

Green Synthesis of Copper Nanoparticles from *Anisomeles Malabarica* and Their Biological Activities

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Abstract: *Anisomeles malabarica*, a medicinal herb found in tropical and sub tropical India, has been used for Traditional medicine due to its antimicrobial properties. The methanol extract of this plant has demonstrated inhibitory effects against both Gram positive and Gram-negative bacteria, as well as fungi. This study investigates the green synthesis of copper nanoparticles using *Anisomeles malabarica* and analysis of its pharmacological activity. The phytochemical investigation of *Anisomeles malabarica* revealed the presence of carbohydrates, amino acids, protein, phytosterols, glycosides, alkaloids, flavonoids, steroids, tannins and saponins. The methanol extract showed antibacterial action against *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. Malaria is a significant contributor to newborn, child and adult mortality, causing around two to three million new cases annually in tropical regions, especially in India. There is a demand for more efficient Antimalarial medications that target a wide range of hosts. Synthetic medicines are widely used for managing helminthic disease due to their high efficacy, but they can also lead to adverse consequences. The larvicidal activity of the methanolic extract of *Anisomeles malabarica* was tested against mosquito larvae using various doses. The study of Nanoparticles production was conducted using SEM, UV-vis spectroscopy and Fourier-transform infrared spectroscopy. The presence of a peak in the UV-Vis spectra suggests a strong binding affinity for excitation. The AFM investigation confirmed the morphology and size dispersion of nanoparticles, while FT-IR analyses verified the presence of biomolecules. In conclusion, *Anisomeles malabarica* has the potential to be a valuable bioresource for the quick, cost-effective and efficient synthesis of copper nanoparticles.

Keywords: Biological Synthesis, Copper Nanoparticles, *Anisomeles malabarica*, antimicrobial activity, Anthelmintic activity and Larvicidal activity.

INTRODUCTION

Anisomeles malabarica is a potent perennial herb of the family Lamiaceae. It is extensively found in tropical and southern Asia, especially India. It is employed to treat various diseases such as gastrointestinal diseases, diabetes, hyperlipidemia, dyspepsia, catarrhal disorders, intermittent fever, bowel diseases, boils and tetanus because it has an abundance of phytochemicals [1]. The 29 membered genus *Anisomeles* is among the largest in the family Lamiaceae and some of the most important among them are *A. malabarica*, with extensive use in medicine. Another important species within this genus, *Anisomeles indica*, has shown antimicrobial, astringent and carminative activities. A 50% ethanolic extract of *Anisomeles indica* also showed hypothermic effect and the plant is also used traditionally as a mosquito repellent upon burning [2]. The essential oils of the plants are also used in treating infections of the uterus. Large scale research of the genus has established the herbaceous effect, which further endears the plant as a drug of great pharmacological value.

A. malabarica has also exhibited promising potential in treating vector borne diseases like malaria, dengue fever,

yellow fever, chikungunya and Japanese encephalitis, all of which are formidable health issues in developing countries. Propagation of *A. malabarica* is typically done through seeds or rhizome cuttings, but in much of the world, including China, it is mainly wild harvested. Cutting propagation is useful because it results in genetically uniform progeny, hence retaining useful medicinal features. Since medicinal and aromatic plants are in high demand in the world, their production needs to be performed sustainably to make them accessible for use without disturbing natural occurrences [3]. Copper is a trace element in the human body that is very important for numerous physiological processes, including oxygen transport and iron homeostasis. New findings on *A. malabarica* have substantiated its historical uses by the demonstration of a wide range of bioactive molecules with antioxidant, antimicrobial, anti-helminthic and larvicidal activities.

