD, such as chromatin condensation,

mitochondrial damage, and an increase in ROS production, which essentially indicates that Gongolarone B could effectively disrupt the parasite's survival mechanisms [45].

The antiamoebic potential of sesquiterpenes isolated from the red alga *Laurencia dendroidea* has also been explored [46]. The sesquiterpene (+)-elatol demonstrated notable activity against trophozoites and cysts of *N. fowleri*. Importantly, the compound's low molecular weight and high lipophilicity make it a powerful candidate for crossing the blood-brain barrier, which is an essential requirement for effective PAM treatment. Further mechanistic studies have identified that (+)-elatol induces PCD in trophozoites and has the potential to serve as a therapeutic agent targeting multiple stages of the parasite life cycle [46]. Furthermore, recent studies have focused on the potential of cyclolaurane-type sesquiterpenes as potential antiamoebic agents. Debromolaurinterol, a compound isolated from the red alga *Laurencia johnstonii*, demonstrated remarkable efficacy against *N. fowleri*, achieving 99.98% inhibition of ATP production in treated cells [20]. Molecular modeling and Structure-Activity Relationship (SAR) analysis indicated that the unique structural features of cyclolaurane metabolites improved their binding affinity and efficacy against the parasite. Further, in silico ADME/Tox analysis also indicated satisfactory pharmacokinetic properties, thus reinforcing the potential of these compounds as chemical models for the development of new PAM treatments [46].

A germacrane sesquiterpene lactone, was isolated from the zoanthid coral, *Palythoa aff. Clavate* [50]. Recently, it has been shown to be a promising antiamoebic compound. In vitro testing showed that anhydroartemorin inhibited *N. fowleri* with an IC_{50} of 23.02 ± 1.26 μM. The compound was also tested for its ability to induce programmed cell death in trophozoites, and showed positive results. Anhydroartemorin possesses properties that allow it to cross the blood-brain barrier, a major hurdle in the development of effective antiamoebic drugs. Hence, due to its ability to penetrate the central nervous system, it is a promising candidate for further development as a PAM therapeutic agent [50].

The diversity and uniqueness of the chemical structures in marine metabolites form a largely untapped reservoir of a large number of potential bioactive compounds, with the potential for innovation in the search for therapeutics against combating *N. fowleri* and other challenging pathogens. As research into marine bioactive compounds continues to expand, there is growing optimism that these natural products may yield effective and targeted solutions for combatting amoebic infections and other emerging diseases.

3.3. Microbial Metabolites in the Quest for Novel Therapies

Microbial metabolites have attracted the interest of researchers as promising candidates for novel therapeutic research. Metabolites produced by microorganisms have shown therapeutic potential for a range of diseases, including cancer and infections [51–54]. Indocarbazoles derived from *Streptomyces sanyensis* showed promising results against *N. fowleri*. These compounds have previously been reported for their ability to induce PCD. Four compounds extracted from mangroves were found to inhibit *N. fowleri* [40]. These findings align with previous studies reporting that parasites use protein kinases for several biological processes and for survival. In support of this, a study conducted by Rizo-Liendo *et al*., the indolocarbazoles derived from *Streptomyces sanyensis* exhibit protein kinase and DNA topoisomerase I inhibitory properties [55]. All these findings point to a possible avenue to developing compounds that are more effective against *N. fowleri*.

In addition to bacterial sources, mangrove fungi can produce a wide range of secondary metabolites that exhibit significant antimicrobial activity. For instance, fungal isolates from *Rhizophora mangle*, *Avicennia germinans*, *Laguncularia racemose*, *Conocarpus erectus*, and *Coccoloba uvifera* have been previously screened for bioactive extracts. From this screening, 34 extracts exhibited greater than 33% amoeba inhibition. Among the compounds identified, lovastatin, a common secondary metabolite produced by various fungal species, was of particular interest [56]. This compound is a known inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a crucial component of cholesterol biosynthesis in humans and ergosterol in protozoa [41]. Given that ergosterol is essential for the integrity of the cell membrane of *N. fowleri*, makes it a good target for antiamoebic compound screening [41]. Notably, lovastatin was tested against *N. fowleri* trophozoites and successfully eliminated the pathogen.

