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Biosynthesis of copper nanoparticles using *Anisomeles malabarica* and its pharmacological activity

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Abstract
Traditional medicine has made use of a herb known as *Anisomeles malabarica*. Medicinal plant extracts and their components have been shown to be biodegradable, and their effects are similar to those of prescription drugs. *Anisomeles malabarica* (Family - Lamiaceae) is a medicinal plant found in tropical and sub-tropical regions of India that is used to treat a wide range of disorders such as dementia, anorexia, allergies, cancer, and inflammatory diseases. Malaria is one of the main causes of direct or indirect infant, child, and adult mortality in the tropics, accounting for two to three million new cases annually, mostly in India. More potent antimalarial drugs with broad host specificity are required. Helminthic infections can damage millions of livestock and result in a large financial loss in domestic animals. Worldwide, helminthic illnesses are managed with synthetic drugs. Although these drugs are highly effective in treating helminthiasis, they also have a number of unfavorable side effects. Larvicidal activity is an important consideration when testing mosquito larvae against *Anisomeles malabarica* methanolic extract at different dosages.

Keywords: *Anisomeles malabarica*, copper nanoparticles, antimicrobial activity, larvicidal activity and antihelminthic activity

Introduction

Anisomeles malabarica L. is a fragrant perennial herb that is found throughout Southern and Tropical Asia. It is a member of the Lamiaceae family (Gupta and Tandon, 2004) [1]. According to Kotha *et al.* (2017) [2], the leaves of *Anisomeles malabarica* are used in traditional medicine to treat hyperlipidemia, diabetes, and gastrointestinal disorders. A powerful medication used in Siddha and Ayurvedic therapy is *Anisomeles malabarica*. According to Gupta *et al.* (2008) [3], it helps with intestinal worms, halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, fever from teething children, intermittent fever, gout, edema, and diarrhea. Numerous biological benefits, including anti-inflammatory, anti-allergic, antiviral, and anti-cancer properties, have been identified for flavanoids. Alkaloids are one type of chemical that plants produce, and because of their remarkable effects on people, potent painkillers have been developed (Saranraj *et al*., 2024) [4].

In many underdeveloped nations, vector-borne diseases rank among the leading causes of sickness and mortality. Millions of people worldwide are impacted by mosquitoes, which are capable of spreading more diseases than any other species of arthropods. Infecting approximately 700,000,000 people annually worldwide, including 40,000,000 Indians, mosquito-borne diseases are common in more than 100 nations. According to Anupam Ghosh *et al.* (2012) [5], they are a nuisance to people and the main vector for the spread of deadly illnesses such Japanese encephalitis, dengue fever, yellow fever, chikungunya fever, lymphatic filariasis, and malaria.

In India, Anopheles is the primary vector of malaria. Larvicides are essential for controlling mosquitoes effect on places with beneficial and non-target organisms, in their breeding grounds (Sritabutra *et al.*, 2011) [6]. Because of their exceptional pharmacological activity and therapeutic value, medicinal and aromatic plants are highly sought after on the market, requiring improved mechanical and medical care. Asexual reproduction has the benefit of producing plants that are almost identical to the plants they are derived from. In China, *Anisomeles malabarica* is mostly collected from the wild, though it can be cultivated from seed or rhizome cuttings (Ling *et al.*, 2001) [7].

Fig 1: *Anopheles albimanus*

Many copper-containing enzymes support various bodily processes, including iron homeostasis and oxygen transport (Vimbela *et al.*, 2017) [8]. Furthermore, skin, bones, and various body organs contain copper (Al-Hakkani, 2020) [9]. Although vector-borne diseases are present everywhere in the world, they have an influence on human health and can result in a loss of labor and commercial productivity, especially in poor nations with tropical and subtropical climates (Fradin and Day, 2002) [10]. Many diseases, including Japanese encephalitis, dengue fever, yellow fever, filariasis, schistosomiasis, and malaria, are spread mostly by mosquitoes. One of the leading direct or indirect causes of new born, child, and adult death in India is malaria.

