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# Phytochemical Profiling and Biological Activities of Flavonoid-Rich Extracts from *Anisomeles malabarica* (L)

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**This study revealed the antioxidant, antimicrobial, GC-MS, FTIR, and UV-Vis. of ethanol derived from Anisomeles malabarica leaves to identify a new resource with potential therapeutic applications. This study used well-known extraction and significant separation techniques to focus on flavonoid compounds' preparative isolation. The Anisomeles malabarica leaf extract inhibited the DPPH by approximately 62.10% at increasing concentrations (500 µg/ml), 38.47% at minimal concentrations (10 µg/ml), and IC50 at 79.07 µg/ml, separately. The ABTS+ assay revealed that 84.1 per cent of the concentration at the lowest concentration (10 µg/ml) and 91.04% at increasing concentrations (500 µg/ml) showed inhibition. According to the H2O2, the IC50 was 51.67 µg/ml, and the inhibition was approximately 77.8% at higher concentrations (500 µg/ml) and 38.5% at lower concentrations (10 µg/ml). The antimicrobial activity of an Anisomeles malabarica leaf extract was examined against gram-positive, gram-negative, and fungi. Staphylococcus aureus (15.25±0.35mm), Corynebacterium diphtheria (14.25±0.35 mm), followed by Klebsiella pneumonia (14.5±0.7mm), was achieved at 500 µg/ml. While Proteus vulgaris (50 µg /ml, as 5.25±0.7 mm). The antifungal activity against Phialophora verrucosa was achieved at (500 µg/ml, 18.5±0.35 mm) and (400 µg /ml, 17.25±0.7 mm). Whereas Sporothrix schenckii (200 µg /ml, as 4.5±0.7 mm). The leaf extract of Anisomeles malabarica, analysed using GC-MS and FTIR, contained 40 flavonoid compounds, with five peak compounds identified, indicating its potential for phytochemical screening. The study reveals that A. malabarica leaves possess antioxidant properties, effectively treating bacterial degenerative diseases in conventional medicine and validating their use in pharmaceutical formulations.**

**Keywords:** Antioxidant Properties; Antimicrobial Properties; Conventional Medicine; GC-MS analysis; Therapeutic Efficacy.

Recent medical research has shifted its attention to conventionally used medicinal plants, which are rich in therapeutic elements that could be utilized in the creation of medications. Natural plants remain the best source of bioactive chemicals and pharmaceuticals because the researcher's main objective was to reduce the adverse effects of synthetic treatments.[2]

The therapeutic potential of medicinal plants as antioxidants in preventing such free radical-induced tissue damage has received more attention recently. Free radicals are metastable chemical elements that have contributed to the domestication of numerous unusual and unique plant species. Pure chemicals extracted from

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biological reactions frequently appropriate electrons from molecules nearby to develop current pharmaceutical drugs. A rising number of diseases, including atherosclerosis, heart failure, neurological disorders, ageing, cancer, diabetes mellitus, hypertension, and several others are being related to free radicals and tissue damage.[12] The study explores the use of FTIR in identifying antimicrobial compounds in plants, animals, and microorganisms, particularly in folk medicines. It aims to identify biomolecules of *Anisomeles malabarica*, a plant, and develop a platform for treating various illnesses by screening bioactive ingredients.[13]

*Anisomeles malabarica* is a shrub of the Lamiaceae family that is found throughout much of tropical and sub-tropical India. The shrub is thought to possess emmenagogue, diaphoretic, and antiperiodic properties. According to ethnobotany, the plant's leaves can treat tetanus, colic, boils, anorexia with dyspepsia, rheumatism, and convulsions. The plant is also thought to relieve inflammation, uterine problems, colds, coughs, itching, and stomach aches. It has also been demonstrated that *A. malabarica* has anticonvulsant, diuretic, anticancer, antispasmodic, and antifertility properties. *A. malabarica* has been shown to help with a variety of ailments, including halitosis, epilepsy, hysteria, dementia, anorexia, intestinal worms, colic, flatulence, teething children, gout, swelling, diarrhoea, and wound healing.[23] The much-admired herbaceous plant characteristics have gained popularity recently. Medicinal plants have long been used to treat illnesses, and the growing resistance to antibiotics is driving researchers to continue their hunt for novel medications.[5]

By evaluating the flavonoid compounds' antimicrobial and antioxidant capabilities and demonstrating their biological activities using GC-MS, FTIR, and UV-Vis, the study sought to validate the potential of these compounds as natural preservatives. The flavonoid compounds were extracted from the ethanolic extract of *A. malabarica*. This work creates a platform for evaluating a variety of bioactive ingredients for use in treating various illnesses.

## MATERIALS AND METHODS

### Plant collection and authentication

*Anisomeles malabarica* (L) R. Br. ex-Sims Fresh, young, and seemingly healthy leaves were collected from the dry, rocky terrain of Peramangalam in the Tiruchirappalli region of Tamil Nadu, India. Rapinat Herbarium Voucher number: V.N. 001 at St. Joseph College in Tiruchirappalli identified and verified the plant material's authenticity.

### Extraction Flavonoids Compounds

250 mL of ethanol and 20 g of *Anisomeles malabarica* leaves were combined in the Soxhlet device. Then it was left for three hours. Following this, the crude extract (AMLE) was filtered and evaporated. The later crude extract was made by using ethanol as a solvent and separating the flavonoid components at an acidified pH. First, the ground plant material (crude extract) was diluted and treated with lead acetate 3g for one hour. Then, add 10% HCl and continue to boil for about an hour. Then add 10ml of alcohol and leave for 1 hour. [3] Acetate-free flavonoid molecules were extracted with alcohol and dark green fractionally crystallized, as illustrated in Figure 1A. Following filtration, it manifests as a pale green precipitate that is most likely tannin.

### GC-MS analysis

The chemical composition of *Anisomeles malabarica* flavonoids leaf extract and the presence of active ingredients were determined using gas chromatography and mass spectrometry. The Shimadzu QP-2020 plus GC/MS system, equipped with the TD 20 thermal desorption system, was used to analyse the *Anisomeles malabarica* extract. The SGE BX-30 column had dimensions of 30 m x 0.25 mm x 0.25 m. After 0 minutes of isothermal heating at a rate of 0°C per minute, the oven's starting temperature was kept at 50°C and the final temperature was 250°C. A 2-minute isothermal heating cycle was performed at a rate of 6°C per minute. Helium was the carrier gas used in this case. The injection volume for the split mode was 0.11. The source is heated to 250°C, while the intake line is heated to 200°C. Mass spectra ranging from 45 to 450 amu were recorded using a 70-eV electron impact ionisation energy. The

sample was run for 40 minutes in total. There was a solvent delay of 0–2 minutes. The compounds were identified by comparing the mass spectra of the unknown peaks generated to those stored in Wiley and NIST's mass spectral electronic libraries.

