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ORIGINAL ARTICLE

Phytochemical Analysis and Pharmaceutical Description of *Anisomeles malabarica* (L) Leaves: Phenolic Compounds Driving Bioactivity

V Nanditha[1], **D Muthuselvam**[1]*

1 Department of Botany, Bishop Heber College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli-620017, Tamil Nadu, India

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* **Corresponding author**.

muthuselvam.by@bhc.edu.in

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Abstract

Objectives: A perennial herb used for many years in traditional medicine, *A. malabarica* is the subject of this study, which compares the relative safety and economic viability of many naturally occurring Phenol compounds combined for therapeutic purposes. **Methods:** The current study utilises GC-MS, FTIR, and UV-vis spectroscopy to evaluate the antioxidant and antimicrobial properties of phenolic compounds from *A. malabarica* leaf extracts. Using the latter crude extract, AMLE was extracted at an acidic pH in the presence and separation of phenolic components using ethanol as the solvent. Antioxidants were studied using H_2O_2, DPPH, and ABTS. An array of bacterial and fungal species was used to assess the antimicrobial capabilities using the agar well diffusion method. The purpose of characterisation was to determine the presence of functional groups and bioactive compounds. **Findings:** The Phenol leaf extract from *A. malabarica* exhibited dose-dependent antioxidant properties during this study, with IC_{50} values of 110.2 μg/mL for DPPH, 93.01 μg/mL for ABTS+, and 143.4 μg/mL for H_2O_2. HRBC membrane stability by 81.43%. A maximum of 15.5$\pm$0.7 mm of antibacterial activity and 8.4$\pm$0.56 mm of antifungal activity was found against *P. acnes* and *A. niger*. Twenty phenol extracts were detected by GC-MS analysis. The functional groups, amine salt, alkene, sulfonyl chloride, aromatic ester, and cycloalkane, were represented by the FTIR peak values. The best protection against *A. aegypti* and *A. stephensi* for 180 minutes at a dosage of 500 ppm of 100% dead larvae, 40%-90%, followed by 150 and 120 min. **Novelty:** The Phenol components of the leaf extract of *A. malabarica* possess antibacterial and anti-inflammatory qualities, making them possible targets for various diseases.

Keywords: Antimicrobial; *Anisomeles malabarica*; aromatic ester; hepatoprotective; Phenol

1 INTRODUCTION

Anisomeles malabarica has a long history as an important aspect of Tamil religious rites in Tamil Nadu, India, and this is the first to provide insight into how its right application enhances mental health and personal attainment of success. *A. malabarica* is used medicinally across the world, including Asia, the Maltese Islands, and other Mediterranean countries. This has added a challenge to extraction operations, implying that employing a single step or an effective extraction strategy may alter the extraction of phenolic components from plant crude samples.[(1)]. It belongs to the Lamiaceae, or mint family,[(2)] also known as 'Malabar catmint' and 'Peyameratti' in local Tamil[(3)], which is important in medicine and recognised for its pleasant scents. The plant's leaves, flowers, stems, and essential oils include a variety of key phytochemical moieties and biological activities, including antimicrobial, antioxidant, and antimalarial effects.[(4)], *A. malabarica* leaf infusion is used in traditional medicine to treat fever, epilepsy, and teething in children. Fresh and dried *A. malabarica* leaves are used as wicks, while rolled leaves are steeped in holy oils before being burned in lamps. The odour released by *A. malabarica* leaves is supposed to encourage excellent thinking, improve pharmaceuticals, induce harmonious senses, and decrease criticism. Volatile substances may interfere with brain signals.[(5)] Traditional medicine has long employed a wide range of therapeutic and aromatic plants. Strong-smelling essential oils, tannins, saponins, and organic acids are common medicinal compounds found in plants. Traditional medicinal medications include herbal teas, liquids, ointments, injections, and powders.[(6)]

Effective and aromatic plants are in high demand in the market due to their high therapeutic value and pharmacological activity, requiring more effective mechanical and medical treatments. They are utilised as medicinal herbs in Indian and Sri Lankan traditional medicine. They are used to manufacture medications, perfumes, and cosmetics. They are also used to treat a wide range of conditions, including congenital mental abnormalities, teething fevers, and oedema.[(7)] Furthermore, observational grounds should be integrated into arguments that support the use of herbs in traditional medicine. Herbal remedies were related to the separation, purification, and identification of biological molecules from plant materials. Identifying the structures of active compounds from extracts proved troubling, depending on the complexity of the content.[(8)]

A variety of studies have demonstrated the efficacy of bioactive ingredients derived from *A. malabarica*, highlighting its potential for current medical uses. Considering the traditional therapeutic and medical importance of these plants, there is an urgent need to examine their phytochemical profiles, functional group identification using FTIR[(9)], antioxidant, and antimicrobial activities[(10)],[(11)]. anti-inflammatory effects of plant extracts[(12)]. *A. malabarica* leaves contain a variety of bioactive chemicals, including flavonoids and phenolic acids, which have strong antioxidant capabilities and help to protect cells from oxidative damage, reducing the risk of chronic illnesses, including tumour and coronary artery disease[(13)]. A recent review aims to provide information on the bioactive substances, chemical profiles, biological activities, pharmacological methods, and anticipated therapeutic applications of *A. malabarica* leaves, integrating traditional use with scientific inquiry to bridge the gap between ethnomedicine and evidence-based research.[(14)],[(15)].

The current research additionally utilises GC-MS analysis to detect phytoconstituents in therapeutic plants, with an emphasis on phenolic components and the UV-vis and FTIR spectrum profiles of *A. malabarica* for a variety of ailments and disorders, as well as the anti-inflammatory and antioxidant activity, a medicinal plant with antimicrobial, larvicidal activities, and pharmacology, will be very appealing as a therapy for *A. malabarica* to develop effective medicines.

2 METHODOLOGY

2.1 Collection of Plant Materials and Authentication

Healthy, young leaves, and a fresh harvest of *Anisomeles malabarica* (L) R. Br. ex-Sims were sourced from the dry, rocky Peramangalam Musiri area of Tiruchirappalli District, Tamil Nadu, India. (11.0111108°N, 78.659052°E).[(16)]. The identity of the plant was authenticated by the Rapinat Herbarium at St. Joseph College in Tiruchirappalli, and the verified plant material is deposited as Voucher No. V.N. 001.

2.2 Extraction of Phenolic Compounds

Twenty grams of ground leaves were taken in the Soxhlet apparatus, and 250 mL of ethanol was added. After this time, it is filtered and evaporated to obtain a crude extract performed using the presence and separation of Phenol compounds as a solvent at acidic pH. At first, the Crude Extract was taken, then 10% of HCl was added after 1 hr. Extraction of polyphenols was performed with 10 mL of ethyl acetate [Figure 1]. The Extraction was repeated in triplicate. Finally, the ethyl acetate was evaporated to dryness in a rotary evaporator, and the residue was stored at -20°C for subsequent analyses.[(17)],[(18)]. The organic solvent (ethyl acetate) was evaporated, and the Phenolic compounds were dried and collected.