Botanical Distribution of *Anisomeles malabarica*

Anisomeles malabarica is a floral herb that reaches heights of 1.2 to 2.0 meters. It is heavily pubescent. Simple, opposite leaves that are very thick, aromatic, oblong-lanceolate, acute, pale above, white below, crenate-serrate, and woolly are belonging to this plant. The fruits are nutlets that contain compressed, ellipsoid seeds, and the purple blooms have thick whorls of somewhat interrupted spikes. Aruvaachadachi is a common name for the Labiatae family plant *Anisomeles malabarica* (L) R.Br. that is widespread throughout India (Saranraj *et al.,* 2024) [11]. The Lamiaceae family includes *Anisomeles malabarica*, which is widely distributed in the tropical and sub-tropical regions of India.

Phytochemical Constituents

Phytochemical components of the plant, including tannins, polyphenols, alkaloids, and flavonoids, were quantitatively examined. The presence of alkanes, halogens, alcohols, nitro compounds, esters, aldehydes, acid anhydrides, amino acids, amines, and amides allowed for the observation of the stem sample's absorption spectra. Sudha and Srinivasan (2014) [12] extracted the bioactive chemicals 3, dihydroxy benzoic acid, and 4', 5, 7-trihydroxyflavone from the leaves of *Anisomeles malabarica* using chloroform.

Pharmacological activities of *Anisomeles malabarica*

1. Antioxidant activity

Strong antioxidant properties are possessed by members of the genus *Anisomeles malabarica*. Among the several plants analyzed for antioxidant properties, *Anisomeles malabarica* showed notable antioxidant properties relative to other plants. Using free radical scavenging tests, such as hydroxyl and superoxide anion radicals, as well as 2,2-diphenyl-1-azinobis-(3-ethylenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, the antioxidant properties of the ethyl acetate extract of *Anisomeles malabarica* leaves were evaluated. The extract was found to be an efficient scavenger of DPPH, ABTS, and superoxide anion radicals. Leaf extract has potent antioxidant and free radical-scavenging properties at all dosages; its preventive effects were dose-dependent. Comparatively, the methanolic extract of the entire *Anisomeles malabarica* plant has significantly more antioxidant principles than the other solvent extracts. *Anisomeles malabarica* plant was evaluated for its antioxidant activity using the FRAP assay, hydroxyl radical scavenging, and nitric oxide radical scavenging. The results showed that the methanolic extract had significantly more free radical scavenging activity than the standard. The ethanolic extract of *Anisomeles malabarica* demonstrated a significant antioxidant potential *in vitro* (Subramanian and Vedanarayanan 2012) [13]. *In vitro* antioxidant experiment was performed to examine the DPPH free radical scavenging activity and reduction ability of *Anisomeles malabarica* leaf extracts. The methanol and hexane extracts were shown to have potent antioxidant activity. Good antioxidant activity and efficacy were demonstrated by *Anisomeles malabarica* leaf extracts in methanol and hexane. After comparing the crude extracts, it was found that methanol extract shows better results than hexane. Methanol extract had good reductive capability and antiradical activity (Vinod *et al.*,2014) [14].

2. Antibacterial activity

The ethanolic extract of *Anisomeles malabarica* showed the highest antibacterial and antifungal activity when compared to the other two extracts in tests of petroleum ether, ethyl acetate, and methanol extracts for antimicrobial efficacy against gram positive and gram negative bacterial and fungal species. Boiling leaf extracts from *Anisomeles malabarica* produced silver nanoparticles that significantly impeded the growth of *Pseudomonas* sp. Using the Agar well diffusion method, the concentration of the bacteria dictated the outcome of *Anisomeles malabarica's in vitro* antimicrobial activity against the multidrug resistant strains of clinically significant bacteria, specifically *Staphylococcus aureus, Bacillus subtilis*, and *Klebsiella pneumoniae* (Packialakshmi and Nisha, 2014) [15]. The bactericidal properties of petroleum ether, methanol, ethanol, and an aqueous extract from boiling and *Anisomeles malabarica* leaves were examined by researchers. The largest zone of inhibition against *S. aureus* was found in polar investigations. Non-polar investigations, on the other hand, discovered the highest zone of inhibition against *Pseudomonas aeruginosa*. *Anisomeles malabarica* leaf extracts were tested for their *in vitro* antibacterial activity against *Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa,* and *Escherichia coli.* The results of the investigation showed that at the same doses, diethyl ether extract produced a 30 mm zone of inhibition against *Staphylococcus aureus*, whereas ethanol produced a 25 mm zone (Singh *et al.*,2010) [16].

