Antibacterial Activity of Anisomeles Malabarica Against Tooth Decay Pathogens

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***Abstract*— The medicinal plant Anisomeles malabarica has been used in traditional medicine to treat floric diseases [1]. The aim of this work is to examine the antibacterial activity of the leaf extract that was prepared using analytical standard polar and non-polar solvents there were three distinct solvents used ethyl acetate, acetone, and chloroform. The antibacterial properties against some pathogenic gram positive and gram negative bacteria collected from Saveetha Dental College and Hospital Chennai of tooth decay-causing agents such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutants, Klebseilla pneumoniae, Bacillus pumilus, Enterococcus faecalis, and Serratia sp. Maximum zones of inhibition were seen by Anisomeles malabarica acetone extracts against Staphylococcus aureus, Klebsiella pneumoniae, and Bacillus pumilus. Anisomeles malabarica chloroform extracts demonstrated the highest zone of inhibition against Pseudomonas aeruginosa, Enterococcus faecalis, and Streptococcus mutants. Additionally, Anisomeles malabarica ethyl acetate extracts demonstrated a maximal zone of inhibition against Serratia sp and E.Coli.**

***Index Terms*— Antimicrobial activity, Anisomeles malabarica, tooth decay pathogens, phytochemical activity**

I. INTRODUCTION

As they have a high concentration of bioactive chemicals and have minimal side effects, herbal plants have a lot of medical promise. The phytoactive compounds in the herbal plant reduce the pathogenicity of bacteria, preventing and guarding against diseases. India's Ayurvedic treatments are prominent throughout the entire world [2].

Plants in the Lamiaceae family include Anisomeles malabarica (Malabar catmint) Cogn.Syn. Nepeta malabarica L. With 45 genera and 574 species, including 256 endemic species, Anisomeles are members of the Lamiaceae family. This aromatic perennial herb, which stands 1.22 meters tall and is a member of the Lamiaceae family, has abundant growth [3].

A wide range of disorders have been treated with the bioactive chemicals present in plants used for human health. These compounds have been employed as immune-modulators, anti-allergenic, antidiabetic, antioxidant, antimutagenic, and anticarcinogenic agents [4]. Medicinal plants along with Anisomeles malabarica widely used for the treatment by indigenous people [5]. This plant contains flavonoids, which have been shown to have remarkable biological effects on humans, including anti-inflammatory, anti-allergic, antiviral, and potent analgesic properties [6].

Plant-derived antimicrobial chemicals may prevent the growth of bacteria in ways different from those that are currently used [7]. The antibacterial activity of ethanol extract from leaves of Anisomeles malabarica was carried out against bacterial pathogens such as Staphylococcus aureus, Bacillus subtilis, and Proteus vulgaris [8]. The studies aim to identify and isolate the main active ingredients in the plants responsible for this antimicrobial activity against tooth decay causing pathogens.

II. MATERIALS AND METHODS

2.1 Collection of plant materials

Fresh leaves of Anisomeles malabarica free from the disease were collected from Tiruvannamalai district of Vandavasi (Figure No.1). The taxonomic identification of this plant was determined by Hubert Enviro Care Systems Private Ltd, Chennai.

2.2 Preparation of plant extract

The leaves of Anisomeles malabarica were washed thoroughly 2-3 times with running water and air dried

in the shade for five days and then powdered with the help of a warring blender. 20gram of shade-dried powder filled in a thimble and extracted with acetone, chloroform, and ethyl acetate solvents in Soxhlet apparatus [9] (Figure No.2). The solvent extracts were concentrated under reduced pressure and stored in the airtight bottle until further use.

2.3 Extraction procedure

The dried and powdered Anisomeles malabarica materials were dried at 55°C in the oven for 1 hour. 10g of each powdered sample was soaked in 100 ml of each solvent contained in a sterile conical flask. The solvents such as Ethyl acetate, acetone, and chloroform.

