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Therapeutic Potentials of Neem Against Malaria Parasite: A Review

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Abstract
Malaria presents a significant public health threat, affecting both developed and developing nations. The rise of drug resistance in regions with high malaria prevalence has posed challenges in effectively managing the disease. Azadirachta Indica, commonly known as the neem plant, has long been recognized as a medicinal plant in Asia and Africa, with historical usage in treating malaria parasites. The neem plant contains various bioactive compounds, making it a popular ingredient in traditional remedies. This study aimed to assess the effectiveness of neem leaf and seed extracts in combating malaria parasites. The information used in this review was obtained from previously published studies sourced from various journals. The findings suggest that neem (Azadirachta indica) seeds or leaves show promise as a potential treatment for inhibiting the malaria parasite. However, further research is required to ascertain the antimalarial potency of the neem plant before confidently recommending it as a treatment for malaria parasites.

Keywords: *Azadirachta Indica,* Neem Leaves, Malaria, Parasite, Plasmodium

INTRODUCTION
African communities are still greatly burdened by malaria, which has significant social and economic implications (Ifijen *et al*., 2018). Current efforts to control malaria transmission typically focus on adult primary vectors through the use of interventions like bed nets and indoor residual spraying, which can have a substantial impact on vectorial capacity (Ifijen *et al*., 2020). However, these methods are vulnerable to insecticide resistance, vector behavioral adaptation, and challenges with reaching vulnerable populations (Casimiro *et al*., 2006; Ifijen *et al*., 2019). In the past, larval control methods were effective in reducing malaria transmission by targeting the environmental conditions needed for mosquito larval development (Keiser *et al*., 2005). However, these methods fell out of favor with the introduction of synthetic insecticides and bed nets, which are not dependent on site-specific knowledge as required for larval control (Walker & Lynch, 2007). Integrated vector management programs that incorporate a variety of interventions, including larval control, may be the most effective in

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reducing malaria transmission rates (Walker & Lynch, 2007). Furthermore, targeting mosquito larvae has the potential to be a cost-effective and environmentally friendly approach (Gianotti *et al*., 2008). As researchers continue to search for new treatments for malaria, certain plants with antimalarial properties, such as the neem tree (*Azadirachta indica*), have been studied.
Neem, also known as *Azadirachta indica*, is an evergreen tree from the Meliaceae family that is widespread across the globe (Sithisarn *et al*., 2011). It has an average height of 25 meters and a semi-straight trunk. This flowering tree typically starts bearing fruit after 3-5 years and becomes productive within a decade (Maragathavalli *et al*., 2012). The bark of the neem tree is rough and gray while its leaves can grow up to 30 centimeters long with 10-12 serrated leaflets that are 7 centimeters by 2.5 centimeters in size. The neem tree thrives in regions with low rainfall (Mohashine *et al*., 2009). The different parts of this plant have been used as a remedy for various ailments and diseases.

Neem (*Azadirachta indica*) is a plant that has been used in traditional medicine for centuries due to its various therapeutic properties. The plant is native to the Indian subcontinent and has been widely used in India, Nepal, Bangladesh, and other neighboring countries (Khine *et al*., 2013). As mentioned, neem leaves and their paste have been traditionally used for their antiviral and anti-inflammatory properties to treat skin reactions and smallpox and chickenpox. The use of neem twigs for cleaning teeth is also a common practice in many parts of South Asia, as it is believed to have antimicrobial properties that can help prevent dental problems (Kumar *et al*., 2009). Neem juice is used as a tonic to stimulate appetite and as a vermifuge to remove intestinal worms. It is also used to control fever and has hypoglycemic, hypolipidemic, and hypotensive effects. Additionally, neem leaf extract has been found to have antimicrobial properties against dental pathogens, making it useful in the treatment of various dental problems (Chaturvedi *et al*., 2011). In Ayurvedic medicine, neem has been used to treat malarial fever, which is a common disease in many tropical and subtropical regions (Owusu-Boadi *et al*., 2021). The plant's bitter taste and cooling properties are believed to help reduce fever and alleviate other symptoms of malaria. Neem oil has also been found to be effective in repelling mosquitoes, making it useful in the preparation of mosquito-repellent products such as sprays and candles (Al-Hashemi & Hossain, 2016). Overall, neem is a versatile plant with a wide range of therapeutic uses, and its continued use in traditional medicine is a testament to its efficacy (Al-Hashemi & Hossain, 2016). Among these compounds, azadirachtin, nimbin, and nimbidine are considered to be the most active. These compounds have been shown to possess a range of therapeutic properties, such as anti-inflammatory, antimicrobial, antifungal, antiviral, antidiabetic, and antitumor activities. They have also been found to be effective against a range of ailments, including cancer, skin diseases, digestive disorders, and AIDS (Wylie *et al*., 2022).