2.3 Characterisation of *A. malabarica* leaf extracts on phenolic compounds

2.3.1. Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis was performed on the various fractions generated from the phenolic compounds and A. malabarica leaf extract using the SHIMADZU GC-MS QP-2020 system. This system uses a polar capillary Dd 30.0 column (0.25 m diameter, 0.25 m thickness; 30 m < 0.25 mm). After 0 minutes of isothermal conditions at 50°C, the oven reached 280°C in 2 minutes at a rate of 6°C per minute. Helium was utilised as a carrier gas. The injection volume in split mode was 0.1μL. The temperature differential between the source and inlet lines is 200 degrees Celsius. The electron impact ionisation energy in mass spectra spanning 450 amu was 70 eV. The overall running time of a sample was 40 minutes. Solvent delay: 1–2 minutes.

2.3.2. UV- VIS spectroscopic analysis

The phenolic compounds of *A. malabarica* leaf extracts were evaluated using a UV-visible spectrophotometer (Perkin Elmer, USA, Model: UV-2600 Series, 5.0 nm slit width). For a proximate test of the extract, visible and UV light with wavelengths ranging from 220-800 nm were utilised. After centrifuging at 3000 rpm for 10 minutes, filter it through Whatman No. 1 filter paper and dilute it 1:10 with the same solvent.

2.3.3. FTIR analysis

The phenolic compounds in the *A. malabarica* leaves extract were analysed using the potassium bromide (KBr) pellet (FTIR grade) method. A Jasco FTIR-6300 Fourier transform infrared spectrometer with a transmittance mode was used to record the spectra at a 1 cm resolution.

2.4 Anti-inflammatory activity

The antiinflammatory properties of phenolic compounds in *A. malabarica* leaf extracts were assessed using the HRBC membrane stabilisation technique[19],[20]. Before centrifugation with isosmotic saline solution, an equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride, and 100 ml distilled water) was added to the blood. 1 mL of 0.7% distilled water and 1 mL of 10% HRBC solution were mixed. Diclofenac sodium served as both a standard and a positive control. The test compounds were added in equal amounts at five concentrations (500, 250, 100, 50, and 10 μg/mL). The phenol extract was mixed with 1.0 mL of 10% RBCs. After heating to 56°C for 30 minutes, the solution was centrifuged at 2500 rpm for 10 minutes before being cooled to room temperature. Each test combination was centrifuged at 37 degrees Celsius for 30 minutes. A spectrophotometer calibrated to 560 nm was used to measure the amount of haemoglobin in the supernatant solution.[21].

$$\textbf{Per cent of protection} = \textbf{100} - \textbf{Percentage of Hemolysis}$$

2.5 Antioxidant activity

2.5.1. DPPH radical scavenging assay

A few minor adjustments were made to the approach for the DPPH radical scavenging experiment[22]. The test sample was prepared at various concentrations (500, 250, 100, 50, and 10 μg/mL), and a 0.1 mM DPPH solution was prepared in methanol. After adding 100μL of DPPH solution to the test specimens, they were incubated at room temperature for 30 min. During the incubation time, the absorbance of the test samples at 517 nm was measured using a UV-VIS spectrophotometer. The percentage of inhibition was calculated using the formula.

$$\textbf{Inhibition } \% = \textbf{ Absorbance of Control} - \textbf{Absorbance of Sample } \backslash \textbf{ Absorbance of Control } \times \textbf{100}$$

One of the controls was ascorbic acid. A non-linear regression technique was employed to estimate the IC_{50} values, utilising the per cent inhibition vs. concentration plot as a basis. The percentage of inhibition was computed using the standard graph equation.

2.5.2. ABTS radical cation scavenging assay

The ABTS assay was carried out using the approach, with a few minor adjustments. The stock ABTS solution was prepared by combining 7 milligrams of ABTS solution with 2 milligrams of potassium persulfate solution. A workable solution was created by combining 1 millilitre of stock ABTS with 60 millilitres of methanol. Test samples (500, 250, 100, 50, and 10 μg/mL) were

treated with 1 mL of ABTS working solution. The absorbance was measured at 734 nm after 7 minutes of incubation. The percentage of inhibition was calculated using the formula shown below.

$$\textbf{Inhibition}\% = \textbf{Absorbance of Control Absorbance of Sample} \setminus \textbf{Absorbance of Control} \times \textbf{100}$$

2.5.3. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay was carried out using the approach described with minor changes [23]. A 43 mM hydrogen peroxide solution is created using phosphate buffer. Incubate test samples (500, 250, 100, 50, and 10 μg/ml) in 0.6 ml of 43 mM hydrogen peroxide for 10 minutes. Measure absorbance at 230 nm. The formula for determining the percentage of inhibition

$$\textbf{Inhibition}\% = \textbf{Absorbance of Control} - \textbf{Absorbance of Sample} \setminus \textbf{Absorbance of Control} \times \textbf{100}$$

2.6 Antimicrobial activity

The eight bacterial species, four Gram-positive and four Gram-negative, were initially examined for antibacterial activity by the phenol leaf extract of *A. malabarica*. (*Staphylococcus aureus- 902, Streptococcus pyogenes-1928, Propionibacterium acnes-1951, Corynebacterium diphtheria, Pseudomonas aeruginosa- 424, Proteus vulgaris-426, Aeromonas hydrophilla, and Klebsiella pneumoniae)* and fungus-fighting ability against the four fungus species (*Candida albicans, Aspergillus niger, Sporothrix schenckii and Phialophora verrucosa*) from MTCC in Chandigarh, India, was bought. The antimicrobial capacity of phenolic compounds in *A. malabarica* leaf extract was tested at different concentrations (50, 100, 200, 300, 400, and 500 μg/ml). Employing the agar well diffusion method. Bacteria were cultivated in Petri plates for 24 hours in 20 millilitres of nutrient agar medium with bacterial strain cultures, and the OD value was set to 0.5 using the McFarland standard. Following that, the plates were incubated for another 24 hours at 37°C. Gentamicin and Amphotericin B provided positive controls. After that, the plates were incubated for another 72 hours at 28 degrees Celsius. The diameters of the inhibition zones for leaf extracts against various bacteria and fungi were measured in mm.