**UV- VIS spectroscopic analysis**

A UV-visible spectrophotometer (Perkin Elmer, USA Model: UV-2600 Series) with a 5.0 nm slit width was employed to examine the flavonoid leaf extract of *Anisomeles malabarica.* The extract was subjected to visible and UV light with wavelengths ranging from 220-800 nm for proximate analysis. The extract was filtered through Whatman No. 1 filter paper after being centrifuged for 10 minutes at 3000 rpm. Using the same solvent, the sample is diluted 1:10 times.

**FTIR analysis**

The *Anisomeles malabarica* flavonoid leaf extract was subjected to an FTIR analysis using the potassium bromide (KBr) pellet (FTIR grade) method. A Jasco FTIR-6300 Fourier transform infrared spectrometer, operating at a resolution of 1 cm, was utilized to record the spectrum. It was outfitted with a JASCO IRT-7000 Intron Infrared Microscope and operated in transmittance mode.

**Potential biological properties in antioxidant activity**

**DPPH radical scavenging activity**

The DPPH radical scavenging assay was performed based on the method with slight modifications[28]. 0.1 mM DPPH solution was prepared using the methanol and the test sample was prepared at various concentrations (500, 250, 100, 50, and 10 ìg/ml). 100ìl of DPPH solution was added to the test samples and incubated for 30 minutes at room temperature. After the incubation, the absorbance of the test samples was measured at 517nm using a UV-VIS spectrophotometer. Using ascorbic acid as the standard, the study examined the absorption of a control sample that contained ethanol and DPPH solution. Its radical-scavenging activity was calculated using the formula to determine the percentage of inhibition.

$$\text{Impairment percentage} = (\text{abs control} - \text{abs sample} / \text{abs control}) \times 100$$

Whereas abs sample is the absorbance of the DPPH solution in the presence of the sample extract, abs control is the absorbance of the DPPH solution in the absence of the sample extract.

**ABTS Radical cation scavenging activity**

ABTS assay was done according to the methodology with slight modifications[28] with slight modifications. The stock ABTS solution was prepared by using 7 mM ABTS solution and 2.4 mM potassium persulfate solution. 1 ml of stock ABTS was added to 60 ml of methanol and used as a working solution. Test samples (500, 250, 100, 50, and 10 ìg/ml) were added with 1 ml of ABTS working solution and allowed to react with ABTS and incubated for 7 minutes, and the absorbance was measured at 734 nm. The percentage of inhibition was determined using the following equation:

$$\text{Inhibition (\%)} = \text{abs control} - \text{abs sample} / \text{abs control} \times 100$$

Abs control represents the rate of methanol ABTS radical absorption, whereas abs sample denotes the absorbance of the ABTS radical solution with the sample extract or standard.

**Hydroxyl Radical Scavenging Activity**

Hydroxyl radical scavenging assay was performed using the methodology followed by slight modifications[14.] 43 mM hydrogen peroxide solution is prepared using phosphate buffer. Test samples (500, 250, 100, 50, and 10 ìg/ml) were added with 0.6 ml of 43 mM hydrogen peroxide and incubated for 10 minutes and the absorbance was measured at 230 nm. The formula for calculating the percentage of inhibition was:

$$\%\ \text{inhibition}\ H_2O_2 = 1 - \text{Abs standard} / \text{Abs control} \times 100$$

Where the Abs sample represents the absorbance at 560 nm when the extract is present, and Abs control represents the absorbance of the control sample at 560 nm (without extract). The experiment was conducted three times.

**Assessment of Antimicrobial Activity**

The antibacterial activity of flavonoids from *Anisomeles malabarica* leaf extract was studied in eight bacterial species, including four gram-positive bacteria. (*Staphylococcus aureus- 902, Streptococcus pyogenes-1928, Propionibacterium*

*acnes-1951, Corynebacterium diphtheria)* four-gram negative bacteria (*Pseudomonas aeruginosa-424, Proteus vulgaris-426, Aeromonas hydrophilla, and Klebsiella pneumonia*) and anti-fungal activity in four fungal species (*Candida albicans, Aspergillus niger, Sporothrix schenckii and Phialophora verrucosa)*. Was purchased from MTCC, Chandigarh, India. Agar well diffusion was divided into smaller sections with different concentrations (50, 100, 200, 300, 400, and 500 µg/ml). The antibacterial and antifungal activity was assessed using the agar well diffusion method, with gentamicin and amphotericin B serving as positive controls. Plates were incubated at 37ºC for 24 hours. The zone of inhibition was measured (mm) and calculations were performed using Graph Pad Prism 6.0 software (USA).

## RESULTS

### Identification of flavonoid compound from crude extract

According to these results, the identification of flavonoid compounds in the *A. malabarica* leaves confirms that the ethanol crude extract is mainly related to the presence of these flavonoid compounds. Figure 1A Shows the crude extract of the flavonoid compounds extracted from the *A. malabarica* leaves. The addition of a few drops of 20% NaOH and 70% HCL to the crude extract caused the colours to change from yellow formation to yellow disappearance, indicating that the samples contained flavonoid compounds in *A. malabarica* leaves. Its appearance is white-creamy yellow, indicating the presence of flavonoids. Figure 1B. Depicts the results of identifying flavonoid compounds with an alkaline reagent containing 20% NaOH and 70% HCL.

### GC-MS analysis

The GC-MS analysis was utilized to identify the flavonoid compounds in Anisomeles malabarica leaf extract, resulting in the identification of 40 flavonoid compounds. The GC-MS chromatogram is shown in Figure 2. The peak compound was phthalic acid, di(2-propylpentyl) ester, which had a 19.34% area. Table 1. shows the top five peaks. In addition to GC-MS analysis, Naphthalene (11.87%), 1,2,3-propane tricarboxylic acid, 2-hydroxy-, triethyl ester (6.5%), and 1-undecanol (5.51%) Cyclononasiloxane, Octadecamethyl (4.62%) were identified.

