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IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF *ANISOMELES MALABARICA* (L.)SIMS LEAF AGAINST IMPORTANT SOIL BORNE BACTERIA

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ABSTRACT

Antibacterial activity of aqueous extract of leaves of *Anisomeles malabarica* (L.)Sims were tested against six soil borne bacteria. *B.subtilis* and *S. aureus* recorded a maximum inhibition of 30.0mm at 50 µl concentration followed by *P. fluorescens* which was recorded 28.0mm inhibition, *E. tracheiphila* recorded 24.0mm, *E. coli* recorded 23.0mm and *X. campestris* recorded 22.0mm inhibition at 50 µl concentration. Moderate activity was observed in 20 and 30 µl concentration in all the test bacterial species. Compared to synthetic antibiotics Gentamicin and Tetracycline, highly significant activity was observed in *B.subtilis, S. aureus* and *P. fluorescens.*

Key words: Antibacterial activity, *Anisomeles malabarica,* Aqueous extract, Synthetic antibiotics

INTRODUCTION: Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The use of medicinal plants as a source for aid from illness can be traced back over five millennia to written documents of the early culture in China, India and the Near east, but it is, without a doubt, an art as old as mankind. The prospective of higher plants as basis of new drugs is still largely uncharted [1]. Among the

anticipated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller [2]. People all over the world have used plants as medicines from time immemorial. It is estimated by WHO that 80% of the population, majority of this in developing countries, still rely on plant-based medicine for primary health care[3] .There are more than 1,340 plants known to be potentially sources of antimicrobial compounds but few have been systematically studied scientifically[4]. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties[1]. The abundance of medicinal plants in nature and the traditional knowledge increase the understanding of the medicinal plants properties, safety and efficacy[5]. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors [6]. This concern has been expressed because of the resistance of clinically pathogenic microorganisms to be antibiotics that have produced in the last decades [7,5]. In the present study, leaves of *Anisomeles malabarica* (L.)Sims. Belongs to family Lamiaceae were investigated for antibacterial activity against six bacterial species

MATERIALS AND METHODS

Test plant: Fresh and healthy leaves of *A. malabarica* collected from Mysore. The leaves were shade dried and washed thoroughly two to three times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter, and used for the preparation of extracts.

EXTRACTION

Aqueous extract: One hundred grams of the thoroughly washed and air dried healthy leaves of *A. malabarica* were macerated with 100 ml of sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for five minutes. The macerate was filtered

through double-layered muslin cloth, and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120^0 C for 10 minutes, which served as 100% aqueous mother extract. The extract was preserved aseptically in a sterile brown bottle at 5^0 C until further use[8].

Test organisms: Five pathogenic bacteria namely *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive), *Erwinia tracheiphila* (Gram Negative), *Escherichia coli* (Gram Negative), *Xanthomonas campestris*(Gram Negative) and *Pseudomonas fluorescens* (Gram Negative) were isolated from soil samples following the procedure of [9]. The obtained cultures were subcultured on nutrient agar medium. After 24 hours of incubation at 37°C the cultures were preserved aseptically in refrigerator until further use.

Preparation of Inoculum: A loopful of all the test bacteria were taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test tubes were incubated at 37°C for 24 hours. The broth was standardized using sterile normal saline to obtain a population of 10 cfu/ml.

ANTIBACTERIAL ACTIVITY:

Preparation of standard culture inoculums of test organism: Three or four isolated colonies of all the test bacterial species were inoculated into 2 ml of Nutrient broth and incubated at 37^oC for 24 hours till the growth in the broth was equivalent with Mac-Farland standard(0.5%) as recommended by WHO.

Agar cup diffusion method: Agar cup diffusion method described by [10] was employed. An overnight culture of *S. aureus, B. subtilis,, E. tracheiphila , E. coli , X.campestris* and *P. fluorescens* was standardized to contain approx.107cfu/ml and inoculated into 20 ml of nutrient broth. The culture medium was allowed to set. Thereafter, all the inoculum was swabbed over the surface of nutrient agar medium plate using sterile cotton swab. Using a sterile cork borer of 5 mm diameter, five wells were made in solidified sterile nutrient agar medium plate (one in the centre and four wells at the corner). The agar plugs were removed with a flamed and cooled wire loop. Then 10,20,30,40 and 50µl of all the test oil samples were placed in the wells made in inoculated plates. The treatment also includes 50 µl of absolute alcohol served as control. All the plates were incubated for 24hours at 37^oC and zone of inhibition if any around the well were measured in millimeter (mm). For each

treatment ten replicates were maintained. The same procedure were followed for standard antibiotics Gentamicin (25mg) and Tetracycline (25mg) to compare the efficacy of plant extract against test organisms.