2.7 Larvicidal activity

To assess the larvicidal activity range of phenolic compounds in *A. malabarica* leaf extracts, mosquito larvae were first exposed to a range of test concentrations (ppm) and a dis.H_2O control solution. After evaluating the death rate of larvae at various concentrations, the 50% lethal concentration (LC_{50}) values were calculated using a limited range of 100, 200, 300, 400, and 500 ppm. The number of dead larvae was counted after being exposed to each solution for 30 min, 60 min, 90 min, 120 min, 150 min, and 180 min. As a control, the larvae were exposed to 0.1 ml of ethanol, and the 20 ml plastic bottles were stored in a temperature-controlled environment at 28°C $\pm$ 2. The control group was given dechlorinated water. The aqueous medium (10 ml of dechlorinated water + 1 ml of plant extract) and the appropriate amount of plant extract were added to 20 ml plastic bottles containing 10 ml of the mosquito species. Each test concentration was replicated. The number of dead larvae was counted after 24 hours of exposure, and the percentage of mortality was computed as the average of five repeats.

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

2.8 Statistical Analysis

The values were presented as means $\pm$ SD of determinations. For unpaired comparisons, the data were statistically analysed using Student's test. Significance was determined at $p < 0.05$. These figures were calculated using the GraphPad Prism 6.0 application (USA). The average larval mortality data were subjected to probit analysis to calculate the LC_{50} and other statistics at 45% fiducial limits, and chi-square values were calculated using the Excel software. P-values < 0.05 indicated statistical significance.

3 RESULTS AND DISCUSSION

3.1 Identification of Phenol compounds from Crude drug extract

According to these observations, the extraction of phenolic compounds from *A. malabarica* results in a crude extract using the solvent. [Figure 1(A)] illustrates a crude extract of phenolic chemicals produced from *A. malabarica*. The addition of $FeCl_3$ the crude extract induces a colour transformation from dark green and black to greenish-black, indicating the presence of phenol chemicals in *A. malabarica*. Figure 1(B) displays the results of identifying phenolic chemicals using $FeCl_3$.

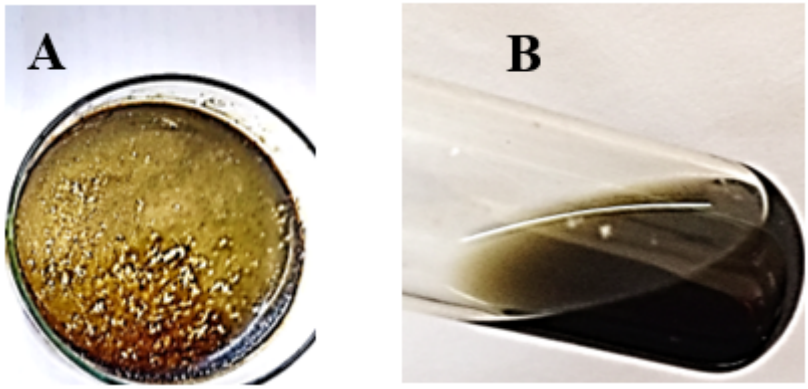
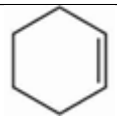
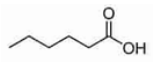
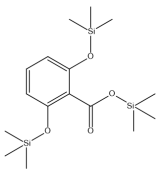


Fig 1. Extraction of Phenolic compounds from *A. malabarica* leaves (A) and Positive $FeCl_3$ test (B).

3.2 GC-MS analysis

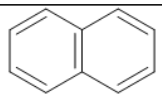
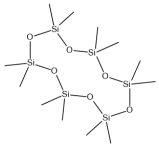
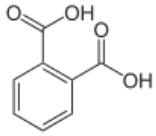
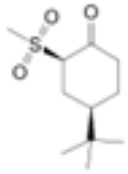
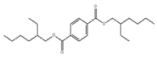
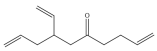
A GC-MS study of the phenolic compounds from *A. malabarica* leaves revealed the presence of twenty phenol components. Four unique phytochemical components dominated by four peaks in [Table 1]. The GC-MS analysis revealed that the phenolic compounds contained naphthalene (48.66%), 1,2-benzenedicarboxylic acid (12.23%), 1,4-benzenedicarboxylic acid bis(2-ethylhexyl) ester (10.19%), cyclohexasiloxane, and dodecamethyl (3.18%).

Table 1. GC-MS analysis of Phenolic compounds in *A. malabarica* leaves

S. No	Mass Peak	Ret. Time	Ret. Index	SI	Area%	compound name	Molecular Formula	Molecular Weight	Molecular Structure	Biological activity
1.	2	6.245	0	98	1.3	cyclohexene	$C_6H_{10}S$	114		Precursors to produce adipic acid and caprolactam.
2.	9	6.855	0	90	2.85	hexanoic acid, ethyl ester	$C_8H_{16}O_2$	114		Antimicrobial activity.
3.	3	10.025	0	84	1.39	benzoic acid, 2,6-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370		Fungal infection of the skin.

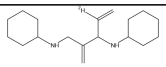
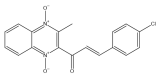
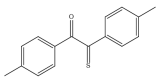
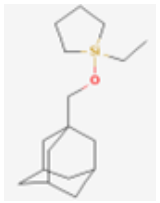
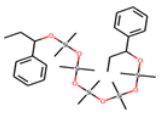
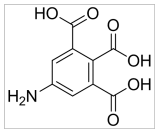
Continued on next page

Table 1 continued

4.	17	11.345	0	97	48.66	naphthalene	$C_{10}H_8$	128	 Acute effect- cataracts, hemolytic anticar- cino- genic effects of moth- balls.
5.	7	13.955	1240	82	3.18	cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	 Emollient, hair condi- tioning agent.
6.	14	34.935	0	92	12.23	1,2- benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	 Anti- inflammatory, antibac- terial activity.
7.	16	37.290	0	63	1.52	cis-4-tert-2- (methylsulfonyl)cyclohexanone	$C_{11}H_{20}O_3S$	232	 antimicrobial poten- tial
8.	26	37.555	2704	87	10.19	1,4- benzenedicarboxylic acid, bis(2- ethylhexyl) ester	$C_{24}H_{38}O_4$	390	 antifungal activity
9.	16	38.195	0	58	0.91	3-allyl-1,8- nonadiene-5- one	$C_{12}H_{18}O$	178	 Flammable hydro- carbon fuel

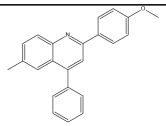
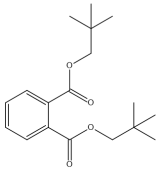
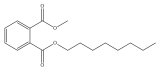
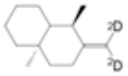
Continued on next page