### UV-VIS analysis

Because of the sharp peaks and acceptable baseline, the qualitative UV-VIS profile of the flavonoid extract of *Anisomeles malabarica* leaves was obtained at wavelengths between 220 and 800 nm. Table 2. shows the absorption values of 0.772, 1.282, and 2.007 corresponded to the peaks in the profile at 230, 273, and 312 nm. The absorption spectra of *Anisomeles malabarica* flavonoid leaf extract Figure 3. demonstrate that it is almost transparent in the 220-800 nm wavelength range.

### FTIR analysis

The active components functional group was identified by FTIR analysis. Figure 4. displays the FTIR spectrum of the *Anisomeles malabarica* flavonoid leaf extract as a KBr pallet. The functional groups of the constituents were separated based on the ratio of the peak when the plant extract was added to the FTIR spectrum. The FTIR peak values for the following functional groups are displayed in Table 3. alcohol, primary alcohol, carboxylic acid, aromatic ester, aromatic mono-substituted amine salt, and carbodiimide. The Hydroxyl group was measured at the absorption range of 3379.44 cm-1. C-H stretching alkane group was obtained at 2900.73 cm-1. The vibrational absorption of the methyl band was identified at 1925.00cm-1 and 1649.83cm-1 The C-O stretching alkyl aryl ether band was measured at 1272.07 cm-1 C-C stretching cycloalkane functional group was measured at 434.42 cm-1.

### Biological evaluation of Antioxidant activity

### DPPH scavenging activity

The antioxidant activity of *Anisomeles malabarica* extract flavonoids was evaluated for DPPH scavenging using ascorbic acid as a standard. *Anisomeles malabarica* exhibited an $IC_{50}$ value of 79.07 µg/ml for DPPH. 62.10% inhibition was measured at higher concentrations of 500ìg/ml and 38.47% inhibition at minimal concentrations of 10ìg/ml Figure 5. and Table 4. This demonstrated the antioxidant activity of *Anisomeles malabarica* flavonoid extract against DPPH.

### ABTS radical cation scavenging activity

The antioxidant activity of the flavonoid extract from *Anisomeles malabarica* was determined using the ABTS test. Ascorbic acid was used as a standard to compare antioxidant

properties. *Anisomeles malabarica* exhibited an $IC_{50}$ value of 70.55 ìg/ml against $ABTS^{+}$. *A. malabarica* leaf extract inhibited $ABTS^{+}$ by 91.04% at a higher concentration of 500 µg/ml and 84.1% at the minimal concentrations of 10 ìg/ml, while ascorbic acid inhibited by 95.04% Figure 6. and Table 5. This confirms *Anisomeles malabarica* antioxidant properties.

**Hydrogen peroxide scavenging activity**

This technique works on the principle that when hydrogen peroxide oxidizes, it loses some of its absorbance. In addition to the naturally occurring hydrogen peroxide, immune cells can actively produce it to neutralize foreign objects. *Anisomeles malabarica* flavonoids extract had an $H_2O_2$ $IC_{50}$ value of 51.67 ìg/ml. At 500ìg/ml, $H_2O_2$ inhibition was achieved at a higher concentration of 74.9%, while 11.2% at minimal concentrations (10ìg/ml). Ascorbic acid was used as a standard, demonstrating 88.56% inhibition—figure 7. and Table 6.

**Antimicrobial activity**

The antimicrobial activity of flavonoids in *Anisomeles malabarica* was determined by the agar well diffusion method by measuring the zone scale, A zone scale was used to quantify the inhibition diameter following 48 hours of incubation, gram-positive bacteria (5-15mm) Figure 8. were subject to the same degree of inhibition as gram-negative bacteria (5–15 mm) Figure 9. *Propionibacterium*

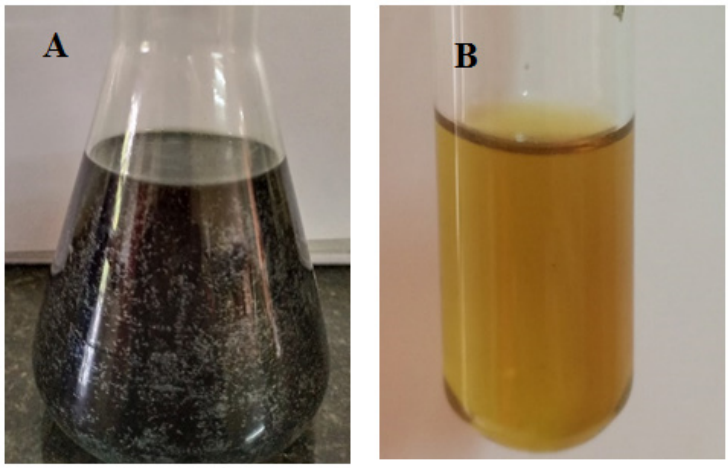



**Fig. 1.** Extraction of flavonoids from *Anisomeles malabarica* leaves (A) and Positive alkaline reagent confirmative tests (B) results

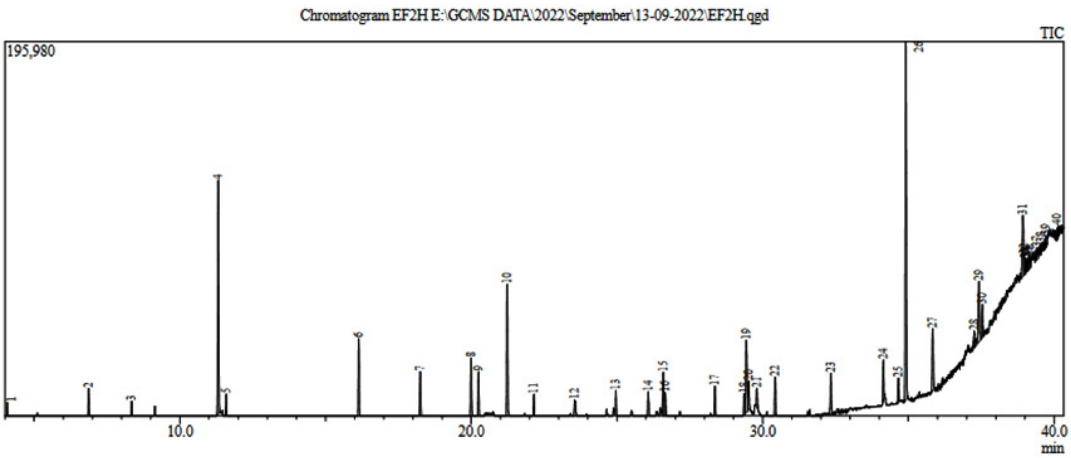
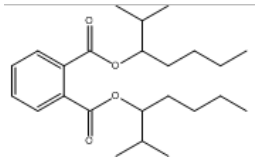
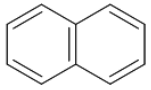
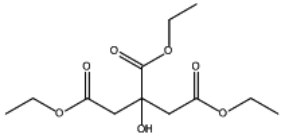
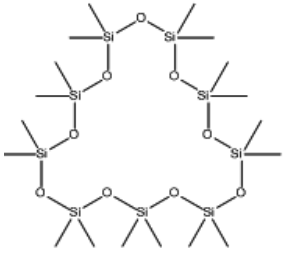



