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Full Length Research Article

Antimicrobial Activity of Various Fractions of Different Extracts from *Anisomeles malabarica* (L). R.Br.

N. Thavapudalvi*[1], S. Vasantha[2], V. Ambikapathy[3], A. Panneerselvam[4], P. Prakash[5], A. Kanmani[6] and D. Rathna[7]

[1-4] P. G. and Research Department of Botany, A. V. V. M. Sri Pushpam College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli - 24), Poondi, Thanjavur - 613 503, Tamil Nadu, India
[5-7] Indian Biotrack Research Institute, Thanjavur - 613 005, Tamil Nadu, India

Abstract

The current study deals with the antimicrobial activity of various fractions of different extracts from *Anisomeles malabarica* and was analyzed by using the agar well diffusion method. To determine the zone of inhibition in bacterial and fungal organisms using plant extracts of aqueous and benzene solvents. The concentration of extracts in (standard, 25, 50, 75, 100µg/ml) was tested against bacteria such as *Streptococcus pyogenes*, *E. coli*, *Salmonella* sp and *Klebsiella pneumoniae*. For antibacterial activity, the zone of inhibition (mm) of extracts was compared to standards such as streptomycin. The extracts of *Anisomeles malabarica* were tested against fungi such as *Aspergillus niger, A. flavus, A. terreus* and *A. fumigatus* as well as standards like fluconozole for antifungal activity. The maximum zones of inhibition for leaves, flowers and seeds were aqueous in bacteria and benzene in fungi. When all parts of *Anisomeles malabarica* were compared, the maximum concentration was 100 µg/ml. The results support the use of *A. malabarica* to treat a variety of infectious illnesses. Finally, these results suggest that *Anisomeles malabarica* can be used as a source of antibiotic compounds for potential bacterial infection treatment and medication development. Recent research has identified the most valuable *Anisomeles* species for phyto-pharmaceuticals.

***Key words*:** *Anisomeles malabarica*, Plant extracts, Solvents, Streptomycin, Fluconozole, Pharmaceuticals

The large percentage of antibiotics have traditionally come from natural sources products produced by microorganisms, but as herbal medicine becomes more commonly recognized as a complementary form of treatment, it has become essential to search for active compounds in medicinal plants, as these could be potential sources of new antibiotic prototypes [1]. New antimicrobial compounds have been obtained for from a variety of sources, including microorganisms, animals, and plants. Folk medicines are one of these resources. Systematic testing of them could lead to the discovery of fresh, powerful chemicals [2]. In recent years, extracts and biologically active chemicals obtained from plant species used in herbal medicine have received a lot of attention due to the negative side effects and antibiotic resistance that pathogenic microorganisms develop [3]. The concept of food that provides both nutritional and therapeutic benefits is especially popular at the moment. Many organic substances that have been extracted from plants have shown a wide range of biological activities. Essential oils from aromatic and medicinal plants are given special consideration as potential natural food preservation agents among these diverse types of organic substances [4]. The Lamiaceae family includes the genus *Anisomeles* L. R. Br., which has approximately 20 species with a major range in tropical Asia and Australia. These are arid, sunny, semi-bushy, stony, and rocky areas annual or perennial aromatic plants. This genus many members are well known for having aromatic and medicinal properties [5]. Root, stem, flower, fruit, twig exudates, and modified plant organs are among the various parts that are used. Many other raw drugs are collected in larger quantities and traded on the market as the raw material for many herbal industries, while other of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use [6]. Newer antibacterial have increased therapeutic options, increasing the need for in vitro testing of some organic medicines antibacterial susceptibility. Therefore, there is a need to create more potent and safe antibacterial agents

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Correspondence to: N. Thavapudalvi, P. G. and Research Department of Botany, A.V.V.M. Sri Pushpam College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli - 24) Poondi, Thanjavur - 613 503, Tamil Nadu, India, E-mail: biorese2016@gmail.com

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derived from plants [7]. Bioactive compounds, which have been used as herbal medicines since ancient times, are found within plants. The majority of the population, especially in developing nations, has relied on medicinal plants for their primary healthcare needs for hundreds of generations [8-12]. In the past, aerial parts were used to treat a variety of conditions like rheumatism, pregnancy, paralysis, epilepsy, convulsions, and spasms. *A. indica* leaves are applied to snake bites as well as persistent skin eruptions, and they are chewed for dental problems [13-15]. The plant is used to treat intermittent fever, intestinal mucus layer thickness, stomach infection, and gastric catarrh [16].