Futhermore, tyrocidin-derived peptides have shown promising activities against *N. fowleri.* Tyrocidines are naturally occurring antimicrobial produced by B*acillus spp*. found in the soil [43]. When tested against *N. fowleri in vitro*, this compound exhibited potent inhibitory effects on trophozoites and encystation. Although the mechanism of action was not reported in this study, a previous study reported the ability of tyrocidine to disrupt membrane integrity and lead to cell death [57].

3.4. Pathways Targeted by Natural Agents in N. Fowleri

Natural compounds and their nano-formulated derivatives target multiple vital mechanisms in *N. fowleri*. In the last decade, several *in vitro* and *in vivo* studies have confirmed their effectiveness through molecular, cellular, and biochemical analyses. These studies

demonstrate how natural agents modulate cellular pathways, suppress virulence factors, and induce programmed cell death.

3.4.1. Inhibition of Cytolytic Activity

Cytolytic activity is the fundamental mechanism by which *N. fowleri* invades host tissues. The amoeba produces cytolytic enzymes, such as phospholipases and neuraminidases, which degrade host cell membranes, enabling tissue invasion and feeding on cellular debris [7]. Phospholipases hydrolyze phospholipids, disrupting cell membrane integrity, whereas neuraminidases cleave sialic acids from host cell surfaces, enhancing adhesion and phagocytosis [58]. Natural compounds, such as quercetin and kaempferol, both flavonoids, have shown promise in inhibiting phospholipase activity, thereby reducing tissue destruction caused by *N. fowleri* [34,35]. Additionally, saponins found in various plants can disrupt cell membranes, potentially contributing to the immune responses that protect against amoebic infections. These findings suggest that natural compounds may be effective in limiting the cytotoxic effects of *N. fowleri* [59].

3.4.2. Adhesion and Phagocytosis Inhibition

Adhesion is critical for *N. fowleri* infection in host tissues. Amoebas use structures called food cups during phagocytosis to engulf host cells [60]. It relies on adhesion molecules, such as lectins and surface glycoproteins, to attach to epithelial cells, such as those in the nasal cavity. Lectins bind to specific sugars on host cells, facilitating attachment, whereas surface glycoproteins assist in interacting with host cell receptors, promoting invasion [55]. Natural compounds have demonstrated efficacy in disrupting adhesion mechanisms. For example, plant-derived polysaccharides, such as those from *Aloe vera*, can inhibit the ability of *N. fowleri* to adhere to host cells, preventing the amoeba from accessing the central nervous system (CNS) [20]. Resveratrol, a polyphenol found in grapes, affects cytoskeletal dynamics, hindering the formation of food cups necessary for phagocytosis. This disruption of adhesion reduces the ability of *N. fowleri* to cause infection and to propagate within host tissues [61].

3.4.3. Induction of Programmed Cell Death

Programmed cell death or apoptosis is a promising strategy for controlling the growth of *N. fowleri* [40,62]. Apoptosis results in cellular self-destruction without causing an inflammatory response, making it a desirable mechanism for reducing the viability of pathogens. Natural compounds can induce apoptosis in *N. fowleri* via pathways such as caspase activation and mitochondrial dysfunction [35,63]. Caspases are enzymes critical for executing apoptosis, and their

activation can trigger cell death in amoebas. Kaempferol induces apoptosis-like morphological changes in *N. fowleri*, including chromatin condensation and membrane blebbing [35,64]. Similarly, curcumin, a compound found in turmeric with known anti-inflammatory properties, has been demonstrated to activate apoptotic pathways. Curcumin is believed to generate reactive oxygen species (ROS) and disrupt mitochondrial function, thereby promoting cell death in *N. fowleri*. These natural compounds may serve as potent agents for inducing cell death in amoebas, limiting their pathogenic potential [7].

3.4.4. Inhibition of Enzymatic Activity

The survival and proliferation of *N. fowleri* depend on various enzymatic activities such as those of acetylcholinesterase (AChE) and proteases [65]. AChE is responsible for the breakdown of acetylcholine, a neurotransmitter essential for neuronal signalling. By inhibiting AChE, natural compounds can disrupt neural communication in *N. fowleri* and impair its motility [65]. Proteases that facilitate degradation are vital for cellular function and growth. Laurinterol, a compound derived from marine algae, has been identified as an AChE inhibitor that effectively disrupts neurotransmitter signalling in *N. fowleri* and hinders its motility [20]. Furthermore, plant-derived protease inhibitors have been shown to impair the proteolytic activity of *N. fowleri*, further limiting its ability to grow and spread within host tissues. These findings underscore the potential of enzymatic inhibition as a therapeutic approach for treating *N. fowleri* infections [59].