Table 1 continued

10.	16	38.435	0	64	1.66	1,5-dicyclohexyl-2-(1-deuterioethenyl)-3-methylene-1,5-diazapentane	$C_{18}H_{31}DN_2$	277	 -
11.	16	38.565	0	62	2.12	2-methyl-3-(p-chlorocinnamoyl)quinoxaline-1,4-dioxide	$C_{18}H_{13}C_1N_2O_3$	340	 Sterilisation and growth-promoting of animals.
12.	17	38.660	0	56	0.77	ethanone, bis(4-methylphenyl)thioxo-	$C_{16}H_{14}OS$	254	 Acetic anhydride from acetic acid.
13.	24	39.280	1586	55	0.56	1-(1-adamantylmethyloxy)-1-ethyl-1-silacylopentane	$C_{17}H_{30}OSi$	278	 Analgesics.
14.	22	39.430	2835	53	2.2	1,11-di(1-phenylpropyl)-2,2,4,4,6,6,8,8,10,10-decamethyl-1,3,5,7,9,11-hexaoxa-2	$C_{28}H_{52}O_6Si_5$	624	 -
15.	19	39.500	0	57	0.83	1,1-dimethoxy octadecane	$C_{20}H_{42}O_2$	314	 Anti-corrosion agent.
16.	15	39.655	0	60	1.91	5-amino-1,2,3-benzenetricarboxylic acid.	$C_9H_7NO_6$	255	 Intermediate for resins and dyes.

Continued on next page

Table 1 continued

17.	20	39.735	2901	54	2.29	6-methyl-2-(4-methoxyphenyl)-4-phenyl-quinoline	$C_{23}H_{19}NO$	325		Pharmacological effects
18.	25	39.830	2066	55	2.75	dineopentyl phthalate	$C_{18}H_{26}O_4$	306		Epidemiology, metabolic effects
19.	23	40.130	2136	57	1.67	methyl octyl phthalate	$C_{17}H24O_4$	292		pvc plasti-cizers
20.	19	40.280	0	52	1.01	naphthalene, decahydro-1,4a-dimethyl-2-(methylene-d2)-	$C_{13}H_{20}D_2$	180		Anti-inflammatory activity

3.3 UV-VIS analysis

A sufficient baseline and strong peaks enabled the collection of the UV-VIS profile of the phenolic compounds of *A. malabarica* leaves at wavelengths ranging from 220 to 800 nm (Figure 2). Peaks were seen in the profile at 229, 274, 328, and 640 nm, with corresponding absorption values of 2.091, 2.161, 0.932, and 0.055.

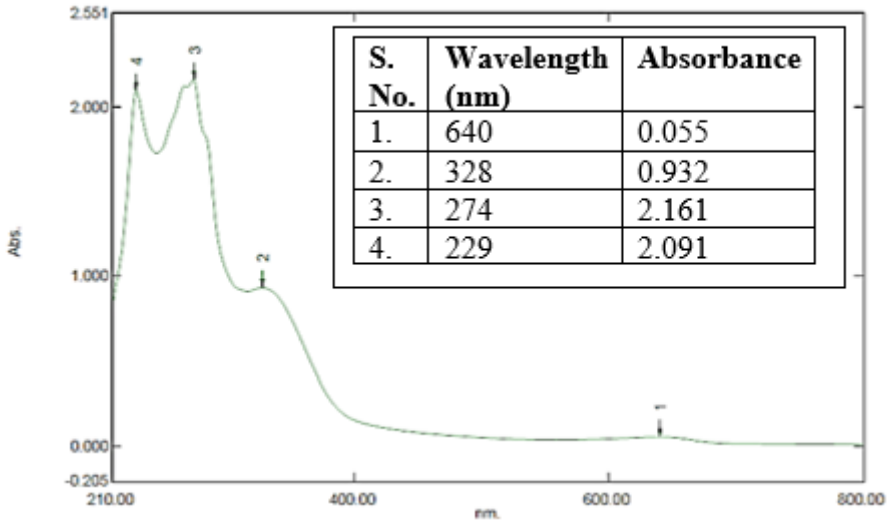


Fig 2. UV-visible spectrum of phenolic compounds in *A. malabarica* leaf extracts.

3.4 FTIR analysis

The FTIR analysis was performed on phenolic compounds of *A. malabarica* leaves, functional groups such as alcohol, amine salt, alkyne, alkene, sulfonyl chloride, cycloalkane, and aromatic ester [Table 2]. The Hydroxyl group was measured at the absorption range of 1450.66 cm^{-1}. C-H stretching alkane was obtained at 1643.57 cm^{-1} and 1925.95 cm^{-1}. The methyl band exhibited vibrational absorption at 1251.35 cm^{-1}. The C-O stretching alkyl aryl ether band was measured at 1271.99 cm^{-1}, while the C-C stretching cycloalkane was measured at 436.70 cm^{-1}.

Table 2. Infra-Red Spectrum Analysis by *A. malabarica* leaves

S.no	Peak value	Stretching	Interpretation
1.	3659.28cm-1	O-H stretching	Alcohol
2.	3407.26cm-1	O-H stretching	Alcohol
3.	2976.15cm-1	N-H stretching	amine salt
4.	2901.67cm-1	N-H stretching	amine salt
5.	2126.51cm-1	C≡C stretching	Alkyne
6.	1925.95cm-1	C=C=C stretching	Allene
7.	1643.57cm-1	C=C stretching	Alkene
8.	1450.66cm-1	C-H bending	Alkane
9.	1405.84cm-1	S=O stretching	Sulfate
10.	1385.27cm-1	S=O stretching	sulfonyl chloride
11.	1251.35cm-1	C-O stretching	aromatic ester
12.	1229.55cm-1	C-N stretching	Amine
13.	1049.61cm-1	CO-O-CO stretching	Anhydride
14.	880.48cm-1	C-H bending	1,2,4-trisubstituted
15.	669.53cm-1	C-H bending	1,3-disubstituted
16.	436.70cm-1	C-C stretching	Cycloalkane

3.5 Antiinflammatory activity

The antiinflammatory properties of phenolic compounds from *A. malabarica* leaves vary with concentration [Table 3]. It produced an IC_{50} of 0.4393±0.0127 in the control group and displayed the maximum inhibition of 81.43% at 500 μg/mL. While sustaining haemolysis, concentration increases, and membrane protection of the sample is lowered by 2.6301%. The concentration of diclofenac sodium was 2.92±0.022 μg/mL. The IC_{50} value of phenol extracts from *A. malabarica* leaves was 278.0 μg/mL.

3.6 DPPH free radical scavenging activity

The antioxidant activity of *A. malabarica* phenolic compounds was evaluated for DPPH scavenging activity [Figure 3]. The IC_{50} value of 75.84 μg/mL for DPPH. The ascorbic acid inhibited at 76.4%, 70.7% at 500μg/mL, and 27.7% at 10μg/mL.