MATERIALS AND METHODS

Collection of plant materials

The *Anisomeles malabarica* plant leaves, flowers and seeds were collected from A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India. The leaves were separated from the collected plant and dried under shade. After drying it was pulverized to powder in a mechanical grinder for further studies [17].

Preparation of plant extract

The plants and inflorescence were washed with running water before being air dried in the shade at room temperature. Also, with help of a commercial electrical stainless-steel blender, the dried plant material was mechanically grinded. Using a hot continuous percolation procedure, the powdered plant material was extracted for 24 hours in a Soxhlet apparatus, aqueous and benzene (80°C). The plant extracts were filtered using a Buchner funnel and Whatmann number 1 filter paper.

Antimicrobial activity

Using pathogenic microorganism: In the experiment, four bacterial and fungal strains were used for antimicrobial activity. The preserved strains were obtained from the Indian Biotrack Research Institute, Thanjavur.

Agar well – diffusion method: It was followed for determination of antimicrobial activity [18]. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old-broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About standard, 25, 50, 75 and 100µg/ml of aqueous and benzene extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions and the plates were incubated in an upright position at 37˚C ± 2˚C for 24 h for bacterial and 28˚C ± 2˚C for fungi. Results were recorded as the presence or absence of inhibition zone. Triplicates were maintained and the average values were recorded for antimicrobial activity.

Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

RESULTS AND DISCUSSION

The *Anisomeles malabarica* antibacterial activities revealed that leaf extract had the best antimicrobial properties. Better zone of inhibition was produced by the extract against *Staphylococcus aureus, Pseudomonas* and *E. coli*. The least zone of inhibition against *Klebsiella* sp and *Streptococcus epidermis* [17]. Both polar and non-polar extracts to analyze the solvent extracts. The results indicate that, in comparison to non-polar chemicals, the plant leaf provided the greatest zone of inhibition for polar compounds. *Anisomeles malabarica* leaf methanol extract has good antibacterial activity, whereas extract of four pathogenic bacteria, K. *pneumoniae, S. aureus, V. cholerae* and *Aeruginosa* had less antibacterial activity [19]. The antimicrobial properties of *Anisomeles malabarica* leaf extracts are also examined in this research. The antibacterial activity of extracts was tested using the agar well diffusion method against clinically important multidrug resistant bacterial strains such *Staphylococcus aureus, Bacillus subtillis* and *Klebsiella pneumoniae*. It displayed antimicrobial activity that was concentration-dependent. With the largest inhibitory zone when compared to the common antibiotic drug tetracycline, high antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* bacterial strains [20].