3.5. Combination with Nanotechnology: A Promising Strategy

In recent years, nanoparticles have emerged as a revolutionary tool in drug delivery, owing to their unique physiochemical properties [57]. Metals such as silver, gold and zinc have been extensively studied and utilized due to their exemplary antimicrobial properties alongside its compatibility with preexisting medical applications [66,67]. Offering several advantages over traditional systems, such as targeted drug delivery to specific tissue, controlled drug release and enhanced stability of the drug [68]. The nanoscale interaction of biological systems at a molecular level is astounding with reports of increased bioavailability and precision therapeutic intervention [69]. This coincides perfectly to the needs of *N. fowleri* afflictions as this circumvents the known challenges of poor solubility, degradation of natural compounds, and mitigating other potential unintended side effects [70]. The adjudication of nanoparticles with natural compounds aims to augment the existing treatments by increasing efficacy and efficiency against *N. fowleri* [71].

Further expanding upon the pertinence of the metallic components of nanoparticles, such as silver and gold due to their small size and large surface area [72], they are more readily able to

interact effectively with microbial cells, including amoebic parasites such as *N. fowleri* doing so by inhibiting cell growth and cellular functions [13]. The reports of enhanced amoebicidal activity with silver nanoparticles coated in amphotericin B serves as the basis for improvement of natural compounds with nanoparticles against *N. fowleri* [73]. The conjugation of nanoparticles also enables targeted and controlled drug delivery, with the ability to be engineered to target specific cells by attaching functional groups or ligands that bind to receptors on diseased cells [74], This allows for more controlled drug release, reduced frequency of dosing and the reduction of drug concentration for treatment. As for the question of biocompability reports detail that this can be addressed with functionalization of gold and zinc with organic polymers to enhance human cell compatibility [75]. The added benefit of nanoconjugated compounds having a reduced cytotoxic effect on human cells while maximizing pathogen damage, this is underscored with the study done by Sani, 202 [76].

Table 2 shows the recent studies that have explored the use of nanoparticles to enhance the efficacy of existing drugs against *N. fowleri*. Notably, the clinically approved antifungal drugs amphotericin B, fluconazole, and nystatin were tested against *N. fowleri* alone and in conjugates with silver nanoparticles. Nanoconjugates of these drugs showed potent amoebicidal activity at 10 μM, which was significantly more effective than when the drugs were tested alone [66,77]. *trans*-Cinnamic acid and curcumin are both natural anti-microbial that possess a wide range of biochemical properties. Both were conjugated with gold nanoparticles to enhance their anti-microbial activity against *N. fowleri*. The conjugates demonstrated enhanced anti-Fowleri activity compared to that of the compounds alone [78].

Our group reported the effects of oleic acid and terpenes andrographolide, borneol, and forskolin-conjugated silver nanoparticles against *N. fowleri.* Significant activity was exhibited at a concentration of 10 μM when the trophozoites were treated with the nanoconjugates, and low toxicity was recorded against host cells [79,80]. The exact mechanism is unknown; however, silver nanoconjugated oleic acid has been previously reported to cause cytoplasmic destruction in bacteria, leading to cell death. Oleic acid is a fatty acid that occurs naturally in living organisms and possesses anti-inflammatory and immune-system-boosting properties in humans. Flavonoids, a diverse group of plant-derived secondary metabolites, have garnered significant attention owing to their wide range of biological activities and therapeutic potential [81]. However, the clinical applications of these compounds are limited owing to their poor bioavailability. Hesperidin and naringin, two flavonoids derived from citrus fruits, have been conjugated with gold and silver nanoparticles and stabilized by plant gums [81]. This unique approach resulted in drastically enhanced amoebicidal activity with the inhibition of encystation and excystation. The integration of nanotechnology offers a promising avenue for the

development of effective therapies against *N. fowleri*, addressing the urgent need for new and more effective treatments [82].

Table 2. Summary of the natural compound nanoconjugates and their efficacy.