**Fig. 2.** A chromatogram of flavonoid compounds from *Anisomeles malabarica* leaf

**Table 1.** Recognized compounds in Anisomeles malabarica leaves by GC-MS

| S. No | Mass Peak | Ret. Time | Ret. index | SI | Area % | Compound Name | Molecular Formula | Molecular Weight | Molecular Structure | Biological Activity |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 29 | 34.910 | 2704 | 95 | 19.34 | phthalic acid, di(2-propyl pentyl) ester | $C_{24}H_{38}O_4$ | 390 |  | (Ling Huang.,2021) |
| 2. | 16 | 11.320 | 0 | 97 | 11.87 | naphthalene | $C_{10}H_{18}$ | 128 |  | (Heriberto Robles,2023) (Y.B. Rokade and R.Z sayyed,2009) |
| 3. | 10 | 21.230 | 0 | 93 | 6.5 | 1,2,3-propane tricarboxylic acid, 2-hydroxy-, triethyl ester | $C_{12}H_{20}O_7$ | 276 |  | (Emmanuel Nyongesa Waswa.,2022) |
| 4. | 20 | 29.435 | 1357 | 85 | 5.51 | 1-undecanol | $C_{11}H_{24}O$ | 172 |  | (Hesselberg RJ, Seelye JG.,1982) |
| 5. | 22 | 37.420 | 1860 | 81 | 4.62 | cyclononasiloxane, octadecamethyl- | $C_{18}H_{54}O_9Si_9$ | 666 |  | (Saad S. Alqahtani., 2022) |

*acnes* (15.5±0.7 mm), *Streptococcus pyogenes* (14.25±0.35 mm), *Corynebacterium diphtheria* (14.25±0.35 mm), *Proteus vulgaris*, and *Klebsiella pneumonia* (14.5±0.7 mm), were the bacteria that were most effectively inhibited by the antibacterial action, which was acquired at 500 µg/ml. However, *Staphylococcus aureus* (8.5±0.7 mm), *Streptococcus pyogenes, Pseudomonas aeruginosa* (7.5±0.7 mm), and *Aeromonas hydrophilla* (7.5±0.7 mm) showed the greatest suppression at 50 µg/ml as the maximum inhibitor. Table7. While *Proteus vulgaris* (50 µg/ml, as 5.25±0.7 mm) and *Corynebacterium diphtheria* (50 µg/ml, as 5.25±0.7 mm) showed the least action in comparison to the other pathogens under investigation.

**Table 2.** UV-VIS peak values of *A. malabarica*

| S. No. | Wavelength (nm) | Absorbance |
|---|---|---|
| 1. | 312 | 2.007 |
| 2. | 273 | 1.282 |
| 3. | 230 | 0.772 |

A zone scale was utilized to assess the inhibition diameter to examine the antifungal effects of the flavonoid leaf extracts of *A. malabarica*

