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Phytochemical profiling and antimicrobial evaluation of zinc oxide nanoparticles from the leaves of *Anisomeles malabarica* (L) R. Br (lamiaceae)

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Abstract The results of qualitative phytochemical analysis reflected the occurance of alkaloids, flavonoids, terpenoids, phenols and tannins, whereas the quantitative HPLC analysis showed the occurence of compounds such as luteolin, β-Thujone, carveol, p.coumaric acid, quercetin, β-pinene and myrtenol. UV-visible spectrophotometer analysis of green synthesized ZnO NPs showed the absorption peak maximum at 368 nm, FTIR analysis result showed the N=O stretch nitrogen groups, C=O stretch anhydrides, =C-N stretch alkenes. SEM images reflected a cluster of spherical evenly distributed ZnO NPs with the size of 50.4 nm in size and EDX pattern exhibited strong emission energy at 1.743keV. The antibacterial activity of ZnO NPs were tested against *E. coli, Enterobacter, Bacillus* resulted the maximum zone of growth inhibition on all the bacterial strains. The outcome of the present study revealed that *A. malabarica,* mediated ZnO nanoparticles is found to be more effective than crude extracts. Further research is needed to explore the molecular action, which will be benefited to the scientific communities as well as pharmaceutical industries for developing plant-based drugs in future.

Keywords: *Anisomeles malabarica,* Phytochemicals, ZnO nanoparticles, Antimicrobial properties

Introduction

Anisomeles malabarica (L) R. Br (Figure 1) is a medicinal, aromatic herb belongs to mint family or Lamiaceae, commonly called as "Malabar catmint" and "Aruvaachadachi" and “Peimirrati” in Tamil language (Rameshprabu *et al*., 2013). The Malabar catmint is an indigenous herb to Sri Lanka, tropical and subtropical regions of India. Because of its anti-diabetic, anti-allergic, anti-

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inflammatory, anti-cancer efficacy, catmint is considered as a medicinally significant herb (Bhuvaneshwari and Anandhan, 2024).

It helps to treat anorexia, fever, swellings, rheumatism, and amenorrhoea, anti-edemic, anti-inflammatory, anti-allergic, anti-nociceptive, antiplasmodial, antiseptic, and antiperotic qualities.

Numerous biological benefits, antiviral properties, which have been linked to flavonoids (kaempferol and luteolin) and the phenolic compounds (caffeic acid and chlorogenic acid and) (Choudhary and Swarnkar, 2011) (Tharani *et al*., 2025) of this plant.

In the present research, the aqueous leaves extracts of *A. malabarica* (containing various phyto constituents) were used to prepare and stabilize zinc oxide nanoparticles (ZnONPs) (Abomuti *et al*., 2021). In addition, the NPs synthesized from the plant extracts are more stable, scalable and nontoxic than the chemically derived materials. (Prabhahar *et al.,* 2022).

Figure 1. *Anisomeles malabarica* (L) R. Br

Bionano science is one of the essential fields in the molecular drug delivery process, because of its cost-effectiveness, green biosynthesis is employed for the fabrication of nanoparticles (NPs). Abomuti *et al.* (2021) specified that, when comparision with other metal nanoparticles, ZnO nanoparticles exhibits significant potential and advantages for both clinical and environmental applications. The major phytochemical composition of *A. malabarica* leaves is reported to contain flavonoid glycosides, alkaloids, phenols, tannins, terpenes/terpenoids, and carbohydrates (Vinod *et al*., 2014) which have been isolated from its aqueous extract. Similarly, the methanolic extracts of the *A. malabarica* contains higher amount of phenolic acids such as 3-

caffeoylquinic, caffeic acid and the pharmacological benefits were reported by Bhuvaneshwari and Anandhan (2024). More than 120 chemical components have been characterized from the aerial parts of *A. malabarica.* Flavonoids and phenolic complexes of *A. malabarica* reported as stimulating agents, which can adhere to the surface of nanoparticles when combined into aqueous leaf extracts (kumar *et al*., 2024).