2.4 Phytochemical Screening of Anisomeles malabarica

The dry methanolic extract of Anisomeles malabarica was screened for the presence of various phytoconstituents or secondary metabolites that are responsible for the therapeutic values of the drug, for example, the presence of tannins, alkaloids, glycosides, carbohydrates, tannins, proteins & amino-acids, gums, mucilage, flavors, flavonoids, saponins and steroids and Sterols [10] (Figure No.3-5). The resulting data obtained were recorded in the (Table No.9).

2.5 Collection of Bacterial Culture

Collection of bacterial cultures of gram positive such as Staphylococcus aureus, Streptococcus mutants, Bacillus pumilus, Enterococcus faecalis, and cultures of gram negative such as Escherichia coli, Serratiasp, Pseudomonas aeruginosa, and Klebsiella pneumonia obtained from Dr.S. Rajeshkumar young scientist (DST-SERB) department of pharmacology, Saveetha Dental College and Hospital Chennai-600077.

2.6 Determination of Antibacterial activity by Kirby-Bauer

Known quantities of extracts or fractions are dissolved in solvents DMSO (Dimethyl Sulfoxide): Methanol of 1:1 ratio and then the extract is used for the next level process. The Petri plates containing 20 ml Muller Hinton Agar were seeded with the 24-hour culture of bacterial strains. Except for the control well, wells were cut, and 20µg of various concentrations of 500, 1000, and 2000 mg were added, respectively. The extracts (namely Acetone, Chloroform, and ethyl acetate) were added and incubated at room temperature for 2hrs. Then the plates were incubated at 37ºc for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition Zone formed around the well. Distilled water was used as a positive control [11].

III. RESULTS AND DISCUSSION

3.1 Confirmation of the Pathogenic Microorganisms

The pathogenic microorganism was obtained from Saveetha Medical College. Chennai was confirmed as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutants, Klebseilla pneumoniae, Bacillus pumilus, Enterococcus faecalis, and Serratia sp., on the basic of Gram staining and biochemical tests and their results.

3.2 In vitro Antimicrobial Assay by Well Diffusion Method

The in vitro antimicrobial activity of acetone, chloroform, and ethyl acetate extracts Anisomeles malabarica leaf against eight pathogenic microorganisms was carried out by a well diffusion method. It was found that the plant extract showed good inhibitory activity on almost all the pathogenic microbes tested. (Figure No. 6-13).

3.3 Antibacterial Activity of Anisomeles malabarica Leaf Extracts

The bioactivities of various solvent extracts vise were tested against pathogenic bacteria (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutants, Klebseilla pneumonia, Bacillus pumilus, Enterococcus faecalis, and Serratia sp). Three different solvents vise chloroform, acetone, and ethyl acetate were used for the extraction of Anisomeles malabarica. Overall view, the acetone extracts of Anisomeles malabarica showed a maximum zone of inhibition against Staphylococcus aureus, Klebsiella pneumonia, and Bacillus pumilus. The chloroform extracts of Anisomeles malabarica showed a maximum zone of inhibition against Streptococcus mutants, Enterococcus faecalis, and Pseudomonas aeruginosa. The ethyl acetate extracts of Anisomeles malabarica showed a maximum zone of inhibition against E. coli and Serratia sp. The present findings are presented below (Table 1-8).

Table No.1 Antimicrobialactivityof Anisomelesmalabaricaleavesextractsagainst Bacterial cultures

Bacterial cultures	Acetone	Chloroform	Ethyl acetate	Control
	500 mg ppm	1000 mg ppm	2000 mg ppm	
E. coli	6	12	15	10
	6	8	12	20
	8	12	18	12
Serratiasp	3	5	8	12
	3	5	7	10
	9	11	16	20
Staphylococcus aureus	3	15	17	21
	3	8	10	22
	4	8	9	12
Streptococcus mutants	2	8	15	18
	8	10	16	22
	10	12	17	18
Klebsiellapneumonia	8	12	13	19
	3	5	6	13
	3	5	6	13
Enterococcusfaecalis	5	7	9	12
	6	8	9	11
	5	6	7	9
Pseudomonas aeruginosa	4	5	7	20
	6	8	10	15
	3	5	8	10
Bacillus pumilus	4	15	16	15
	6	8	12	14
	5	7	9	16