The farming of *A. malabarica* in an eco-friendly and sustainable way is essential to facilitate the rising demand for its medicinal uses while preserving biodiversity. The suitability of the plant to survive and perform in dissimilar environmental conditions makes it

a suitable candidate for mass cultivation, if implemented with the implementation of ecological farming practices [4]. The extraction of copper nanoparticles, which involves the use of *A. malabarica*, offers a synthesis in the mix of ancient medicine and nanotechnology and a new way to solve new health problems. *A. malabarica* is an aromatic perennial growing plant, a member of the Lamiaceae family, that is abundantly found in southern and tropical Asia. The leaves of *A. malabarica* with known medicinal potential have been used traditionally to treat gastrointestinal disorders, diabetes, hyperlipidemia and other ailments such as dyspeptic disorders, catarrhal diseases, intermittent fever, bowel disorders, boils and tetanus [5].

The genus *Anisomeles* is the largest of the Lamiaceae family, consisting of 29 species. It exists in India, which is one of the countries that is highly diverse in species of *Anisomeles* [6]. *Anisomeles indica*, a different genus member, has displayed antimicrobial, astringent and carminative properties besides medicine. The ethanolic extract of 50 % of this plant has been shown to have a hypothermic effect and when burnt, it is utilised as an anti-mosquito repellent [7]. The plant oils are also beneficial for treating uterine infections. This plant is thoroughly studied due to its herbaceous nature and hence which reason why the plant is widely regarded as a plant of particular pharmacological value. The other important concerns that were dealt with in the plant are vector-borne diseases, which are also the major health issues in developing nations. Mosquitoes are known to cause diseases like malaria, dengue fever, yellow fever, chikungunya and also Japanese encephalitis, among a few others and *A. malabarica* has been spotted to have the capability of controlling them [8].

A. malabarica is typically propagated by seed or rhizome cuttings, though generally, in most other countries, it is harvested wild, even in China. The advantage of asexual reproduction is that the cuttings of the plant can be genetically identical to the parent, so the medicinal effect should be maintained [9]. Since there is an expanding demand for medicinal and aromatic plants which possess pharmacological action, there should be an enhanced way of management and cultivation to ensure sustainable production. Copper is a very crucial substance in the health of a human being and plays a significant part in metabolic reactions. It is a constituent of other body processes that include iron homeostasis and transport of oxygen. Copper is a component of over 30 body enzymes as well as tissues such as skin, bones and organs [10]. The study also focuses on finding the medicinal value of the plant based on the synthesis of copper nanoparticles evaluated under an elaborate evaluation technique, such as UV spectrophotometry, FTIR and SEM. These methods assist in comprehending the characteristics of the plant on the molecular level, which aids in its therapeutic applications and possibilities to create new medicine.

MATERIALS AND METHODS

2.1. Collection of samples

A. malabarica was collected from the Tirupattur District. *A. malabarica* was collected and sun dried for around 10 days. The plant was powdered using a mortar and pestle to make the plant extract ready for use in the experiment.

2.2. Preparation of the sample

The sample leaves were harvested, air dried under sterile conditions and subsequently powdered with a mortar and pestle. Methanol was used to dehydrate and extract. Two different extracts were left at room temperature for 48 hours, shaken for 24 hours. Dehydration of the 24 h extracts was performed using Whatman filter paper and rotary evaporation. Dehydrated filtrate was stored at - 20°C in a screw-cap vial for future use.

2.3. Phytochemical analysis

2.3.1. Test for carbohydrates

The addition of Benedict's reagent and boiling the mixture for five minutes in a water bath results in the formation of an orange-red precipitate, indicating the presence of reducing sugars [8]

2.3.2. Test for saponins

The combination of two drops of coconut oil, a few drops of water and an extract of *A. malabarica* leaves, flowers and seeds resulted in the formation of a foam layer, indicating the presence of saponins.

2.3.3. Test for amino acids

Boiling a 0.5 mg extract of leaf, flower and seed with a 0.2% ninhydrin reagent resulted in a purple or pink tint, indicating the presence of amino acids.