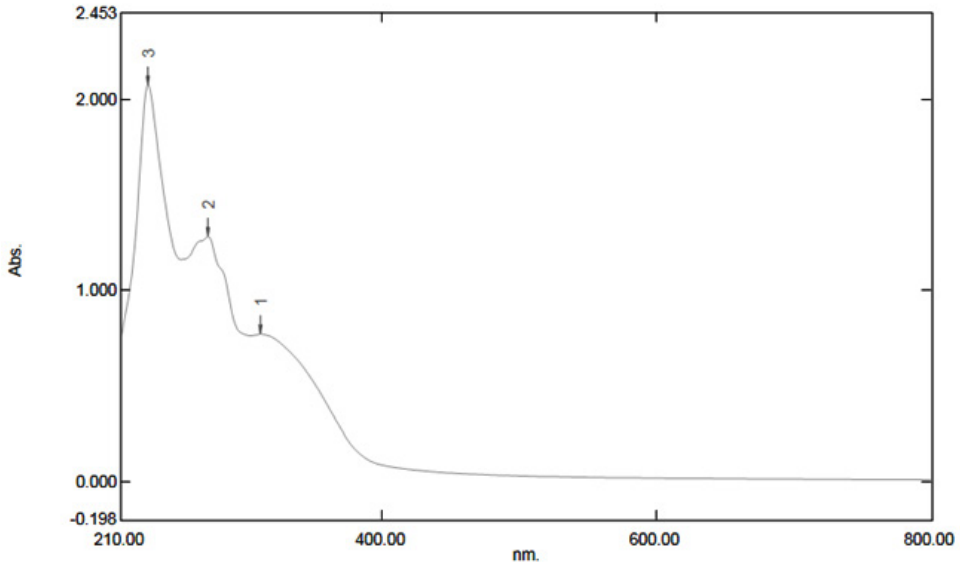



**Fig. 3.** UV-VIS spectra of pure ethanolic *Anisomeles malabarica* leaf

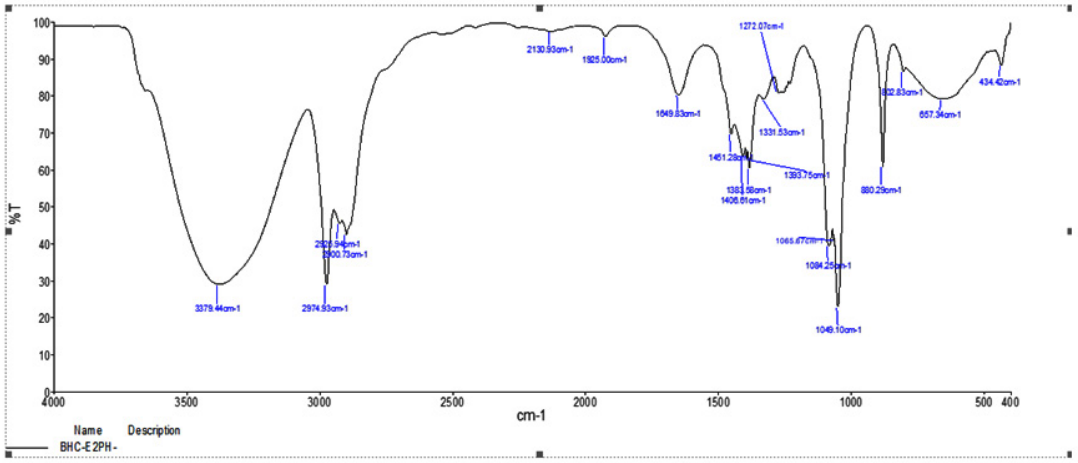



**Fig. 4.** FTIR spectra of pure Ethanolic *Anisomeles malabarica* leaf

following a 72-hour incubation period. Figure 10. shows that the highest level of antifungal activity was attained at *Candida albicans* (14.5±0.7mm) and *Phialophora verrucosa* (18.5±0.35mm) showed the highest antifungal activity when diluted to 500 µg/ml and 400 µg/ml, respectively Table 8. However, against *Candida albicans* (100 µg/ml, as 9.5±0.7 mm) and *Phialophora verrucosa* (200 µg/ml, as 14.5±0.7 mm), 50 µg/ml demonstrated the best suppression. As opposed to *Aspergillus niger*, *Candida albicans*, and *Sporothrix schenckii* (50 µg/ml, 6.5±0.7 mm, 200 µg/ml, 4.5±0.7 mm, respectively. Comparatively speaking to the other infections under investigation, *Aspergillus niger*, *Phialophora verrucosa*, and *Sporothrix schenckii* as 0 mm, 50 µg/ml, and 100 µg/ml all showed

**Tables 3.** Infra-Red Spectrum Analysis BY *A. malabarica*

| No. | Peak value | Stretching | Interpretation |
|---|---|---|---|
| 1. | 3379.44cm-1 | O-H stretching | alcohol |
| 2. | 2974.93cm-1 | N-H stretching | amine salt |
| 3. | 2925.94cm-1 | N-H stretching | amine salt |
| 4. | 2900.73cm-1 | C-H stretching | alkane |
| 5. | 2130.93cm-1 | N=C=N stretching | carbodiimide |
| 6. | 1925.00cm-1 | C=C=C stretching | allene |
| 7. | 1649.83cm-1 | C=C stretching | alkene |
| 8. | 1451.28cm-1 | C=O stretching | Carboxylic Acid |
| 9. | 1406.61cm-1 | S=O stretching | sulfonyl chloride |
| 10. | 1393.75cm-1 | S=O stretching | sulfate |
| 11. | 1383.58cm-1 | S=O stretching | sulfonyl chloride |
| 12. | 1331.53cm-1 | S=O stretching | sulfone |
| 13. | 1272.07cm-1 | C-O stretching | alkyl aryl ether |
| 14. | 1084.25cm-1 | C-O stretching | primary alcohol |
| 15. | 1065.67cm-1 | S=O stretching | sulfoxide |
| 16. | 1049.10cm-1 | CO-O-CO stretching | anhydride |
| 17. | 880.29cm-1 | C-H bending | 1,3-disubstituted |
| 18. | 802.83cm-1 | Ar-C stretching | Aromatic mono-Substituted |
| 19 | 657.34cm-1 | C-Br stretching | halo compound |
| 20 | 434.42cm-1 | C-C stretching | Cycloalkane |

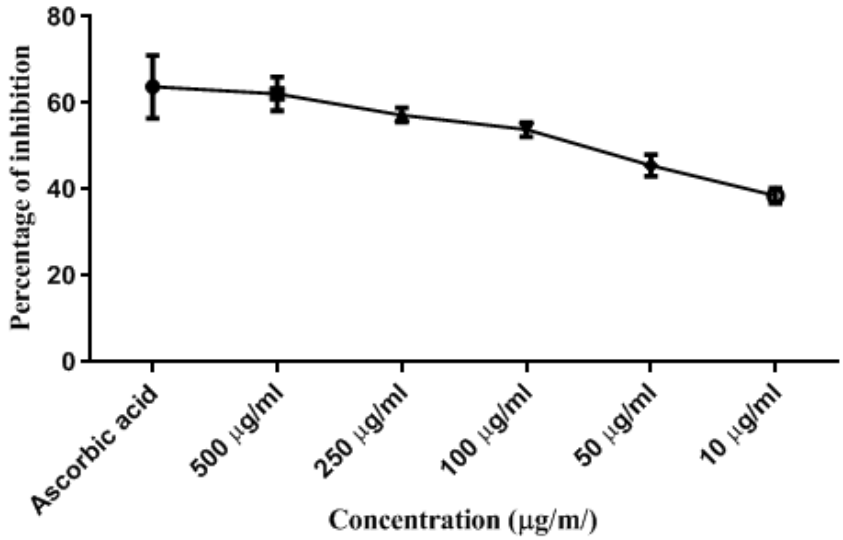



**Fig. 5.** *Anisomeles malabarica* was tested for its capacity to scavenge DPPH free radicals

the least amount of activity. Utilizing Graph Pad Prism 6.0 software (USA), the antifungal activity was assessed by measuring the diameter of the inhibitory zone that developed around the wells.

## DISCUSSION

Traditional medical practices have gained importance in the past decade due to their safety and cultural significance. Many developing countries rely on herbal healers and medicinal plants, while developed countries increasingly use complementary therapies.[2] This study aims to identify Indian plants with strong antioxidant activity, particularly *A. malabarica*, a medicinal herb used for various illnesses, by analyzing its leaf extract for antioxidant and antibacterial compounds.[16] Phytochemicals known

