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Extraction of Bioactive Alkaloids from *Anisomeles Malabarica* (L) and Their Biological Applications

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ABSTRACT:
Anisomeles malabarica is a perennial herb with an extensive history of use in traditional medicine. This summary examines the potential of combining multiple naturally occurring alkali compounds for therapeutic purposes against vector-borne diseases, which have been used to treat illnesses for generations. The current study aims to characterize the antioxidant and antimicrobial properties of alkaloids from AMLE using GC-MS, FTIR, and UV-Vis. AMLE was extracted with the Soxhlet apparatus, and the alkaloid compounds were separated with a separating funnel. Antioxidant properties were investigated with DPPH, ABTS, and H_2O_2. Antimicrobial properties were tested against a variety of bacterial and fungal species using the agar well diffusion method. Characterization was carried out to determine the presence of bioactive compounds and functional groups. In this study *Anisomeles malabarica* alkaloids leaf extract exhibited anti-oxidantproperties in dose-dependent manner with the IC_{50} value of 110.2 µg/ml (DPPH), 93.01 µg/ml (ABTS) and 143.4 µg/ml (H_2O_2). The results revealed maximum anti-bacterial activity and anti-fungal activity against *Propionibacterium acnes* (15.5±0.7 mm) and *Aspergillus niger* (8.4±0.56 mm) respectively. GC-MS analysis andFTIR peak values were used to identify the presence of the therapeutic compound chloroxylenol. The functional groups carboxylic acid, aromatic mono-substituted alkene, and aromatic ester were represented. Based on this study, we conclude that alkaloids from AMLE have antioxidant and antimicrobial properties and could serve as a potential target against various diseases.

Keywords: Anisomeles Malabarica, Alkaloid, DPPH, ABTS, GC-MS.

1. INTRODUCTION

Anisomeles malabarica is one of these indigenous plants used for traditional medicine that may be found in India's tropical and subtropical regions. It belongs to the Lamiaceae family, which has 45 genera and 574 species, including 256 endemics. It is an aromatic, densley pubescent perennial herb that grows to a height of 1.2-2 m. It is an upright plant that also known as "Peyimarutti" and is commonly found in India's western ghats from Maharashtra to Kerala and Tamil Nadu. It is also called as "Malabar catmint." These plants are common in Asia, the Maltese Islands, and other Mediterranean countries. It works as an effective Ayurvedic and Siddha medicine [1]. These plants are usually herbs or shrubs with a sweet aroma. Many Lamiaceae species are used in traditional and alternative medicine, accounting for a significant portion of the population. They are also used as culinary and ornamental plants, Mint, thyme, Tulsi, spearmint, and coleus are some examples of culinary and ornamental plants. [2]. Several properties of the plant have been reported which includes allergenic, anthelmintic, anaphylactic, antibacterial, anticarcinoma, antiedemic, antihistaminic, anti-inflammatory, anti-leukemic, antinociceptive, anti-plasmodial, antiseptic, and antiperotic properties [3]. Traditional remedies for rheumatism, dementia, anorexia, fevers, swellings, and other ailments have been developed based on the therapeutic properties of this plant. Anisomeles malabarica is effective in treating wounds caused by Anorexia, dyspepsia, colic, flatulence, intestinal worms, fever in teething babies, intermittent fever, gout, edema, and diarrhea. A recent study investigates plant herbaceous characteristics [4].

The phytoconstituents in medicinal plants can be found and evaluated using a variety of techniques. The goal of the current study is to use the GC-MS analysis to find the various Phyto-constituents that may be useful in the treatment of a variety of diseases and disorders. This study analyses the alkaloid compounds and characterizes using UV-Vis and FTIR, the spectrum profiles of *Anisomeles malabarica* using visible or close light. These analysis focuses on the structure of isolated biomolecules, with wavelength being a key distinguishing feature. In alternative medicine, antioxidants are thought to be important compounds with considerable health benefits that come from a range of medicinal plants. The ability of medicinal plant components to prevent or control disease has been linked to their antioxidant qualities and therefore Anisomeles malabarica leaf can be used in pharmaceuticals, complementary treatments, and natural therapies for its antibacterial properties.

2. MATERIALS AND METHODS

Collection of Plant Materials

Fresh, young, and healthy *Anisomeles malabarica* (L) R. Br. ex-Sims leaves were collected from Peramangalam Musiri (11.011108^{o}N, 78.659052^{o}E), a dry rocky region in Tiruchirappalli district, Tamil Nadu, India. Rapinat Herbarium Voucher number: V.N. 001 at St. Joseph College, Tiruchirappalli, identified and confirmed the plant material's authenticity.

Extraction of Alkaloid Compound

AMLE was extracted using the Soxhlet extraction using ethanol as solvent. Soxhlet extraction was performed by the methodology described by Jeyakumar *et al*., with slight modifications [5] The crude extract was obtained from the Soxhlet extract through filtration and concentration in rotary evaporator under reduced pressure, which was taken for the extraction of alkaloid compound using separating funnel “Figure.1”. 10% HCL and 10% Ammonia, 50 ml of chloroform were added to the crude extract and allowed for layer separation, after the layer separation chloroform solvent was evaporated using the Rotary vacuum evaporator and the final substance was stored at -20^{o}C for further analysis. The entire separating funnel

experiment was performed based the methodology described by Ji Y et al., with slight modifications [6]. Mayer's reagent was used to confirm the presence of alkaloid compound "Figure.2".