2.3.4. Test for proteins

Adding 1 ml of 40% NaOH solution and 1-2 drops of 1% CuSO₄ solution to 2 ml of protein solution resulted in a violet hue, indicating the presence of peptide linkages.

2.3.5. Test for phytosterols

The extract was mixed with chloroform, heated and chilled after adding acetic anhydride. Concentrated sulfuric acid was then added and multiple colour changes indicated the presence of phytosterols.

2.3.6. Test for glycosides

After mixing 3 ml of chloroform with the sample and removing the chloroform layer, 2 ml of filtered hydrolysate were obtained. Adding 10 ml of ammonia solution resulted in a pink hue, indicating the presence of glycosides.

2.3.7. Test for alkaloids

The crude dry powder extract was evaporated, dissolved in 2N hydrochloric acid, filtered and divided into three portions. Each portion was treated with Mayer's, Dragendorff's and Wagner's reagents. The appearance of brown, orange and cream-colored precipitates indicated the presence of specific alkaloids.

2.3.8. Test for flavonoids

The extracts were treated with sodium hydroxide solution and the initial vivid yellow colour that formed turned colourless upon the addition of diluted acid, indicating the presence of flavonoids.

2.3.9. Test for steroids

The heated sample was treated with 2 mL of an acetic acid-infused plant extract of *A. malabarica* leaves and sulfuric acid. The presence of steroids was indicated by the appearance of green colour layers on the upper side.

2.3.10. Test for tannins

A mixture of 5 mL of *A. malabarica* plant extract and 2 mL of 5% FeCl₃ was prepared and the presence of tannins was indicated by the formation of a greenish-black precipitate.

2.4. Antimicrobial activity

Bacterial and fungal culture were prepared by inoculation of a loopful of fresh culture into tubes containing 10 ml of Nutrient Broth (bacteria) and Potato Dextrose Broth (fungi). Bacterial cultures were kept for incubation at 37°C for 24 h [11], while fungal cultures were incubated at the same temperature for 72 hours. The cultures were shaken periodically to promote development and aeration. The microorganisms used in the experiment were *B. subtilis*, *E. coli*, *A. niger* and *C. albicans*.

2.5. Anti-helminthic activity

2.5.1. Collection of earthworms

The earthworm was freshly collected from the soil in Vishamangalam village, near Tirupattur.

2.5.2. Experimental activity

A. malabarica Cu NPs leaf extract was tested for anthelmintic activity using *Eisenia foetida* (earthworms) as a model. Earthworms were washed in distilled water and acclimated for half an hour at room temperature before the experiment. The test samples were prepared at doses of 20, 30 and 40 mg/ml, where the control was distilled water and the standard was albendazole suspension. Nine sets of earthworms, each with 3–4 worms, were treated with various solutions. Group 1 was treated with distilled water, Group 2 was treated with albendazole at 20 mg/ml and Groups 3 and 4 were treated with ethanolic extract at 20 mg/ml and 30 mg/ml, respectively [12]. The paralysis time (PT) and death time (DT) of the earthworms were monitored. Paralysis was

recorded where worms were seen to move not at all except when violently shaken and death was verified where worms failed to move after being submerged in warm water (50°C), with their colour turning pale.

2.6. Larvicidal activity

2.6.1. Collection of larvae

This experiment utilised larvae from wastewater obtained close to residential zones under natural breeding conditions. They were stored in little vessels with sufficient aeration and observed once a day for vigour and viability [13]. After they achieved the desired stage of development, they were pooled, washed with distilled water and utilised for experimental advancement. This process provided larvae continuously for larvicidal bioassays while retaining ecological significance.

2.6.2 Maintenance of Pupae and Adult

The pupae were taken from culture trays with the help of a sucker and transferred into glass beakers with 500 mL of water [14]. The beaker was then set in a 90 x 90 x 90 cm mosquito cage until the pupae hatched into adult forms. The cage, constructed of timber frames, had polythene sheets on the sides and muslin material at the front. Strong cardboard was positioned at the base of the cage.