3. Anti-inflammatory activity

Anisomeles members possess strong anti-inflammatory qualities. Comparing ethanol and *Anisomeles malabarica* aqueous extract to the popular medication indomethacin, significant anti-inflammatory effects were observed in a dose-dependent manner. The anti-inflammatory effects of several *Anisomeles malabarica* extracts were assessed using rats cotton pellet granuloma and paw edema brought on by carrageenan. Out of all the investigated extracts, the ethanol and aqueous extracts from the leaves showed the most anti-inflammatory effects, and these effects were dose-dependent. *In vitro* research revealed that *Anisomeles malabarica* leaves possess anti-inflammatory, antiplatelet, and anti-arthritic properties (Lavanya *et al.*,2011) [17].

4. Anti-cancer activity

After ingesting an oral dose of 100 mg/kg body weight of the ethanolic extract of *Anisomeles malabarica*, lower levels of total protein glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, acid phosphatase, and alkaline phosphatase were seen in the liver and blood (Jeyachandran *et al.*,2007) [18]. Extracts of *Anisomeles malabarica* in n-hexane and chloroform exhibited cytotoxicity toward cervical cancer cells that was dependent on both duration and dose. The cells' typical apoptotic characteristics demonstrated the impact of the therapy. Early indicators of phosphatidyl serine translocation, apoptosis, and loss of mitochondrial transmembrane potential were observed in the treated cells. DNA damage was validated by the comet test.

Anisomelic acid's (AA) cytotoxicity and capacity to cause apoptosis in breast and cervical cancer cells were studied. AA is cytotoxic to all types of cell lines investigated in a

fashion that is depending on dosage and duration, according to the MTT assay for cell viability. Constricted chromatin and the development of apoptotic structures were evident when AA-treated cells were stained with Acridine Orange and Ethidium Bromide (AO & EB) and Hoechst 32558. One or more DNA strand breaks were found by the comet assay, proving that AA oxidizes DNA and causes apoptosis. Research has been done on the impact of *Anisomeles malabarica* whole plant methanol extract on the cellular redox state during the hamster buckle pouch carcinogenesis process. *Anisomeles malabarica* increased GSH-dependent antioxidant enzyme activities and reversed the buckle pouch's susceptibility to lipid per oxidation; in contrast, the liver and erythrocytes showed reduced lipid per oxidation and elevated antioxidant levels. The results of the study suggest that the plants flavonoids, an antioxidant and anti-proliferative phytochemical, may provide protection against the high-BP carcinogenic effects. (Ranganathan and Vijayalakshmi, 2012) [19].

5. Anti-proliferative activity

Research has demonstrated that *Anisomeles malabarica's* anti-proliferative qualities in n-hexane and chloroform extracts can inhibit the growth of cervical cancer cells and even eradicate HPV16-positive cervical cancer cells **(**Zahir *et al*.,2012) [20].

6. Antiplasmodial activity

The leaf methanol extracts of *Anisomeles malabarica* and *Ricinus communis* shown strong antileishmanial activity in *Leishmania donovani* among the ten experimental plant extracts (Kavitha *et al.*,2012) [21].

7. Anti-pyretic activity

Rats with Brewer's yeast-induced pyrexia were used to test the anti-pyretic properties of several *Anisomeles malabarica* extracts. Antipyretic tests were conducted using the extracts at oral dose levels of 50 mg/kg, 100 mg/kg, and 200 mg/kg. According to Pappu *et al.* (2010) [22], all three extracts had a good antipyretic efficacy at the utilized doses.