The active chemical compounds in neem are slightly hydrophilic, which means that they have some affinity for water. However, they are more freely lipophilic, which means that they have a greater affinity for fats and oils. This property makes them more soluble in organic solvents, such as water, alcohol, ketones, and esters (Sarkar *et al*., 2021).

Overall, neem is a promising source of bioactive compounds with potential therapeutic applications. However, more research is needed to fully understand the mechanisms of action of these compounds and their potential side effects ((Al-Hashemi & Hossain, 2016).
The aim of this research is to explore the potential antimalarial effects of neem leaf extract (*Azadirachta indica*). Despite previous investigations into neem leaf's antimalarial properties, there is a scarcity of comprehensive and up-to-date review studies available. As a result, this

study endeavors to review a broad range of research conducted on the antimalarial properties of neem leaf over an extended period, up to the present day.

Malaria

Malaria is a disease caused by a parasite that is commonly transmitted through the bite of certain mosquitoes that feed on humans, and it can be a severe and even fatal illness (World Health Organization, 2021). Symptoms typically include high fevers, shaking chills, and flu-like symptoms, and there are four main types of malaria parasites that infect humans: *Plasmodium falciparum, P. vivax, P. ovale,* and *P. malariae.* In some regions, a type of malaria known as *P. knowlesi,* which usually infects macaques in Southeast Asia, can also infect humans, leading to a type of malaria that is transmitted from animals to humans (Centers for Disease Control and Prevention, 2022). Of these types, P. falciparum is the most likely to cause severe infections and death if not treated promptly. Nevertheless, preventing illness and death from malaria is generally possible, despite the potentially deadly nature of the disease (Centers for Disease Control and Prevention, 2022).

The World Health Organization has estimated that in 2020, there were 241 million clinical cases of malaria worldwide, resulting in 627,000 deaths, with a majority of them being children in Africa (World Health Organization, 2021). Malaria is a significant burden on numerous national economies due to its high mortality and morbidity rates. As the disease primarily affects poorer countries, it creates a vicious cycle of poverty and illness (World Health Organization, 2022).

Malaria is commonly contracted when a female Anopheles mosquito, which is the only type capable of transmitting the disease, bites a person and transmits the malaria parasites from a previous blood meal (World Health Organization, 2022). These parasites are mixed with the mosquito's saliva and injected into the person during the next blood meal, usually about a week later. Aside from mosquito bites, malaria can also be transmitted through blood transfusions, organ transplants, or the sharing of contaminated needles or syringes. Additionally, a pregnant woman can pass on malaria to her unborn baby (Mayo Clinic, 2021). Symptoms of malaria typically include fever, chills, and headaches, and in some cases, the disease can become severe or life-threatening (Mayo Clinic, 2021).

Impact of Neem leaves (*Azadirachta indica*) on Malaria parasite

In the past, controlling malaria vectors in their larval stage was an effective method for reducing malaria transmission. However, this approach lost popularity after the introduction of synthetic insecticides and bed nets (Keiser & Singer, 2019). Nonetheless, an integrated approach to malaria control that includes larval control methods is still the most promising strategy, given the challenges of insecticide resistance, changes in vector behavior, and difficulty in reaching vulnerable populations (World Health Organization, 2021). Recent laboratory studies have looked at the impact of neem seed (*Azadirachta indica*) extracts on Anopheles larvae, and have found that it leads to high rates of larval mortality, reduces adult longevity, and has a low potential for resistance development (Mebrahtom *et al*., 2019).