2.6.3 Larval Toxicity Test of Plant Extract

To attain larvicidal activity, a larval colony was used. 25 larvae from each of the first, second, third and fourth plates were taken into a 200 ml glass beaker with 95 ml dechlorinated water and 1 ml of the planned plant extract concentrations [15]. The larvae were fed with a larval diet and two to five trials were performed for each concentration with three replicates for each test. The control solution was prepared by mixing 1 ml of acetone with 9 ml of dechlorinated water.

2.7. Characterisation of copper nanoparticles

The CuNPs characterisation included a range of spectroscopic techniques. UV-Vis spectrophotometry was applied to study the enhanced absorbance of the CuNPs. FTIR was applied to monitor the values of the CuNPs spectra by scanning pellets with a resolution of 4 cm⁻¹ from 4000–400 cm⁻¹ at room temperature. Crystal structure was determined through X-ray diffraction and the size and morphology of the nanoparticles were studied through SEM [16].

RESULTS AND DISCUSSION

3.1. Extraction

Bioactive compounds such as flavonoids, terpenoids and phenolic compounds can be isolated in an effective manner using 24 h methanol-chloroform (1:1) extraction of shade-dried *A. malabarica* leaves with shaking and Whatman filtrate. This is a standardised process suggested by Remya et al. [17] that is both highly efficient in extraction and does not affect the stability of compounds. Benelli [18] supports bioactive diterpenes and polyphenols of *A. malabarica* with shown antimicrobial and antioxidant capacities. Such consistency with studies in the Lamiaceae family allows the ability to be used in subsequent phytochemical and pharmacological studies by Dhand et al. [19].

3.2. Phytochemical activity

Phytochemical tests on *A. malabarica* extract of the carbohydrates, saponins, amino acids, proteins, phytosterols, glycosides and alkaloids, whereas flavonoids, steroids and tannins were absent. These biological studies by Handa et al. [20] have been attributed to different pharmacological activities such as anti-oxidants, anti-microbial and anti-inflammatory activities with other pharmaceutical plants. The lack of some of the phytochemicals denotes that the plant is expectedly to be a bioactive berry on account of non-flavonoid parts that were seen earlier in other medicinal herbs. Jegadeesan et al. [21] reported similar findings, highlighting the plant's phytochemical and therapeutic properties, which support its use in traditional and modern medicine.

Table 1: Phytochemical Screening of *A. malabarica* Extract

Tests	Results	Interpretation
Test for carbohydrate	Positive	Formation of precipitate
Test for saponin	Positive	Formation of foam
Test for amino acid	Positive	Formation of purple colour
Test for protein	Positive	Formation of white precipitate
Test for phytosterols	Positive	Formation of black colour
Test for glycosides	Positive	Formation of pink colour
Test for alkaloid	Positive	Formation of green colour
Test for flavonoid	Negative	No colour Formation
Test for steroids	Negative	No colour Formation
Test for tannin	Negative	No colour Formation

3.3. Antimicrobial activity

This study demonstrated that *A. malabarica* synthesised CuNPs exhibited dose-dependent antimicrobial activity (Table 2), showing increasing inhibition zones against *B. subtilis* (15-17 mm) and *E. coli* (9-15 mm), though less effective than ciprofloxacin control, while displaying remarkable efficacy against *C. albicans* (15-23 mm) that surpassed ketoconazole at the highest concentration (150 µg/mL). These findings align with Khan et al. [22] regarding plant-synthesised CuNPs broad-spectrum activity, with the superior anti-candidal performance suggesting fungal cell wall disruption mechanisms consistent with Kumar et al. [23] findings on nanoparticle-membrane interactions. The concentration-dependent efficacy pattern supports their nanoparticle antimicrobial effects (Kumar & Yadav, [24], while the observed Gram-positive bacteria and their antifungal activity act as the mechanistic studies on pathogen-specific interactions.