STATISTICAL ANALYSIS: The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULT AND DISCUSSION

Among the six bacterial species tested, *B. subtilis* recorded 30.0mm inhibition at 50µl concentration. At 40µl concentration it was recorded 26.0mm, at 30µl, it was recorded 17.0mm, at 20µl concentration it was 10.0mm and at 10µl, 4.0mm inhibition was observed. *S. aureus* recorded 30.0mm at 50µl, 26.0mm in 40µl and 21.0mm in 30µl, 14.0mm at 20µl and 6.0mm inhibition at 10µl concentration. Moderate activity was observed in *P. fluorescens* and recorded 28.0mm at 30µl concentration, 22.0mm at 40µl and least inhibition was observed in 10µl concentration and recorded 3.0mm respectively. *E. tracheiphila* recorded 24.0mm inhibition at 50µl concentration , 20.0mm at 40 µl concentration and 2.0mm inhibition at 10µl concentration. In *E. coli,* maximum inhibition was recorded at 50µl concentration and recorded 23.0mm inhibition. Least inhibition was recorded at 10µl concentration and recorded 2.0mm inhibition. *X.campestris* recorded 22.0mm inhibition in 50µl concentration and 2.0mm in 10µl concentration (Table 1). Compared to synthetic antibiotics Gentamicin and Tetracycline tested at recommended dosage of 25mg, *B.subtilis* recorded 32.0mm and 31.0mm, *E. coli* recorded 36.0mm and 33.0mm, *P. fluorescens* recorded 35.0mm and 34.0mm, *E. tracheiphila* recorded 32.0mm and 33.0mm, *X. campestris* recorded 34.0mm and 34.0mm and *S. aureus* recorded 30.0mm and 28.0mm in Gentamicin and Tetracycline respectively.

Table 1: Antibacterial activity of aqueous extract of *Anisomeles malabarica* (L.)Sims against six soil borne bacterial species.

Bacteria	Zone of inhibition(mm)						
	Concentration						
	Plant extract					Synthetic antibiotics	
	10 µl	20 µl	30 µl	40 µl	50 µl	Gentamicin (25mg)	Tetracycline (25mg)
B.subtilis	4.0^{a} ±0.0	10.0^{b} ±0.1	17.0^{c} ±0.1	26.0^{d} ±0.1	30.0^{e} ±0.0	32.0^{g} ±0.1	31.0^{f} ±0.1
E. coli	2.0^{a} ± 0.0	7.0^{b} ±0.1	13.0^{c} ±0.1	18.0^{d} ±0.0	23.0^{e} ±0.0	36.0^{g} ±0.2	33.0^{f} ±0.2
P. fluorescens	3.0^{a} ±0.1	8.0^{b} ±0.0	15.0^{c} ±0.1	22.0^{d} ±0.0	28.0^{e} ±0.0	35.0g ±0.0	34.0^{f} ±0.0
E. tracheiphila	2.0^{a} ±0.1	6.0^{b} ±0.0	14.0^{c} ±0.1	20.0^{d} ±0.0	24.0^{e} ± 0.0	32.0^{f} ±0.0	33.0^{g} ±0.0
X. campestris	2.0^{a} ±0.0	5.0^{b} ±0.0	11.0^{c} ±0.1	19.0^{d} ±0.0	22.0^{e} ±0.0	34.0^{f} ±0.0	34.0^{f} ±0.0
S. aureus	6.0^{a} ±0.1	14.0^{b} ±0.0	21.0^{c} ±0.0	26.0^{d} ±0.0	30.0^{e} ±0.0	30.0^{g} ±0.1	28.0^{f} ±0.1

- Values are the mean of ten replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.

The extensive use of synthetic drugs, excessive unwanted medication will cause increasing side effects in the body, sometimes, the toxic effects produced by the administration of drugs is much more a serious problem than that of the disease itself [11]. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare [12]. In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested

scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents[13]. Increasing bacterial resistance is prompting a resurgence in research of the antimicrobial role of herbs against resistant strains [14]. Hence an alternative approach is used to cure bacterial diseases which is ecofriendly approach and which can reduce the ill effects by using synthetic antibiotics.

CONCLUSION

From the above observation, it was noted that leaves of *A. malabarica* showed a promising result against six bacterial species. In the present study, aqueous extract were evaluated and observed a maximum inhibition at 30, 40 and 50µl concentration. A further evaluation of solvent extracts is need against many species pathogenic bacteria. Based on the result in solvent a further isolation of bioactive principle is needed and its characterization is needed.

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