



Review Article

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Neem derivatives in integrated control of vectors and pathogens of leishmaniasis: Current knowledge and computational insights for future perspectives

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ABSTRACT

Leishmaniasis remains a major neglected tropical disease affecting millions of people worldwide, particularly in resource-limited regions where effective prevention and treatment options are often inaccessible. Current control strategies primarily rely on chemical insecticides for sandfly management and antileishmanial drugs for disease treatment. However, the emergence of insecticide and drug resistance, treatment-associated toxicity, environmental contamination, and limited affordability has created an urgent need for safer and more sustainable alternatives. In this context, plant-derived bioactive compounds have gained increasing attention as eco-friendly tools for the management of vector-borne diseases. *Azadirachta indica* A. Juss. (Neem) is a medicinal plant extensively used in traditional healthcare systems and recognized for its diverse pharmacological properties. This review critically examines the available literature on the efficacy of neem-derived products against sandfly and *Leishmania* spp. Growing evidence highlights neem as a unique dual-action intervention capable of targeting both the parasite and its vector, making it an attractive candidate for integrated leishmaniasis control. Nevertheless, challenges related to standardization, bioavailability, safety evaluation, and clinical validation remain substantial barriers to translation. Future integration of computational drug discovery, nanotechnology-based formulations, and multidisciplinary experimental approaches may facilitate the development of neem-based therapeutics and vector control strategies for sustainable leishmaniasis management.

KEYWORDS: *Azadirachta indica*; Neem derivatives; Leishmaniasis; Antileishmanial agents; Sandflies; Vector control; Molecular docking and simulation

1. Introduction

Leishmaniasis are parasitic diseases with diverse clinical outcomes, ranging from self-healing cutaneous lesions to fatal visceral forms marked by splenomegaly. They are caused by intracellular protozoa of the genus *Leishmania* and transmitted by female sandflies. The epidemiology is complex, involving more than 20 *Leishmania* species, nearly 90 sandfly vectors, and over 70 reservoir hosts, including humans. Transmission spans tropical and subtropical regions, affecting 99 countries worldwide[1]. Globally, cutaneous leishmaniasis is the most common form, with 0.6–1.0 million new cases[2], while visceral leishmaniasis (VL) is the most lethal form, which accounts for 50 000 to 90 000 cases per year[3]. The principal vectors are *Phlebotomus* in the Old World and *Lutzomyia* in the New World. Pathogenic species such as *Leishmania major* (*L. major*), *Leishmania tropica*, *Leishmania donovani* (*L. donovani*), *Leishmania infantum* (*L. infantum*), *Leishmania chagasi*, *Leishmania amazonensis*, *Leishmania mexicana*, and *Leishmania braziliensis* thrive near human settlements, contributing significantly to the global disease burden[4].

Despite control measures—indoor residual spraying with pyrethroids like permethrin, insecticide-treated bed nets, topical repellents such as N,N-diethyl-meta-toluamide[5], and antileishmanial drugs

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including liposomal amphotericin B (AmBisome), miltefosine, and antimonials—leishmaniasis remains a major public health challenge. Effectiveness is undermined by resistance[6,7], drug toxicity, bioaccumulation, and ecological damage[8]. Among the VL cases, 50% of affected people are children[9], posing a major concern for treatment, which demands local therapies (*e.g.*, plant-based therapies) in combination with standard drugs (miltefosine) to achieve a positive outcome[10]. This highlights the urgent need for safer, sustainable alternatives.

Plant-based interventions are increasingly recognized as eco-friendly solutions due to affordability, accessibility, low toxicity, and reduced risk of resistance. Extracts and essential oils from species such as *Citrus*, *Derris*, *Allium*, *Myrtus*, *Melia*, *Eucalyptus*, and *Acalypha* have shown insecticidal activity against *Phlebotomine* and *Lutzomyia* sandflies under laboratory and field conditions[11]. Likewise, phytochemicals have demonstrated efficacy against infectious diseases, including leishmaniasis[12–17]. Yet, pharmacological exploration of plants for vector-borne disease control remains limited.

Among these, *Azadirachta indica* (neem), a member of the Meliaceae family, is widely distributed in tropical regions endemic for leishmaniasis. Neem contains diverse bioactive compounds with antimicrobial and insecticidal properties[18], including azadirachtin, nimbin, salannin, nimbidin, sodium nimbinate, meliantriol, thionemon, nimbolide, azadiradione, and quercetin[19]. These exhibit varied biological activities: nimbin is anti-inflammatory, nimbidin inhibits bacterial growth and ulcers, nimbiol shows antiprotozoal and antimycobacterial activity, gedunin demonstrates antiplasmodial and antifungal effects, and salannin functions as a potent insect repellent[20–24]. The dual functionality (toxic to both vectors and pathogens by sparing the host) of neem grabbed our attention to synthesize this review article with the current knowledge on its derivatives in controlling sandfly vectors and *Leishmania* parasites, limitations in translational potential, and future directions for integrated management of leishmaniasis.

2. Methodology

A systematic search was conducted to identify studies on neem-based products with antileishmanial or sandfly-repellent activity. Databases (PubMed, Scopus, Google Scholar) were searched using the Boolean string: “(*Azadirachta indica* OR neem) AND (leishmania OR leishmaniasis) AND (sandfly OR phlebotomine)”. In PubMed, both Medical Subject Headings (MeSH) and free-text terms were applied to capture indexed and non-indexed articles.