8. Larvicidal activity

The sheep internal parasite *Paramphisto mumcervi* at 2,000 ppm, the bovine tick larvae *Rhipicepha lusmicroplus*, and the star larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* at 1,000 ppm were tested against the dried leaf, flower, and seed extracts of *Anisomeles malabarica* and other plants. All plant extracts had mild effects after 24 hours/ The highest parasite mortality rates against the *Culex tritaeniorhynchus* larvae were shown by the *Anisomeles malabarica* leaf methanol extract, leaf acetone and chloroform extract, and flower chloroform extract (LC_{50} = 68.27, 95.98, 59.51, and 93.94 ppm; LC_{90} = 306.88, 393.83, 278.99, and 413.27 ppm). The larvicidal effectiveness of two natural plants against the larvae of *Anopheles stephensi*, an economically significant malarial vector, was evaluated in a laboratory setting. According to Jayakumar *et al.* (2014) [23], the results showed that the methanol extracts of both plants had significant larvicidal action, and that the combined (synergistic) extracts had the maximum larval mortality.

9. Anti-anaphylactic activity

Anaphylaxis is one of the severe allergic reactions that can happen very quickly and fatally. Anaphylaxis is a potentially fatal severe allergic reaction that can occur rapidly after coming into touch with an allergen. It is estimated that 0.05 – 2 % of people may experience anaphylaxis at some point in their lives, and the incidence rate is growing (Lavanya *et al.*,2011) [24]. Plant study is receiving more attention these days all across the world, to show the vast potential of medicinal herbs used in many different traditional systems. *Anisomeles malabarica* contains essential chemical components that have anti-anaphylactic properties.

10. Anti-epileptic activity

Antiepileptics are one family of medications used to treat epileptic seizures. The total flavonoid fraction of *Anisomeles malabarica* has demonstrated efficacy as a single dose pre-treatment against maximal electroshock and pentylenetetrazole convulsions. It is also associated with a significant decrease in motor activity performance and locomotor activity, which is similar to the neurotoxic consequences of diazepam therapy. Long-term treatment of total flavonoids fraction at lower dosages (6.25 and 12.5 mg/kg, 1 week) has similarly produced significant antiepileptic activity without any neurotoxic effects **(**Choudhary *et al.*,2007) [25].

***In vitro* and *In vivo* Antihelminthic and Larvicidal activity**

Compounds had on the earthworm *Pheritima posthuma*, most early *in vitro* investigations on plant anthelmintics, their different extracts, or oils, were conducted (Siddiqui and Garg 1990) [26]. The essential oils of *Gardenia lucida* (Rubiaceae), *Cyperus rotendus* (Cyperaceae), *Inulara cemosa* (Compositae), *Psitacia integrrima* (Anacardiaceae), *Litsea chinensis* (Lauraceae), and *Randiadu metorum* (Rubiaceae) seeds have been shown to have good anthelmintic efficacy against tapeworms and earthworms. Most of these substances are poisonous to earthworms and first agitate or distress them, causing them to escape the vicinity of the toxin. Studies on *in vivo* have been conducted to evaluate the anthelmintic activity of various plant components. *In vivo* tests have also been carried out on domestic animals, such as cattle, sheep, and goats, to evaluate the anthelmintic activity of various medicinal plants and the active component in them. In these studies, the efficacy of test compounds has usually been assessed by counting the number of Eggs Per Gram of Excrement (EPG) that the infected hosts pass following treatment with plant-derived chemicals, or by counting the worms that are expelled from the hosts. This characteristic means that when the concentration is not high enough to kill the worm, antihelminthics most likely regularly drive the parasite away (Yuvalakshmi *et al.*, 2024) [27].

Ascaris lumbricoides, hookworms, and tapeworms have been used by a number of researchers to evaluate the *in vitro* Antihelminthic effectiveness of different plant materials. It is common practice to evaluate the effects of plant products on Haemonchuscontortus eggs using a modified egg hatch experiment. Several other research projects involving *in vitro* investigations have made use of a variant of the Larval Development Assay (LDA) or Larval Motility Tests, which

are commonly used to screen for parasite resistance to anthelmintics.

Bany *et al*. (2003) [28] studied the effects of alchinal, a complex combination of three components, on the formation of *T. spiralis* in mice. The ingredients are cocoa, *Allium sativum* extract, and *Echinacea purpurea* extract using a mouse model of *T. spiralis*, quinolines with potent *in vitro* action have been studied *in vivo*. The anticestodal properties of a few other plants, such as *Butea minor*, *Trifoliumrepens*, *Strobilanthes discolor*, and *Gladiolus gandavensis*, have been well-determined using purposefully created *H. diminuta* in albino rats. Extracts from *Cucurbi apepo* (Cucurbitaceaea), *Calotropis gigantean* (Asclepiadaceae), *Juglans regia* (Juglandaceae), *Momordica charantia* (Cucurbitaceae), *Musa paradisaca* (Musaceae), and *Scindapsus offcinalis* (Araceae) have demonstrated significant anthelmintic activity on the goat-origin Haemonchuscontortus (Vedha *et al*., 2023) [29].