Characterization of *Anisomeles Malabarica* Alkaloid Leaves Extract

GC-MS Analysis: GC-MS analysis was used to determine the presence of active components and the chemical makeup of *Anisomeles malabarica* alkaloid leaf extract. The GC/MS Shimadzu QP-2020 plus with thermal desorption system TD 20 was used for analysis, and the column was SGE BX-30, measuring 30m X 0.25mm X 0.25m. The oven's beginning temperature was kept at 50°C for isothermal heating for 0 minutes, with a rate of 0°C/min, and the end temperature was 250°C isothermal heating was performed for 2 minutes, with a rate of 6°C/min. Helium was used as the carrier gas. In the split mode, the injection volume was 0.11. The intake line is heated to 200°C, and the source to 250°C. Mass spectra in the 45-450 amu range were captured using an electron impact ionization energy of 70 eV. The sample ran for 40 minutes. The solvent delay was about 0 to 2 minutes. By comparing the generated mass spectra of unknown peaks with those stored in the Wiley and NIST (National Institute of Standards and Technology) mass spectral electronic libraries, the compounds were identified.

UV- VIS Spectroscopic Analysis

Anisomeles malabarica alkaloid leaf extract was subjected to a UV-visible spectrophotometric analysis using a 10-mm cell at room temperature and Perkin Elmer, USA Model: UV-2600 Series with a slit width of 5.0 nm. The extract was examined using visible and UV light with a wavelength range of 220-800 nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper. The sample is diluted to a 1:10 ratio with the same solvent.

FTIR Analysis

The potassium bromide (KBr) pellet (FTIR grade) method was used to conduct an FTIR analysis of the *Anisomeles malabarica* alkaloid leaf extract. The spectrum was recorded using a Jasco FTIR-6300 Fourier transform infrared spectrometer equipped with a JASCO IRT-7000Intron Infrared Microscope in transmittance mode and operated at a resolution of 1 cm.

Antioxidant Potential Anisomeles Malabarica

DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was performed based on the method of Thaipong et al., with slight modifications [7]. 0.1 mM DPPH solution was prepared using the methanol and test sample was prepared at various concentrations (500, 250, 100, 50, and 10 µg/ml). 100µl of DPPH solution was added to the test samples and incubated for 30 minutes at room temperature. After the incubation, absorbance of the test samples was measured at 517nm using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the formula,

$$\text{Inhibition } \% = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Ascorbic was used as a standard. The standard graph equation was used to compute the percentage of inhibition, and a non-linear regression approach was used to estimate the IC50 values from the percent inhibition versus concentration plot.

ABTS Radical Cation Scavenging Assay

ABTS assay was done according to the methodology of Thaipong *et al*., with slight modifications [7] 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 sample (500, 250, 100, 50, and 10 µg/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 calculated using the formula,

$$\text{Inhibition } \% = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging assay was performed used the methodology followed by Purushothaman et al., with slight modifications [8]. 43 mM hydrogen peroxide solution is prepared using phosphate buffer. Test sample (500, 250, 100, 50, and 10 µg/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 percentage of inhibition was calculated using the formula,

$$\text{Inhibition } \% = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Antimicrobial Activity

Antibacterial activity of alkaloids from *Anisomeles malabarica* leaf extract was studied in eight bacterial species, which includes 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*). The antibacterial and antifungal activity was evaluated using the agar well diffusion method with Gentamicin and Amphotericin B as positive control. The plates were incubated for 24 hours at37ºC and the zone of inhibition was measured and the calculations were computed using the Graph Pad Prism 6.0 software (USA).

3. RESULTS

Extraction of Alkaloids Compounds from Crude Extract

The total alkaloid present in the *Anisomeles malabarica* leaves were extracted from the ethanolic crude extract by using a separating funnel as shown in "Figure 1A". The addition of Mayer's reagent to the alkaloid extracts showed the colour change from Greenish-Black and Greenish-Brown respectively to black-brown as shown in "Figure1 (B1)". Similarly, Wagner's reagent showed white, creamy precipitate or reddish-brown in Colour suggests the presence of alkaloids in the sample "Figure. 1(B2)".

Characterization of Alkaloid from *Anisomeles Malabarica*

GCMS: GCMS analysis was used to find the alkaloid compounds present in the crude extract. In GCMS characterization of *Anisomeles malabarica* leaves extract depicted 45 alkaloid compounds. The GCMS chromatogram is shown in "Figure 2" Chloroxylenol was the peak compound with the area of 32.23%. The top 5 peaks were tabled in Table 1. Apart from the chloroxylenol, other compounds like 1,3-bis-(2-cyclopropyl,2-methylcyclopropyl)-buthen-2, -one-1 (8.14%), ricinoleic acid (5. Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-4a. alpha.,7. alpha.,8a. - (4.94%), and 2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (4.17%) were obtained in GCMS analysis.