Alrajhi *et al*., 2024 stated that the accurate synthesis technique with controlled parameters and careful characterizations of the plant mediated ZnONPs are essential for tailoring the molecular properties. Among the various phytochemicals, coumarins are the benzopyrone compounds belong to flavonoid groups of secondary metabolites. Todorov and Kostova (2024) reported coumarins having antiviral, antimicrobial, antioxidant, anti-inflammatory (Araniti *et al.,* 2015), antiadipogenic (Jia *et al*., 2019; Pisani *et al*., 2022) antiprolilferative (Waheed and Mustafa, 2022), antitubercular and cytotoxicity agent (Witaicenis *et al*., 2010), free radical scavenging capacity (Molnar *et al.,* 2017) and acetylcholinesterase inhibitors property which plays a significant role in Alzheimer patients (Akhondzadeh *et al.,* 2003). Due to these wide range of pharmacological importance, coumarins and its derivatives have more attention for synthesis and production (Govindhan *et al.,* 2015). The present research was focused to analyse the phytochemicals of *A. malabarica* leaves and tested their antimicrobial property using ZnO NPs synthesized from *A. malabarica* leaf extracts under *invitro* condition.

Materials and methods

The young and disease-free leaves of *A. malabarica* were collected from forest area in the month of May 2024 from Salem district and authenticated by the Botanical Survey of India, Coimbatore, Tamilnadu with the certificate number BSI/SRC/5/23/2024-25/Tech.533. It was allowed to dry at room temperature for a period of one week. After dried, the leaves were cut into pieces and ground to make powder by means of an electric grinder. Then, the resulting powdered leaf samples were kept in airtight containers at low temperature for future experimental purpose.

Preparation of crude extract and test its phytochemical nature

Totally 10g of powdered leaves were separately dissolved in 100ml of methanol and chloroform separately using airtight conical flasks. The mixtures were maintained on a mechanical orbital shaker for 24 hours at room temperature

to increase the yield. Similarly, the aqueous solution was filtered through muslin cloth, and the resulting filtrate was collected and stored.

Phytochemical analysis for A. malabarica extracts

The phytochemical nature of the methanol and chloroform extract of leaves of *A. malabarica* was done as per the modified procedure of Hanif *et al*. (2023).

Alkaloids assessment: (Hager's test)

About 0.25ml of picric acid was dissolved in 25ml of distilled water and taken 1ml from this solution and added to 1ml of plant extract. The occurrence of alkaloid was shown by the formation of yellow precipitate.

Terpenoids assessment: (H_2SO_4 Test)

A small amount of concentrated sulphuric acid (H_2SO_4) about 1ml was added to 1ml of plant extract, adding a few drops. The yellow color of the solution indicates terpenoids.

Phenols and tannins assessment: (Lead acetate test)

One milliliter of plant extract was mixed with a few drops of sodium hydroxide (NaOH) and heated to 50^0C for three to five minutes in water bath. Add a few drops of Folin's phenol reagent, or lead acetate solution. The appearance of tannins and phenols is indicated by the development of a black color.

Flavanoid assessment (Alkaline reagent test)

To two milliliters of plant extract, two to three drops of sodium hydroxide were added. A deep yellow color initially developed, but with the addition of a few drops of diluted HCL, it gradually turned colorless, signifying the presence of flavonoids.

Protein and amino acid assessment :(Ninhydrin test)

About 0.2 g of Ninhydrin was dissolved in 10ml of 70% ethanol. Take 1ml of this solution add 1ml of plant extract. The formation of violet color shows the presence of protein and amino acid.

Assessment of glycosides: (Keller killani test)

The concentrated glacial acidic acid (4.0ml) with 1drop of 2.0% ferric chloride ($Fecl_3$) was mixed with 2ml of test sample and 1ml of sulfuric acid ($H_2SO_{4)}$ was added. The solution contains the brown ring denotes the presence of glycosides.

Assessment of carbohydrates

Molisch's test

The test solution was mixed thoroughly after adding two to three drops of Molisch's reagent. A few drops of sulfuric acid were then added. When a purple ring forms, it indicates that there are carbohydrates present.

Benedict's test

One millilitre of plant extract was mixed with 2ml of Benedict's reagent and heated for 3-5 minutes. The development of red brick color specifies the presence of carbohydrate.

