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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS OF LEAF AND WOOD OF *Anisomeles malabarica*

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ABSTRACT

Water is essential to life, but many people do not have access to clean and safe drinking water and many die of waterborne bacterial infections. Antibacterial properties of various extracts of *Anisomeles malabarica* (L) were investigated against both clinical and laboratory isolates of bacteria using the disc diffusion method. Aqueous extracts (10 mm zone diameter of inhibition, MIC 10μg/mL) demonstrated the highest activity, followed by Ethanolic extract (8 mm zone diameter of inhibition, MIC 20μg/mL) methanolic extract demonstrated the least activity against the test bacteria (4 mm zone diameter of inhibition, MIC 40 μg/mL). Generally the antibacterial activities of *Anisomeles malabarica* are comparable to that of standard oflaxcin to a certain extent. Finally, these results suggested that *Anisomeles malabarica* (L) can be used to source antibiotic substances for possible treatment of bacterial infections and for the development of new drugs.

INTRODUCTION

Natural products from microorganisms have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of healthcare, the screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes Koduru *et al*., 2006. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds Tomoko *et al.,* 2002. The side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine Essawi and Srour 2000. Further to that, natural extracts are normally perceived as "safer" and less harmful, if compared with artificial products; all these reasons caused an increase in their demand Bianco and Uccella 2000. Wood is composed of three main macromolecular components; cellulose, hemicellulose and lignin, which are present in all woods Fengel and Wegener 1984. The relative proportion of these components is variable within tree species and can range for cellulose between 28 and 50 %; hemicelluloses, 20 and 30 %; and for lignin, 18 and 30 % relative mass fraction Fengel and Wegener 1984. Significant variations in the concentration of these components can also be found within the same tree species Kenney, Sennerby-Forsseand Layton, 1990. The heartwood of trees has long been known to produce and accumulate biologically active compounds from a variety of chemical classes Rennerfelt and Nacht, 1955; Rudman, 1963; Scheffer and Cowling, 1966 that protect them from microbial decay. Alaska yellow-cedar (Callitropsis nootkatensis; previously Chamaecyparis nootkatensis; Little, 2007; Little and Schwarzbach, 2004 heartwood is an excellent example. In recent years multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminative use of commercial antimicrobial drugs commonly used in the treatment of diseases. This situation forced the scientists for searching new antimicrobial substances from various sources of novel antimicrobial chemotherapeutic agent. *Anisomeles malabarica* R.Br., (Lamiaceae) is distributed in major parts of India and especially in South India as a traditional medicinal plant commonly known as Peymarutti (Tamil), Gouzaban (Hindi), Chodhara (Marathi), Karithumbi (Kannada) and Malabar catmint (English). It is used for the traditional treatment of snakebite as Antidote Perumalsamy *et al*., 2008. The herb is reported to the herb is reported to possess anticancer, allergenic, anthelmintic, antiallergic, antianaphylactic, antibacterial, anticarcinomic, antiedemic, antihistaminic, antiinflammatory, antileukemic, antinociceptive, antiplasmodial, antiseptic and antiperotic properties Jeyachandran *et al*., 2007. The present work was set to investigate the antimicrobial activity of leaf and wood extracts of *Anisomeles malabarica* against some selected bacteria.

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Experimental Section

Collection of sample

Anisomeles malabarica (L) were collected from kolli hills in Namakkal, District, Tamilnadu, India and were authenticated in Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai. The leaf were separated from stem, both wood and leaf were washed in clean water, leaf were dried at room temperature Eloff, 1998. The dried plant leaf were milled to a fine powder, The wood was debarked and chipping was done with chipper. Both leaf powder and wood chip were stored in the dark at room temperature in closed containers until required.

Extraction

10g of powdered leaf and woodchips of *Anisomeles malabarica* extracted with 100ml of Ethanol, Aqueous and Methanol separately using soxhlet apparatus.