UV-VIS Analysis

The qualitative UV-VIS profile of the alkaloid extract of *Anisomeles malabarica* leaves was taken at the wavelength range of 220 nm to 800 nm due to the sharpness of the peaks and acceptable baseline. The profile showed peaks at 230, 280, 420, and 654 nm, with corresponding absorption values of 4.485, 1.537, 0.157, and 0.044 in Table 2. The alkaloid leaves extract of Anisomeles malabarica is nearly transparent in the wavelength range of 220-800 nm, as shown by "Figure 3" absorption spectra.

FTIR Analysis:

The functional group of the active components was identified by the FTIR analysis. Fig. 4. displays the FTIR spectrum of the Anisomeles malabarica alkaloid leaf extract in the form of a KBr pallet. The functional groups of the constituents were divided based on the ratio of the peak when the plant extract was transferred into the FTIR spectrum. The FTIR peak values and functional groups carboxylic acid, carbodiimide, primary alcohol, aromatic mono-substituted alkene, and aromatic ester were represented in Table 3. Hydroxyl group was measured at the absorption range of 3380.07 cm^{-1}. C-H stretching alkane group was obtained at 2974.38 cm^{-1}, 2924.82 cm^{-1} and 2900.10 cm^{-1}. The vibrational absorption of methyl band was identified at 1382.08 cm^{-1}. C-O stretching alkyl aryl ether band was measured at 1271.99 cm^{-1} C-C stretching cycloalkane functional group was measured at 434.88 cm^{-1}.

Dpph Free Radical Scavenging Activity:

The antioxidant property of *Anisomeles malabarica* alkaloid extract was evaluated for DPPH scavenging activity with ascorbic acid as standard. The IC_{50} value of *Anisomeles malabarica* leaves extracts against DPPH was identified as 110.2 µg/ml. 62.7% of inhibition was measured at 500µg/ml and 37.7% of inhibition at 10µg/ml "Figure 5" Table 4. This revealed the antioxidant property of *Anisomeles malabarica* alkaloid extract against DPPH.

ABTS Radical Cation Scavenging Activity:

The ABTS test was used to determine the antioxidant activity of the alkaloid extract of Anisomeles malabarica. Ascorbic acid was used as the standard for comparing the antioxidant property. *Anisomeles malabarica* showed the IC_{50} value of 93.01 µg/ml against ABTS. 90.04% of ABTS inhibition was obtained at 500µg/ml and 60.6% at 10µg/ml whereas ascorbic acid showed the inhibition rate of 96.27% "Figure 6" Table 5. This confirms the antioxidant property of Anisomeles malabarica.

Hydrogen Peroxide Scavenging Activity:

This technique works on the principle that when hydrogen peroxide oxidizes, it loses some of its absorbance. In addition to naturally occurring hydrogen peroxide, immune cells may actively produce it to neutralize foreign objects. Hydrogen peroxide scavenging activity of Anisomeles malabarica alkaloid extract was found to be 143.4 µg/ml. At 500µg/ml, 77.8% of H_2O_2 inhibition and 38.5% of H_2O_2 inhibition at 10µg/ml was obtained. Ascorbic acid was used as a standard, and it showed 80.01% of inhibition "Figure 7" Table 6.

Antimicrobial Activity

Antimicrobial activity of alkaloids 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 negative bacteria (4–14 mm) "Figure 8" were subject to the same degree of inhibition as gram-positive bacteria (4-15mm) "Figure 9" The most effective antibacterial effect was attained against Propionibacterium acnes (15.5±0.7 mm), followed by *Aeromonas hydrophilla* (14.5±0.7mm) *Corynebacterium diphtheria*

(13.5±0.7 mm), *Pseudomonas aeruginosa* (12.5±0.7mm), *Streptococcus pyogenes* (11.5±0.7 mm) was achieved at 500 µg/ml Table 7. However, 50 µg/ml exhibited the greatest suppression against *Aeromonas hydrophilla* (10.5±0.7mm) as maximum inhibition. While *Proteus vulgaris* (50 µg/ml, as 5.5±0.7 mm) and *Staphylococcus aureus* (100 µg/ml, as 4.25± 0.35 mm) displayed the least activity compared to the other examined pathogens, respectively. The antifungal effects of *A. malabarica* alkaloid leaves extracts were studied after the 72 hours of incubation, the inhibition diameter was evaluated using a zone scale. The maximum anti-fungal activity "Figure 10" was obtained at *Aspergillus niger* (8.4±0.56 mm), followed by *Candida albicans* (6.5±0.7mm), and *Sporothrix schenckii* (6.25±0.35mm) at 500µg/ml Table 8. However, 50 µg/ml exhibited the greatest suppression against Aspergillus niger (7.25±0.35 mm), whereas Candida albicans (4.25±0.35 mm), *Phialophora verrucosa* (5.25±0.35 mm), and *Sporothrix schenckii* (0 mm) each displayed the least action in comparison to the other studied pathogens. These values were computed using Graph Pad Prism 6.0 software (USA).