Table No.2phytochemicalscreeningofleafextract SAnisomelesmalabarica

S.No	QualitativePhytochemicalTest	Chloroform	Acetone	Ethylacetate
1	TESTFORALKALOIDS			
	a) Mayer'stest	+	-	-
	Wagner'stest	+	-	+
2	TEST FORFLAVONOIDS			
	a) Shinodatest	-	-	-
	Alkalineregent	-	-	+
3	TESTFORCARBOHYDRATES			
	a) Benedict'stest	-	-	-
	Molschtest	-	-	+
4	TEST FORGLYCOSIDES			
	a) Borntrager'stest	-	+	-
	Keller– killanitest	-	+	+
5	TEST FORSAPONINS			
	a) Forthtest	-	+	-
	Leadacetatetest	-	+	+
6	TESTFORTANNIS			
	a) Ferricchloridetest	+	+	-
	Leadacetatetest	+	+	+
7	TESTFORTERPENOIDS			
	Salkowskitest		+	+
8	TESTFORPROTEIN			
	a) Ninhydrintest	-	-	+
	Biurettest	+	+	+
9	TESTFORANTHROQUINONE			
	Ammoniatest	-	-	+



Figure No.1 *Anisomeles malabarica* leaves



Figure No.2 Preparation of extracts by using Soxhlet apparatus

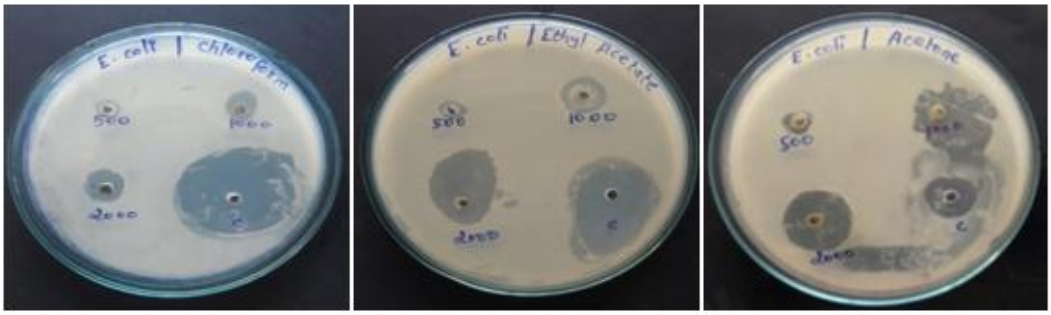


Figure No.3 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *E. coli*



Figure No.4 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Serratia* sp

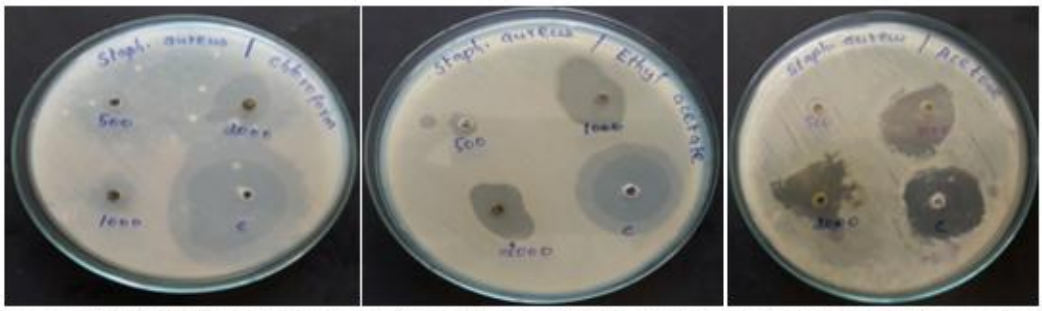


Figure No.5 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Staphylococcus aureus*