In the present study, the human pathogens of bacteria were used, namely *Streptococcus pyogenes, E. coli, Salmonella* sp and *Klebsiella pneumoniae*. The higher concentration of 100g/ml compared with all concentrations excluding standard, *Anisomeles malabarica* leaves in aqueous extract exhibited the maximum zone of inhibition at *Klebsiella pneumoniae* (13.01±0.56µg/ml) and at the benzene extract exhibited at Salmonella sp (6.58±0.11µg/ml) and the minimum zone of inhibition against Streptococcus pyogenes (3.02±0.06µg/ml) and (4.13±0.12µg/ml). The lower concentration of 25µg/ml *Anisomeles malabarica* leaves aqueous and benzene extract exhibited the maximum zone of inhibition against *Klebsiella pneumoniae* (11.2±0.25µg/ml) and (7.14±0.11 µg/ml) and the minimum zone of inhibition against *Steptococcus pyogenes* (5.12±0.15 µg/ml) and (5.00±0.05µg/ml). When compared to all other concentrations, all bacterial pathogens, including the standard streptomycin are presented at their maximum. The standards for streptomycin are highly presented in *E. coli* (14.24±0.05µg/ml) in aqueous extract and in *Klebsiella pneumoniae* (10.48±0.25µg/ml) in benzene extract (Fig 1). The fungi pathogens are used, namely *Aspergillus niger, A. flavus, A. terreus* and *A. fumigatus*. In higher concentration 100 µg/ml, *Anisomeles malabarica* leaves in aqueous extract showed the maximum zone of inhibition at *A. fumigatus* (11.06±µg/ml), and at the benzene extract showed the maximum at *A. flavus* (12.66±0.03µg/ml), and the minimum zone of inhibition against *A. niger* (4.12±0.11µg/ml) and (6.66±0.41µg/ml). The maximum zone of inhibition against *A. fumigatus* (11.3±0.21µg/ml) and (13.2±0.15µg/ml) and the minimum zone of inhibition against *A. niger* (7.00±0.28µg/ml) and (6.00±0.24 µg/ml) were seen at the lower concentration of 25µg/ml *Anisomeles malabarica* leaves aqueous and benzene extract. All bacterial pathogens, including the common strain of fluconozole, are present at their highest concentration when compared to all other concentrations. The standards for fluconozole are very high in *A. flavus* (13.40±0.08µg/ml) in aqueous extract and (14.24±0.02µg/ml) in aqueous extract (Fig 2).

Using methods such as zone of inhibition, the crude extracts of *Anisomeles malabarica* were tested for antimicrobial activity against a number of bacterial pathogens, including those that are Gram positive (*Micrococcus luteus, Bacillus subtilis* and *Staphylococcus aureus*), Gram negative (*Enterobacter aerogens, Salmonella typhimurium, Klebsiella pneumoniae, Shigella flexneri* and *Proteus vulgaris*) bacterial and *fungal* (*Candida albicans, Candida krusei, Candida parapsilosis* and *Malassesia pachydermatis*) *pathogens.* Hexane and ethyl acetate extracts of

the leaf and inflorescence showed the greatest zones of inhibition against Gram positive and Gram-negative bacteria. However, neither the leaf extracts nor the inflorescence demonstrates the ability any appreciable antifungal activity. Hexane and ethyl acetate leaf extracts had minimum inhibitory concentrations of 1000-125 g/ml and 1000-31.25 g/ml, respectively, against Gram-positive and Gram-negative microorganisms. *Anisomeles malabarica* inflorescence extracts in hexane and ethyl acetate exhibited the highest antibacterial activity when compared to leaf extracts [21]. In vitro antibacterial activity of leaf extracts of *Anisomeles malabarica* against *E. coli, S. aureus, P. mirabilis, P. aeruginosa* and *K. pneumoniae*. The diethyl ether extract produced a 30mm zone of inhibition at the same concentration as the ethanolic extract, which exhibited the maximum antibacterial activity at 200g/ml and produced a 25mm zone of inhibition against *S. aureus* [22].