Bogh *et al.* (1996) [30] showed that *Embelias chimperi* extracts were efficient as anthelmintics against *Echinostoma caproni*, *H. polygyrus*, and *H. microstoma* in mice and against *H. diminuta* in rats. The stem bark extract of *Berlinia grandiflora* has been reported to be effective against anthelmintic infections, based on studies against *N. brasiliensis* infections in albino rats. The stems and roots of the following plants were among them: *Amomum aromaticum* (Zingiberaceae), *Ammora wallichii*, *Antho cephalusindicus* (Rubiaceae), *Calamint aumberosa* (Labiatae), *Dalbergi alatifolia* (Leguminosae), the fruit of *Datura quercifolia* (Solanaceae) and *Datura metal* (Solanaceae), *Ficus religiosa* (Urticaceae) plant, the stem and bark of Sentiamyrtina plant, and the leaves of *Sumploco scrataegoides* (Sumplocos). *Commi phoramukul* (Buberaceae), *Anacardium occidentale* (Anacardiaceae), *Buddlea asiatica* (Loganiaceae), *Chloroxylon swientenia* (Rutaceae), and *Callistemon viminalis* (Myrtaceae) are among the plants whose essential oils are said to have potent anthelmintic activity against tape and hookworms. Their efficacy has also been contrasted with that of hexylresorcinol and piperazine phosphate. Other studies involving the essential oils of *Artemisia pallens* (Compositae), *Eupatorium triplinerve* (Compositae), *Artab trysodoratissimus* (Annonanceae), *Capillipedium foetidum* (Poaceae), and the grass of Cymbopogon martini (Poaceae) have reported strong anthelmintic activity against *Taenia solium* and *Ascaris lumbricoides* **(**Garg and Nakhare 1993) [31].

Control of Vectors

Public health professionals have revolutionized the field of vector management with their increased knowledge of the potential advantages of natural products in the battle against disease. Some features of insecticides, such as toxicity and latency (or residual capacity), in order to better identify and constitute an effective control agent. The main performance indices for pesticide evaluation are typically displayed as these two attributes, the LD_{50} and the test period, respectively. While preventing mosquito bites is an admirable goal in and of itself, repellents typically have a low toxicity and a short half-life. Products with high toxicity and those that kill mosquitoes are often highly sought after, but they may also come with unexpected costs. Combining increased toxicity and prolonged latency has been proposed as the most competitive technique to control mosquitoes. In terms of both efficacy and resistance, it has been observed in practice that using mixes to maximize the synergistic effects of substances has been successful. Among the synthetic pyrethroid compounds that are currently available is permethrin. These substances were first employed as compounds that were separated from the blooms of *C. cinerariifolium* (Trev.) Bocc and *Chrysanthemum coccineum* L. (Shaalan *et al.*,2005) [32].

Fig 2: *Culex Pipens*

Mode of Action of Medicinal Herbs against Specific Life Cycle

1. Adult stage

It has long been believed that human protection from this vector is vital since the adult female mosquito bites the host in an effort to draw blood. According to plant essential oils function is by discouraging adult females from biting, which halts the spread of disease. Many of these natural repellents irritate the skin, smell bad, or work for a very limited period of time. The most durable and protective-repellent chemical currently available on the market, N, N-diethyl-3-methylbenzamide (DEET), has demonstrated effectiveness over several decades of commercial use may have adverse side effects (Fradin, 1998) [33].

Repellants are not a long-term solution to the global problems associated with preventing or treating mosquito-borne illnesses since they gradually lose their effectiveness and need to be reapplied. As a fundamental part of the Roll Back Malaria approach, insecticide-treated bed nets are considerably more cost-effective in reducing the prevalence and adult mosquito-borne transmission of malaria (Tolle, 2009) [34]. Bed netting treated with permethrin that mosquitoes to the person sleeping beneath it and employs contact insecticide to kill the vector, is a major component of current prophylactic measures. These tactics help the community as well as the individual and are widely employed.