Table 3. Anti-inflammatory study of phenolic compounds of *A. malabarica*

Concentration (μg/ml)	HRBC Stabilisation (%)	
	% of Hemolysis	% protection of the sample
500 μg/ml	96.856±0.7905	62.6301±0.84429
250 μg/ml	35.663±0.9899	64.337103±0.98
100 μg/ml	28.121±0.4558	71.879±0.4559
50 μg/ml	19.62±0.0906	80.38±0.0906
10 μg/ml	18.5702±0.44148	81.43±0.4415
Control	0.4393±0.0127	
Diclofenac Sodium	2.9213±0.022	

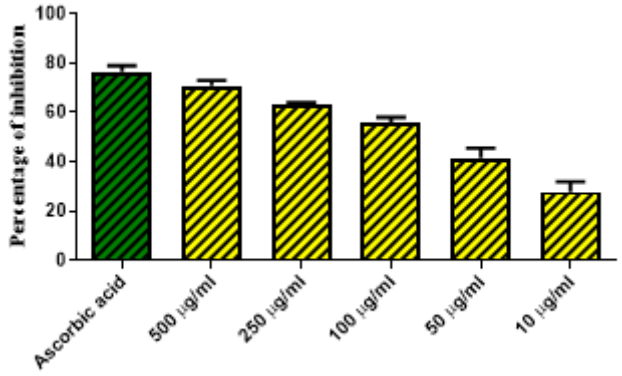


Fig 3. Evaluation of *A. malabarica* leaf extract capacity to scavenge DPPH.

3.7 ABTS radical cation scavenging activity

The antioxidant activity of the *A. malabarica* phenolic compounds was determined using the ABTS test. [Figure 4]. The IC_{50} value for ABTS was 50.70 μg/mL. Ascorbic acid exhibited an inhibition rate of 68.5%, while ABTS was inhibited by 65.8% at 500μg/mL and 33.6% at 10μg/mL.

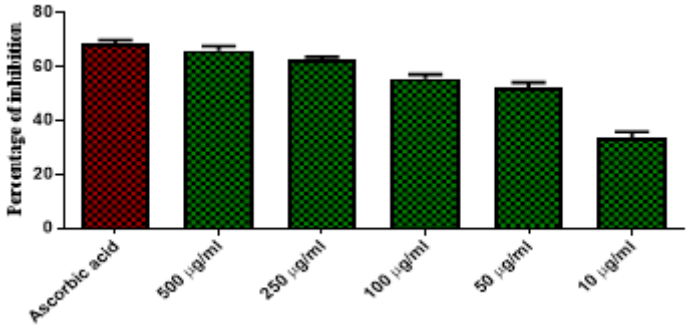


Fig 4. Assessment of the leaf extract of *A. malabarica* ability to scavenge ABTS.

3.8 Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of *A. malabarica* phenolic compounds was found to be an IC_{50} value for 47.77 μg/mL at 500μg/mL, 76.08% of H_2O_2 inhibition and 33.28% of H_2O_2 inhibition at 10μg/mL [Figure 5]. Using ascorbic acid as a reference, 80.01% inhibition was observed.

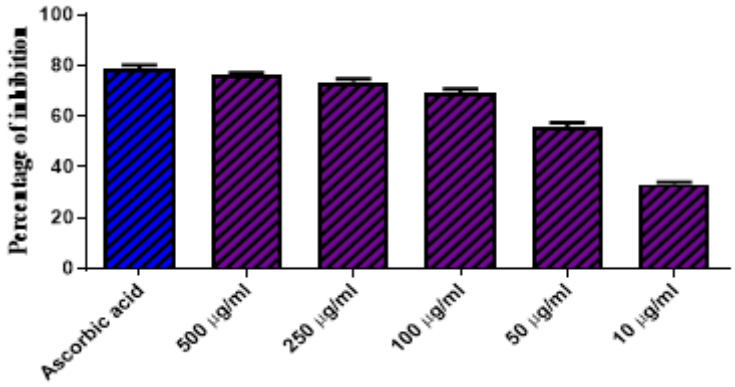


Fig 5. Hydrogen peroxide activity of *A. malabarica* leaf extract.

3.9 Antimicrobial activity

The antimicrobial activity of phenolic compounds, as determined by the most effective, was achieved against *P. vulgaris* (17.25±0.7 mm, *P. acnes* (15.5±0.7 mm), *K. pneumoniae* (14.5±0.7 mm), and *C. diphtheriae* (13.5±0.7 mm) at 500 μg/mL. However, 50 μg/mL exhibited the greatest suppression against *S. pyogenes* (6.5±0.7mm) [Figures 6 & 7]; *K. pneumoniae* (50 μg/mL, as 4.5±0.7 mm) and *S. pyogenes* (100 μg/mL, as 10.25±0.35 mm) exhibited the least activity among the examined pathogens. *C. albicans* (12.5±0.7mm), *S. schenckii* (8.25±0.35mm), and *P. verrucosa* (6.25±0.35 mm) recorded at 500μg/mL [Figure 8]. However, 50 μg/mL exhibited the greatest suppression against *P. verrucosa* (4.5±0.35 mm), *C. albicans* (5.25±0.35 mm), and *P. verrucosa* (100 μg/mL, as 5.25±0.35 mm), as comparison to the other studied pathogens. The foregoing results demonstrate that a portion of *A. malabarica* leaves can be used as a medicine to treat bacterial infections.