In the present study, bacterial pathogens, the higher concentration of 100g/ml compared with all concentrations, *Anisomeles malabarica* flowers in aqueous extract exhibited the maximum zone of inhibition at *Klebsiella pneumoniae* (14.04±0.16µg/ml) and at the benzene extract exhibited at *Streptococcus pyogenes* (16.02±0.17µg/ml) and the minimum zone of inhibition against *Salmonella sp* (10.05±0.26µg/ml) and (5.24±0.47µg/ml). The lower concentration of 25µg/ml *Anisomeles malabarica* flower aqueous and benzene extract exhibited the maximum zone of inhibition against *Klebsiella pneumoniae* (10.06±0.07µg/ml) in aqueous extract and *Streptococcus pyogenes* (9.01±0.13 µg/ml) in benzene extract and the minimum zone of inhibition against *E. coli* (7.01±0.05 µg/ml) in aqueous extract and *Salmonella sp* (2.14±0.28µg/ml). All bacterial pathogens, including the standard streptomycin, are present at their highest levels as compared to all other concentrations. The standards for streptomycin are highly presented in *Streptococcus pyogenes* (8.26±0.05µg/ml) in aqueous extract and in *Klebsiella pneumoniae* (9.22±0.20µg/ml) in benzene extract (Fig 3). In fungi pathogens, higher concentration 100 µg/ml, *Anisomeles malabarica* flowers in aqueous extract showed the maximum zone of inhibition at *A. flavus* (9.66±0.01µg/ml), and at the benzene extract showed the maximum at *A. terreus* (14.60±0.13µg/ml), and the minimum zone of inhibition against *A. fumigatus* (7.06±0.18µg/ml) in aqueous extract and *A. niger* (8.66±0.51µg/ml) in benzene extract. The maximum zone of inhibition against *A. flavus* (7.13±0.31µg/ml) in aqueous and (10.6±0.06µg/ml) in benzene and the minimum zone of inhibition against *A. niger* (3.66±0.18µg/ml) and (7.01±0.34 µg/ml) were seen at the lower concentration of 25µg/ml *Anisomeles malabarica* leaves aqueous and benzene extract. All bacterial pathogens, including the common strain of fluconozole, are present at their highest concentration when compared to all other concentrations. The standards for fluconozole are very high in *A. fumigatus* (8.45±0.85µg/ml) in aqueous extract and (9.05±0.07µg/ml) in aqueous extract (Fig 4).

Using disc diffusion methods along with phytochemical screening, the aqueous leaf, flower, stem, and boiled leaf extracts of *Anisomeles malabarica* were examined for antibacterial activity. *Anisomeles malabarica* extracts are sensitive to five pathogenic organisms using disc diffusion methods, but not *Proteus vulgaris*. These pathogenic organisms include *Staphylococcus aureus, Staphylococcus epidermis, E. coli, Pseudomonas aeroginosa* and *Bacillus substilis*. Proteus vulgaris, in contrast hand, is not being affected by any microbial activity. The bacterial strains revealed the zone of inhibition [23]. The crude methanol extract of the leaves, *Anisomeles malabarica*, was tested for its antibacterial activity using the Disc Diffusion Method. The purpose of this research was to determine the *A. Malabarica* leaves methanol extract's in vitro antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas auregenosa* and *Klebsiella pneumoniae*. The methanol extract was found to provide a 21mm zone of inhibition against both *E. coli* and *P. auregenosa* and to have the highest antibacterial activity at 100 µl/ml [24].