Screening involved title/abstract review followed by full-text evaluation. Inclusion criteria: (i) English-language, full-text

availability; (ii) original laboratory or field studies; (iii) quantitative outcomes (repellency %, insecticidal efficacy, IC_{50}/EC_{50} against *Leishmania*, CC_{50} in host cells). Exclusion criteria: (i) inaccessible full texts; (ii) non-primary literature (books, proceedings, reviews). Of the 248 records, 25 studies met eligibility. Additional supporting literature was retrieved *via* iterative open-access searches across all publication years to maximize comprehensiveness.

3. Evidence on neem interventions

3.1. Neem products in controlling sandfly vectors

Neem-derived products have been widely investigated for their insecticidal and repellent properties against sandfly vectors. Laboratory studies demonstrated that neem fruit and leaf powder (2 g/cm²/day) caused gradual mortality in *Lutzomyia longipalpis* (*Lu. longipalpis*) larvae over 1–3 weeks, with complete death by the third instar stage, though feeding behavior remained unaffected[25]. Neem oil, particularly azadirachtin-based formulations, has shown strong repellency against phlebotomine sandflies, especially in dogs, the primary reservoir hosts of *L. infantum*. While direct evidence of protection against canine leishmaniasis is limited, surveys of Italian veterinarians revealed that 94.4% recommend neem-based products[26]. However, case reports highlight limitations: topical neem oil failed to prevent infection in one dog, attributed to its volatility and short persistence[27].

Experimental models further support neem's efficacy. In mice, neem oil (hexane fraction) provided superior protection against *Phlebotomus papatasi* bites at low concentrations (1%–2%) compared to N,N-diethylphenylacetamide, with both achieving complete protection at 5%[28]. Neem oil formulations (2%–5% in coconut oil) offered up to nine hours of repellency against *Phlebotomus bergeroti* and *Phlebotomus orientalis* in laboratory and field conditions[29]. Field trials demonstrated that 2% neem oil in coconut or mustard oil provided overnight protection against *Phlebotomus argentipes*, outperforming conventional repellents such as N,N-diethyl-met-toluamide and citronella, though protection against *Phlebotomus papatasi* was limited to seven hours[30].

Innovative applications have also been explored. Neem oil (5% in acetone) applied to electric heating mats achieved 100% inhibition of sandfly landing by dispersing volatile compounds into crevices[31]. Ecological studies in West Bengal linked neem tree presence to reduced sandfly density and lower VL risk[32]. These findings suggest both topical and environmental integration of neem as viable control strategies[29].

At the molecular level, neem leaf essential oils contain sesquiterpene hydrocarbons that interact with odorant-binding

protein 4 in *Lu. longipalpis*, contributing to repellency[33]. Azadirachtin A, incorporated into sucrose diets (0.1–10 µg/mg), induced dose-dependent toxicity in adult female *Lu. longipalpis*, disrupting hormonal regulation by inhibiting ecdysteroids[34] and juvenile hormone activity[35,36]. These effects reduced oviposition without impairing egg hatching, suggesting a sterilizing impact[37].

Larval development was similarly disrupted: azadirachtin limited progression to the third instar at low concentrations (0.1–1.0 µg/mg) and to the second instar at higher doses (10 µg/mg)[38]. Neem seed oil (100 mg/mL) inhibited egg hatching (61.56%) and larval development (67.75%), and caused 96.64% adult mortality within 24 hours in *Lu. longipalpis* populations, largely due to triterpenoids such as azadirachtin that interfere with growth and development[39].

Recent studies on *Phlebotomus perniciosus* revealed that azadirachtin (1 µg/mg food) severely impaired larval molting, with only 10% progressing to the second instar, though adult mortality was unaffected. Gene expression analyses showed downregulation of ecdysone signaling genes (*serpent*, *Eip74EF*) and immune-related genes (*attacin*, *defensin 1/2*) in larvae and adults[40]. Ecological observations further suggest fewer sandflies near Meliaceae family trees, including neem, indicating a natural repellent effect[41].

Field interventions in Bangladesh demonstrated neem oil's complementary role in VL control. Although initial interventions (2007–2008) did not directly reduce VL incidence, subsequent surveillance revealed significant declines in blood-fed *Phlebotomus argentipes* populations two years later, suggesting disruption of host-seeking behavior and potential transmission interruption[42].

3.2. Neem constituents as antileishmanial agents

Neem-derived products have been extensively studied for their antileishmanial potential, with efficacy varying according to extract type and constituent profile. Aqueous extracts generally show limited activity, reflected in high IC₅₀ values across species: > 500 µg/mL against *Leishmania guyanensis* AMC promastigotes, 187 µg/mL for *L. major* NADIM5, 247 µg/mL for *L. donovani* GEDII promastigotes, and 451 µg/mL for *L. donovani* BHU814 amastigotes. Cytotoxicity in THP-1 cells (IC₅₀ = 352 µg/mL) further underscores their limited therapeutic potential[43]. In contrast, crude soluble antigens from neem leaves, bark, or seed oil, prepared with water or ethanol, demonstrated pronounced activity: 0.1 mg/mL crude soluble antigens completely inhibited *L. donovani* promastigotes within 48 hours, with axenic amastigotes 1.9-fold more sensitive. Importantly, crude soluble antigens exhibited low cytotoxicity toward peripheral blood mononuclear cells, reducing mitochondrial dehydrogenase activity only 1.3-fold[44].