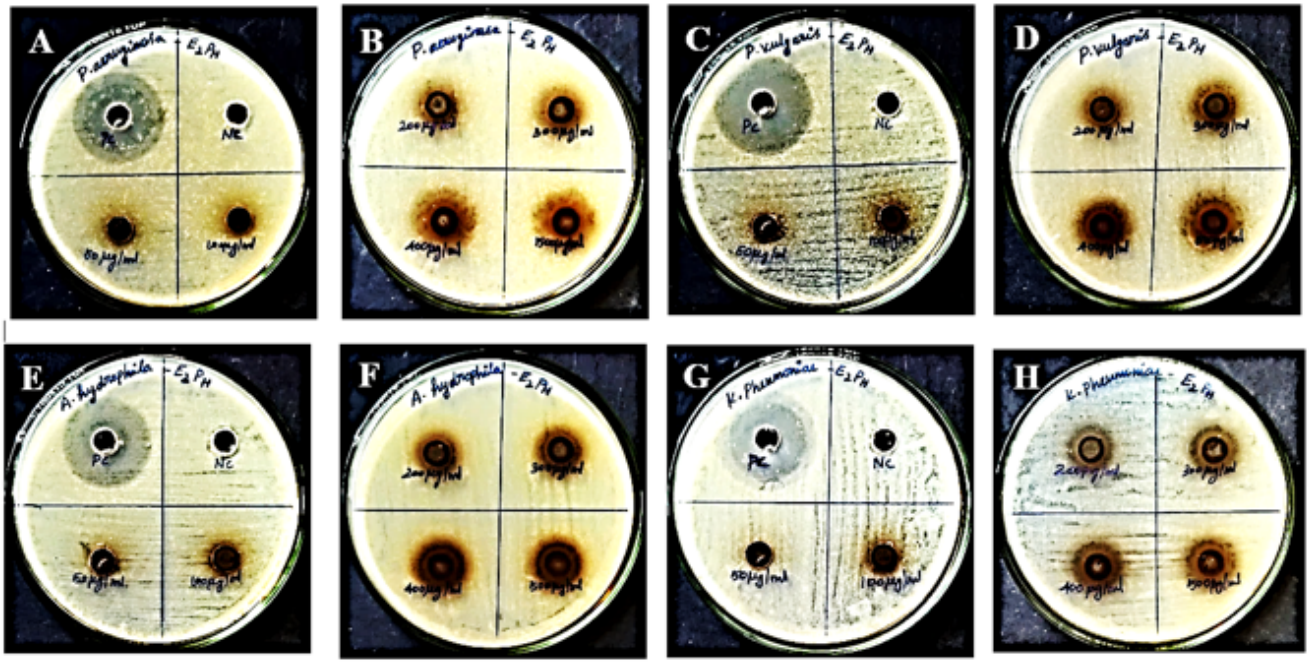


Fig 6. Antibacterial qualities of *A. malabarica* are evaluated against Gram-positive bacteria A and B, *Staphylococcus aureus,* C and D, *Streptococcus pyogenes,* E and F, *Propionibacterium acnes,* G and H, and *Corynebacterium diphtheria.*

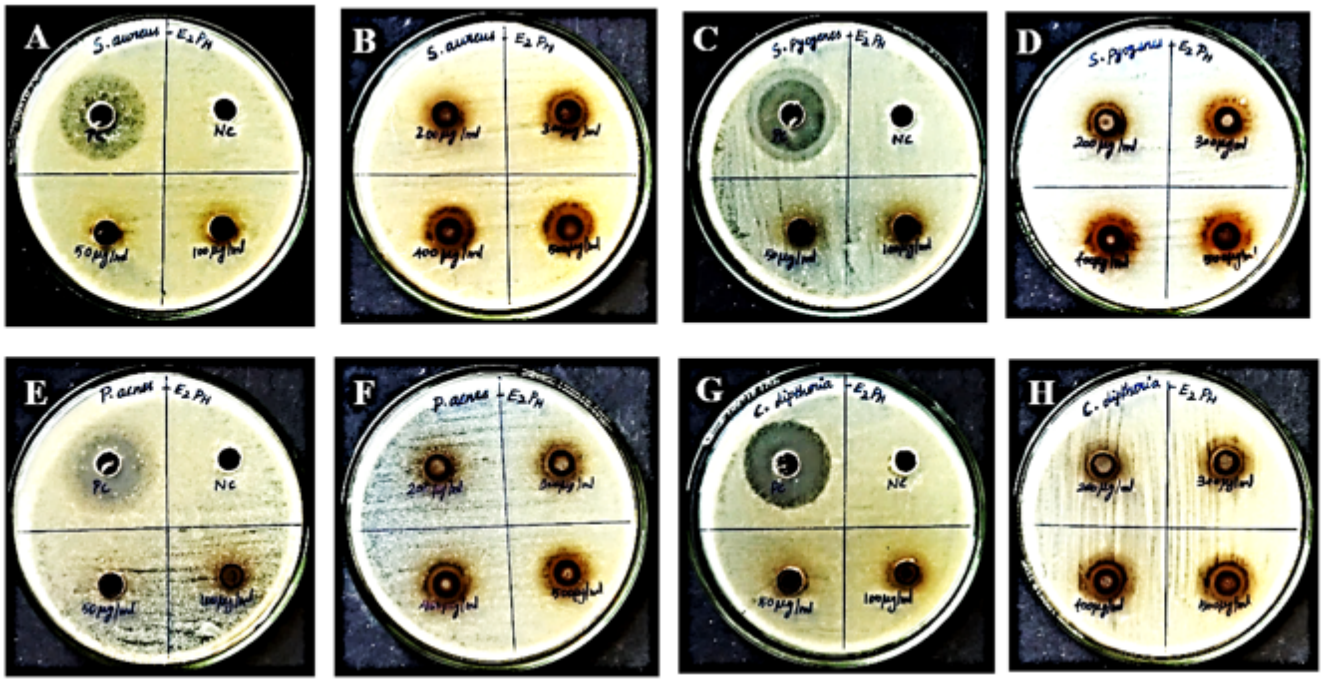


Fig 7. *A. malabarica* antibacterial ability against Gram-negative bacteria, A and B *Pseudomonas aeruginosa,* C and D *Proteus vulgaris,* E&F *Aeromonas hydrophilla,* G and H *Klebsiella pneumoniae.*