High performance liquid chromatography (HPLC) analysis of plant

The HPLC examination was used to evaluate *A. malabarica* methanolic aqueous extracts. It was a two-pump system that included a photodiode array detector (SPD-M20A), a CTO20A column oven, a CBM-20A communications bus module, an in-line degasser DGU-20A3, a SIL-20A auto sampler, and a CTO20A column oven. A Shim-pack CLC-ODS column measuring 25 cm by 4.6 mm was used for data processing and analysis using the LC solution software (version 1.25). Shimadzu, d, 5 μm). The gradient scouting run, which Snyder and Dolan, (1996) defined, was used to guide the first experiments for instrument optimization. An isocratic elution system comprising 90 percent acetonitrile in water and 0–1 percent acetic acid (v/v) at a flow rate of 1–0 ml was one of the optimized conditions following the last adjustments. minute-1. At 201 nm, the absorbance was measured while the column's temperature was maintained at 40°C (Fatma Ebru *et al.,* 2017).

Biosynthesis of ZnO NPs

The synthesis of ZnO NPs with *A. malabarica* were carried out using the technique created by Abomuti *et al.* (2021). 50 ml of distilled water and 10 grams of leaf powder were combined, and the mixture was heated to 60°C. Filter paper was utilized to filter the resultant solution. Zinc sulphate (ZnSO4) was prepared as an aqueous solution at 0.2 M to create zinc oxide

nanoparticles (ZnO NPs). The stabilization of the nanoparticles and the reduction of Zn+ ions to ZnO were made possible by adding *A. malabarica* to 50 ml of zinc sulphate solution and maintaining a pH of 12 with 0–1 M NaOH. In order to lower the possibility of zinc sulphate photoactivation, the mixture was left to stand overnight at 37°C in a dark state. The color of the solution changed from pale green to brown after the incubation period, signifying the formation of ZnO NPs and the reduction reaction. Following centrifugation, the samples were examined, and the results were obtained.

Characterization of ZnO NPs

UV-visible spectroscopy

A SL 159 UV visible spectrophotometer was used to record UV-Vis spectra of the sample, between the range of 200–700 nm at room temperature, the bioreduction of zinc ions to develop creation stability of ZnO NPs in an aqueous colloidal solution was periodically monitored. After completion of the synthesis of ZnO NPs, the reaction mixture was centrifuged at 8000 rpm for 10 min, and the nano pellet was dissolved in sterile distilled water for three times and centrifuged to remove impurities (Bissa *et al*., 2023).

Fourier transform infrared spectroscopy (FTIR)

The most popular tool for analyzing the types of chemical bonds (functional groups) in a solution of nanoparticles is the Fourier Transform Infrared Spectrophotometer (FTIR). The chemical bond is demonstrated by the FT-IR spectrum, which was defined by the wavelength of light absorbed. It is possible to identify the chemical bonds in a molecule by analyzing the infrared absorption spectrum. (Ashokkumar and Ramaswamy, 2014).

Scanning electron microscopy (SEM)

The SEM analysis of *A. malabarica* mediated NPs was done as per the modified technique of Kalaiarasi *et al*. (2015) by placing of a drop of the nanoparticle suspension onto a clean electric stub and allow the water to evaporate. SEM observations of ZnO NPs using VEGA3 TESCAN Electron microscope.

Antimicrobial effects

Inoculam preparation

The fresh inoculum of bacteria was prepared from 24 hours growth culture each time prior to perform the antimicrobial activity. The stock culture for bacterial activity are maintained at 4◦C on of Muller hinton broth, whereas

fungal cultures are retained in Potato Dextrose Broth (PDB), for experiments a loopful of culture mass are transferred from the stock. All the cultures were incubated for 24 h at 37±1.0^{o}C (Soundravalli and Kalaiarasi, 2025).

Antibacterial activity

The biosynthesized zinc oxide nanoparticles were evaluated for its antibacterial action by agar well diffusion technique against three-gram negative *Escherichia coli, Enterobacter* sp., *Klebsiella* sp*., Bacillus* and two-gram positive *Staphylococcus* sp. bacterial strains. Bacteria were grown in nutrient medium and incubated at 37^{0}C for 24 h in petridishes containing Muller-Hinton agar. An amount of 20µL of ZnO NPs, plant extracts, methanol and chloroform aqueous extract was poured into the wells and the diameter of the zone of growth inhibition was analyzed after 24 h by the technique of Ehsani *et al*. (2021) and Euch *et al.* (2019) and all the test samples were performed in triplicates.