2. Larval stage

To reduce mosquito populations at the larval stage has the benefit of controlling the vector before it spreads or becomes infected, thereby interrupting its life cycle before it can do harm. Although resistance has been discovered to each of these in the field, a number of compounds, such as organophophates like temephos and Insect Growth Regulators (IGRs) like methoprene, are directed at mosquito larvae (Rose, 2001) [35]. The market has not yet been impacted by new synthetic compounds. Vertebrate predators have been utilized to control the biological control of mosquito larvae in the case of the mosquito fish, *Gambusia*

affinis, with differing degrees of success. Research on fish used as mosquitoes has revealed both beneficial and detrimental effects. For example, intraguild predation happens when foreign fish consume local aquatic mosquito predators, which does not result in the intended control. Recent studies on invertebrate predatory beetles. Culler and Lamp (2009) [36] have focused on adopting conservation biological control measures that increase the effects of native species. Mathew *et al.* (2009) [37] discusses other biological control techniques. Despite being relatively non-toxic to the environment, the use of *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* bacterial toxins has been shown to be effective against larvae.

Health Benefits of *Anisomeles malabarica*

Halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, intestinal worms, fever linked with teething children, gout, swelling, and diarrhea are a few of the ailments for which *Anisomeles malabarica* is helpful (Saranraj *et al.,* 2024) [38] It is used to cure amenorrhea, rheumatism, fevers, swellings, and anorexia. According to reports, the plant possesses anti-bacterial, anti-carcinomic, anti-edemic, anti-inflammatory, anti-allergic, anti-allergic, anti-nociceptive, antiplasmodial, antiseptic, and antiperotic properties. Flavanoids have been connected to a wide range of biological advantages, such as anti-inflammatory, anti-allergic, antiviral, and anti-cancer effects. Plants contain a family of chemicals called alkaloids, and due to their extraordinary impact on human health, powerful analgesic drugs have been developed (Tharani *et al.,* 2024) [39].

Medicinal values of *Anisomeles malabarica*

Anisomeles malabarica is used in the production of perfumes, cosmetics, and pharmaceuticals. It has been used for many years as a medicinal herb in Indian and Sri Lankan traditional remedies. All parts of the plant are used to treat many ailments such as edema, congenital mental anomalies, fever during teething, and more; however, the leaves and roots are particularly useful. Plants generate alkaloids, and due to their distinct effects on humans, powerful analgesics have been created (Subhalakshmi *et al.,* 2024) [40].

Conclusion

The traditional use of a wide variety of widely available medicinal herbs is important because it provides a convenient and potent source of anthelmintic/larvicidal action. For many years, traditional medical systems in nations like India have made considerable use of *Anisomeles* species. *Anisomeles malabarica* is a species of plant that has been found to have pharmacological importance. The creation of innovative medications for the treatment of various ailments is aided by the existence of bioactive compounds. To create novel drugs of consistently high quality, systematic methods for isolating active ingredients in their purest forms are required. Strong active chemicals with strong anti-inflammatory, anti-cancer, and antioxidant properties are present in the plants. In order to fight cancer, it is currently essential to progress the creation of organic medications. The application of naturally occurring antioxidants from these plants advances the food and pharmaceutical industries. They contain functional components that enable the performance of a wide range of biological processes. *Anisomeles malabarica* is a promising natural remedy that warrants more research into its chemical composition and pharmacological effects because it has enough attributes and protections. Due to its many traditional, therapeutic, and pharmacological uses, the plant is highly valuable. The characteristics of the plant can be extracted, which may help with therapy; however, *in vivo* growth methods must be used. *Anisomeles malabarica* has been used historically to treat a variety of ailments, including HIV, cancer, tumors, inflammation, rheumatoid arthritis, and epilepsy, according to the research findings. The current analysis demonstrates the need for additional molecular research on organic extracts and various plant-derived active components in order to identify new phytochemicals that can take the place of synthetic medications in the treatment of a variety of diseases caused by parasite infections.

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