In their study, Afolabi *et al.* (2021) examined the effectiveness of *Azadirachta indica* in treating malaria, specifically its antiplasmodial activity. The results indicated that the antiplasmodial activity of A. indica varied significantly at different dosages ($P < 0.05$). The highest level of antiplasmodial activity (100%) was achieved when administering 600 mg/kg of the plant extract and 10 mg/kg of chloroquine (Figure 1). When these doses were given, all erythrocytic stages of *P. berghei* were eliminated by the extract in infected mice on day 2 of the plant extract treatment and day 3 of the chloroquine treatment. Additionally, the extract showed a 100%

cure rate in mice treated with 600 mg/kg of the ethanolic plant extract of A. indica for 24 hours and 10 mg/kg of the standard drug (chloroquine) for 48 hours, while the least percentage curative (31.20%) was observed in infected mice treated with 800 mg/kg.

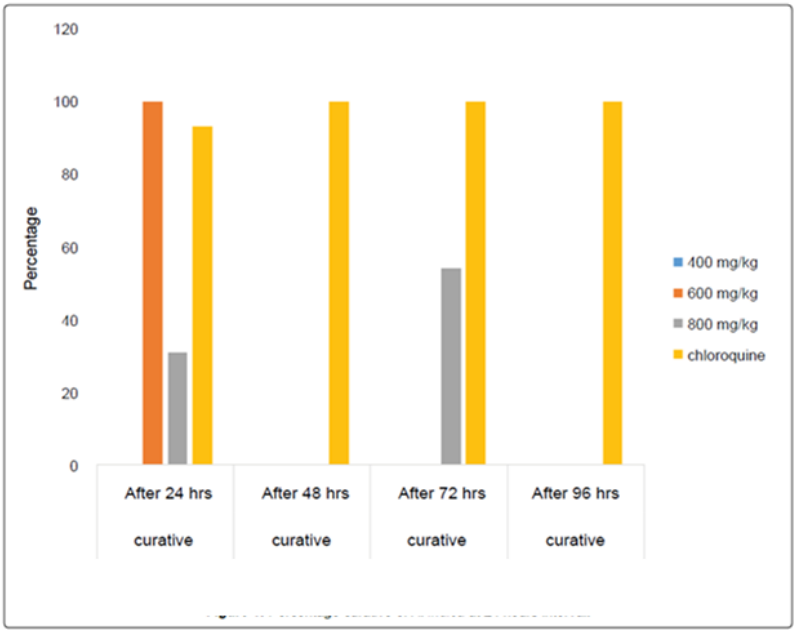

Figure 1: Percentage curative of A. indica at 24hrs interval (Afolabi *et al.* (2021)

Udeinyaa *et al.* (2004) conducted an invitro evaluation of an acetone-water neem leaf extract with antimalarial properties at a concentration of 5µg/ml for inhibition of adhesion of malaria parasite-infected erythrocytes and cancer cells to endothelial cells. The extract was also tested at a concentration of 10µg/ml for protection of lymphocytes against HIV invasion. Additionally, the extract was evaluated in 10 patients with HIV/AIDS at a daily dose of 1000 mg for 30 days. Results showed that in the absence of the extract, infected erythrocytes and cancer cells had a mean binding of 15 and 11, respectively, per endothelial cell. However, in the presence of the extract, the mean binding was reduced to 0 and 2, respectively. The extract also provided 75% protection to lymphocytes, as opposed to 0% protection in the absence of the extract. The treated patients exhibited improvements in haemoglobin concentration, mean CD4+ cell count, and erythrocyte sedimentation rate. Moreover, mean body weight and platelet count also increased, and no adverse effects were observed during the study. The results indicated that the acetone-water neem leaf extract had anti-cytoadhesion activity (Figure 2) and demonstrated a broad-spectrum activity by inhibiting adhesion of malaria-infected erythrocytes and cancer cells, and protecting human lymphocytes from HIV invasion. The extract exhibited antiretroviral activity with a mechanism of action that may involve inhibition of cytoadhesion, thus showing potential for developing novel antimalarial and antiretroviral drugs.