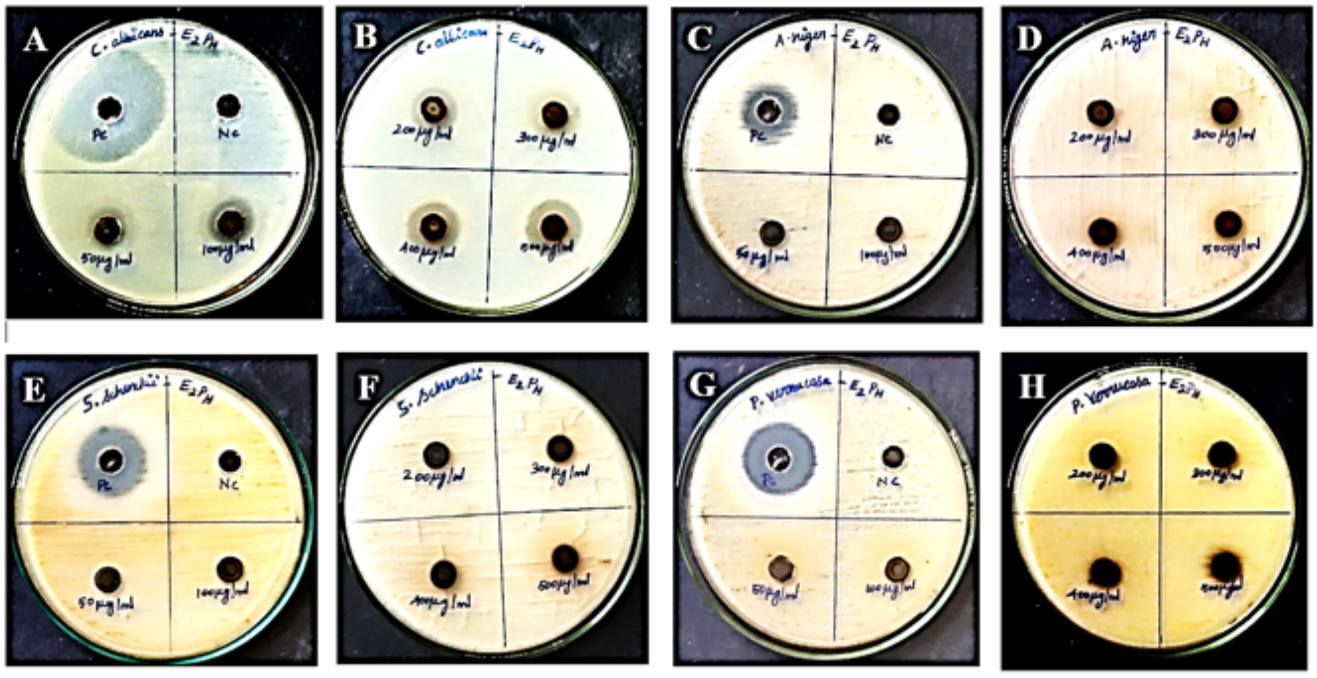


Fig 8. Antifungal property of *A. malabarica* using the Agar diffusion method, A and B *Candida albicans,* C and D *Aspergillus niger,* E and F *Sporothrix schenckii,* G and H *Phialophora verrucosa.*

3.10 Larvicidal action is effective against *A. aegypti* and *A. stephensi.*

The phenolic compounds in the *A. malabarica* leaf utilised in this study demonstrated larvicidal effects. Even at low doses, it displayed the highest larvicidal effectiveness [LC_{50} 63.38 ppm and 74.13 ppm]. The highest concentrations of 100% dead larvae offered the longest protection against *A. aegypti* for 180 minutes. Even with the maximal dose, 40%-90% of the larvae died after 150 and 120 minutes. At the lowest levels of 10% and 20% dead larvae, the protection against *A. aegypti* was 30 and 60 minutes, highest concentrations of 500 ppm, 400 ppm, and 300 ppm provided the most effective protection against *A. stephensi* for 180 minutes, killing all larvae. After 180 minutes of moderate activity, the larval sensitivity was lowest between 100 and 200 ppm, with 90% of the larvae dying [Tables 3 & 4]. The lowest concentrations of dead larvae (10-20%) protected *A. stephensi* for 30 and 60 minutes. At 500 ppm, the control kills 50% of the dead larvae, thereby preventing the spread of *A. aegypti and A. stephensi.*

Table 4. *A. malabarica* Phenolic compounds against *A. aegypti*

Con. (ppm)	No, of Larvae incubated	Mortality in min/hrs. % of repellency						Total Mortality (%)	Larval Mortality (%)
		30 Min	60 min	90 min	120 min	150 Min	180 min		
100 ppm	10	0	1	2	4	6	8	8%	80%
200 ppm	10	0	1	3	5	7	9	9%	90%
300 ppm	10	0	1	2	6	8	9	90%	90%
400 ppm	10	0	2	4	6	9	10	10%	100%
500 ppm	10	1	2	5	7	8	10	10%	100%
Control	10	0	0	1	3	4	5	5%	50%

Table 5. *A. malabarica* Phenolic compounds against *A. stephensi*

Con. (ppm)	No, of Larvae incubated	Mortality in min/hrs. % of repellency						Total Mortality (%)	Larval Mortality (%)
		30 Min	60 min	90 min	120 min	150 Min	180 min		
100 ppm	10	0	1	2	4	7	8	8%	80%
200 ppm	10	0	1	2	5	8	9	9%	90%
300 ppm	10	0	1	2	6	8	10	10%	100%
400 ppm	10	0	2	3	7	9	10	10%	100%
500 ppm	10	1	2	4	6	9	10	10%	100%
Control	10	0	0	1	3	4	5	5%	50%

The metabolic profile of *A. malabarica* revealed a diverse array of bioactive compounds with significant therapeutic implications. Biochemical assays, chromatographic methods, and radiation analysis were used to identify more than 20 chemical components in the ethanolic extract of the leaf included phenol, 2,4-bis(1,1-dimethylethyl), oleic acid, hexadecanoic acid, and coumarin,3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl], all of which have biological activity[24]. The traditional features of an *A. malabarica* formulation (Panchangakshara) with burnt leaf ashes, as well as the physical properties of the sections. Studies have shown that phenolic chemicals can prevent diabetes, cancer, osteoporosis, upper respiratory tract infections, and cardiovascular disease. The Bis(2-ethylhexyl) benzene-1,2-dicarboxylate showed a broader spectrum of antibacterial activity against *S. aureus and E. coli.* 1,2-Benzenedicarboxylic acid, bis (2- ethylhexyl) ester phenolic dimer could be a promising candidate for bioprospecting and medication development in the treatment of diarrhoea and dysentery caused by *Shigella dysenteriae*[25]. FT-IR analysis of *A. malabarica* leaves indicated the presence of aromatic C-H stretch, alkyl carbonate, amide, organic sulphates, aliphatic fluoro compounds (C-F stretch), aliphatic bromo compounds (CBr stretch), and disulphide (S-S stretch).

The antioxidant activity of *A. malabarica* ethanol extract was evaluated using the percentage of DPPH scavenging activity, which was found to be greater at 51.95±0.18 % at 120 μg/mL and an IC50 value of 94.18 μg/mL, compared to the standard (Ascorbic acid, IC_{50} value of 12.83 μg/mL). The maximal ABTS radical cation scavenging activity was determined to be 59.28±0.11 at 60μg/mL, with an IC_{50} value of 40.8 μg/mL, when compared to normal Ascorbic acid (IC_{50} value of 6.43 μg/mL).[26] The ethanol extract of *A. malabarica* reduced RBC haemolysis by up to 70-90 per cent, ethyl acetate, and aqueous extracts. At doses of 10-100 μg/mL, haemolysis increased somewhat, most likely due to increased contact with the RBC phospholipid bilayer. *A. malabarica* leaf extracts may have anti-inflammatory properties

Previous work reveals that *A. malabarica* contains a higher percentage of flavonoids and phenolic chemicals, which have been linked to improved antioxidant activity. The methanol extracts of *A. malabarica* demonstrated potent antioxidant activity in DPPH and ABTS with dose-dependent protective effects. Ascorbic acid and butylated hydroxytoluene (BHT) were tested as antioxidants with variable activity levels. *A. malabarica* is a potent antioxidant with significant anti-free radical action against DPPH, FRAP, and H_2O_2. The ethanol extract of *A malabarica* leaves was tested for antibacterial efficacy against bacterial pathogens such as *S. aureus, B. subtilis,* and *P. vulgaris*. GC-MS analysis was Phenol, 2, 4-bis (1, 1-dimethylethyl)-, Oleic acid, Coumarine, 3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl]-, which indicates considerable therapeutic uses. The findings support the use of *A. malabarica* ethanol extract to treat a variety of infectious illnesses. Furthermore, the ethanol extract could be used to create an effective cancer medication. Phenolic compounds, representing several structural kinds, were found, including hydroxytyrosol,[27] vanillic acid, 3-hydroxybenzoic acid, and cinnamic acid. *A. malabarica* leaves contain monoterpenes and sesquiterpenes, which have strong antioxidant and antimicrobial properties against a variety of pathogenic bacteria and fungi.