4. DISCUSSION

Anisomeles malabarica has various therapeutic properties like anti-inflammatory [9], anti-pyretic [10], anti-cancer [11], antimicrobial properties, and helps in treating various ailments like halitosis, epilepsy, hysteria, dementia, anorexia, dyspepsia, coli, flatulence, and intestinal worms. The presence of various phytocompounds like steroids, flavonoid, and terpenoids was reported by Lavanya et al., these phytocompounds plays a major role in therapeutic property [12] yet in our study we confirmed the antimicrobial and antioxidant activity of alkaloids. Antioxidants play an important role in neutralizing the free radicals and ROS [13]. It prevents the cells damage caused by the free radicals, these damage leads to the various diseases like cardiovascular diseases, cancers, and inflammation [14]. The aim of the present study was to evaluate the presence of alkaloid compound in *Anisomeles malabarica* and their antimicrobial and antioxidant properties. *Anisomeles malabarica* alkaloid compound was extracted using the separating funnel apparatus. To find the antioxidant property of *Anisomeles malabarica* alkaloid extract, DPPH radical scavenging assay, ABTS radical scavenging activity and hydrogen peroxide scavenging activity were performed. The DPPH scavenging activity of *Anisomeles malabarica* alkaloid extract was found to be 110.2 µg/ml. The IC_{50} value of *A. malabarica* against ABTS scavenging activity was measured as 93.01µg/ml and in H_2O_2 scavenging assay, 143.4 µg/ml was measured as the IC_{50} value. Lavanya et al., performed the in-vitro antioxidant property of *Anisomeles malabarica* methanolic extract and concluded the DPPH scavenging activity [12]. In other study Lavanya et al., revealed the anti-inflammatory, anti-platelet, and anti-arthritic activity of Anisomeles malabarica leaf methanolic extract [12]. Vinod et al., revealed that methanolic extract of A. malabarica was most effective than hexane extract in scavenging the DPPH [15]. In our study, alkaloids were extracted from Anisomeles malabarica leaves and they showed the dose dependent inhibition against DPPH, ABTS and H_2O_2. Most of the previous study has been conducted in methanolic extract and few studies in ethyl acetate and other solvents, there were only less amount of works has been done with ethanolic extract of *Anisomeles malabarica* leaves, while comparing with the standard ascorbic acid, our extract showed maximum antioxidant property against DPPH, ABTS and H_2O_2. Characterization like GCMS, FTIR, UV-Vis helped to evaluate the presence of alkaloid compounds in Anisomeles malabarica leaf extract. In our study GCMS analysis revealed the presence of 45 alkaloid compounds, with chloroxylenol as peak compound. This compound was also known as PCMX. it has versatile antibacterial property of eliminating most bacterial and fungal propagules. UV-Vis and FTIR analysis revealed the various functional groups present in *Anisomeles malabarica* alkaloid extract.

Remya mohanraj *et al.*, concluded that the methanol extract of *Anisomeles malabarica* leaf has good antibacterial property against four pathogenic bacteria includes *Klebsiella pneumonia, Staphylococcus aureus, Vibrio cholerae, and Pseudomonas aeruginosa* while the hexane ethylacetate extract has reduced antibacterial activity [16]. In previous study, six soil-prevalent bacteria tested for antibacterial activity using *A. malabarica* leaves aqueous extract. Bacillus subtilis and *Staphylococcus aureus* showed maximum inhibition at 50 µg/ml, followed by *Pseudomonas fluorescens* and *Erwinia tracheiphila* [17] In our study, alkaloid extract of A. malabarica exhibited maximum antibacterial property against *Propionibacterium acnes* (15.5±0.7 mm), *Aeromonas hydrophilla* (14.5±0.7mm) *Corynebacterium diphtheria* (13.5±0.7 mm), Pseudomonas aeruginosa (12.5±0.7mm), *Streptococcus pyogenes* (11.5±0.7 mm) and antifungal property against *Aspergillus niger* (8.4±0.56 mm), Candida albicans (6.5±0.7mm), *Sporothrix schenckii* (6.25±0.35mm) and *Phialophora verrucosa (*5.25±0.35 mm) thereby revealing the antimicrobial property of *A. malabarica.*

5. CONCLUSION

In our study we studied the antimicrobial and antioxidant property of alkaloids from AMLE. Characterization techniques like GCMS, FTIR and UV-VIS revealed the various alkaloid compounds and their functional groups present. GCMS revealed the presence of potential bioactive compound chloroxylenol. Antioxidant assay confirmed the antioxidant property of alkaloids from *A. malabarica* against DPPH, ABTS and H_2O_2. Taken together, these results confirm that the alkaloids of *A. malabarica* leaves could be an excellent antioxidant and therapeutic compound against various diseases.

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Conflicts of Interest
The authors declare that they have no conflict of interest.

Abbreviations:
- AMLE_ Anisomeles malabarica leaf Extract.
- HCl_ Hydrochloric acid.
- GC-MS_ Gas chromatography and mass spectrometry.
- PCMX _ para-chloro-meta-xylenol.
- UV-Vis _ UV- Vis spectrophotometry.
- FTIR _ Fourier Transform Infrared Spectroscopy.
- DPPH_ 1,1-diphenyl-2-picrylhydrazyl
- ABTS_ 3-ethylbenzothiazoline-6-sulfonic acid
- H_2O_2_ Hydrogen peroxide.
- IC_{50}_ Half maximal inhibitory concentration.
- ROS _ Reactive Oxygen Species.