In the present study, bacterial pathogens in 100µg/ml, *Anisomeles malabarica* seeds in aqueous extract demonstrated the maximum zone of inhibition at *Streptococcus pyogenes* (4.78±0.09µg/ml), while the benzene extract demonstrated the maximum zone of inhibition at *E. coli* (17.50±0.15µg/ml), and the minimum zone of inhibition at *Klebsiella pneumoniae* (3.12±0.04µg/ml) in aqueous extract and *Streptococcus pyogenes* (6.10±0.31µg/ml). The diluted 25 µg/ml concentration, the highest zone of inhibition for *Anisomeles malabarica* seeds aqueous and benzene extract against *Streptococcus pyogenes* (3.05±0.02µg/ml) and *E. coli* (9.01±0.13µg/ml) and the minimum zone of inhibition for *Klebsiella pneumoniae* (1.01±0.55µg/ml) and (3.05±0.07µg/ml) respectively. In comparison to all other concentrations, all bacterial pathogens, including the common streptomycin, are present at their highest levels. The standards for streptomycin are highly presented in *Klebsiella pneumoniae* (6.36±0.15µg/ml) and (9.14±0.25µg/ml) in aqueous extract benzene extract (Fig 5). In fungal pathogens, *Anisomeles malabarica* seeds in aqueous extract showed the maximum zone of inhibition at *A. flavus* (15.50±0.10µg/ml), and at the benzene extract showed the maximum at *A. fumigatus* (20.00±0. 31µg/ml), and the minimum zone of inhibition against *A. flavus* (8.36±0.11µg/ml) in the aqueous extract and *A. niger* (9.04±0.22µg/ml) in benzene extract. At the lower concentration of 25 µg/ml *Anisomeles malabarica* seeds aqueous and benzene extract, the maximum zone of inhibition against *A. terreus* (10.61±0.05µg/ml) in aqueous and *A. fumigatus* (12.60±0.36 µg/ml) in benzene, and the minimum zone of inhibition against *A. niger* (8.03±0.15µg/ml) and (6.03±0.34µg/ml). The common strain of fluconozole is present in the highest concentration of all bacterial pathogens compared to all other concentrations. The standards for fluconozole are very high in *A. fumigatus* (9.23±0.41µg/ml) in aqueous extract and (8.91±0.18µg/ml) in aqueous extract (Fig 6).

Anisomeles malabarica flower and stem were tested for their capacity to fight pathogenic gram positive and gram-negative bacteria. Growth of *Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus* and *Escherichia coli* was significantly inhibited. The greatest zones of inhibition were observed in *Bacillus subtilis, Proteus vulgaris* and *Staphylococcus aureus*. *Anisomeles malabarica* antimicrobial effectiveness to test potential antibacterial properties against some pathogenic bacteria, including *Staphylococcus aureus, Streptococcus epidermis, Proteus vulgaris, Klebsiella pneumonia* and *Escherichia coli*, sequential extraction was performed out by using solvents such as ethanol, methanol, petroleum ether, and aqueous extract from the plant's leaf and boiled leaf. In polar studies, the Staphylococcus aureus exhibited the greatest zone of inhibition. In non-polar studies, *Pseudomonas aeruginosa* had the greatest zone of inhibition. All of these discoveries have demonstrated that this plant is effective for treating a variety of bacterial diseases [25]. Hexane, ethyl acetate, and methanol were used to directly extract *Anisomeles malabarica* leaf powder. Depending on the solvent used, various amounts of the substance were extracted. The methanol successfully inhibited every pathogen tested, including *Klebsiella pneumoniae, Staphylococcus aureus, Vibreo cholerae*

and *Pseudomonas aeroginosa*. Because methanol extract inhibited almost all test pathogens more potently than ethyl acetate and hexane extract. The leaf extract inhibited protein synthesis [26].

In the current investigation, the *Anisomeles malabarica* leaves, flowers and seed extracts are maximum against bacterial and fungal pathogens. But the leaves are maximum and great ful then compared with flowers and seeds (Fig 1-6).

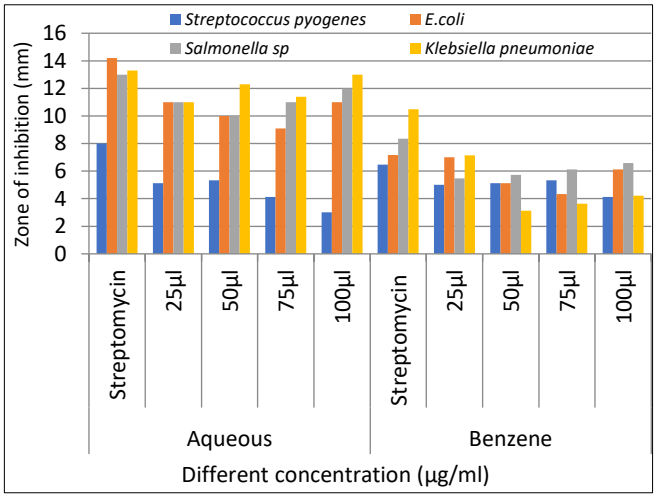

Fig 1 Effect of antibacterial activity of *Anisomeles malabarica* leaves in aqueous and benzene extracts against bacteria

