In-vitro antimicrobial activity and preliminary phytochemical investigation of *Anisomeles malabarica* from Western Ghats, Karnataka

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ABSTRACT-*Anisomeles malabarica* is a traditional medicinal plant distributed throughout Southern India. The aim of this study deals with the investigation of preliminary bioactive phytochemicals present in the leaves extracts obtained by analytical standard hexane and ethanol solvents. The phytochemicals were analysed such as alkaloids, flavonoids, saponins, tannins and terpenoids from both the leaves extracts. This study also extends to evaluate the antimicrobial activity of *Anisomeles malabarica* leaves extracts. The *in vitro* antimicrobial activity was performed by agar well diffusion method against the clinically important multi drug resistant bacterial strains viz., *Staphylococcus aureus* (NCIM 2492), *Bacillus subtillis* (NCIM 2439) and *Klebsiella pneumoniae* (NCIM 2719) with the concentration of extracts ranged from 25 to 75µL. It has shown the concentration dependent antimicrobial activity (MIC). This study shows the powerful antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* bacterial strains with maximum inhibitory zone compared with standard antibiotic drug tetracycline.

Index Terms- *Anisomeles malabarica*, leaves, phytochemicals, agar well diffusion and antimicrobial.

INTRODUCTION

Anisomeles malabarica belongs to family Lamiaceae is found throughout tropical and subtropical regions of India. It is erect shrub commonly known as 'Malabarcatmint' distributed throughout South India (Orient longman 1994)[1]. It is wildly found common at road sides in Western Ghats. The plant is used in folk medicine as a cure for gastric and fever. The essential oil present in herb is used in uterine affection (Kritikar et.al., 1993)[2]. *A.malabarica* is reported to have antibacterial, antipyretic, analgesic, anti-inflammatory, antiallergic, anthelmintic, antispectic activities and it also acts as natural herbicide in wheat fields (Dharmasiri et.al., 2003)[3]. The leaves of *A. malabarica* consist of diterpenoids, ovatodiolide and its derivatives that are used as HIV inhibitors (Alam et.al. 2000)[4]. As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, pathogenic microbial agents have developed resistance to many old and newly produced antibiotic drugs. There is need to develop alternative antibiotic drugs from plants this has led to screening of medicinal plants for their potential antimicrobial activity (Sharma A et al., 2010)[5]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. One such resource is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko N et al., 2002)[6]. There has been growing interest regarding thousands of bioactive compounds that has been produced from plants, compounds are referred to as phytochemicals (Dubey N K *et al.,* 2004)[7]. Systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin (Skinner 1995)[8]. However antimicrobial activities which has indeed formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Reynolds 1996; Lis-Balchin 1997)[9, 10]. In present work an attempt has been carried out to evaluate antimicrobial activity and investigation of phytochemical components present in the leaves of *Anisomeles malabarica* plant.

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MATERIALS AND METHODS

Healthy samples of *Anisomeles malabarica* whole plant was collected from the surroundings of Western Ghats region Karnataka. Plant material is authenticated by Prof. Krishna V, Head and Chairman, Dept of Biotechnology, Kuvempu University and voucher specimen were deposited in the same department. Leaves were separated from the plant and shade dried. Dried material is ground into fine powder filtered through a mesh. Using Soxhlet extractor (50g) dried powdered material is extracted with (500mL) analytical standard varying polarity solvents such as hexane and ethanol. The extracts obtained were evaporated completely and transferred to screw cap vials for further use.

1. PHYTOCHEMICAL SCREENING

Chemical tests were carried on hexane and ethanol leaves extracts of *Anisomeles malabarica*. Standard procedures were employed to identify the presence and absence of metabolites such as alkaloids (Mayers test), glycosides (Keller-Killani test), flavonoids (Shinodas test), tannins (Lead acetate test), saponins (Foam test), steroids

(Libermann-Buchard test) and terpenoids (Salkowski test) (Sofowara (1993); Trease and Evans (1989); Harborne (1973); Edeoga et *al.*, (2005))[11,12,13,14].

2. ANTIMICROBIAL ACTIVITY

2.1 Determination of antimicrobial activity

Test microorganisms used

Antimicrobial activity of *Anisomeles malabarica* hexane extract and ethanol extract were tested against the following bacterial strains *Staphylococcus aureus* (NCIM 2492), *Bacillus subtilis* (NCIM 2439), *Klebsiella pneumoniae* (NCIM 2719) were obtained from Institute of Microbial Technology, Chandigarh and maintained on Nutrient agar (NA).

Bioassay for bacterial strains

Secondary metabolites	Hexane extract	Ethanolic extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	-
Steroids	-	-
Glycosides	-	-

Staphylococcus aureus (NCIM 2492), *Bacillus subtillis* (NCIM 2439), *Klebsiella pneumoniae* (NCIM 2719) were inoculated by streaking using a loop onto plates containing Nutrient Agar (NA) media and incubated at 32^{o}C for 24 hr. To carry out agar diffusion assay the bacterial suspensions were prepared in sterile distilled water.