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Figure legends

Fig. 1: Extraction of alkaloid from *Anisomeles malabarica* leaves (A) and positive results of Mayer's (B1) and Wager's (B1) confirmative tests

Fig. 2: Chromatogram of Ethanolic extract of *Anisomeles malabarica* leaves on alkaloids compounds.

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

Fig. 4: FTIR spectra of pure Ethanolic *Anisomeles malabarica* extract

Fig. 5: Evaluation of DPPH free-radical scavenging activity of *Anisomeles malabarica* leaf extra6t.

Fig. 6: Evaluation of ABTS radical cation scavenging activity of *Anisomeles malabarica* leaf extract.

Fig. 7: Evaluation of *Anisomeles malabarica* leaf extract hydrogen peroxide activity
Fig. 8: Antibacterial property of *Anisomeles malabarica* against Gram-positive bacteria using the Agar diffusion Method, A&B *Staphylococcus aureus*, C&D *Streptococcus pyogenes*, E&F *Propionibacterium acnes*, and G&H *Corynebacterium diphtheria*
Fig. 9: Antibacterial property of *Anisomeles malabarica* against Gram-Negative bacteria using the Agar diffusion Method, A&B *Pseudomonas aeruginosa*, C&D *Proteus vulgaris*, E&F *Aeromonas hydrophilla*, and G& H *Klebsiella pneumoniae*
Fig. 10: Antifungal property of *Anisomeles malabarica* using the Agar diffusion Method, A&B *Candida albicans*, C&D *Aspergillus niger*, E&F *Sporothrix schenckii*, and G& H *Phialophoraverrucosa*

List of figures

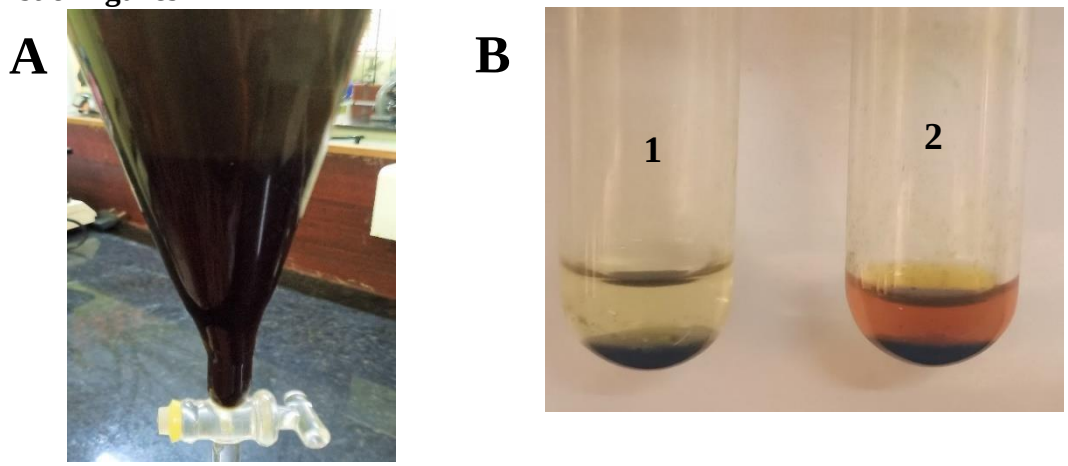


Fig. 1: Extraction of alkaloid from *Anisomeles malabarica* leaves (A) and positive results of Mayer's (B1) and Wager's (B2) confirmative tests

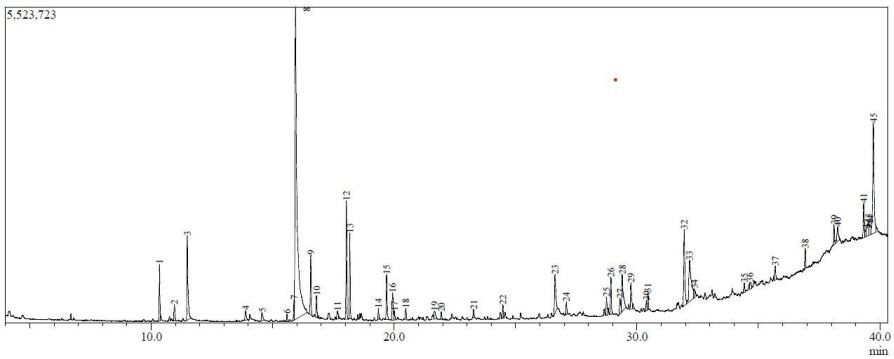


Fig. 2: GCMS Chromatogram of Ethanolic extract of *Anisomeles malabarica* leaves on alkaloids compounds.