Antifungal activity

Preparation of potato dextrose agar (PDA) media

The PDA medium was prepared using fresh potatoes washed and peeled outer skin, cut into small pieces added with distilled water and held in boiling water bath for 10 to 15 mins in boiling water bath. The boiled potatoes were smashed, filtered using muslin cloth and potato liquid were collected in a beaker added with 2g of dextrose and 2g of agar for the preparation of 150 ml of PDA medium.

Screening of antifungal activity

Two fungal cultures (*Aspergillus flavus and A. niger*) were used throughout investigation. The young fungal cultures were prepared before the screening procedure (Maliki *et al.,* 2021). The antifungal action of bio-synthesized against *Aspergillus flavus* and *A. niger* isolates was determined. The PDA medium plates were streaked with *Aspergillus flavus* and with *A. niger*. About 20 µL of ZnO NPs, antibiotics (chloramphenicol), methanol and chloroform aqueous extract was administrated into the wells and the diameter of the zone of growth inhibition was measured after 24 h (Ehsani *et al*., 2021).

Results

Preliminary phytochemical test

The initial phytochemical investigation from the methanolic aqueous extract of *A. malabarica* leaves showed the occurrence of alkaloids, glycosides,

terpenoids, flavonoids, phenols and tannins compounds compared to the chloroform aqueous extract (Table 1).

Table 1. Preliminary phytochemical analysis of *Anisomeles malabarica* (L) R. Br leaves extracts

Phytochemical Test	Methanolic extracts	Chloroform extracts
Alkaloid	+	+
Terpenoids	+	+
Phenols and Tannins	+	-
Flavanoids	+	-
Protein and Amino acid	-	-
Carbohydrate- Molisch's test	+	-
Carbohydrate- Benedict's test	+	-
Glycosides	+	+

(+) Presence (-) Absence

High performance liquid chromatography (HPLC) analysis

The HPLC examination result showed methanolic extract of *malabarica* leaves contains various bioactive compounds like as luteolin, β-Thujone, carveol, p.coumaric acid, quercetin, β-pinene and myrtenol. Based on their peak values (Figure 2).

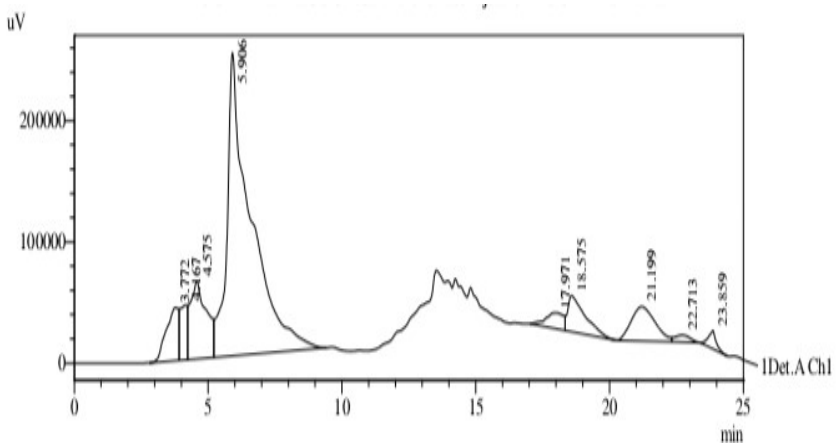


Figure 2. HPLC chromatogram of methanolic leaves extract of *A. malabarica*

Biosynthesis and characterization of NPs

The *A.malabarica* leaf extract was added to 0.2 M ($ZnSo_4$) aqueous solution, the color change was observed from green to brown colour led to the formation ZnO

NPs after overnight incubation in dark and it was further characterization using the following methods.

UV visible spectrophotometer

The methanol aqueous leaf extract of *A.malabarica* serves as a rich source of various biological components, particularly abundant in phytochemicals such as flavonoids and phenolic compounds. The flavonoid components facilitate the capping of Zn2+ ions, while the phenolic compounds form multiple chelating bonds, thereby stabilizing the ZnO nanoparticles following nucleation. The ZnO NPs synthesized using the extract of *A.malabarica* were analyzed using an SL 159 UV-visible spectrophotometer, revealing an absorption peak at 368 nm.