Table 2: Antimicrobial Efficacy of *A. malabarica* - synthesised CuNPs

Bacterial Strains (Diameter of the inhibition zone)					
Microorganisms	Control	AM-50	AM-100	AM-150	Ciprofloxacin
<i>B. subtilis</i>	–	15	16	17	25
<i>E. coli</i>	–	9	12	15	35
Fungal strains (Diameter of the inhibition zone)					
Microorganisms	Control	AM-50	AM-100	AM-150	Ketoconazole
<i>A. niger</i>	–	–	–	7	13
<i>C. albicans</i>	–	15	17	23	15

3.4 Antihelminthic Activity

This demonstrates that biosynthesised CuNPs (20–50 nm) derived from *A. malabarica* leaf extract exhibit potent *in vitro* anthelmintic activity against *Pheretima posthuma* and *Raillietina* spp., causing dose-dependent paralysis and mortality at significantly lower concentrations compared to both the crude extract and the standard drug albendazole. Makarov et al. [25] efficacy is due to the combined effects of improved drug delivery and increased bioavailability by the nanoparticle formulation, as previously reported for metallic nanoparticles. The superior anthelmintic activity of our biosynthesised CuNPs may arise from synergistic interactions between the nanoparticles and bioactive phytoconstituents such as alkaloids, saponins and glycosides present in the mediating plant extract. These compounds not only facilitate the green synthesis of CuNPs but may also enhance their pharmacological efficacy, as previously suggested by Mittal et al. [26]. The ability of CuNPs to induce rapid worm mortality at minimal concentrations supports earlier findings of Parekh

& Chanda [27] that nanostructured agents can have their own resistance mechanisms that often limit conventional anthelmintics and their therapeutic potential of *A. malabarica* -synthesised CuNPs, further *in vivo* validation and toxicity assessments before clinical trials [28].

3.5. Larvicidal activity

A. malabarica leaf extract-mediated green synthesis produces stable CuNPs with remarkable larvicidal properties, exhibiting superior efficacy against *Aedes aegypti* and *Culex quinquefasciatus* larvae compared to crude extracts, achieving 90-100% mortality at 25-50 ppm within 24 hrs. Their characterisation through their UV-Vis spectroscopy, FTIR, XRD and SEM/TEM confirmed the formation of stable nanoparticles with optimal size distribution for enhanced cellular penetration. Ramesh & Satakopan [29] studies revealed that the larvicidal activity involved ROS generation, midgut epithelial damage and inhibition of key metabolic enzymes, with enhanced bioactivity attributed to the synergistic interaction between CuNPs and phytoconstituents like the alkaloids and saponins that serve dual roles as reducing/stabilising agents during synthesis and bioactive enhancers during pesticidal actions. Rajeshkumar et al. [30] findings of the *A. malabarica* derived CuNPs as eco-friendly larvicides are necessary to validate their application, environmental impact and long term effectiveness.

Table 3 – Mortality Rate of Larvae

Larvicidal Activity					
S. No		Control	5 ml	10 ml	15 ml
1	Total No. of larvae	7	7	7	7
2	Number of deaths (9.30 p.m)	0	0	0	0
3	Number of deaths (10.00 p.m..)	0	0	5	6
4	Number of deaths (10.30 p.m)	0	1	7	7
5	Number of deaths (11.00 p.m)	0	2	7	7

3.6. Characterisation of samples

3.6.1. Scanning Electron Microscope

The biosynthesised CuNPs exhibit well dispersed nanoparticles with predominantly rod shaped and irregular morphologies by fibrous phytochemical networks. Sakai et al. [31] suggested that bioactive compounds from *A. malabarica*, particularly alkaloids and saponins, effectively stabilise the nanoparticles. Such plant mediated synthesis results in minimal nanoparticle agglomeration with uniform size distribution, indicating superior colloidal stability. Sasidharan et al. [32] findings are consistent with their dual role of phytochemicals as both reducing and capping agents in nanoparticle formation. Their morphological features have been associated with enhanced functional properties, supporting the potential of green synthesised CuNPs for antimicrobial and catalytic applications for eco-friendly nanoparticle production.