Organic solvent extracts have shown superior efficacy. Ethyl acetate fractions[45] and ethanolic extracts[46] induced apoptosis in

L. donovani promastigotes and triggered reactive oxygen/nitrogen species-mediated killing of intracellular amastigotes. These extracts produced lower IC₅₀ values for amastigotes compared to promastigotes and stimulated robust Th1/Th2 cytokine responses. In BALB/c mice, ethanolic extracts (200 mg/kg) conferred protection comparable to amphotericin B, inducing delayed-type hypersensitivity, proliferation of CD4⁺/CD8⁺ T cells, and upregulation of macrophage co-stimulatory molecules CD80/86[46]. Gas chromatography-mass spectrometry analyses identified azadiradione, nimbin, nimbolide, gedunin, and salannin in ethyl acetate fractions[45], while ethanolic extracts contained stigmasterol acetate, ergosterol acetate, linoleic acid, and phthalic acid derivatives[46].

Complementary *in silico* studies highlight molecular targets: isonimocinolide, nimbolide, and meldenin inhibit *L. major* N-myristoyltransferase[47], while isorhamnetin, meliantriol, and quercetin target *L. infantum* trypanothione reductase[48]. These findings support neem compounds as promising drug candidates.

Among individual constituents, azadiradione has shown therapeutic potential against *L. donovani* by targeting parasite peroxidases and modulating host immunity. It demonstrated a moderate selectivity index (SI ≥ 4.83) and reduced IL-10 expression in infected macrophages, warranting further evaluation in animal models[49]. Quercetin, a flavonoid, has exhibited strong activity against *Leishmania braziliensis* promastigotes (IC₅₀ = 25 µM) and amastigotes (IC₅₀ = 21 µM); it induced reactive oxygen species in macrophages with a high SI (≥ 22). Oral administration reduced parasite burden and lesion size in hamsters[50]. In addition, quercetin achieved IC₅₀ values of 0.27 µM (promastigotes) and 0.85 µM (amastigotes) against *L. major*, outperforming Glucantime. Treatment also reduced IL-6/TNF-α, enhanced collagen deposition, fibroblast proliferation, and antioxidant status, promoting tissue recovery[51].

Formulation advances have improved efficacy. A quercetin nanoemulsion enhanced activity against *L. donovani* clinical isolates, inducing apoptosis and modulating immune responses while maintaining host biocompatibility[52]. Gedunin-type limonoids—kotschyienone A/B, 7-deacetylgedunin, and 7-deacetyl-7-oxogedunin—isolated from neem fruits[53] showed activity against *L. major* promastigotes (IC₅₀ = 2.86–14.9 µM). Kotschyienone A displayed SI ≥ 10 and potent activity against intracellular amastigotes (EC₅₀ = 1.51 µM)[54]. Gedunin itself has been identified as a heat shock protein inhibitor, broadening its therapeutic relevance[55].

Alcoholic extracts of neem leaves also induced dose-dependent mortality in *Leishmania tropica* promastigotes (30–150 µg/mL), with a minimum inhibitory concentration of 90 µg/mL comparable to Pentostam and complete mortality at 150 µg/mL within 72 hours[56]. Ethanolic extracts and dichloromethane/chloroform fractions from neem leaves and seed tegument exhibited potent activity against *Leishmania amazonensis* promastigotes and amastigotes, with

significantly lower IC_{50} values and SI ranging from 15–72. The chloroform fraction (2 $\mu\text{g/mL}$) eliminated intracellular amastigotes *via* a nitric oxide-independent mechanism. Phytochemical analysis revealed terpenoid richness, though azadirachtin itself was ineffective against *Leishmania amazonensis* promastigotes[57].

Methanolic extracts have consistently demonstrated strong activity. Neem leaf extracts showed an IC_{50} of 10.2 $\mu\text{g/mL}$ [58], while stem bark extracts yielded an IC_{50} of 11.5 $\mu\text{g/mL}$ [59] against

L. major promastigotes. Comparable efficacy was observed against amastigotes (IC_{50} = 11.5 $\mu\text{g/mL}$). In BALB/c mice, leaf extract (10 mL/kg) has reduced parasite burden and splenomegaly[60]. Topical methanolic extract (0.1 g/mL) achieved complete healing of cutaneous lesions in 83.3% of Sudanese patients within ~22 days, comparable to Pentostam[61].