Scanning electron microscopy & EDX

The results of SEM analysis of sample authorized the creation of ZnO NPs in the reaction mixture. NPs started to precipitate while adding 0.2 M aqueous zinc sulphate solution to the plant extract at room temperature. Under a microscope, the surface appearance of NPs revealed these precipitates. VEGA3 TESCAN images of ZnO NPs generated from boiled leaf extracts are displayed in the images. The ZnO NPs shape and size were examined using SEM-EDX and SEM pictures at various magnification. A cluster of spheres, evenly distributed ZnO NPs with the average size of 50.4 nm in size was analysed (Figure 3) using image J software by utilizing SEM- EDX, the chemical profile of the synthesized ZnO NPs was assessed, the ZnO NPs EDX pattern exhibits strong emission energy at 1.743keV (Figure 4).

Fourier transform infrared spectroscopy (FTIR)

The FTIR results of the *A. malabarica* leaves nanoparticles revealed distinct absorption bands at the following wavelengths: 643 cm^{-1} for alkyl halides (C-Cl stretch) and alkyl halides (C-Br stretch); 852 cm^{-1} for primary and secondary amines (N-H Wag) and aromatics (C-H); 978 cm^{-1} for alkenes (=C-H bond); 1291 cm^{-1} for nitro compounds (N-O Symmetric stretch); 1636 cm-1 for primary amines (N-H bond); 2748 cm^{-1} for carboxylic acids (O-H stretch); 2845 cm^{-1} for carboxylic acids (O-H stretch); 3448 cm^{-1} for alcohol (O-H stretch); and phenols (H-Bonded) (Figure 5).

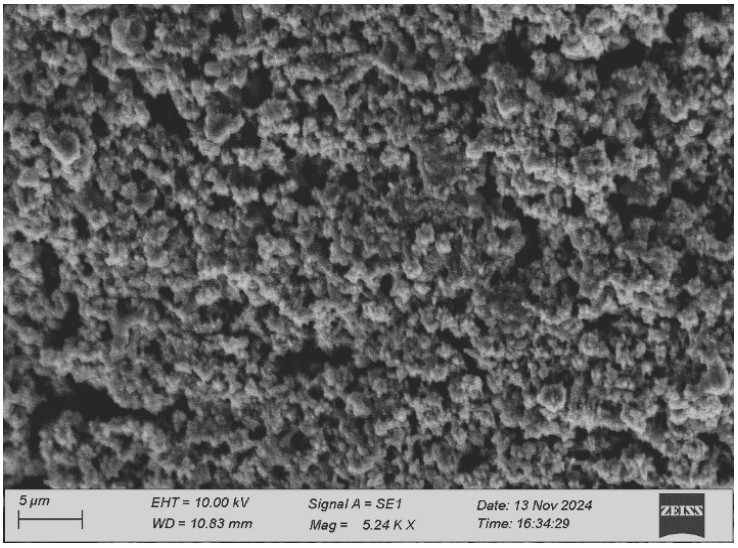
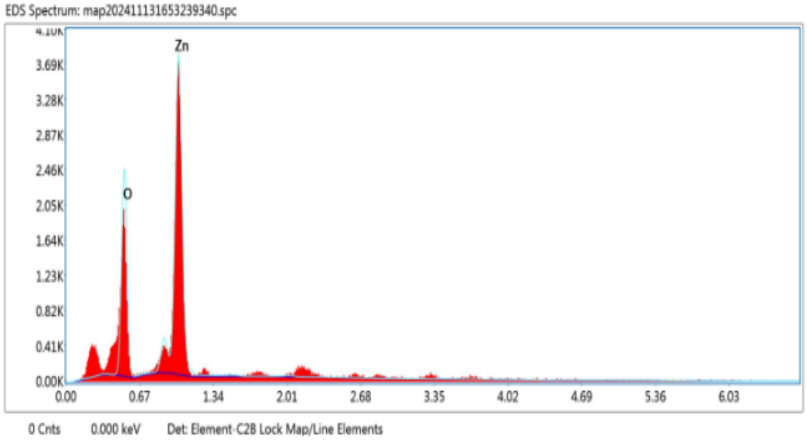


Figure 3. SEM image of green synthesized ZnO NPs using leaf extracts *A.malabarica*



Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	25.38	58.15	183.93	6.81	0.1938	1.2656	0.6035	1.0000
ZnL	74.62	41.85	249.34	3.28	0.6318	0.9111	0.9293	0.9999

Figure 4. EDX analysis of green synthesized ZnO NPs using leaf extracts *A. malabarica*