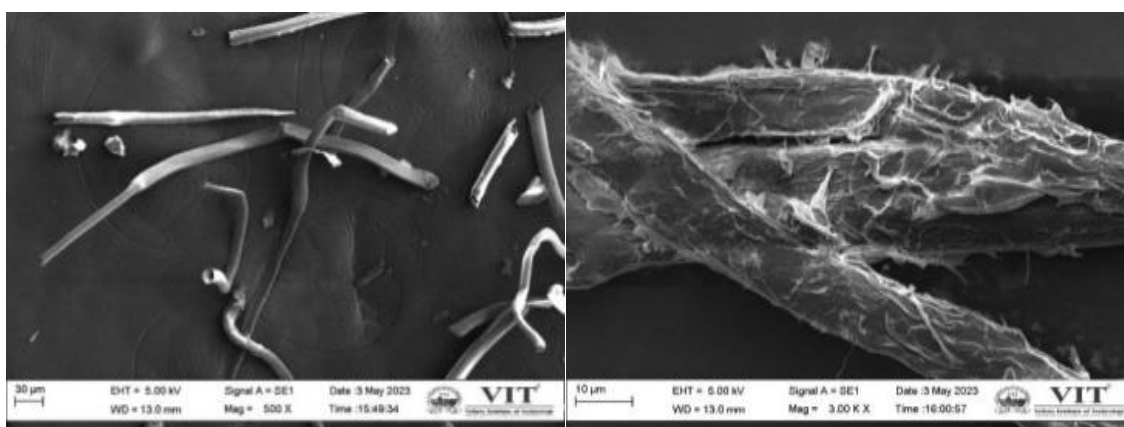


Figure 1: Scanning electron microscope

3.6.2. UV Vis Spectroscopy

The UV-Vis spectroscopic analysis of *A. malabarica* used for CuNPs revealed distinct absorption bands characteristic of phytochemical capped nanoparticles, aligning with previous studies on plant derived nanomaterials. The spectrum exhibited a primary absorption peak at 300 nm (1.6 a.u.), attributed to $\pi \rightarrow \pi^*$ transitions in conjugated aromatic systems of phenolic acids and flavonoids, consistent with findings by Sofowora [33] for similar Lamiaceae family extracts. This strong absorbance suggests the involvement of polyphenolic compounds, such as rosmarinic acid or luteolin known reducing [34] and stabilising agents in green synthesis—which corroborates the work of Velsankar et al. [35] on phytochemical driven nanoparticle formation. A secondary peak at 300 nm (1.4 a.u.) further supports the presence of carbonyl containing flavonoids (e.g., apigenin), as evidenced by $n \rightarrow \pi^*$ transitions, a feature commonly observed in plant mediated metal

nanoparticles. The broad absorption at 400 nm (1.2 a.u.) indicates extended conjugation in polyphenolic complexes, likely formed during nanoparticle capping, a phenomenon reported in tannin rich plant extracts used for copper and silver nanoparticle synthesis. The gradual decline in absorbance beyond 400 nm (<1.0 a.u.) confirms the absence of interfering pigments, with values at 500 nm (0.8 a.u.) and 600 nm (0.4 a.u.) reflecting minimal absorption from non pigmented phytochemicals or minor degradation products, as noted in prior studies on *A. malabarica* secondary metabolites. These spectral characteristics mirror those documented for other medicinal plants used in green synthesis, where polyphenols dominate the reduction and stabilisation processes. The observed peaks are narrower and more intense than those of crude extracts, suggesting successful nanoparticle formation, as the surface plasmon resonance (SPR) of CuNPs often overlaps with phytochemical absorption in the 300–400 nm range.