Neem oil studies, though limited, suggest promising antileishmanial activity. Against *L. infantum* and *Leishmania tropica*, neem oil exhibited

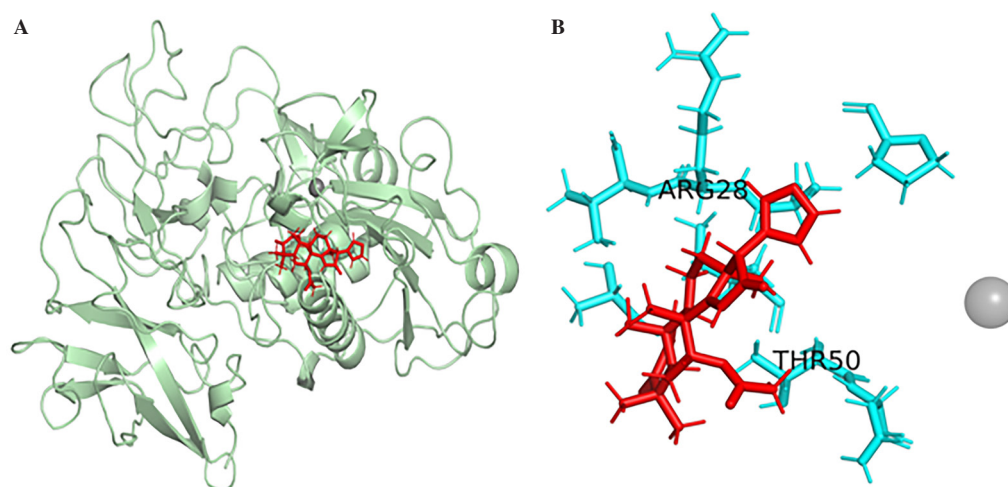


Figure 1. Structural overview of *Leishmania* glycoprotein 63 (GP63, also known as leishmanolysin)-azadiradione complex (created *via* PyMOL open-source software). (A) Docked conformation showing azadiradione (red sticks) in GP63 catalytic cavity (PDB: 1LML). (B) Binding pocket close-up with key residues (cyan) and catalytic Zn^{2+} (grey).

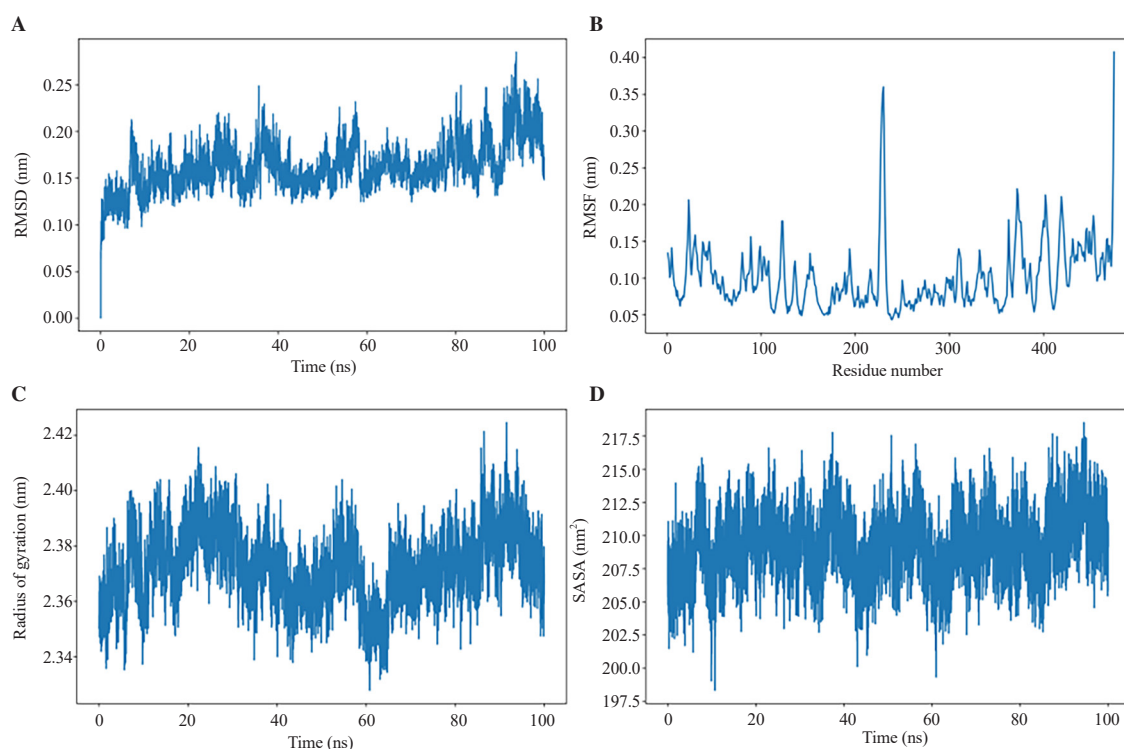


Figure 2. Molecular dynamics trajectory of GP63-azadiradione complex (100 ns): (A) root mean square deviation (RMSD) of Ca atoms. (B) Per residue root mean square fluctuation (RMSF). (C) Radius of gyration. (D) Solvent accessible surface area (SASA). All parameters consistently demonstrate stable and compact binding throughout the simulation[71].

IC₅₀ values of 15.3–17.6 µg/mL for amastigotes versus >200 µg/mL for promastigotes. Its low cytotoxicity (CC₅₀ > 700 µg/mL) in THP-1 macrophages yielded a high SI (~40), comparable to miltefosine[62].

4. Computational insights for drug development

Leishmania glycoprotein 63 (GP63), or leishmanolysin, remains a classical therapeutic benchmark due to its central role in host-parasite interactions and virulence[63]. Complementing this, the recently identified tetratricopeptide repeat-kinase-ubiquitin ligase (TKUL) protein has emerged as a novel druggable target; inhibition of its kinase domain disrupts parasite infection while sparing mammalian cells, underscoring its selective pharmacological potential[64].