The antibacterial activity of five different *A. malabarica* extracts were assessed using the agar well diffusion method. The best antibacterial activity was found against *A. baumannii* (21 mm), followed by *E. coli* (20 mm), *S. typhi* (19 mm), *B. subtilis* (17 mm), and *S. aureus* (16 mm). The methanol extract outperformed the aqueous, acetone, and petroleum ether extracts in terms of antibacterial activity against Gram-negative bacteria, with *C. albicans and P. aeruginosa* exhibiting the most activity.[28]. Because it is extracted from *A. malabarica* may provide an option in treatment protocols for antibiotic resistance, which is becoming an increasingly important issue. The previous findings indicate that the ethanol extract of *A. malabarica* can be utilised as a broad-spectrum antibiotic to treat bacterial infections. Plant-derived secondary metabolites had strong antioxidant and antibacterial activities.[29].

The phytochemical assessment of *A. malabarica* leaves has used a variety of methods, including GC-MS, FTIR, molecular docking, and others. These procedures have enabled the identification and characterisation of various bioactive chemicals, including flavonoids, phenols, and terpenoids, which exhibit outstanding antioxidant and antibacterial properties. New methodologies improve our understanding of the phytochemical genetic makeup of *A. malabarica* leaves, but they also help to standardise their pharmacognostic properties for medicinal purposes[30]. This approach will enable quantitative assessment of phytoconstituents, antioxidant properties, and functional group recognition[31]. The leaf extract of *A. malabarica* may help stabilise red blood cell membranes by inhibiting the release of lytic enzymes and inflammatory mediators. Phytochemical research has yielded steroids, flavonoids, alkaloids, and terpenoids, which are well-known for their potent anti-inflammatory and antioxidant properties.

The dried leaves of *A. malabarica* are frequently utilised for traditional herbal medicines, with *A. malabarica* leaves and inflorescence extracts *A. stephensi* larval dead rate. The larval toxicity of *A. malabarica* leaf extract on the percentage of *A. stephensi* mortality following *A. malabarica* treatment of I to IV instar larvae and pupae at concentrations of 20, 40, 60, 80, and 100 ppm. AMLE treatment at 20 ppm caused 26% mortality in I instar larvae, which escalated to 88% at 100 ppm. At 20 ppm, larval mortality was 15%, but at 100 ppm, it increased to 84%. The first instar had an LC_{50} of 64.76%, then the third instar at 71.46% and the fourth instar at 74.55%. The LC_{90} readings for the first instar were 118.22%, the second instar 121.60%, the third instar 123.65%, and the fourth instar 119.94%. The methanol extract of *A. malabarica* had an LC_{50} value of 20.5 mg/mL. *A. malabarica* leaves and inflorescence extracts affect the larval duration of *A. stephensi*. The therapy extended larval duration (I to IV instars) by 20.8% at 20 ppm. *A. malabarica* leaves originating from methanol extracts provide a plentiful supply of harmless, biodegradable compounds that can be tested for mosquito repellent and insecticidal effects.[32].

4 CONCLUSION

Secondary metabolites found in *A. malabarica* leaf extracts include tannins, alkaloids, flavonoids, and phenols. The findings will serve as a foundation for extracting phenolic molecules, which have been linked to several medical effects, including antioxidant and anti-inflammatory properties. Based on the observations of the preceding study, the phenolic compounds in the filtrate of the crude extract from *A. malabarica* leaves form a variety of secondary metabolites, and attempts to purify these pharmaceutically relevant chemicals are currently underway. However, these studies suggest that *A. malabarica* leaves

contain many bioactive compounds with medicinal potential, highlighting their therapeutic and commercial possibilities. The haemolysis assay confirmed these findings by demonstrating the ability of phenolic compounds to stabilise red blood cell membranes under hypotonic and heat stress, implying lysosomal membrane integrity and the prevention of inflammatory mediator release. HRBC haemolysis was significantly raised at 500 μg/mL. While the *A. malabarica* leaf extract contains phenolic compounds, its antioxidant activity has also been shown to scavenge H_2O_2, DPPH, and ABTS. The accurate isolation of active ingredients could aid in identifying potential lead substances effective in treating diseases mediated by free radicals. FT-IR spectroscopy provided detailed chemical profiles by identifying key functional groups that play protective roles and have potential agricultural applications. Finally, *A. malabarica* leaf extract was prepared and purified at 400 ppm and 500 ppm, maintaining 100% of the dead larvae. Various concentrations were harmful to different *A. aegypti* and *A. stephensi* larval instars in laboratory toxicity tests. At the highest concentration tested (100 ppm), larvae treated had a higher death rate (80%), indicating that more research is needed into the larvicidal efficacy of dengue and malaria treatments. These findings emphasise the pharmacological, medical, and economic importance of these plants, warranting further research into their applications. It is critical to identify and explain bioactive compounds that have a variety of biological activities and can be used for both human and environmental benefits.

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6 Abbreviations

AMLE *Anisomeles malabarica* leaf Extract, *A. malabarica* - *Anisomeles malabarica* **HCl** Hydrochloric acid, **DPPH** 1,1-diphenyl-2-picrylhydrazyl, **ABTS** 3-ethylbenzothiazoline-6-sulfonic acid, $\mathbf{H_2O_2}$ Hydrogen peroxide, $\mathbf{IC_{50}}$ Half maximal inhibitory concentration. **GC-MS**: Gas Chromatography-Mass Spectrometry; **FTIR**: Fourier Transform Infrared Spectroscopy; **UV-VIS**: Ultraviolet-Visible Spectroscopy; **OD**: Optical Density; **rpm**: revolutions per minute; **BHT**: Butylated hydroxytoluene; **MTCC**: Microbial Type Culture Collection; **E2PH**: Extract 2 Phenol compounds **V.N.:** Voucher Number; **SD:** Standard Deviation; **DST-FIST:** Department of Science and Technology - Fund for Improvement of Science and Technology Infrastructure; **DBT:** Department of Biotechnology; **UGC:** University Grants Commission; **R. Br.:** Robert Brown.

7 CONFLICT OF INTEREST

The authors declare that no conflict of interest.

FUNDING No funding.

Compliance with ethical standards

Ethical approval for research involving human participants and/or animals: if applicable, None.

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