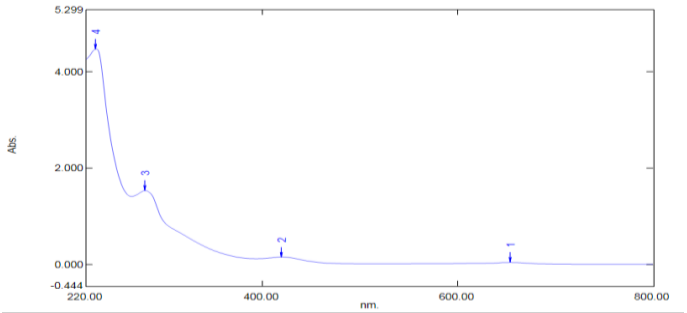


Fig. 3: UV-VIS spectra of pure ethanolic *Anisomeles malabarica* extract

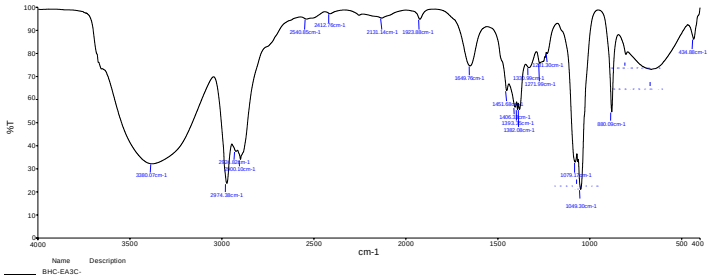


Fig. 4: FTIR spectra of pure Ethanolic *Anisomeles malabarica* extract

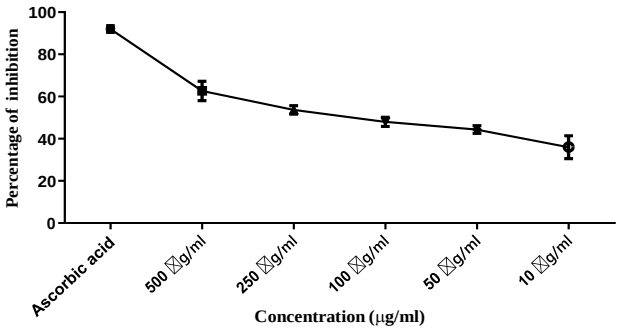


Fig. 5: Evaluation of DPPH free-radical scavenging activity of *Anisomeles malabarica* leaf extract.

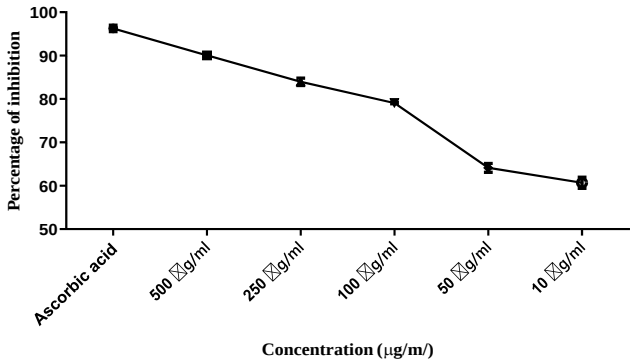


Fig. 6: Evaluation of ABTS radical cation scavenging activity of *Anisomeles malabarica* leaf extract.

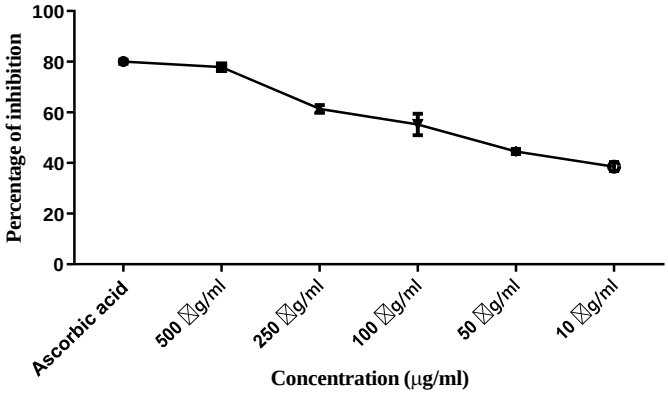


Fig. 7: Evaluation of *Anisomeles malabarica* leaf extract hydrogen peroxide activity

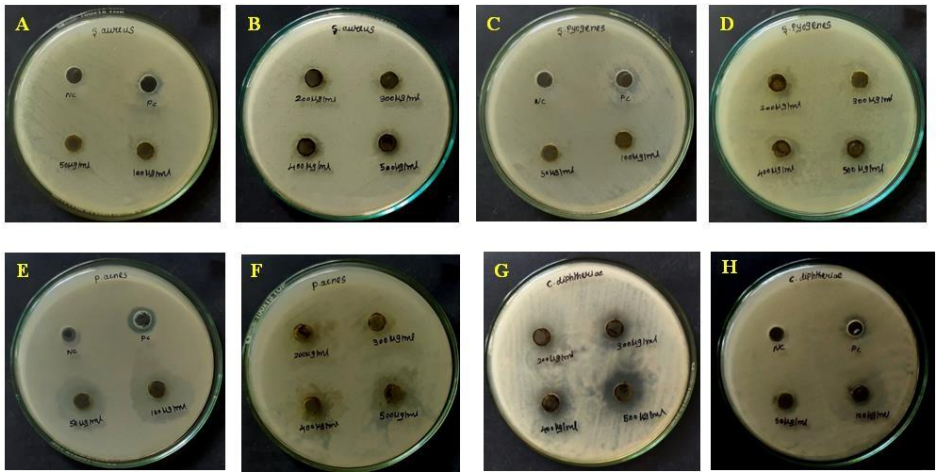


Fig. 8: Antibacterial property of *Anisomeles malabarica* against Gram-positive bacteria using the Agar diffusion Method, A&B Staphylococcus aureus, C&D Streptococcus pyogenes, E&F Propionibacterium acnes, and G&H Corynebacterium diphtheria