Protein structures of GP63 (*L. major*, PDB: 1LML) (Figure 1A) and TKUL (*Leishmania mexicana*, PDB: 9GMP) (Figure 3A) were retrieved from the RCSB Protein Data Bank[65], prepared in PyMOL[66] with polar hydrogens added, and docked with 18 neem-derived phytoconstituents and five reference drugs using AutoDock Vina[67] in PyRx[68]. Docking grids targeted the GP63 zinc-binding cleft (His165, His169, His235, Glu166) and TKUL nucleotide-binding pocket. Protein-ligand contacts were analyzed with LigPlot+[69], and the most promising complexes—GP63-azadiradione and TKUL-nimbidinin—were advanced to 100 ns molecular dynamics simulations in GROMACS[70] using AMBER ff19SB and GAFF2 force fields.

4.1. GP63–azadiradione

Azadiradione, despite ranking fourth in docking score (−9.0 kcal/mol), was prioritized for its superior interaction profile. LigPlot+

revealed hydrogen bonds with Ala128, His165, and His235, plus nine hydrophobic contacts including Glu166 and the catalytic Zn²⁺ ion (Figure 1B). Simultaneous engagement of both catalytic histidines may be characteristic of effective zinc-metalloprotease inhibition[63]. Other compounds, such as margosinoline and epoxyazadiradione, showed favorable scores but lacked comparable catalytic residue recognition.

Molecular dynamics simulations confirmed stable binding: root mean square deviation (RMSD) equilibrated at 0.15–0.20 nm, rising slightly to ~0.25 nm after 80 ns without dissociation (Figure 2A). Root mean square fluctuation (RMSF) remained < 0.15 nm except for localized loop flexibility near residue 230 (Figure 2B). Radius of gyration (Rg; 2.33–2.43 nm) and solvent accessible surface area (SASA; 200–218 nm²) indicated preserved compactness and solvent exposure (Figure 2C–D). Azadiradione was stably accommodated within the catalytic pocket throughout the simulation.

4.2. TKUL–nimbidinin

Nimbidinin, with a docking score of −7.3 kcal/mol, was selected for its strong mechanistic relevance. LigPlot+ identified four hydrogen bonds, including a triple interaction with Ser160—the key β-phosphate recognition residue—and one with Gln102 (Figure 3B). This exceeded the engagement of native ATP/ADP (−6.6 kcal/mol). Other high-scoring compounds lacked direct Ser160 contacts, suggesting weaker specificity.

Molecular dynamics simulations validated stability: RMSD plateaued at 0.25–0.32 nm [mean (0.28 ± 0.02) nm; Figure 4A], with elevated fluctuations confined to peripheral loops (residues 151–156, RMSF up to 0.505 nm). The nucleotide-binding core remained rigid [mean RMSF (0.12 ± 0.07) nm; Figure 4B]. Rg (~2.00 nm) and

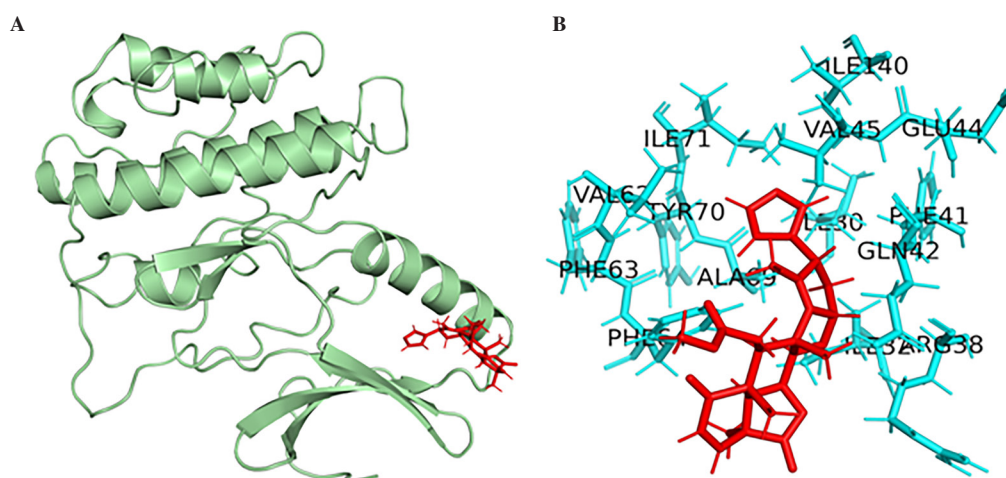


Figure 3. Structural overview of tetratricopeptide repeat-kinase-ubiquitin ligase (TKUL)-nimbidinin complex (created *via* PyMOL open-source software). (A) Docked conformation showing nimbidinin (red sticks) in TKUL nucleotide binding cleft (PDB: 9GMP). (B) Binding pocket close up with key residues (cyan).

SASA (140–148 nm²) confirmed compactness and gradual interface consolidation (Figure 4C–D). Nimbidinin remained docked within the ATP-binding cleft for the full trajectory.

Docking and molecular dynamics simulations identified azadiradione and nimbidinin as promising neem-derived inhibitors of GP63 and TKUL, respectively. Both compounds engaged critical

active-site residues and maintained stable complexes, supported by bounded RMSD, localized RMSF, conserved compactness, and stable solvent exposure. These findings warrant further validation through MM-PBSA/GBSA free-energy calculations and experimental assays, positioning neem phytoconstituents as potential next-generation antileishmanial agents.