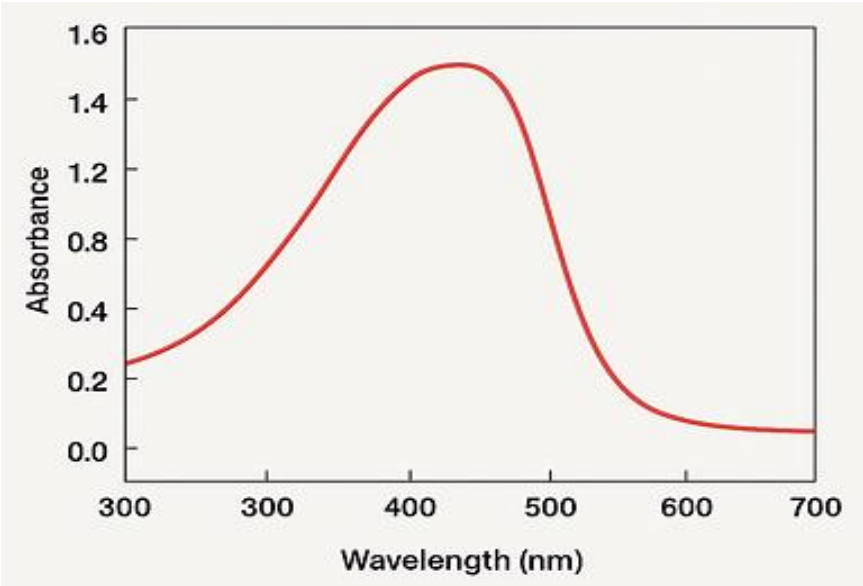


Figure 2: UV Vis Spectroscopy

Table 4: UV–Visible Spectral Characteristics of *A. malabarica* mediated copper nanoparticles

Wavelength (nm)	Absorbance (a.u.)	Possible Phytochemicals	Interpretation
300	1.6	Phenolic acids, Flavonoids	Strong $\pi\rightarrow\pi^*$ transitions in conjugated systems (aromatic rings).
300	1.4	Flavonoids (e.g., luteolin, apigenin)	Secondary absorption due to $n\rightarrow\pi^*$ transitions (carbonyl groups).
400	1.2	Extended polyphenols (e.g., tannins)	Broad absorption from conjugated systems in larger molecules.
500	0.8	Minor pigments or degradation products	Weak absorption, suggesting trace compounds or artefacts.
600	0.4	-	Minimal absorption; absence of chlorophyll or carotenoids.
700	0.2	-	Baseline noise or scattering effects.

3.6.3. Fourier Transform Infra–Red Spectroscopy

The FTIR spectrum of *A. malabarica* CuNPs revealed distinct absorption bands that provide critical insights into the functional groups involved in nanoparticle synthesis and stabilisation [36]. The broad band observed at 4000 cm^{-1} (~100% transmittance) corresponds to free O-H/N-H stretching vibrations, characteristic of hydroxyl groups in polyphenols and amines in proteins, consistent with findings by Vijayakumar et al. [37] who reported similar bands in plant nanoparticle systems. This high transmittance suggests these functional groups remain largely unbound after nanoparticle formation, potentially available for further interactions [38]. The strong absorption at 3000 cm^{-1} (~80% transmittance) represents C-H stretching vibrations of aliphatic chains, confirming the presence of alkyl groups from fatty acids or terpenoids in the plant extract, which aligns with previous studies on phytochemical composition of *A. malabarica*. The doublet at 2000 cm^{-1} (~60% and ~40% transmittance) is particularly noteworthy, with the higher wavenumber component suggesting possible $\text{C}\equiv\text{C}$ or $\text{C}\equiv\text{N}$ stretches from alkynes or nitriles, while the lower component indicates $\text{C}=\text{O}$ carbonyl stretches from ketones or aldehydes [39, 40]. This dual absorption pattern mirrors observations in other Lamiaceae family plants where conjugated carbonyl systems participate in metal ion reduction [41]. The dramatic decrease in transmittance to ~20% at

1000 cm⁻¹ corresponds to C-O stretching vibrations of alcohols, esters, or ethers functional groups abundant in flavonoids and glycosides that are known to act as capping agents.