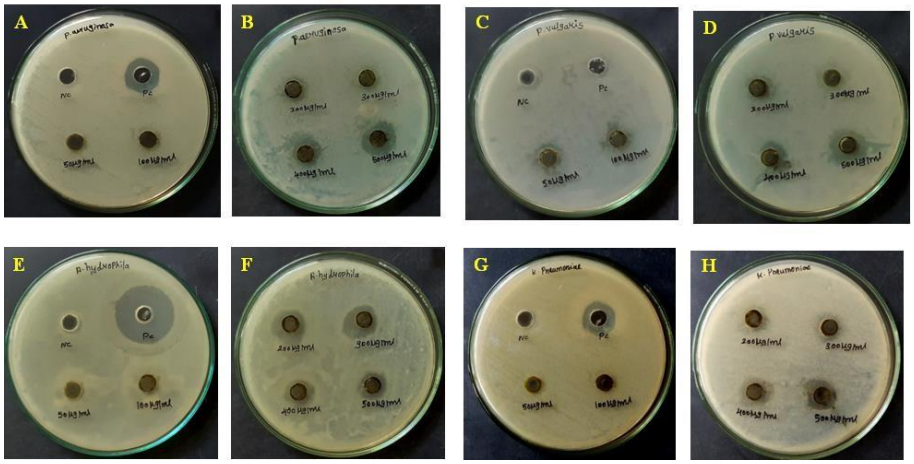


Fig. 9: Antibacterial property of *Anisomeles malabarica* against Gram-Negative bacteria using the Agar diffusion Method, A&B Pseudomonas aeruginosa, C&D Proteus vulgaris, E&F Aeromonas hydrophilla, and G& H Klebsiella pneumoniae

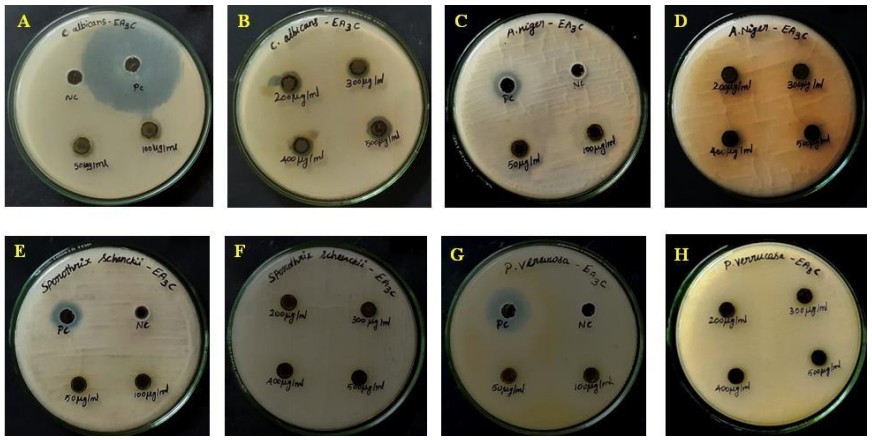


Fig. 10: Antifungal property of *Anisomeles malabarica* using the Agar diffusion Method, A&B Candida albicans, C&D Aspergillus niger, E&F Sporothrix schenckii, and G& H Phialophora verrucosa

List of Tables

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

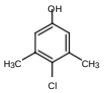
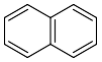
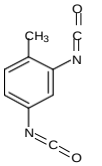
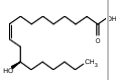
S.No	Mass Peak	Ret. Time	Ret. index	SI	Area %	Compound Name	Molecular Formula	Molecular Weight	Molecular Structure	Biological Activity
1.	84	15.945	1307	94	32.23	Chloroxylenol	C8H9C10	156		(Keke Ma et al.,2021)
2.	28	18.040	1469	98	4.94	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta)	C15H24	204		(ATSDR.,2005).
3.	177	31.945	0	86	4.17	2,4-diisopropenyl-1-methyl-1-vinylcyclohexane	C15H24	204		(Xue Feichen et al.,2023)
4.	138	32.165	2337	96	5.08	Ricinoleic acid	C18H34O3	298		(Vieira et al., 2000). (Sai H.S. Boddu et al.,2015)
5.	269	39.730	0	76	8.14	1,3-bis-(2-cyclopropyl,2-methylcyclopropyl)-buthen-2,-one-1	C18H26O	258		(Sofia Ayari-Guentri et al.,2017)