Compound	Conjugated with	*In vivo* efficacy on trophozoites (% inhibition)	Reference
Oleic acid	Silver	66	[80]
***trans*-Cinnamic Acid**	Gold	84	[78]
Hesperidin **Naringin**	Silver Gold	99 75	[81]
Curcumin	Gold	69	[83]
Andrographolide **Borneol** **Forskolin**	Silver	63 70 73	[79]

4. Future Directions

The future of treating amoebic infections, especially PAM, lies in the advancement of innovative technologies as well as multidisciplinary methodologies to combine biochemistry, synthetic chemistry, pathology, nanotechnology, and physiology. The effectiveness of these bioactive compounds should be enhanced through auspicious structural modifications. A semi-synthetic derivative approach can be applied for these terpenes, flavonoids, and oxasqualenoids to tailor their structures, which will further improve their ability to cross the BBB, selectivity, transportation, and potency. This could enhance the effectiveness of these compounds against PAM infection. Furthermore, other computational methods, such as dynamic simulation and molecular docking, should be employed to assist in the discovery and fine-tuning of compounds that target critical cellular pathways.

This novel nanotechnological approach offers a highly promising strategy, particularly in coating drug-loaded nanoparticles designed for tissue-specific targeting and precise drug release. Functionalized nanoparticles can cross the BBB and are likely to considerably improve the effectiveness of both existing and newly identified therapeutic drugs and compounds against PAM infection. Likewise, the conjugation of nanoparticles, biologically active plant-derived and marine compounds, and other anti- microbial compounds can produce synergistic effects. Thus,

reducing the likelihood of resistance and ensuring the complete eradication of both trophozoite and cyst stages of *N. fowleri*. Effective and meaningful advancement in these areas necessitates interdisciplinary teamwork among researchers in pharmacology, chemistry, natural compounds, and nanomedicine to develop novel and effective treatments for this crucial health challenge.

5. Conclusion

N. fowleri is a highly pathogenic and often fatal, bi-phasic, free-living amoebic parasite. The search for potent therapeutic drugs against this fatal amoeba has advanced significantly through two major strategies, which is the use of natural products, particularly purified compounds, and the application of nanotechnologies. Among these natural sources, plant-derived bioactive compounds such as kaempferol, terpenes, and diosgenin, along with marine metabolites like sesquiterpenes and oxasqualenoids, have shown strong and promising antiamoebic activity. These properties are achieved by various mechanisms of action, such as triggering PCD, disruption of cellular membranes, and inhibition of different enzymes. Likewise, microbial bioactive metabolites, such as tyrocidines and lovastatin, have demonstrated significant efficacy by targeting crucial cellular processes and pathways of these amoebic parasites. However, this approach faced critical challenges that were overcome by a novel strategy. That is by incorporating bioactive compounds with various nanoparticles. This enhanced their stability, precise transportation, concentration, and bioavailability, while reducing their potential toxicity to human cells due to their small size. This integrated novel approach shows great promise in overcoming current challenges and combating pathogenic *N. fowleri*, highlighting the crucial role of naturally derived compounds and advanced technologies in the management and treatment of fatal PAM infections.

Author Contributions: Conceptualization, Data curation, methodology, investigation, writing—original draft preparation, formal analysis, review, ACM.; RN; UA; KR; Formal Analysis, Investigation, writing—original draft preparation, ACM; RN; NKK; YZ; NSAL; Formal Reviewing and editing; YSW; SCBG; Investigations and resources were provided by all authors, project administration, supervision, KR; DA; AA; Funding, visualization KR; AA.

Funding: This research was supported by Sunway University and Sunway University internal grant scheme (GRTIN-RAG-DADTP-03-2024.).

Acknowledgements: We thank Alexia Chloe Meunier, Reetashini Nair, Lee Yeong Zher, Natasha Sura Anak Lubau, Usman Ahmed, Niwasini Krishna Kumar, Yuan Seng Wu and Subash CB Gopinath for their contribution to the completion of the review manuscript. Special thanks to Kavitha Rajendran and Ayaz Anwar for their support and valuable insight into the final review of the manuscript for submission.

Conflict of interests: The authors declare that there is no conflict of interest.