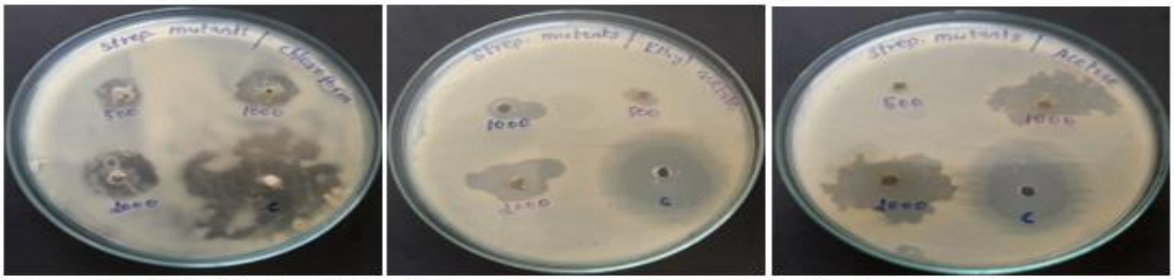


Figure No.6 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Streptococcus mutants*

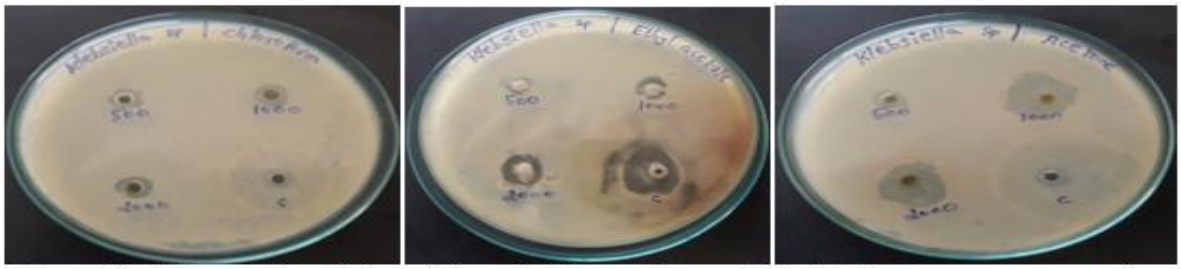


Figure No.7 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Klebsiella pneumoniae*



Figure No.8 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Enterococcus faecalis*



Figure No.9 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Pseudomonas aeruginosa*

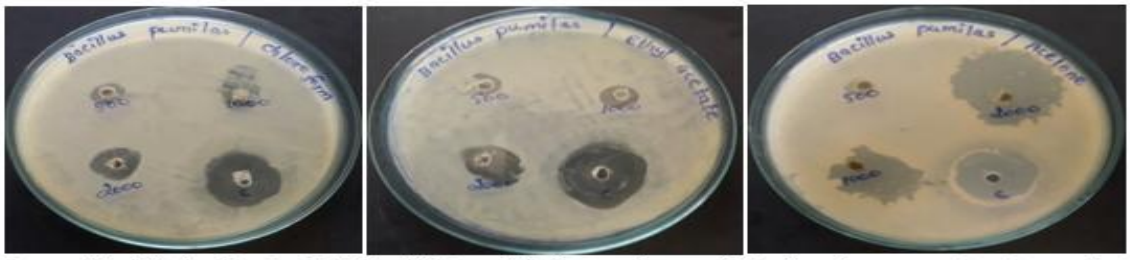


Figure No.10 Antimicrobial activity of *Anisomeles malabarica* leaves extracts against *Bacillus pumilus*

IV. CONCLUSION

The selection of Anisomeles malabarica for the present study was based on their traditional use to treat infectious bacterial diseases mainly Tooth decay. Due to lake of appropriate health facilities, these diseases are commonly treated with local herbs. Our results against the microbes showed that Anisomeles malabarica leaves have some potential compounds that could provide a scientific base for the treatment of stated infections by herbal practitioners.The result of the present study indicates Phytochemical screening of Anisomeles malabarica shows the presence of various chemical substances such as phenolic compounds, oils, saponins, flavonoids, Alkaloids, and tannins. Flavonoids and many phenolic compounds act as antimicrobial agents against many pathogenic bacteria. With the results obtained in the present study, we conclude that Anisomeles malabarica leaves can be used as a good source for medicinal treatment against pathogens causing tooth decay.

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