Culture media

The cultures were subcultured regularly (every 30 days) and stored at 40˚C as well as 800˚C by preparing suspensions in 10% glycerol.

Microorganisms

In this study *Escherichia coli, Salmonella typhi, Shigella dysenteriae, Campylobacter jejuni, Enterobacter aerogenes* were used.

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 370˚C for 4 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1-2×108 colony-forming units per milliliter (cfu/ml). This 2-h grown suspension was used for further testing.

RESULTS

The Results are represented in Table 1 2 3. Of the tested plant extract, aqueous extract of *Anisomeles malabarica* showed antibacterial activity against most of the bacterial species investigated including its wide spectrum of activity, while Ethanolic and Methanolic extract of *Anisomeles malabarica* showed less activity compared to that of Aqueous extract.

Table 1. Antimicrobial screening of *Anisomeles malabarica* extract by pour plate method

Species	Control	Leaf	Wood
E.Coli	+	-	-
Salmonella typhi	+	-	-
Shigella dysenteriae	+	-	-
Campylobacter jejuni	+	-	-
Enterobacter aerogenes	+	-	-

Table 2. Minimum inhibitory concentration (MIC) of extracts of *Anisomeles malabarica* (L)

Organisms	MIC (µg/ml)							
	Leaf				Wood			
	EE	AE	ME	Ofx	EE	AE	ME	Ofx
E.Coli	20	30	50	30	20	30	60	10
Salmonella typhi	20	10	60	20	30	20	50	30
Shigella dysenteriae	30	10	40	40	20	10	50	50
Campylobacter jejuni	50	50	70	40	40	30	60	30
Enterobacter aerogenes	40	40	60	60	50	40	90	40

EE-Ethanol Extract; AE-Aqueous Extract; ME-Methanol Extract; Ofx- Ofloxacin.

Table 3. Synergistic activity of extracts of *Anisomeles malabarica* (L) (30µg/ml) with antibiotics (30µg/ml)

	Zone of Inhibition (mm)					
	Leaf			Wood		
	E	O	EO	E	O	EO
E.coli	7	10	13	4	7	10
Salmonella typhi	9	11	17	6	9	12
Shigella dysenteriae	10	14	19	5	8	11
Campylobacter jejuni	-	7	9	-	5	9
Enterobacter aerogenes	-	-	7	-	4	7

E-Extract; O-Ofloxacin; EO-Extract/Ofloxacin.

DISCUSSION

The MIC of the extracts ranged from 10-100 µg/mL, with the aqueous extract of leaf demonstrating the lowest values (MIC 10 µg/ml) against *Salmonella typhi* and *Shigella dysenteriae*, followed by the ethanolic extract against *E. coli* (MIC 20 µg/mL) and *Shigella dysenteriae* (MIC 30 µg/ml) (Table 2). The methanolic extract of *Anisomeles malaborica* also showed inhibitory activity for Gram-positive and negative bacteria Boobalan Raja and Jeganathan Manivann, 2010. The aqueous extract of wood shows lowest values (MIC 10 µg/ml), followed by ethanolic extract against *E.Coli* (MIC 20 µg/ml) and *Salmonella typhi* (MIC 30 µg/ml). Most of the MIC values were lower, it indicating that the extracts could be bactericidal in action and high efficacy. The active extracts are reported to play a role in the prevention of colonization by bacteria and fungi Chiang, 2003. Lower MIC values (Table 2) and higher zones of inhibition (Figure 1 and 2) for aqueous extracts connotes higher solubility of phytoconstituents in the aqeuous compared to the other solvents used. Members of the bacterial genera *Actinomadura, Microbispora, Microtetraspora and Sphingobacterium* were also found in similar environments to wood chip piles Novinscak *et al.*, 2009; Adams and Frostick 2009 and were able to degrade hemicellulose compounds, eg. xylan, under aerobic conditions Holtz, 1991; Berens, 1996; Pankratov, 2007. Table 3 shows the effect of combination of extracts and antibiotic agents on the test organisms. Results revealed an increased activity of ofloxacin (30 µg/mL) in the presence of both leaf and wood extract (30 µg/mL). At 30 µg/mL ofloxacin extracts had no effect on *Enterobacter*, but when combined, there was a remarkable activity (7 mm zone diameter of inhibition). At 30 µg/mL the activity of the extract and ofloxacin against *Shigella dysenteriae* were 10 and 14 mm (zone diameter of inhibition), respectively but this increased to 19 mm when the extract and the antibiotics were combined. There has been much interest in recent years on compounds derived from plants and herbs for their medicinal properties or biological activities Al-dabbas *et al.*, 2006. Throughout human history, infectious diseases are known to have been treated with herbal remedies Ozturk and Ercisli, 2006.