References

1. Debnath A. Drug discovery for primary amebic meningoencephalitis: from screen to identification of leads. Expert Rev Anti Infect Ther 2021;19:1099–106.
2. Ahmed U, Anwar A, Ong S, *et al*. Applications of medicinal chemistry for drug discovery against *Acanthamoeba* infections. Med Res Rev 2022;42:462–512.
3. Ahmad Zamzuri M 'Ammar I, Abd Majid FN, Mihat M, et al. Systematic Review of Brain-Eating Amoeba: A Decade Update. Int J Environ Res Public Health 2023;20:3021.
4. CDC. About Naegleria fowleri Infections. EmNaegleria Fowleri EmInfection 2024. https://www.cdc.gov/naegleria/about/index.html (accessed May 5, 2025).
5. Alanazi A, Younas S, Ejaz H, *et al*. Advancing the understanding of *Naegleria fowleri*: Global epidemiology, phylogenetic analysis, and strategies to combat a deadly pathogen. J Infect Public Health 2025;18:102690.
6. Chow FC, Glaser CA. Emerging and Reemerging Neurologic Infections. The Neurohospitalist 2014;4:173–84.
7. Güémez A, García E. Primary Amoebic Meningoencephalitis by Naegleria fowleri: Pathogenesis and Treatments. Biomolecules 2021;11:1320.
8. Siddiqui R, Khan NA. Primary Amoebic Meningoencephalitis Caused by Naegleria fowleri: An Old Enemy Presenting New Challenges. PLoS Negl Trop Dis 2014;8:e3017.
9. Visvesvara GS. Free-Living Amebae as Opportunistic Agents of Human Disease. J Neuroparasitology 2010;1:1–13.
10. Bellini NK, Santos TM, Da Silva MTA, *et al*. The therapeutic strategies against Naegleria fowleri. Exp Parasitol 2018;187:1–11.
11. De Jonckheere JF. A century of research on the amoeboflagellate genus Naegleria. Acta Protozool 2002;41:309.
12. Chao-Pellicer J, Arberas-Jiménez I, Sifaoui I, *et al*. Exploring therapeutic approaches against *Naegleria fowleri* infections through the COVID box. Int J Parasitol Drugs Drug Resist 2024;25:100545.
13. Rajendran K, Anwar A, Khan NA, *et al*. Brain-Eating Amoebae: Silver Nanoparticle Conjugation Enhanced Efficacy of Anti-Amoebic Drugs against Naegleria fowleri. ACS Chem Neurosci 2017;8:2626–30.
14. Cárdenas-Zúñiga R, Silva-Olivares A, Villalba-Magdaleno JDA, *et al*. Amphotericin B induces apoptosis-like programmed cell death in Naegleria fowleri and Naegleria gruberi. Microbiology 2017;163:940–9.

15. Idrissi YE, Elouafy Y, Moudden HE, *et al*. Evaluation of Antioxidant and Antimicrobial Activity of Saponin Extracts from Different Parts of Argania spinosa L. Skeels. Prog Microbes Mol Biol 2023;6.

16. Elouafy Y, Mortada S, Yadini AE, *et al*. Bioactivity of Walnut: Investigating the Triterpenoid Saponin Extracts of Juglans regia Kernels for Antioxidant, Anti-diabetic, and Antimicrobial Properties. Prog Microbes Mol Biol 2023;6.

17. Malaquias G, Cerqueira GS, Ferreira PMP, *et al*. Utilização na medicina popular, potencial terapêutico e toxicidade em nível celular das plantas Rosmarinus officinalis L., Salvia officinalis L. e Mentha piperita L. (Família Lamiaceae) 2014.

18. Kingston DGI. Modern Natural Products Drug Discovery and Its Relevance to Biodiversity Conservation. J Nat Prod 2011;74:496–511.

19. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001;109:69–75.

20. Arberas-Jiménez I, García-Davis S, Rizo-Liendo A, *et al*. Laurinterol from Laurencia johnstonii eliminates Naegleria fowleri triggering PCD by inhibition of ATPases. Sci Rep 2020;10.

21. Wehelie YI, Nasih AL, Anwar A, *et al*. Natural Products for Targeting *Acanthamoeba* spp. Anti-Infect Agents 2022;20:e191121198116.

22. Chtibi H, Harboul K, Benali T, *et al*. Comparative Study of Antibacterial Activity of Cistus ladanifer L. Leaves Extracted by Ultrasound-Assisted Extraction and Maceration. Prog Microbes Mol Biol 2023;6.

23. Bouaouda K, Elagdi C, Hachlafi NE, *et al*. HPLC-UV-MS/MS Profiling of Phenolics from Euphorbia nicaeensis (All.) Leaf and Stem and Its Antioxidant and Anti-Protein Denaturation Activities. Prog Microbes Mol Biol 2023;6.