Table 2: UV-VIS peak values of extract of *Anisomeles malabarica*

S. No.	Wavelength (nm)	Absorbance
1.	654	0.044
2.	420	0.157
3.	280	1.537
4.	230	4.485

Table 3: Structural features of the *Anisomeles malabarica* by FTIR spectrum

S.no	Peak value	Stretching	Interpretation
1.	3380.07cm-1	O-H stretching	alcohol
2.	2974.38cm-1	C-H stretching	alkane
3.	2924.82cm-1	C-H stretching	alkane
4.	2900.10cm-1	C-H stretching	alkane
5.	2540.85cm-1	C≡N stretching	nitrile
6.	2412.76cm-1	C-H stretching	alkane
7.	2131.14cm-1	N=C=N stretching	carbodiimide
8.	1923.88cm-1	C=C=C stretching	allene
9.	1649.76cm-1	C=N stretching	imine / oxime
10.	1451.68cm-1	N–O asymmetric stretch	nitro compounds
11.	1406.33cm-1	S=O stretching	sulfonyl chloride
12.	1393.35cm-1	S=O stretching	sulfonyl chloride
13.	1382.08cm-1	C-H bending	alkane
14.	1330.99cm-1	O-H bending	Phenol
15.	1271.99 cm-1	C-O stretching	alkyl aryl ether
16.	1231.30cm-1	C-N stretching	amine
17.	1079.17cm-1	C-O stretching	primary alcohol
18.	1065.40cm-1	C-O stretching	primary alcohol
19	1049.30cm-1	CO-O-CO stretching	anhydride
20	880.09cm-1	C=C bending	alkene
21.	803.07cm-1	C-H stretching	1,2,3,4-tetrasubstituted
22.	666.25cm-1	C=H bending	alkynes
23.	434.88cm-1	C-C stretching	Cycloalkane

Table 4: DPPH radical scavenging activity

Concentration (µg/mL)	% of inhibition
Control	1.299±0.2403269
10 µg/ml	37.733333±2.9156189
50 µg/ml	44.4133±1.81575
100 µg/ml	48.0333 ±2.14262
250 µg/ml	53.6767±1.99189
500 µg/ml	62.7367±4.59165
Ascorbic acid	92.0433±1.60288

Table 5: ABTS radical scavenging activity

Concentration (µg/mL)	% of inhibition
Control	2.774±0.10964
10 µg/ml	60.6767±1.04936

50 µg/ml	64.1267±0.84231
100 µg/ml	79.0633±0.14817
250 µg/ml	83.9633±0.70693
500 µg/ml	90.0433±0.27084
Ascorbic acid	96.27±0.61188

Table 6: Hydrogen peroxide scavenging activity

Concentration (µg/mL)	% of inhibition
Control	1.89±0.64272934
10 µg/ml	38.566667±1.78500233
50 µg/ml	44.51±0.996243
100 µg/ml	55.2133±4.267626
250 µg/ml	61.3867±1.519254
500 µg/ml	77.8967±0.780534
Ascorbic acid	80.0167±0.80077

Table 7: Antibacterial activity of *A.malabarica*

S.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	0	4.25±0.35	5.25±0.35	6.5±0.7	7.5±0.7	8.5±0.7	7.5±0.7
2.	Streptococcus pyogenes	0	0	7.5±0.7	9.5±0.7	10.5±0.7	11.5±0.7	13.5±0.7
3.	Propionibacterium acnes	9.25±0.35	10.25±0.35	11.25±0.35	12.25±0.35	14.5±0.7	15.5±0.7	7.5±0.7
4.	Corynebacterium diphtheria	0	4.5±0.7	7.5±0.7	9.5±0.7	10.25±0.35	13.5±0.7	5.5±0.7
5.	Pseudomonas aeruginosa	0	4.25±0.35	4.5±0.7	6.5±0.7	10.5±0.7	12.5±0.7	14.5±0.7
6.	Proteus vulgaris	5.5±0.7	6.25±0.35	6.25±0.35	6.5±0.7	8.5±0.7	11.5±0.7	4.5±0.7
7.	Aeromonas hydrophilla	10.5±0.7	11.5±0.7	12.25±0.35	13.25±0.35	14.25±0.35	14.5±0.7	28.5±0.7
8.	Klebsiella pneumoniae	0	0	6.5±0.7	7.25±0.35	8.5±0.7	10.5±0.7	10.5±0.7

Table 8: Antifungal activity of *A. malabarica*

S.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	4.25±0.35	5.25±0.35	5.5±0.7	5.5±0.7	6.25±0.35	6.5±0.7	38.5±0.7
2.	Aspergillus niger	7.25±0.35	8.25±0.35	8.25±0.35	8.3±0.42	8.35±0.49	8.4±0.56	10.5±0.7
3.	Sporothrix schenckii	0	0	5.25±0.35	5.5±0.7	6.25±0.35	6.5±0.7	9.5±0.7
4.	Phialophora verrucosa	5.25±0.35	5.5±0.7	6.25±0.35	6.3±0.42	6.4±0.56	6.5±0.7	35.5±0.7