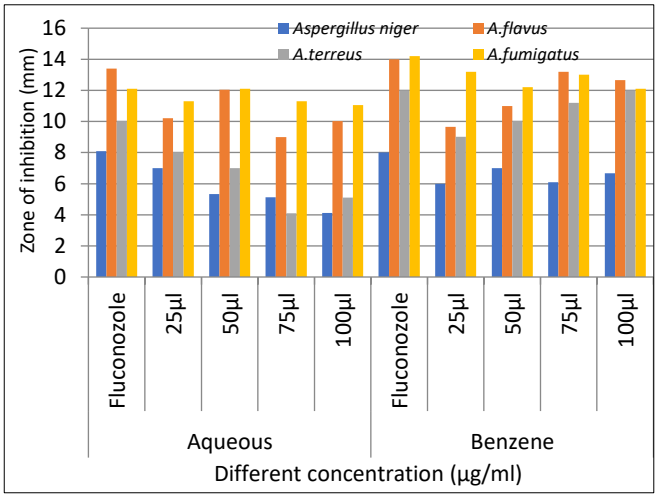

Fig 2 Effect of antifungal activity of *Anisomeles malabarica* leaves in aqueous and benzene extracts against fungi

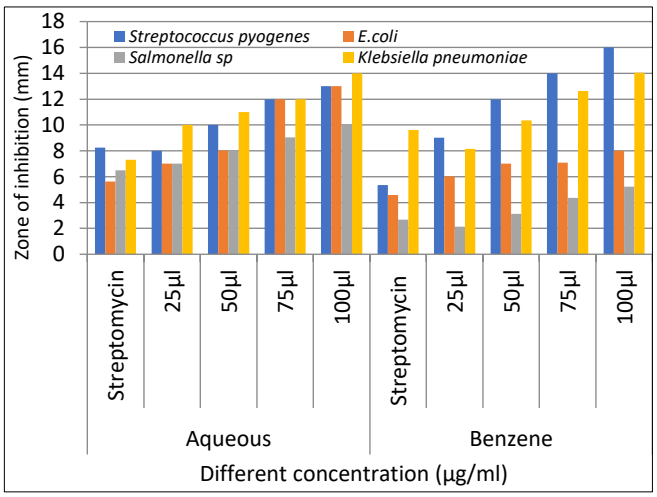

Fig 3 Effect of antibacterial activity of *Anisomeles malabarica* flowers in aqueous and benzene extracts against bacteria

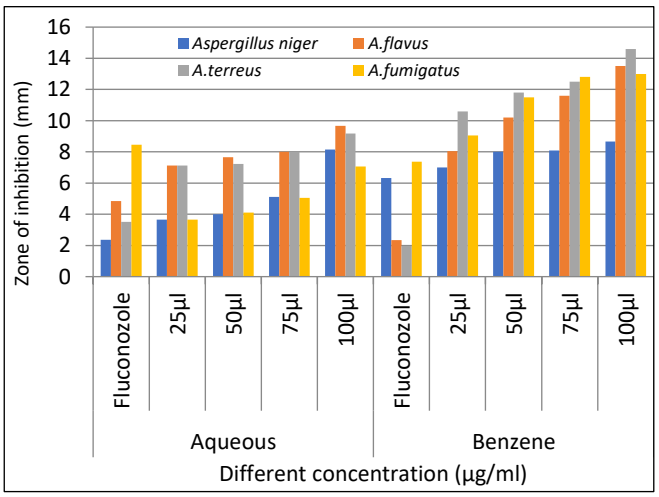

Fig 4 Effect of antifungal activity of *Anisomeles malabarica* flowers in aqueous and benzene extracts against fungi