24. Dilfuza E, Dilfuza J. Plant-Derived Antimicrobial Compounds. New Find. Nat. Subst., 2022, p. 104-115 (12).

25. Jadimurthy R, Jagadish S, Nayak SC, *et al*. Phytochemicals as Invaluable Sources of Potent Antimicrobial Agents to Combat Antibiotic Resistance. Life 2023;13:948.

26. Qassadi FI, Zhu Z, Monaghan TM. Plant-Derived Products with Therapeutic Potential against Gastrointestinal Bacteria. Pathogens 2023;12:333.

27. Komal R, Kumari R, Singh AK. Momordica charantia L.: A Medicinal Plant. Bioact. Pharmacol. Med. Plants, Apple Academic Press; 2022.

28. Cho J, Choi H, Lee J, *et al*. The antifungal activity and membrane-disruptive action of dioscin extracted from Dioscorea nipponica. Biochim Biophys Acta 2013;1828:1153–8.

29. Wang G-X, Han J, Zhao L-W, *et al*. Anthelmintic activity of steroidal saponins from Paris polyphylla. Phytomedicine Int J Phytother Phytopharm 2010;17:1102–5.

30. Wang Y-J, Pan K-L, Hsieh T-C, *et al*. Diosgenin, a plant-derived sapogenin, exhibits antiviral activity in vitro against hepatitis C virus. J Nat Prod 2011;74:580–4.

31. Rabablert J, Tiewcharoen S, Auewarakul P, *et al*. ANTI-AMEBIC ACTIVITY OF DIOSGENIN ON NAEGLERIA FOWLERI TROPHOZOITES. Southeast Asian J Trop Med Public Health 2015;46:827–34.

32. Bashyal B, Li L, Bains T, *et al*. Larrea tridentata: A novel source for anti-parasitic agents active against Entamoeba histolytica, Giardia lamblia and Naegleria fowleri. PLoS Negl Trop Dis 2017;11:e0005832.

33. Zeouk I, Sifaoui I, Rizo-Liendo A, *et al*. Exploring the Anti-Infective Value of Inuloxin A Isolated from *Inula viscosa* against the Brain-Eating Amoeba (*Naegleria fowleri*) by Activation of Programmed Cell Death. ACS Chem Neurosci 2020;12:195–202.

34. Lê HG, Võ TC, Kang J-M, *et al*. Antiamoebic activities of flavonoids against pathogenic free-living amoebae, Naegleria fowleri and Acanthamoeba species. Parasites Hosts Dis 2023;61:449–54.

35. Lê HG, Kang J-M, Võ TC, *et al*. Kaempferol induces programmed cell death in Naegleria fowleri. Phytomedicine 2023;119:154994.

36. Rajendran K, Ahmed U, Meunier AC, *et al*. Natural Terpenes Inhibit the Cytopathogenicity of *Naegleria fowleri* Causing Primary Amoebic Meningoencephalitis in the Human Cell Line Model. ACS Chem Neurosci 2023;14:4105–14.

37. Mungroo MR, Anwar A, Siyadatpanah A, *et al*. Anti-Naegleria fowleri and anti-Balamuthia mandrillaris activities of propolis. Nat Prod J 2022;12.

38. Ben Youssef M, Omrani A, Sifaoui I, *et al*. Amoebicidal thymol analogues against brain-eating amoeba, *Naegleria fowleri*. Bioorganic Chem 2025;159:108346.

39. Lê HG, Kim W, Kang J-M, *et al*. The anti-amoebic activity of Pinus densiflora leaf extract against the brain-eating amoeba Naegleria fowleri. Parasites Hosts Dis 2024;62:169–79.

40. Rizo-Liendo A, Sifaoui I, Cartuche L, *et al*. Evaluation of Indolocarbazoles from Streptomyces sanyensis as a Novel Source of Therapeutic Agents against the Brain-Eating Amoeba Naegleria fowleri. Microorganisms 2020;8:789.

41. Zhou W, Debnath A, Jennings G, *et al*. Enzymatic chokepoints and synergistic drug targets in the sterol biosynthesis pathway of Naegleria fowleri. PLOS Pathog 2018;14:e1007245.

42. Rizo-Liendo A, Sifaoui I, Reyes-Batlle M, *et al*. In Vitro Activity of Statins against Naegleria fowleri. Pathogens 2019;8:122.

43. Akbar N, Kaman WE, Sarink M, *et al*. Novel Antiamoebic Tyrocidine-Derived Peptide against Brain-Eating Amoebae. ACS Omega 2022;7:28797–805.