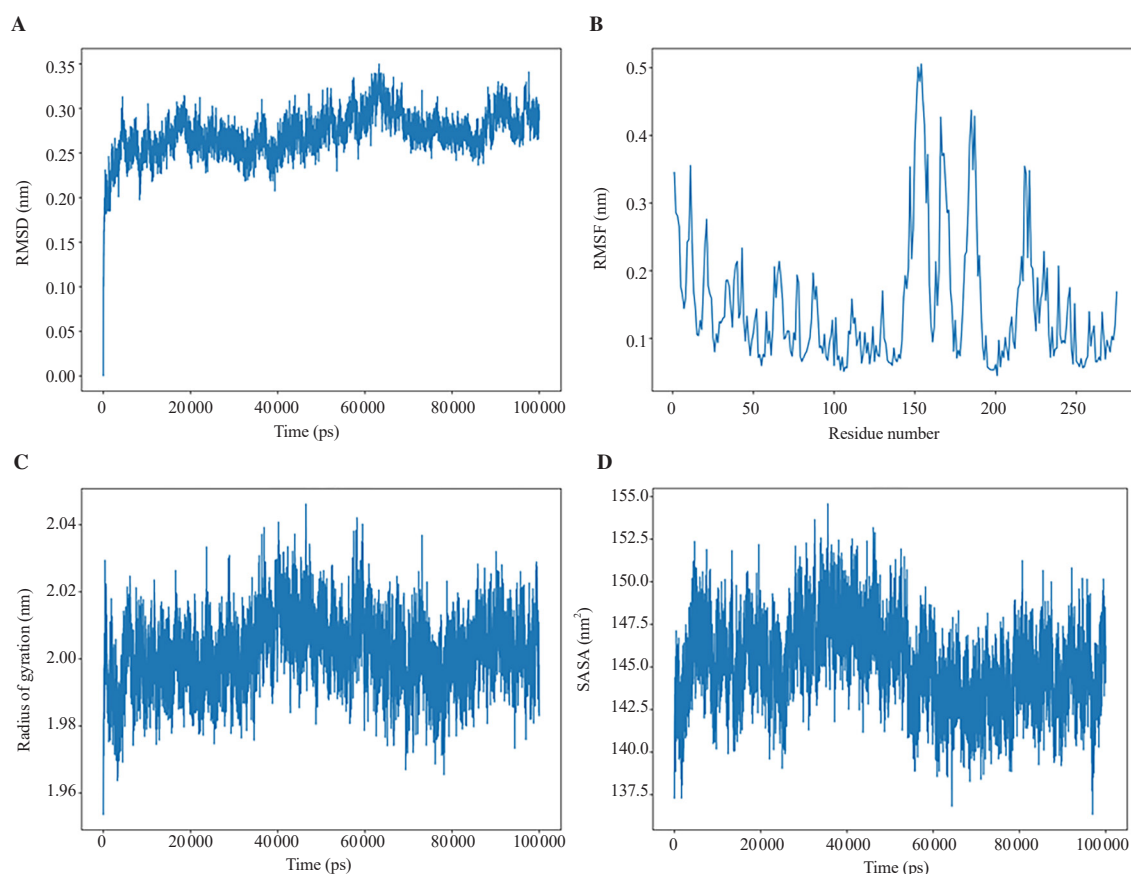


Figure 4. Molecular dynamics trajectory of TKUL-nimbidinin complex (100 ns): (A) RMSD of C α atoms. (B) Per residue RMSF, with peak at residues 151–156 (0.505 nm) indicating loop flexibility. (C) Radius of gyration. (D) SASA. Metrics confirm stable and compact binding[64].

Table 1. List of studies on neem bioactive fractions and compounds against different sandfly species' development and mortality.

Neem/its derivatives	Sandfly spp.	Efficacy	Key finding	Reference
Neem oil (5% in acetone, heating mats)	<i>Phlebotomus papatasi</i>	Repellency	100% inhibition of landing in endemic area trials	[31]
Neem oil (2% in coconut/mustard oil)	<i>Phlebotomus argentipes</i> , <i>Phlebotomus papatasi</i>	Repellency	Overnight protection, superior to DEET	[30]
Neem oil (hexane fraction)	<i>Phlebotomus papatasi</i>	Repellency	Greater protection than DEPA at low concentrations	[28]
Neem oil (2%–5% in coconut oil)	<i>Phlebotomus bergeroti</i> , <i>Phlebotomus orientalis</i>	Repellency	Up to 9 h protection in Ethiopia field trials	[29]
Neem oil	<i>Phlebotomus argentipes</i>	Vector control	Reduced blood-fed sandfly populations in Bangladesh	[42]
Neem fruit/leaf powder	<i>Lutzomyia longipalpis</i> larvae	Larvicidal	Complete mortality by 3rd instar stage	[25]
Azadirachtin	<i>Lutzomyia longipalpis</i> larvae	Developmental disruption	Arrested progression to later instars	[38]
Azadirachtin	<i>Lutzomyia longipalpis</i> adults	Hormonal interference	Reduced oviposition, sterilizing effect	[37]
Azadirachtin	Sandflies (general)	Endocrine disruption	Inhibition of oogenesis and ovarian steroid synthesis	[34,35]
Neem leaf essential oils	<i>Lutzomyia longipalpis</i>	Molecular repellency	OBP4 protein interaction, repellent activity	[33]
Neem oil	<i>Phlebotomus</i> spp.	Veterinary prophylaxis	94.4% veterinarians recommend; efficacy variable	[26]
Neem spray	<i>Phlebotomus</i> spp.	Preventive	Limited persistence; failed protection	[27]

DEET: N,N-diethyl-meta-toluamide; DEPA: N,N-diethylphenylacetamide; OBP4: odorant-binding protein 4.