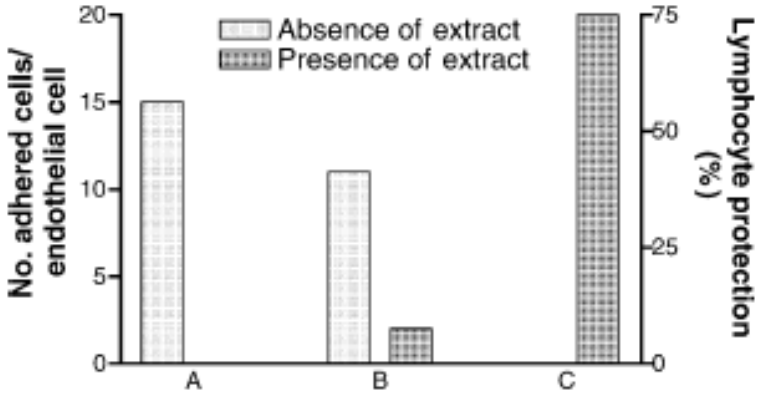

Figure 2: Comparing adhesion: Malaria-infected erythrocytes (A) and metastatic cancer cells (B) adhering to cultured human endothelial cells, while neem extract shows potential protection (C) for lymphocytes cultured with HIV (Udeinyaa *et al.,* 2004)

In the study conducted by Achi *et al*. (2018), the phytochemical content of *A. indica* was determined, and its therapeutic effects on malaria-induced male Wistar rats were investigated with important clinical indices. The plant material was extracted with ethanol and the lethal dose (LD50) on the rats was determined prior to the study. The rats were divided into eight groups with normal and *Plasmodium berghei* infected rats, and *lumartem* and plant extract were administered orally at therapeutic doses of artemether/lumefantrine (2/12 mg/kg) and 300 and 500 mg/kg body weight, respectively. The animals were sacrificed for experimental analysis after five days of treatment. The LD50 of the plant extract was found to be greater than 5000 mg/kg body weight, indicating that it was safe. Alkaloids, tannin, and terpenoids were found to be present in high concentrations through quantitative phytochemical studies. Treatment with both A. *indica* extract and lumartem in malaria-infected rats resulted in a slight decrease in triglycerides, while total cholesterol, HDL, and LDL levels increased significantly ($p < 0.05$). The increase in body weight of rats treated with A. indica was dependent on the concentration of the extract administered. Treatment with the plant extract and lumartem resulted in a slight restoration of the hematological values. The study concludes that both *Azadirachta indica* and lumartem were well-tolerated and safe for use.

In 2008, Udeinya and colleagues conducted an invitro study to evaluate the effects of crude extract of neem leaves (IRAB), which was composed of 50% acetone and 50% water, on both the asexual and sexual forms of the malarial parasite *Plasmodium falciparum*. The results of the study showed that the neem leaf extract was highly effective against both forms of the parasite, with a median inhibition concentration of less than 0.5 mg/mL (Figure 3). The extract's effectiveness was dose-dependent and resulted in complete parasite elimination within 72 hours at doses lower than 5.0 mg/mL that are pharmacologically relevant. Moreover, the neem extract demonstrated strong activities against gametocytes, the form of the parasite involved in mosquito-based malaria transmission (Figure 4). The extract caused lysis and disintegration of gametocytes in treated cultures but not in controls. The study's findings suggest that further investigations of the neem extract's structure-activity relationships may facilitate the development of effective *antigametocyte* drugs. This study uncovered the encouraging characteristics of neem leaf extracts, indicating the potential for future investigations that could result in the advancement of novel treatments for malaria and other illnesses.

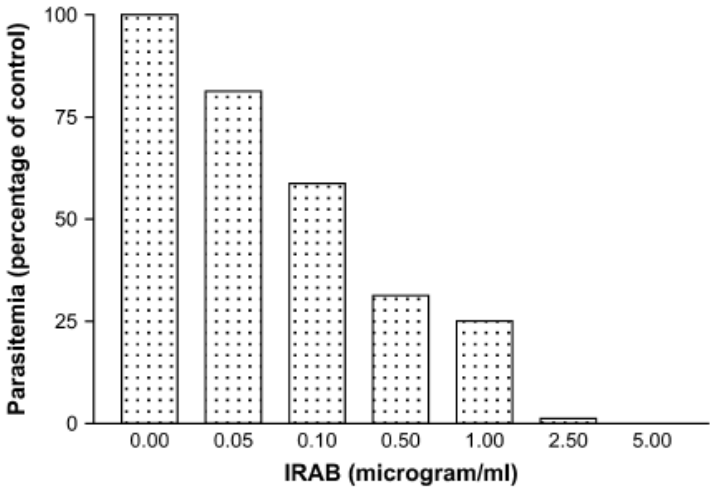

Figure 3: Effect of neem leaf extract (IRAB) on the malaria parasite, *P. falciparum,* in 72 hours (Udeinya *et al.,* 2008).