Agar diffusion assay

The modified agar well diffusion method was employed (Perez C et al., 1990)[15]. The bacterial suspensions were inoculated onto nutrient agar media by spread plate technique. Once it is dried, 4mm diameter wells were punched onto the media. Tetracycline was used as a standard antibiotic drug (1mg/mL), 10 mg/mL leaves extracts of *Anisomeles malabarica* were dissolved in DMSO (Dimethyl Sulphoxide) from which 25, 50 and 75 microlitres of each extract was added into agar wells. The plates were sealed and incubated at 32^{o} C for 24 hr. The inhibition zone diameter was recorded after the incubation period along the two cardinal diameters and averaged. Same procedure was repeated with the extract and inhibition zone diameters were recorded (Perez C et al., 1990; Makari H K et al., 2009; Rojas J.J et al., 1990) [15,16,17]. All the experiments were conducted in quadruplicates.

RESULTS AND DISCUSSIONS

1. Phytochemical analysis

The Western Ghats is considered as one of the richest biodiversity in the world (Myers N et al., 2000)[18]. In the present study, we have collected *Anisomeles malabarica* plant because of its popular uses in different ailments. The ingredients of " Vishakallu" stone, which is used as an antidote for snakebite are made of different medicinal plants along with *Anisomeles malabarica* widely used for the treatment by indigenous people (Nampoothiri K et al., 2010)[19]. This investigation revealed that the *Anisomeles malabarica* plant leaves strongly contains bioactive

Substances such as alkaloids, flavonoids, saponins and tannins in hexane and ethanolic extracts. Terpenoids was screened in hexane extract. Similar results were also observed in recent studies. All these estimated phytochemical analysis of *Anisomeles malabarica* leaves extracts is presented in Table 1.1. Secondary metabolites including flavonoids and alkaloids seem to be typical in *Anisomeles malabarica* plant. Flavonoids have been reported for their amazing effect on humans in biological effects such as anti-inflammatory, anti-allergic, anti-viral and powerful pain killer medicine present in this plant (Raffuf R F 1996)[20]. Tannins are used in the treatment of diarrhea and dysentery (Subhuti Dharmanaada 2003)[21]. The findings of this study encourage the potency of antimicrobial activity.

Table 1.1 Phytochemicals screened in *Anisomeles malabarica* leaves exctract

(+presence, - absence) secondary metabolites

2. Determination of Antimicrobial Activity

The antimicrobial activity of *Anisomeles malabarica* leaves extracts against the multi drug resistant bacterial strains viz., *Staphylococcus aureus, Bacillus subtillis and Klebsiella pneumoniae* as shown in Table 2.1 and Figure 2.2. It has shown that concentration dependent antimicrobial activity in all the assays minimum inhibitory concentration (MIC). Hexane extract isolated from *Anisomeles malabarica* have active antimicrobial activity. Ethanolic extract of *Anisomeles malabarica* exhibited good inhibition activity against *S.aureus, B.subltillis* and *K.pneumoniae* comparison with standard drug Tetracycline. The extracts showed pronounced antibacterial activity with their inhibitory zones ranging from 20 to 25mm compared to the standard drug tetracycline 30mm. The extracts demonstrated good antimicrobial potency against the drug resistant strains, hexane extract showed a 23mm zone of inhibition against *S. aureus* and *K. pneumoniae* and 21mm against *B. subtillis*. Ethanolic extract showed powerful antimicrobial potency of 25mm and 24 mm against *S. aureus* and *K. pneumoniae* and 22mm against *B.*

subtillis. Reports from other workers also support the study of *Anisomeles malabarica* plant (Ushir Y V et al.,) [22].

The present study discusses the significance of *Anisomeles malabarica* plant leaves as a valuable source of secondary metabolites like alkaloids, flavonoids, saponins, tannins and terpenoids from hexane and ethanolic extracts. Further, leaves extracts of *Anisomeles malabarica* revealed the remarkable antimicrobial activity comparison with the standard antibiotic drug. This study would be a base for the further isolation of novel components from this plant material.

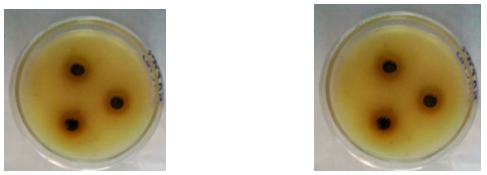
Table 2.1: Antimicrobial activity of *Anisomeles malabarica* leaves extracts

Bacterial strains	Inhibition zone diameterin mm**					
	Concentration in µL/mg					
	Hexane			Ethanol		
	*1	2	3	*1	2	3
S. aureus	15	15	23	20	22	25
B. subtillis	15	20	21	20	20	22
K. pneumoniae	15	22	23	18	20	24

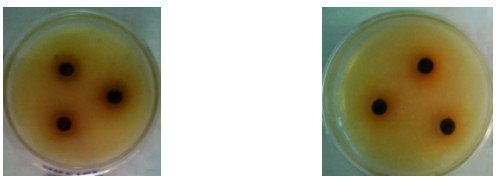
*Concentration of extracts: 25 µL, 50 µL, 75 µL.
** Each value in the table was obtained by calculating the average of three experiments

Figure 2.2: Inhibitory concentration of *Anisomeles malabarica* leaves extracts against bacterial strains.

Hexane extracts Ethanol extracts



Staphylococcus aureus



Bacillus subtillis

Klebsiella pneumoniae

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