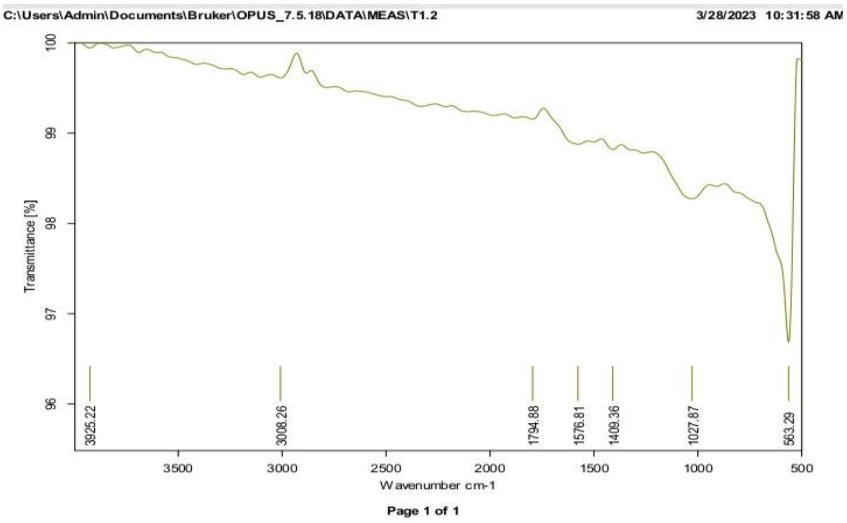
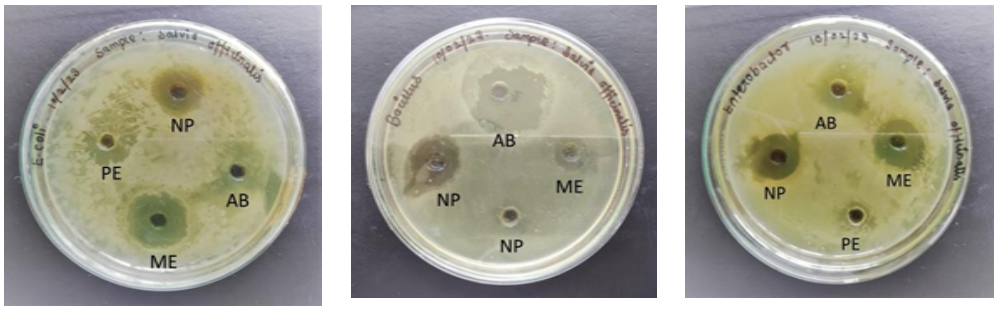


Figure 5. FTIR analysis of ZnO NPs of *A. malabarica* leaves

Antimicrobial activities

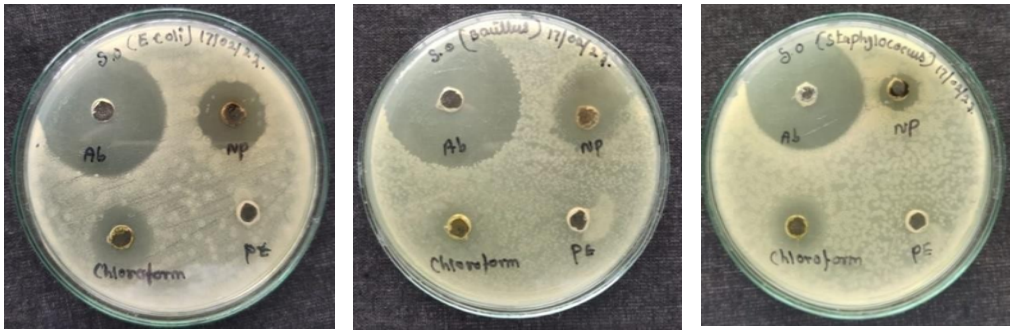
Antimicrobial activities of samples (methanolic aqueous extracts, leaves chloroform aqueous extracts and synthezised ZnO NPs) were performed by agar well diffusion method and the result showed broad spectrum of antibacterial activity against all the bacteria (Figure 6 and 7) namely *E. coli, Bacillus* sp., and *Enterobacter* sp. The ZnO NPs showed better antibacterial effect against *E. coli, Bacillus* sp., and *Enterobacter* sp., and there is no notable zone of growth of inhibition in the fungal cultures namely *Aspergillus flavus* and *A. niger* expect the standard antibiotics (Figure 8).