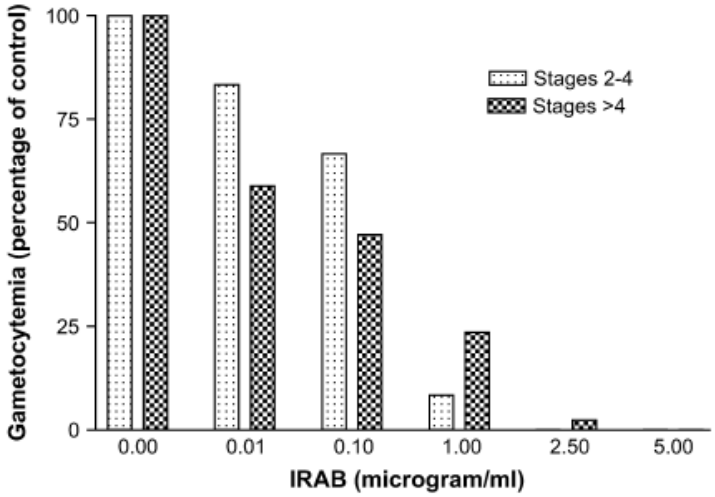

Figure 4: Elimination of cultured *gametocytes* by the neem leaf extract (IRAB) in 72 hours (Udeinya *et al.,* 2008)

In a separate investigation, Farahna *et al.* (2010) explored the potential antiplasmodial and protective effects of *Azadirachta indica* (Neem), a medicinal plant commonly used in traditional medicine, through the administration of an ethanolic extract of its leaves in a mouse model of malaria. *Plasmodium berghei* ANKA strain, a type of rodent malaria parasite, was injected intraperitoneally into Swiss albino mice. The presence of parasites was evaluated by examining blood samples daily under a microscope. The mice were treated with intraperitoneal doses of Neem extract at 300, 500, and 1000 mg/kg for five consecutive days starting from the day *parasitaemia* reached 5% of the parasite inoculation. Standard drug treatments using intraperitoneal chloroquine and artemether were employed as control measures. The mice's symptoms, including neurological or respiratory disorders, mortality, weight, and temperature, were documented. Additionally, the brains of the mice were prepared and examined for apoptotic cells after staining with hematoxylin-eosin and immunohistochemistry. Results revealed that all groups treated with Neem extract displayed gradual increases in *parasitaemia*, leading to signs of terminal illness (e.g., hypothermia, ptosis, and convulsions) within two to four days post-treatment. In contrast, the chloroquine and artemether treatment groups showed no symptoms of cerebral malaria and no fatalities. The mice treated with Neem and chloroquine exhibited Purkinje cell apoptosis, cerebral haemorrhage, and oedema. As per the mouse model, *Azadirachta indica* (Neem) extract was

found to be ineffective in safeguarding against malaria symptoms and signs. However, a difference in the number of apoptotic Purkinje cells between the untreated control group and Neem treatment at 500 mg/kg suggested that Neem may have some neuronal protective effect.

The study conducted by Lucantoni *et al*. (2010) aimed to evaluate the potential of NeemAzal®, an extract of neem seeds enriched with azadirachtin, to block the transmission of *Plasmodium berghei*, a rodent malaria parasite, using the in vivo model of *Anopheles stephensi* mosquitoes feeding on infected mice. The mosquitoes were treated with NeemAzal® intraperitoneally one hour prior to feeding, and the transmission-blocking activity of the product was assessed by examining oocyst prevalence, oocyst density, and the ability to infect healthy mice. In addition, Giemsa-stained mosquito midgut smears were examined to characterize the anti-plasmodial effects of NeemAzal® on early midgut stages. The results showed that NeemAzal® completely blocked the development of *P. berghei* in the mosquitoes at an azadirachtin dose of 50 mg/kg mouse body weight, with none of the examined mosquitoes revealing any oocyst, and none of the healthy mice exposed to their bites developing *parasitaemia.* The examination of midgut content smears also revealed a reduced number of zygotes and post-zygotic forms, as well as the absence of mature ookinetes in treated mosquitoes. Furthermore, post-zygotic forms showed several morphological alterations, suggesting an interference of azadirachtin with the microtubule organizing centers and the assembly of cytoskeletal microtubules, which are essential processes in Plasmodium gametogenesis and ookinete formation. These findings demonstrate the in vivo transmission blocking activity of an azadirachtin-enriched neem seed extract at a dose compatible with 'druggability' requirements. As a result, this study provides evidence of the anti-plasmodial activity of neem products and encourages further research to evaluate their potential for designing novel or improved transmission-blocking remedies.