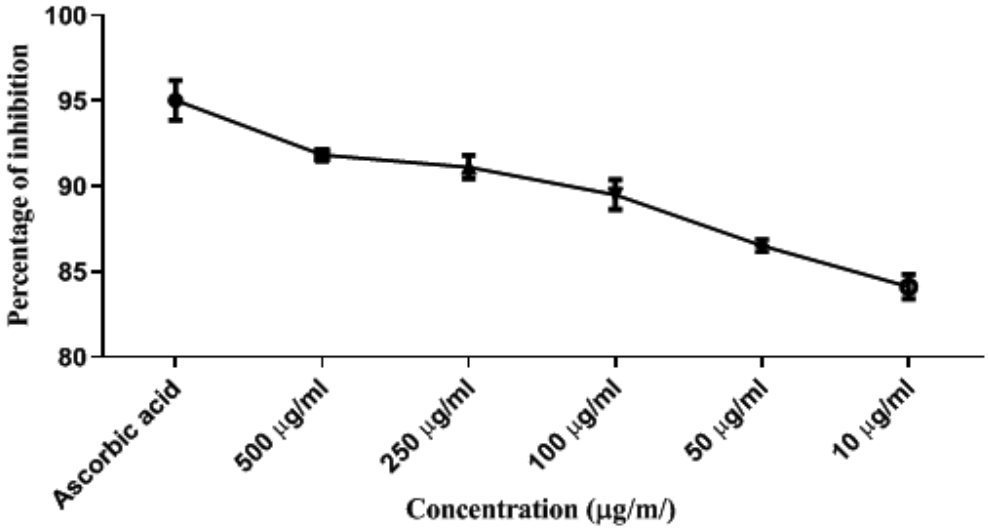



**Fig. 6.** *Anisomeles malabarica* ability to scavenge ABTS radical cations was assessed

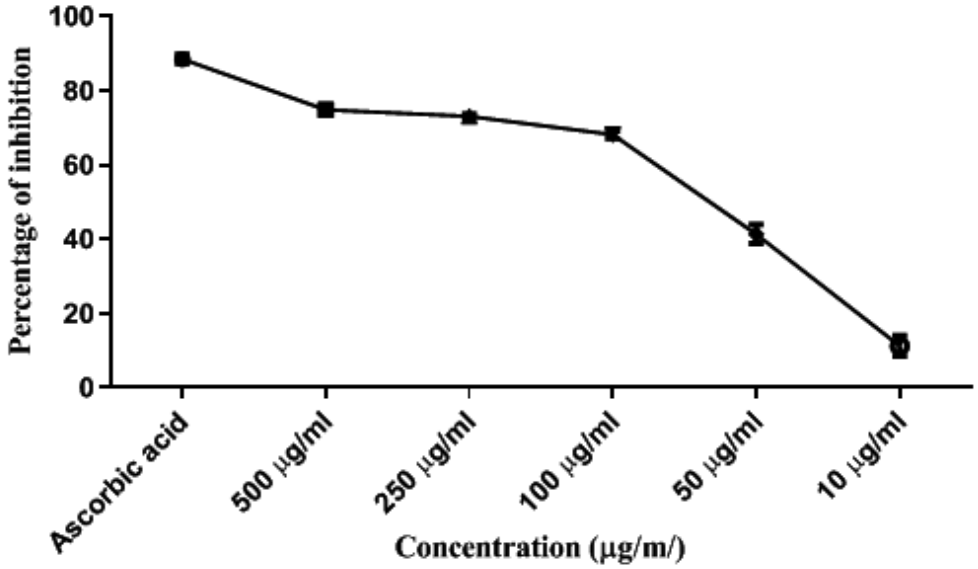



**Fig. 7.** Depicts an evaluation of the hydrogen peroxide activity of *Anisomeles malabarica*

as flavonoids have gained attention lately due to their potent antioxidant properties and potential medical benefits, including the ability to lower blood pressure and treat diabetes.[6,8]

The plant extract's phytochemical analysis revealed flavonoids, which may contribute to its antioxidant activity and have been linked to various biological benefits, including anti-inflammatory, anti-allergic, antiviral, and anti-cancer effects.[11] Flavonoids have biological functions in addition to their function as antioxidants, in the defence against free radicals, hepatotoxins, viruses, bacteria, tumours, and inflammation.[1] Higher plants' antibacterial activity is a relatively new area of study, but their antimicrobial properties have influenced their use in drugs, complementary therapies, and natural therapies.[15] The present study aims to assess the antibacterial activity and explore the phytochemical constituents found in the leaves of the *A. malabarica* plant.[18]

Recent studies highlight flavonoids' high antioxidant properties, potential for wellness improvement, and disease prevention due to their high concentration and abundance. The molecular

**Table 4.** DPPH free radical assay of *A. malabarica*

| Concentration (µg/mL) | % of inhibition |
|---|---|
| Control | 0.285333±0.016653 |
| 10 µg/ml | 38.47953±1.766043 |
| 50 µg/ml | 45.49709±2.489346 |
| 100 µg/ml | 53.80117±1.658183 |
| 250 µg/ml | 57.19298±1.607919 |
| 500 µg/ml | 62.10526±3.907204 |
| Ascorbic acid | 63.74269±7.304087 |

**Table 5.** ABTS radical cation scavenging activity of *A. malabarica*

| Concentration (µg/mL) | % of inhibition |
|---|---|
| Control | 2.03733±0.29455 |
| 10 µg/ml | 84.127±0.70858 |
| 50 µg/ml | 86.2543±0 |
| 100 µg/ml | 89.5107±0.87314 |
| 250 µg/ml | 91.1308±0.69484 |
| 500 µg/ml | 91.8344±0.34481 |
| Ascorbic acid | 95.0417±1.16068 |

**Table 6.** Hydrogen peroxide assay of the activity of *A. malabarica*

| Concentration (µg/mL) | % of inhibition |
|---|---|
| Control | 0.554667±0.021502 |
| 10 µg/ml | 11.2515±2.763168 |
| 50 µg/ml | 41.39591±2.561227 |
| 100 µg/ml | 68.17088±1.026397 |
| 250 µg/ml | 73.16486±0.63391 |
| 500 µg/ml | 74.90975±0.650817 |
| Ascorbic acid | 88.56799±1.228674 |