a) *Escherichia coli* b) *Bacillus subtilis* c) *Enterobacter sp.*

Figure 6. Antibacterial activities of the methanol leaf extract of *A. malabarica* leaves and synthesized ZnO NPs

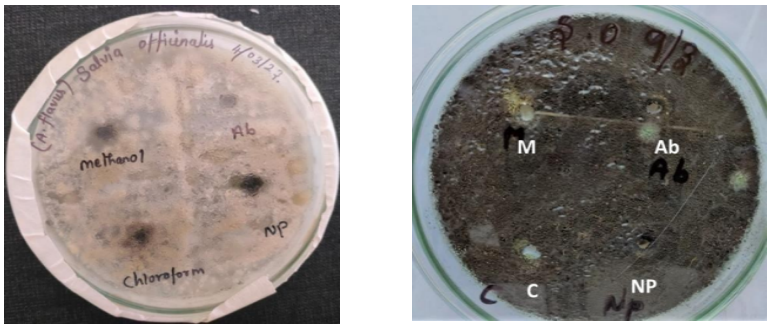
PE-20µl of Plant aqueous Extract, AB-20µl of Chloramphenicol, ME- 20µl of Methanol Extract, NP- 20µl of Nanoparticles



a) *Escherichia coli* b) *Bacillus subtilis* c) *Enterobacter sp.*

Figure 7. Antibacterial activities of chloroform extract and plant mediated ZnO NPs from *A. malabarica* leaves

PE-20µl of Plant Aqueous Extract, AB-20µl of Chloramphenicol, CE- 20µl of Chloroform Extract, NP-20µl of Nanoparticles



a) *Aspergillus flavus* b) *Aspergillus niger*

Figure 8. Antifungal effect of plant crude extract and plant mediated ZnO NPs of A. *malabarica* leaves

Discussion

The methanolic extracts of *A.malabarica* leaves have greater potency on antioxidant properties, due to the presence of bioactive compounds (Jeevanantham and Hussain, 2021). Whereas, Kaviyarasi *et al*. (2023) described that the presence of alkaloids, phenols, tannins and glycosides confirmed with ethanol leaves of this plant and these biomolecules exhibits their molecular potential against antibacterial, antiviral, anticancer and antineuralgic effects. The absence of alkaloids, flavanoids and tannins in the hexane aqueous leaves extracts of *A. malabarica* was reported by Supriya and Growther (2021). Several authors reported that the polar solvent (methanol) has the capability to extract the most of phytochemicals present in the sample. (Mokhtari *et al*., 2023,

Vijayarangan and Durai., 2024). Whereas the HPLC results denoted that the β-Pinene a major compound with the highest peak of a monoterpene molecule responsible for antibacterial, antidepressant, cytotoxic, antimicrobial property and also have the neuroprotective efficacy (Salehe *et al*., 2019). The second major peak was identified as p.coumaric acid a polyphenol, coumarin scaffolds have shown as an excellent potential for AChE inhibition and anti-Aβ aggregation potential (Bhatia *et al.,* 2021).

The compounds containing coumarin as the heterocyclic moiety exhibits strong significant AChE inhibitory activity. The coumarin ring is an vital for optimal activity, and replacement of the coumarin ring by relevant moieties such as chromones associated with loss of AChE inhibitory activity (Anand *et al.,* 2012) and also express various pharmacological activities (Patil *et al.,* 2013)., Previously, Nutan and Veena (2019) reported the HPTLC analysis of methanolic leaf extracts of *A. indica* shows the occurrence of low alkaloids and flavonoids content, whereas it is rich in phenolics like, gallic acid, vanillic acid, catechin, quercetin, ferulic acid and apigenin. Presence of luteolin is responsible for anticancer activity (Ganai *et al.,* 2021) with antiaging properties (Gendrisch *et al.,* 2021) and the flavonoid compound has a potent anti-diabetic property (Yi *et al*., 2023) and these kinds of flavonoid compounds can cause membrane distruption in the bacterial culture causing antibacterial effects (Devarajan *et al.,* 2015). β-Thujone is a terpenoid which exhibits antidiabetic and anti tumourogenic property (Lee *et al*., 2020), carveol is a monoterpenoid which have higher antioxidant property especially against hepatotoxicity boosting the antioxidant mechanisms (Rahman *et al*., 2021) and myrtenol is also a monoterpene with potent pharmacological properties including antimicrobial, anxiolytic, antiapoptotic and antinoceceptive effects (Rahizadeh *et al*., 2020), whereas quercetin is a flavonoid reported to have better anticancer, anti-inflammatory and antioxidant property (Javanbakht *et al.,* 2023).