44. Arberas-Jiménez I, Cen-Pacheco F, Chao-Pellicer J, *et al*. Identification and characterization of novel marine oxasqualenoid yucatecone against Naegleria fowleri. Int J Parasitol Drugs Drug Resist 2023;22:61–71.

45. Arberas-Jiménez I, Rodríguez-Expósito RL, San Nicolás-Hernández D, *et al*. Marine Meroterpenoids Isolated from Gongolaria abies-marina Induce Programmed Cell Death in Naegleria fowleri. Pharmaceuticals 2023;16:1010.

46. Arberas-Jiménez I, Nocchi N, Chao-Pellicer J, *et al*. Chamigrane-Type Sesquiterpenes from Laurencia dendroidea as Lead Compounds against Naegleria fowleri. Mar Drugs 2023;21:224–224.

47. Cheng W, Huang Y, Gao H, *et al*. Marine Natural Products as Novel Treatments for Parasitic Diseases. In: Wainwright CL, Schini-Kerth VB, editors. Nat. Prod. Sources Nov. Drugs, Cham: Springer Nature Switzerland; 2025, p. 325–93.

48. Rajakumar S, S.V. R, D. S, *et al*. A Comprehensive Review on the Role of Chemotype Marine Derived-Drug Discovery. Curr Bioact Compd 2024;20:e220623218182.

49. Mayer AMs, Pierce M, Reji M, *et al*. Marine-Derived Pharmaceuticals in Clinical Trials in 2022. J Pharmacol Exp Ther 2023;385:299.

50. Arberas-Jiménez I, Rizo-Liendo A, Nocchi N, *et al*. Sesquiterpene lactones as potential therapeutic agents against Naegleria fowleri. Biomed Amp Pharmacother 2022;147:112694.

51. Elsalam RM, Goh KW, Mahadi M, *et al*. The Antibacterial Activities of Secondary Metabolites Derived from Streptomyces sp. Prog Microbes Mol Biol 2022;5.

52. Thye AY-K, Letchumanan V, Tan LT-H, *et al*. Malaysia's Breakthrough in Modern Actinobacteria (MOD-ACTINO) Drug Discovery Research. Prog Microbes Mol Biol 2022;5.

53. Law JWF, Tan LT-H, Letchumanan V, *et al*. Streptomyces griseiviridis sp. nov., a Novel "Modern Actinobacteria" isolated from Malaysia Mangrove Soil. Prog Microbes Mol Biol 2023;6.

54. Law JW-F, Letchumanan V, Hong K-W, *et al*. Streptomyces learnhanii sp. nov., unveiling a Mangrove-Derived Novel "Modern Actinobacteria" in Malaysia. Prog Microbes Mol Biol 2023;6.

55. Betanzos A, Bañuelos C, Orozco E. Host Invasion by Pathogenic Amoebae: Epithelial Disruption by Parasite Proteins. Genes 2019;10:618.

56. Shurshalova GS, Yulmetov AR, Sharapova DA, *et al*. Interaction of Lovastatin with Model Membranes by NMR Data and from MD Simulations. BioNanoScience 2020;10:493–501.

57. Troskie AM, De Beer A, Vosloo JA, *et al*. Inhibition of agronomically relevant fungal phytopathogens by tyrocidines, cyclic antimicrobial peptides isolated from Bacillus aneurinolyticus. Microbiology 2014;160:2089–101.

58. Ali U, Lu S, Fadlalla T, *et al*. The functions of phospholipases and their hydrolysis products in plant growth, development and stress responses. Prog Lipid Res 2022;86:101158.

59. Siddiqui R, Boghossian A, Khatoon B, *et al*. Antiamoebic Properties of Metabolites against Naegleria fowleri and Balamuthia mandrillaris. Antibiotics 2022;11:539.

60. Sohn H, Song K, Kang H, *et al*. Cellular characterization of *actin* gene concerned with contact-dependent mechanisms in *Naegleria fowleri*. Parasite Immunol 2019;41:e12631.

61. Pais-Morales J, Betanzos A, García-Rivera G, *et al*. Resveratrol Induces Apoptosis-Like Death and Prevents In Vitro and In Vivo Virulence of Entamoeba histolytica. PloS One 2016;11:e0146287.