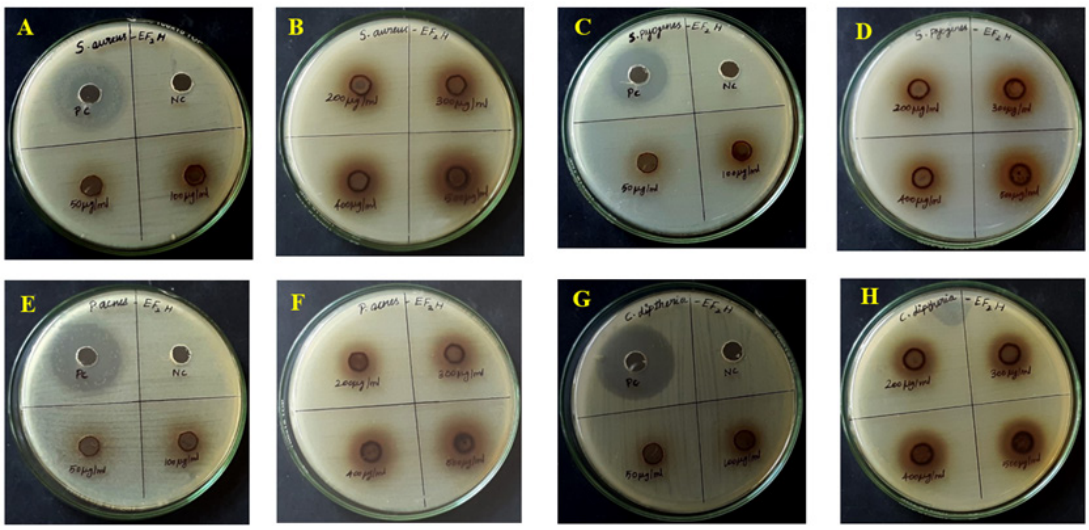



**Fig. 8.** The antibacterial property of *Anisomeles malabarica* against Gram-positive bacteria using the Agar well diffusion, A&B *Staphylococcus aureus,* C&D *Streptococcus pyogenes,* E&F *Propionibacterium acnes,* and G&H *Corynebacterium diphtheria*

structure of flavonoids determines their potential as antioxidants.[3] The effectiveness of flavonoids as antioxidants and free radical scavengers is significantly influenced by the location of their hydroxyl groups and other structural characteristics. Thus, it would be advantageous to generate and utilize more potent antioxidants derived from natural sources. Though there are not many reports of them, these biological activities might be related to *A. malabarica* antioxidant and antibacterial properties. The objectives served as a guide for organizing and conducting the current investigation.[4]

The study utilized an in vitro antioxidant activity assay to identify potential antioxidant components in a plant, revealing that increasing plant extract levels increased DPPH free radical inhibition.[8] Using the FRAP test, nitric oxide

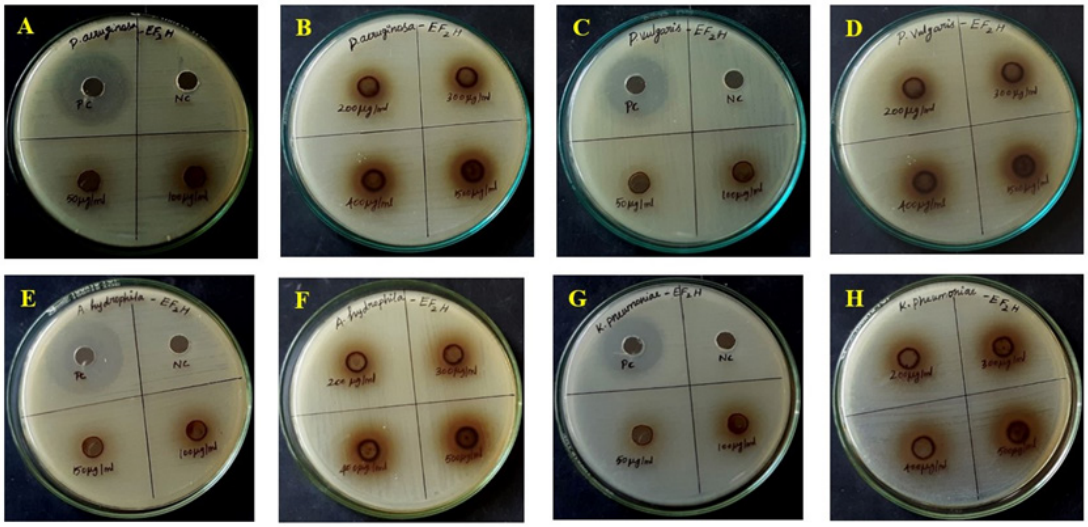



**Fig. 9.** The antibacterial property of *Anisomeles malabarica* against Gram-Negative bacteria using the Agar well diffusion, A&B *Pseudomonas aeruginosa,* C&D *Proteus vulgaris,* E&F *Aeromonas hydrophilla,* and G& H *Klebsiella pneumoniae*

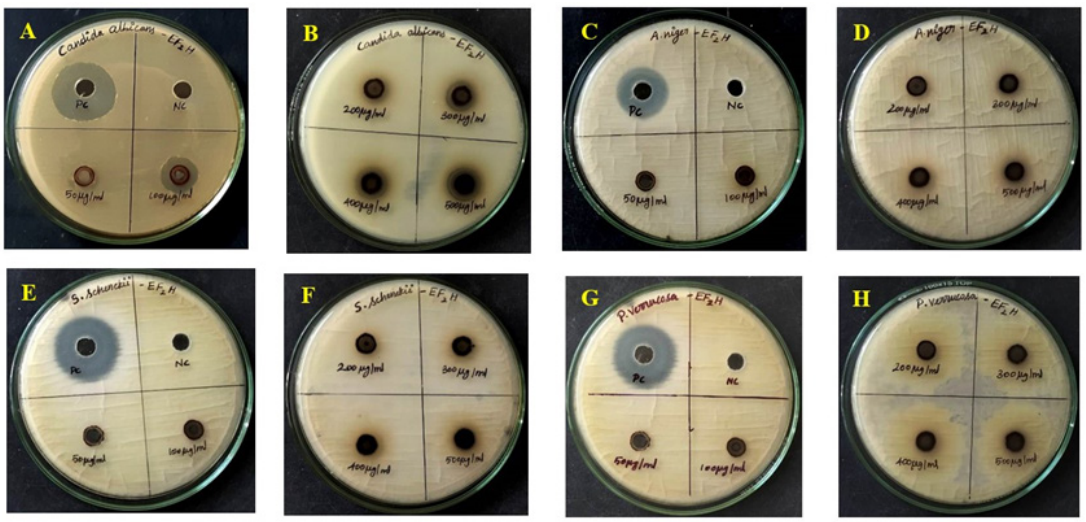



**Fig. 10.** Antifungal properties of *Anisomeles malabarica* using the Agar well diffusion, A&B *Candida albicans,* C&D *Aspergillus niger,* E&F *Sporothrix schenckii,* and G& H *Phialophora verrucosa*