The produced ZnO nanoparticles showed UV spectrophotometer absorption peak targeted at 350 nm is typical for ZnONPs substantial excitation binding strength at optimum temperature, whereas the majority of zinc oxide molecules absorbs till 385 nm has been reported by Nayagam *et al.* (2018) and Shamhari *et al.* (2018) stated that the absorption peak of ZnONPs at 357 nm denotes the intrinsic band gap of zinc and oxygen. The extreme shift absorption of ZnONPs is due to an excessive reduction in particle size (Sekar *et al.,* 2022). SEM- EDX, the chemical profile of the synthesized ZnO NPs peaks below 8.020 keV indicates the existence of a pure zinc ion. The pattern also shows peaks that coincide with the binding energies of carbon and oxygen, which are likely caused by impurities that entered into the nanoparticles during drying. Sekar *et al.* (2022) reported that the ZnONPs are spherical shapes accumulated like grouped

to form flower-shaped bundles. The electrostatic appeal and polarity cause agglomerations of these ZnONPs. However, ZnO-NPs have the ability to involve in bacterial cell wall integrity by direct connection. The NPs structure and appearance are found to be more potent in antimicrobial activity generating reactive oxygen species (Zhou *et al*., 2023).

Synthesized ZnO NPs' FTIR spectra displayed multiple peaks and varying intensities, indicating that the main biological molecules from the plant extract were adhered to the ZnO NP surface. Because of the donor-acceptor mechanism in which the phenolic and flavonoid molecules react with Zn2+ ions, ZnO NPs may be formed using the *A.malabarica* extracts. As the hydroxyl or oxygen molecules in the plant extract tend to ZnO NPs are created. The bactericidal activity denotes the redox chain reaction with the making of reactive oxygen species (ROS) formed by hydroxyl radical and superoxide radical anion of ZnO NPs. The excitation of ions can destabilize the cytoplasmic membrane resulting in their rupture of the cell (Abebe *et al.,* 2020). The outcome of this study express the synthesized nanoparticles showed the maximum inhibitory effect against the microorganisms tested than other extracts. The bactericidal activity is due to the electrostatic interations between the ZnO NPs and the cellular surfaces with peptidoglycan layers creating membrane gradients leading to cell death (Santos *et al*., 2022). Whereas the fungal cultures showed resistance to growth of zone of inhibition, this is being due to the resistance to antimicrobial phytochemicals presence in the plant (Mishra *et al*., 2011), further study has to be carried out with increasing the concentration of phytochemicals along with the ZnO NPs in future to evaluate its antifungal potential.

In this research, *A. malabarica* leaves encompassing various phytochemicals used to prepare and stabilize ZnO NPs. The preliminary phytochemical analysis, result showed methanol and chloroform extracts of *A. malabarica* confirm the presence of alkaloids, terpenoids and glycosides from the leaves extract. However, when compared to chloroform leaves extracts, *A. malabarica* methanolic leaves showed the higher metabolic profile having phenols and tannin compounds which are responsible for various aliments against microbial infections. High Performance Liquid Chromatography (HPLC) of methanol aqueous extract resulted the presence of luteolin, β-thujone, carveol, p.coumaric acid, quercetin and myrtenol. The characterization of biosynthesized ZnO NPs were performed using several techniques, including UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and the energy-dispersive X-ray (EDX) spectra confirmed the presence of zinc and oxygen as primary elements. The ZnONPs with their spherical shaped flower like bundles with minimum nm size with respective lipophilic aromatic and alkyl halides functional groups. The

antibacterial effect of the nanoparticles were assessed through the agar diffusion method against five different bacteria's and the result showed better antibacterial effect against *E. coli* and *Enterobacter sp*. Chloroform extracts exhibited minimum inhibitory efficacy against both *E. coli* and *Klebsiella sp.*, whereas, the nanoparticles displayed a better zone of growth inhibition on tested bacteria compared to other two solvents denotes the surface area binding with the microbes and it showed the metal chelating ability to fight against human pathogenic microorganisms.

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Conflicts of interests

The authors declare no conflict of interest.

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