5. Conclusion

Neem, a versatile medicinal plant, is recognized for its broad biological activities[20]. Its global importance has been acknowledged by the World Health Organization-United Nations Environment Programme (1989) and the U.S. National Academy of Sciences, which ranked it among the top ten plants for health research[72,73]. In leishmaniasis, neem has gained attention for its dual role in

vector control and therapy. Epidemiological studies on neem tree distribution and VL risk remain inconclusive: some report reduced sandfly density and protective effects, while others find no significant association[74,75]. These discrepancies highlight the need for rigorous proof of concept studies to clarify neem's ecological impact on VL transmission.

Most research has focused on crude solvent extracts, which, while biologically active, may cause off-target effects and

Table 2. List of studies on neem bioactive fractions and compounds against different *Leishmania* species and their infection in the host.

Neem extract/derivative	<i>Leishmania</i> spp.	Key finding	Reference
Aqueous extract (Leaves)	<i>Leishmania guyanensis</i>	IC ₅₀ = >500 µg/mL against promastigotes	[43]
	<i>Leishmania major</i>	IC ₅₀ = 187 µg/mL against promastigotes	
	<i>Leishmania donovani</i>	IC ₅₀ = 247 and 451 µg/mL against promastigotes and amastigotes, respectively	
Aqueous extract (Leaves & bark), ethanolic extract (Seed oil)	<i>Leishmania donovani</i>	Crude soluble antigens (0.1 mg/mL) kill 100% promastigotes within 48 hours and axenic amastigotes are 1.9-fold higher susceptible; low PBMC toxicity	[44]
Ethyl acetate fraction (Leaves)	<i>Leishmania donovani</i>	IC ₅₀ = 52.4 µg/mL against promastigotes, induces apoptosis, reduces macrophage infectivity and splenic parasite load, and Th2/Th1 cytokine ratio	[45]
Ethanolic extract (Leaves)	<i>Leishmania donovani</i>	IC ₅₀ = 34 µg/mL against promastigotes and 17.66 µg/mL against amastigotes, and the therapeutic efficacy in BALB/c is 87.76% in the liver and 85.55% in the spleen	[46]
Ethanolic extract (Seeds)	<i>Leishmania donovani</i>	IC ₅₀ = 77.66 µg/mL against promastigotes and 24.66 µg/mL against amastigotes, and the therapeutic efficacy in BALB/c is 85.54% in the liver and 83.62% in the spleen	[46]
Azadiradione	<i>Leishmania donovani</i>	IC ₅₀ = 17.09 µM against promastigotes, EC ₅₀ = 11.67 µM against intracellular amastigotes, and CC ₅₀ = 56.32 µM against human THP-1 macrophages. Targeted peroxidases in <i>Leishmania</i> and IL-10 production from host cells to control the infection	[49]
Quercetin	<i>Leishmania braziliensis</i>	IC ₅₀ = 25 µM against promastigotes and 21 µM against amastigotes, and CC ₅₀ = 478 µM against hamster peritoneal macrophages. Oral treatment of 20 mg/kg body weight for 8 weeks (5 days per week) reduced lesion thickness in the hamster model without inducing toxicity	[50]
Quercetin	<i>Leishmania major</i>	IC ₅₀ = 0.27 µM against promastigotes and 0.85 µM against amastigotes, CC ₅₀ = 27.3 µM against the RAW 264.7 cell line. Oral treatment 50 mg/kg body weight for 4 weeks reduced lesion size and its parasite load	[51]
Quercetin nanoemulsion	<i>Leishmania donovani</i>	IC ₅₀ = 6.6 µM and IC ₉₀ = 26.4 µM against promastigotes; reduced infectivity in macrophages via induced ROS and nitric oxide production	[52]
Gedunin-type limonoids	<i>Leishmania major</i>	MIC and 50% growth inhibition of kotschyienone A (10 µM and 2.86 µM), kotschyienone B (100 µM and 14.9 µM), 7-deacetylgedunin (100 µM and 11.6 µM), and 7-deacetyl-7-oxogedunin (>100 µM and 7.63 µM) against promastigotes. Kotschyienone A also exhibited a 50% growth inhibition value of 1.51 µM against intracellular amastigotes. It might target HSP for therapeutic success	[53,54]
Alcoholic extract	<i>Leishmania tropica</i>	MIC = 90 µg/mL and 100% killing of promastigotes at 150 µg/mL	[56]
Ethanolic fraction (Leaves)	<i>Leishmania amazonensis</i>	IC ₅₀ = 38 µg/mL against promastigotes and 9.8 µg/mL against amastigotes	[57]
Ethanolic extract (Seed coat)	<i>Leishmania amazonensis</i>	IC ₅₀ = 2.7 µg/mL against promastigotes and 0.4 µg/mL against amastigotes	[57]
Dichloromethane fraction (Leaves)	<i>Leishmania amazonensis</i>	IC ₅₀ = 3.9 µg/mL against promastigotes and 1.1 µg/mL against amastigotes	[57]
Dichloromethane fraction (Seed coat)	<i>Leishmania amazonensis</i>	IC ₅₀ = 2.1 µg/mL against promastigotes and 0.6 µg/mL against amastigotes	[57]
Chloroform fraction (Leaves)	<i>Leishmania amazonensis</i>	IC ₅₀ = 1.2 µg/mL against promastigotes and 0.6 µg/mL against amastigotes. Overall, SI for different fractions is ≥ 15-72 against intracellular amastigotes	[57]
Methanolic extract (Leaves)	<i>Leishmania major</i>	IC ₅₀ = 10.2 µg/mL against promastigotes	[58]
		IC ₅₀ = 10.1 and 11.5 µg/mL against promastigotes and amastigotes, respectively	[60]
Methanolic extract (Stem bark)	<i>Leishmania major</i>	IC ₅₀ = 11.5 µg/mL against promastigotes	[59]
Neem oil	<i>Leishmania infantum</i>	IC ₅₀ = 215 µg/mL against promastigotes and 15.3 µg/mL against amastigotes, CC ₅₀ = 710.5 µg/mL against THP-1, and SI ≥ 46	[62]
	<i>Leishmania tropica</i>	IC ₅₀ = 211 µg/mL against promastigotes and 17.6 µg/mL against amastigotes, and SI ≥ 40	