**Table 7.** Anti-Bacterial Activity of *A. malabarica*

| No. | Pathogenic Microorganisms | Zone of inhibition (mm) SD ± Mean | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 50 µg/ml | 100 µg/ml | 200 µg/ml | 300 µg/ml | 400 µg/ml | 500 µg/ml | PC Gentamicin |
| 1. | *Staphylococcus aureus* | 8.5±0.7 | 9.25±0.35 | 10.25±0.35 | 11.25±0.35 | 12.25±0.35 | 15.25±0.35 | 17.5±0.7 |
| 2. | *Streptococcus pyogenes* | 7.5±0.7 | 10.5±0.7 | 11.25±0.35 | 12.25±0.35 | 13.25±0.35 | 14.25±0.35 | 15.5±0.7 |
| 3. | *Propionibacterium acnes* | 6.5±0.7 | 7.25±0.35 | 8.25±0.35 | 10.25±0.35 | 12.5±0.7 | 15.5±0.7 | 19.5±0.7 |
| 4. | *Corynebacterium diphtheria* | 5.25±0.7 | 6.5±0.7 | 9.5±0.7 | 10.25±0.35 | 12.25±0.35 | 14.25±0.35 | 21.5±0.7 |
| 5. | *Pseudomonas aeruginosa* | 7.5±0.7 | 11.5±0.7 | 12.5±0.7 | 13.5±0.7 | 14.25±0.35 | 15.25±0.35 | 22.5±0.7 |
| 6. | *Proteus vulgaris* | 5.25±0.35 | 7.5±0.7 | 8.25±0.35 | 9.25±0.35 | 12.5±0.35 | 14.5±0.7 | 16.5±0.7 |
| 7. | *Aeromonas hydrophilla* | 6.5±0.7 | 8.5±0.7 | 10.25±0.35 | 11.25±0.35 | 13.25±0.35 | 15.25±0.35 | 18.5±0.7 |
| 8. | *Klebsiella pneumonia* | 7.5±0.7 | 8.5±0.7 | 10.25±0.35 | 11.25±0.35 | 13.5±0.7 | 14.5±0.7 | 15.25±0.7 |

SD – Standard Deviation, *Significance - $p< 0.05$

**Table 8.** *Antifungal activity of A. malabarica*

| No | Pathogenic fungi | Zone of inhibition (mm) SD ± Mean | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 50 µg/ml | 100 µg/ml | 200 µg/ml | 300 µg/ml | 400 µg/ml | 500 µg/ml | PC Amphotericin B |
| 1. | *Candida albicans* | 6.5±0.7 | 9.5±0.7 | 8.5±0.7 | 10.5±0.7 | 12.5±0.7 | 14.5±0.7 | 15.5±0.7 |
| 2. | *Aspergillus niger* | 0 | 0 | 4.25±0.35 | 5.5±0.7 | 6.5±0.7 | 8.5±0.7 | 13.5±0.7 |
| 3. | *Sporothrix schenckii* | 0 | 0 | 4.25±0.35 | 4.5±0.7 | 7.5±0.7 | 9.5±0.7 | 19.5±0.7 |
| 4. | *Phialophora verrucosa* | 0 | 0 | 14.5±0.7 | 15.25±0.35 | 17.5±0.7 | 18.5±0.7 | 16.25±0.35 |

SD – Standard Deviation, *Significance - $p< 0.05$

radical scavenging activities, and hydroxyl radical scavenging activities, the antioxidant activity of the entire *A. malabarica* plant was evaluated. The methanolic extract exhibited substantially greater free radical scavenging activity than the standard extract. With concentration, the extract's capacity to neutralize free radicals grew. Significant in vitro antioxidant activity was observed in the ethanolic extract of *A. malabarica.*[21]

*E. coli* (9mm), *S. aureus* (24mm), *P. mirabilis* (10mm), *P. aeruginosa* (20mm), *and K. pneumoniae* (19mm), were some of the gram-positive and gram-negative bacteria that *A. malabarica* antibacterial efficacy was tested against. The inhibitory effects of the two extracts varied. The inhibitory effects of extracts were inversely correlated with the concentration of leaves from plants grown in fields. *A. malabarica* wide ethanolic and methanolic extract showed lower activity in comparison to the aqueous extract when its broad spectrum of activity was tested.[8] These phytochemicals may be related to the observation that aqueous extracts exhibited the highest level of activity against the bacterial strains. The active ingredients in plants typically negatively impact the growth and metabolism of microorganisms.[11]

Studies have been conducted on the potential antibacterial properties of methanol extract against gram-positive and gram-negative bacteria. There have been reports of antibacterial properties in ethanol extract.[15] The study found that ethanol and A. malabarica extract showed significant antibacterial activity, with dose-dependent effects, compared to commonly used indomethacin in illness and infection management.[17]

## CONCLUSION

The current study's findings revealed that the ethanol extract of *A. malabarica* leaves contained a significant number of flavonoids. GC-MS analysis was used in this work to identify 40 chemical constituents from flavonoids leaf extract, with 5 peak compounds. Numerous bioactive compounds found in the plant support traditional practitioners' use of it to treat a range of illnesses. These factors might be the reason for the plant's high level of antioxidant activity. Comparing the different antioxidant capacities with the common antioxidant ascorbic acid. As a result, the results of this investigation indicate that *A. malabarica* ethanol extract may be a promising natural source of antioxidants. An ethanolic extract of *A. malabarica* leaves was the most effective at combating fungal species, after gram-positive and gram-negative bacteria. According to the study, the flavonoid medication ethanol extract was very effective at getting rid of Gram-positive bacteria. *Staphylococcus aureus* and *Streptococcus pyogenes* and moderately effective against *Pseudomonas aeruginosa* and *Aeromonas hydrophilla* Gram-negative bacteria, but useless against *Corynebacterium diphtheria* and *Proteus vulgaris.* Fungi were also successfully inhibited by the highest amount of ethanol extract. The most powerful antifungal action against *Phialophora verrucosa* and *Candida albicans*. According to the results of the study, the leaves of the *A. malabarica* plant had antibacterial and antifungal properties. This also serves as scientific evidence for the use of this herb to treat wounds. Therefore, additional research and careful separation of the active principles may aid in the discovery of new lead compounds that are efficient against diseases caused by free radicals.

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The authors do not have any conflict of interest.

**Data Availability Statement**

This statement does not apply to this article.

**Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Informed Consent Statement**

This study did not involve human

participants, and therefore, informed consent was not required.

**Author Contributions**

Nanditha V and Dr. Muthuselvam. D participated in the design of the workplace.; Data gathering, findings analysis and interpretation, and draft manuscript production; Formal analysis, outcome evaluation, manuscript editing, and finalization; Study design, formal analysis, outcome evaluation, editing, and manuscript finalization; The completed manuscript has been read and approved by the authors.

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