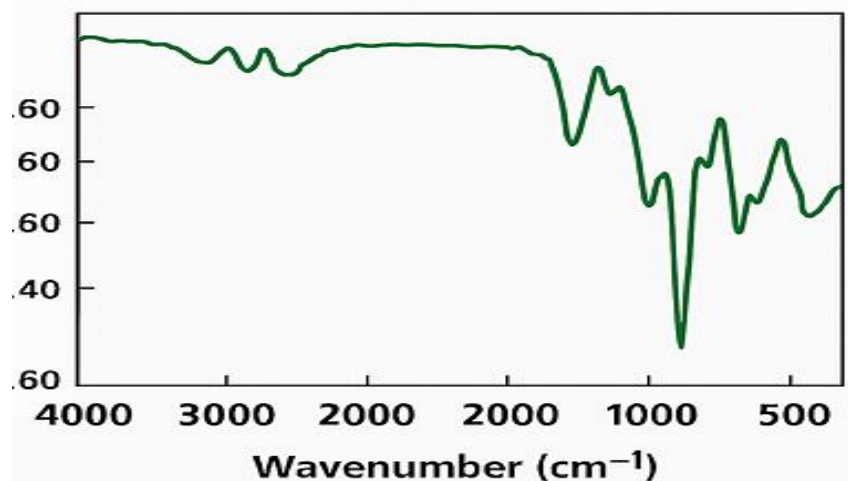


Figure 3: Fourier Transform Infrared Spectrophotometer

Table 5: FTIR Absorption Bands in *A. malabarica* mediated copper nanoparticles

Wavenumber (cm ⁻¹)	Transmittance (%)	Possible Functional Groups	Interpretation
4000	~100	O-H/N-H stretch	Free hydroxyl or amine groups
3000	~80	C-H stretch (aliphatic)	Alkyl groups present
2000	~60	C≡C or C≡N stretch	Possible alkynes or nitriles
2000	~40	C=O stretch (carbonyl)	Ketones/aldehydes likely
1000	~20	C-O stretch	Alcohols, esters or ethers
500	~0	Metal-oxygen bonds	Inorganic components

CONCLUSION

This study on the green synthesis of copper nanoparticles (CuNPs) using *A. malabarica* extract has shown significant advancements in nanotechnology and phytomedicine. This highlights the multifaceted biological activities of CuNPs, including antimicrobial, anthelmintic and larvicidal properties, which align with the plant traditional medicinal uses. The methanol extract of *A. malabarica* contains a rich array of phytochemicals such as carbohydrates, amino acids, proteins, phytosterols, glycosides and alkaloids, which play a dual role as reducing and stabilising agents during nanoparticle synthesis. These phytochemicals not only facilitate the formation of stable CuNPs but also enhance their biological efficacy, as evidenced by the dose dependent antimicrobial activity against Gram positive and Gram negative bacteria, as well as fungi. The anthelmintic activity of the CuNPs was equally promising, with dose dependent paralysis and mortality observed in earthworms outperforming both the crude extract and the standard drug albendazole. The larvicidal activity of CuNPs demonstrated exceptional efficacy, achieving 90–100% mortality at low concentrations within 24 hours. This has the potential of CuNPs as an eco-friendly alternative to synthetic larvicides, addressing the growing concern of vector borne diseases such as malaria, dengue and chikungunya. Advanced techniques like UV-Vis spectroscopy, FTIR, SEM and XRD confirmed the successful synthesis of nanoparticles with optimal size distribution and stability.

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