SI: selectivity index; MIC: minimum inhibitory concentration; PBMC: peripheral blood mononuclear cell; ROS: reactive oxygen species; HSP: heat shock protein.

toxicity. Advanced phytochemical characterization using thin-layer chromatography, gas chromatography-mass spectrometry, high-performance liquid chromatography, nuclear magnetic resonance, and X-ray diffraction is essential to isolate specific bioactives responsible for therapeutic effects. Although purified neem constituents have been studied against mosquitoes and parasites, their evaluation against sandflies and *Leishmania* remains limited. For instance, deacetylnimbin disrupts *Plasmodium berghei* transmission[76], while azadirachtin has been computationally linked to gephyrin E in cerebral malaria[77]. Neem kernel oil compounds—azadirachtin A, salannin, nimbin, nimbolide—exhibit mosquitocidal activity[78,79]. Azadirachtin is a well-studied biopesticide[34,35,80], while salannin acts as a potent antifeedant[35]. However, targeted evaluation of these compounds against sandflies and *Leishmania* is still lacking.

Practical application of neem products faces challenges of environmental sensitivity, rapid biodegradability, and variable toxicity. Toxicity depends on plant part, extraction method, administration route, and biological model[81]. To overcome these limitations, novel formulations are being explored. Encapsulation of neem oil volatiles *via* protein-polysaccharide coacervation enhances stability and prolongs repellency, particularly in dogs[82]. Nanoformulations, such as quercetin nanoemulsions, improve antileishmanial efficacy by increasing bioavailability and half-life[52]. The antigen-encapsulated neem-based nanoparticles with calcium phosphate adjuvant may demonstrate as an effective vaccine delivery system for leishmaniasis, as reported with poly (ϵ -caprolactone) nanoparticles against *L. infantum* infection[83]. Similar to poly (ϵ -caprolactone), the neem derivatives are also biodegradable and biocompatible in nature, and exhibit a low risk of inducing adverse immune responses.

Neem's eco-friendly profile and public acceptability have led to its inclusion in vector control programs[84,85]. Future strategies should integrate neem with chemical insecticides, biological agents, and antileishmanial drugs to strengthen disease management. Neem oil's safety for non-target organisms, such as copepods used in mosquito control, supports compatibility with biological approaches[79,86].

Overall, neem-based products demonstrate considerable promise as eco-friendly agents for the integrated management of leishmaniasis. Their multifaceted mechanisms—repellency, disruption of sandfly development, and interference with reproduction—highlight their potential in vector control (Table 1), while bioactive constituents also exhibit therapeutic activity against *Leishmania* parasites (Table 2). Despite encouraging laboratory and field evidence, practical application remains constrained by volatility, short residual activity, and variability in efficacy. Addressing these limitations will require rigorous validation, standardization of active compounds, optimization of delivery systems, and establishment of effective

application intervals. To fully realize neem's translational potential, interdisciplinary collaboration, financial investment, and partnerships between academia and industry are essential. With such coordinated efforts, neem and its phytoconstituents could be developed as sustainable tools for both sandfly control and antileishmanial therapy, contributing significantly to the fight against vector-borne diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

The authors declare that AI tools were employed solely to improve the clarity, grammar, and readability of the English language in this manuscript. No AI tools were used for data analysis, interpretation of results, or generation of scientific content. The authors take full responsibility for the accuracy, integrity, and originality of the manuscript.

Authors' contributions

PNS, AK, and DA developed the concept. DA and AHB designed the study framework. PNS, DA, and SK carried out data collection. AHB, DA, and AK performed data analysis and interpretation, including computational studies. PNS, AK, and SK drafted the manuscript. DA and AHB critically revised the manuscript and approved the final version.

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