A similar trend was observed with wood extract-ofloxacin combination against the bacterial species. At30 µg/mL, both ofloxacin and the wood extracts had very less effect on *Enterobacter and Enteromonas*, but when combined, there was a remarkable activity (7 mm and 6 mm zone diameter of inhibition respectively). At 30 µg/mL the activity of the extracts and ofloxacin against *Salmonella typhi* were 6 and 9mm (zone diameter of inhibition), respectively but this increased to 12 mm when the extracts and the antibiotics were combined. Many reports are available on the antiviral, antibacterial,

antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants Samy and Ignacimuthu, 2000; Palombo and Semple, 2001; Kumaraswamy, 2002; Stepanovic, 2003; Bylka, 2004; Behera and Misra, 2005; Govindarajan, 2006.

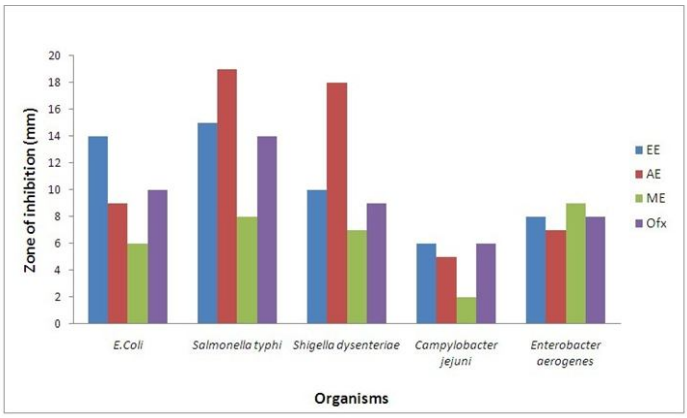

Figure 1. represents antibacterial activity of *Anisomeles malabarica* leaf extract

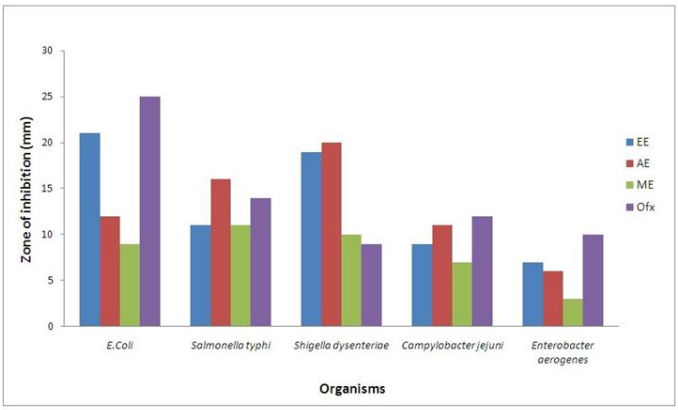

Figure 2. represents Antibacterial activity of *Anisomeles malabarica* wood extract

Conclusions

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergency of new bacterial strains that are multi-resistant. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The use of natural products as antibacterials seems to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food. Extract showed varying of inhibitory effects. The plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Thus, the study ascertains the value of *Anisomeles malabarica* plant used in ayurveda, which could be of considerable interest to the development of new drugs.

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