62. Ahmed U, Ong S-K, Khan KM, *et al*. Effect of embelin on inhibition of cell growth and induction of apoptosis in Acanthamoeba castellanii. Arch Microbiol 2023;205:360.

63. Eugin Simon S, Ahmed U, Saad SM, *et al*. New synthetic phenylquinazoline derivatives induce apoptosis by targeting the pro-survival members of the BCL-2 family. Bioorg Med Chem Lett 2022;67:128731.

64. Ahmed U, Ong S-K, Tan KO, *et al*. Alpha-Mangostin and its nano-conjugates induced programmed cell death in Acanthamoeba castellanii belonging to the T4 genotype. Int Microbiol 2023;27:1063–81.

65. Grace E, Asbill S, Virga K. Naegleria fowleri: Pathogenesis, Diagnosis, and Treatment Options. Antimicrob Agents Chemother 2015;59:6677–81.

66. Bruna T, Maldonado-Bravo F, Jara P, *et al*. Silver Nanoparticles and Their Antibacterial Applications. Int J Mol Sci 2021;22:7202.

67. Muddapur UM, Alshehri S, Ghoneim MM, *et al*. Plant-Based Synthesis of Gold Nanoparticles and Theranostic Applications: A Review. Molecules 2022;27.

68. Yusuf A, Almotairy ARZ, Henidi H, *et al*. Nanoparticles as Drug Delivery Systems: A Review of the Implication of Nanoparticles' Physicochemical Properties on Responses in Biological Systems. Polym Basel 2023;15.

69. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 2013;65:36–48.

70. Kim K, Khang D. Past, Present, and Future of Anticancer Nanomedicine. Int J Nanomedicine 2020;15:5719–43.

71. Xu M, Han X, Xiong H, *et al*. Cancer Nanomedicine: Emerging Strategies and Therapeutic Potentials. Molecules 2023;28.

72. Yin IX, Zhang J, Zhao IS, *et al*. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. Int J Nanomedicine 2020;15:2555–62.

73. Wang X, Mohammad IS, Fan L, *et al*. Delivery strategies of amphotericin B for invasive fungal infections. Acta Pharm Sin B 2021;11:2585–604.

74. Tiwari H, Rai N, Singh S, *et al*. Recent Advances in Nanomaterials-Based Targeted Drug Delivery for Preclinical Cancer Diagnosis and Therapeutics. Bioeng Basel 2023;10.

75. Huang H, Liu R, Yang J, *et al*. Gold Nanoparticles: Construction for Drug Delivery and Application in Cancer Immunotherapy. Pharmaceutics 2023;15.

76. Sani A, Cao C, Cui D. Toxicity of gold nanoparticles (AuNPs): A review. Biochem Biophys Rep 2021;26:100991.

77. Anwar A, Siddiqui R, Hussain MA, *et al*. Silver nanoparticle conjugation affects antiacanthamoebic activities of amphotericin B, nystatin, and fluconazole. Parasitol Res 2018;117:265–71.

78. Rajendran K, Anwar A, Khan NA, *et al*. trans-Cinnamic Acid Conjugated Gold Nanoparticles as Potent Therapeutics against Brain-Eating Amoeba Naegleria fowleri. ACS Chem Neurosci 2019;10:2692–6.

79. Rajendran K, Ahmed U, Meunier AC, *et al*. Nanoparticle-Terpene Fusion: A Game-Changer in Combating Primary Amoebic Meningoencephalitis Caused by *Naegleria fowleri*. ACS Omega 2024;9:11597–607.

80. Rajendran K, Anwar A, Khan NA, *et al*. Oleic Acid Coated Silver Nanoparticles Showed Better *in Vitro* Amoebicidal Effects against *Naegleria fowleri* than Amphotericin B. ACS Chem Neurosci 2019;11:2431–7.

81. Anwar A, Masri A, Rao K, *et al*. Antimicrobial activities of green synthesized gums-stabilized nanoparticles loaded with flavonoids. Sci Rep 2019;9.

82. Rajendran K, Anwar A, Khan NA, *et al*. Brain-Eating Amoebae: Silver Nanoparticle Conjugation Enhanced Efficacy of Anti-Amoebic Drugs against Naegleria fowleri. ACS Chem Neurosci 2017;8:2626–30.

83. Mungroo MR, Anwar A, Khan NA, *et al*. Gold-Conjugated Curcumin as a Novel Therapeutic Agent against Brain-Eating Amoebae. ACS Omega 2020;5:12467–75.