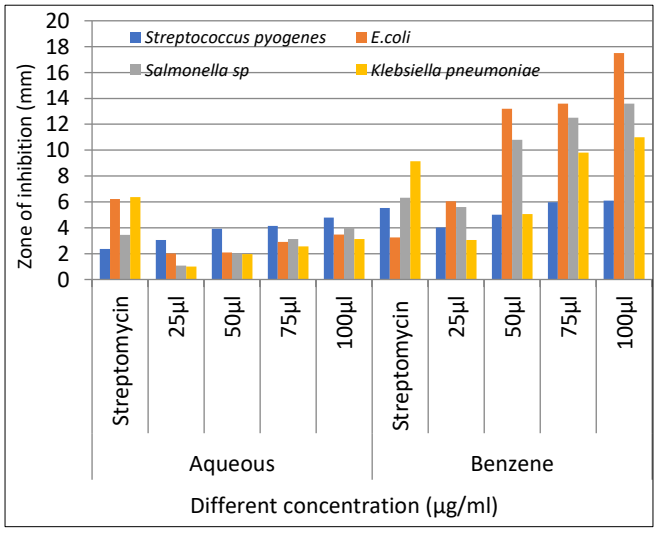

Fig 5 Effect of antibacterial activity of *Anisomeles malabarica* seeds in aqueous and benzene extracts against bacteria

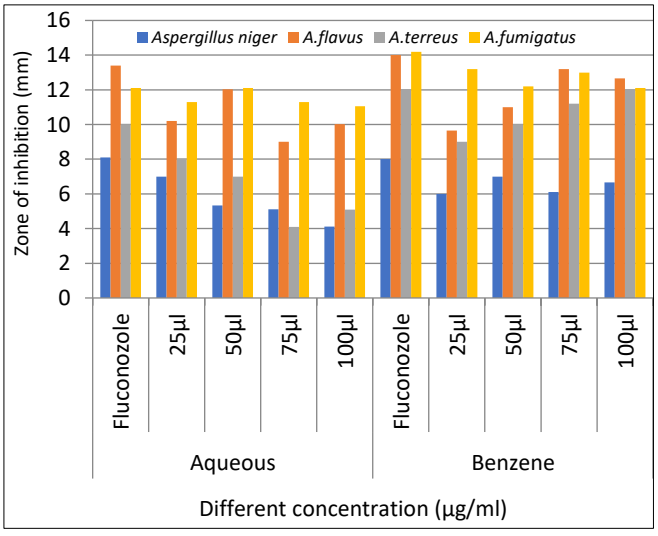

Fig 6 Effect of antifungal activity of *Anisomeles malabarica* seeds in aqueous and benzene extracts against fungi

CONCLUSION

In recent research, the antibacterial and antifungal activities of various solvents from *Anisomeles malabarica* leaves, flowers and seed extracts against pathogenic organisms have been demonstrated. As a result, this plant can be used in traditional medicine to treat coughs, fevers, colds, liver disorders, and stomach ailments. The people with the condition continued to drink the juice for two days. That problem was recovered immediately. It has also been proposed that the antimicrobial compounds in *A. malabarica* may inhibit bacteria in a different manner than current approaches. *Anisomeles malabarica* has tonic, astringent, and carminative properties. The dried or fresh material is used as a wash for skin issues like snakebites, eczema, and pruritis. Mosquitoes are repelled by burning the plant. In Sri Lanka, a decoction prepared from the plant's aerial parts is used as an analgesic. Hence, it is clear that although *Anisomeles malabarica* holds good prospects for treating a variety of ailments, its other biological effects are still unknown. In this study, *Anisomeles malabarica* leaves extract had better results compared with flower and seed extracts from aqueous and benzene solvents. According to my research, the leaves of the plant can be used as medicine to fight against pathogens. In depth research is needed to separate the pure compounds from this plant extract and establish which isolated molecules function.

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