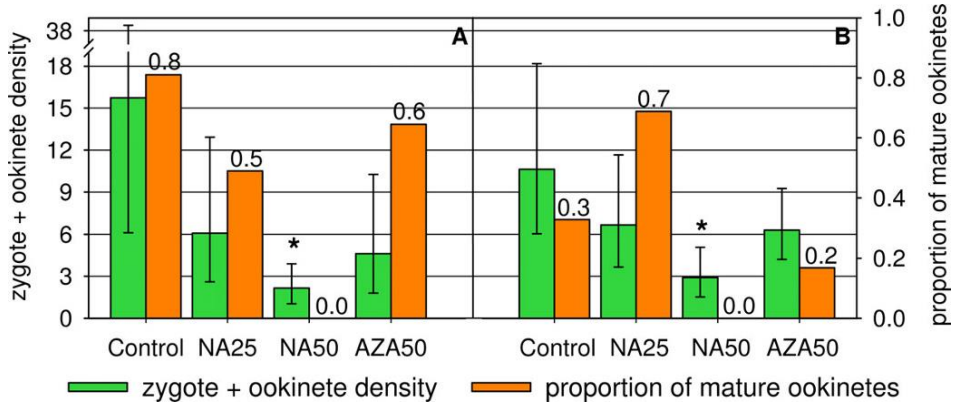

Figure 5: Effects of the neem products on *P. berghei* midgut stages (zygotes to ookinetes).

Parasites were counted on 300 microscopic fields (1000× magnification) of midgut content smears of A. stephensi females that had fed 18 (A), and 20 (B) hours before on mice treated with the indicated neem products and doses. NA25, NeemAzal® 25 mg/kg; NA50, NeemAzal® 50 mg/kg; AZA50, azadirachtin 50 mg/kg. Geometric means of zygote + ookinete densities, evaluated over ten smears (30 mosquitoes), with 95% confidence intervals (green bars; left axis). Mature ookinetes/total midgut forms ratio (orange bars; right axis). * Means differ significantly from control (Student's t test; $p \leq 0.002$).

Challenges of using Neem as an antimalaria agent

The use of neem as a natural alternative for malaria control has been explored for many years. However, there are several challenges associated with using neem to combat malaria parasites:

1. Standardization: The chemical composition of neem extracts can vary depending on factors such as geographical location, climate, and extraction method. This variability can make it difficult to standardize the efficacy of neem-based products.
2. Dosage: The appropriate dosage of neem required to achieve a therapeutic effect against malaria is not well-defined. The dosage can also vary depending on the form in which neem is administered, such as neem oil, neem leaves or neem bark.
3. Resistance: The emergence of resistance to neem-based products is a concern, just as it is with conventional chemical insecticides. Malaria parasites may adapt to the use of neem and develop resistance over time.
4. Availability: Neem trees grow in tropical regions, and the production of neem-based products may be limited in areas where neem is not grown or easily available.
5. Sustainability: Neem-based products may not be sustainable over the long term. Large-scale neem farming can lead to the destruction of natural habitats and ecosystems.
6. Side effects: Although neem is generally considered safe, it may cause side effects such as vomiting, nausea, and diarrhea in some individuals. The long-term effects of neem on human health are not well-understood, and more research is needed to establish its safety for widespread use against malaria.

CONCLUSION

This study conducted a review of various investigations into the effectiveness of *Azadirachta Indica* in treating malaria. Although the studies on the leaves' antimalarial properties are insufficient to draw a definitive conclusion, all of the reviewed investigations suggested that neem leaves have the potential to be used as a therapeutic agent for treating malaria parasites. To further support the use of this plant as a natural remedy for malaria, more primary studies are recommended to strengthen the existing findings.

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