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Targetting Vancomycin Resistant *Enterococci* With Some Indian Medicinal Plants

Corresponding Author

Neeraj Khullar[1,†]
[1]Department of Biotechnology Panjab University, Chandigarh -160014 (INDIA)
[†]3358 Euclid Avenue, Vancouver BC,V5R 5G2, (CANADA)
E-Mail: n_khullar@yahoo.com

Co-Author

Nikhil Kirtipal
Department of Biotechnology Panjab University, Chandigarh -160014 (INDIA)

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ABSTRACT

Reports on vancomycin resistant enterococci (VRE) infections in hospitals have been increasing worldwide in recent years. The emergence of these resistant bacteria has created a major concern and an urgent need for new anti-bacterial agents. In the past, a variety of plants growing in Indian forests have been used as medicines to prevent and cure several diseases. The use of natural products as anti-VRE agents would therefore be promising, in order to prevent these infections. In this study, the antimicrobial activities of methanolic as well as aqueous extracts of the medicinal plants. *Aristolochia indica*, *Andricus fecundator*, *Trapa bispinosa* and *Rheum emodi* against vancomycin resistant strains of *Enterococci* were evaluated. *Andricus fecundator* and *Aristolochia indica* showed promising results.

KEYWORDS

Vancomycin;
Antibiotic resistance;
Enterococci;
Aristolochia indica;
Andricus fecundator;
Trapa bispinosa;
Rheum emodi;
Medicinal plants and
Antimicrobial activity.

INTRODUCTION

Enterococci have emerged as important nosocomial pathogens. They have been demonstrated to be the third most common blood strain and urinary isolates, the most common isolate from surgical site infections and the fourth most common isolate from all sites[1]. Enterococci are generally not regarded as highly virulent bacterial pathogens. But they have been able to survive in the hospital environment because of their intrinsic resistance to currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons[2]. Till recently, vancomycin

was virtually the only drug that could provide consistent relief for the treatment of multidrug resistant *enterococci*[3]. Vancomycin is also the antibiotic of choice for serious infections with ampicillin-resistant *enterococci*. However, over the years, reports indicate that enterococcal resistance to vancomycin is increasing throughout the world[4]. In 1988, Uttley et al. were the first to report the isolation of vancomycin resistant *Enterococci* (VRE) in England[5]. Since then, VRE have spread with unanticipated speed to the west. However, these organisms are difficult to eradicate, because of their inherent and acquired resistance to several antibiotics[6]. In a recent study[7] on the occurrence and relatedness of VRE in animals, humans, and the environment in different European regions, it was concluded that animal associated VRE probably reflect the former use of avoparcin in animal production, whereas VRE in human associated samples may be a result of antibiotic use in hospitals. *Enterococcus fecalis* and *Enterococcus faecium* have recently got much attention, because of their high level of resistance to several antimicrobial agents including glycopeptides, thus emphasizing the importance of controlling the spread of such organisms[8].

The emergence of these resistant bacteria has created a major concern worldwide, as multiple drug-resistant organisms such as VRE and MRSA (Methicillin resistant *Staphylococcus aureus*) are becoming a common cause of hospital-acquired infections[9], thus creating an urgent need for new anti-bacterial agents[10].

The usage of plants in curing illnesses has deep roots in man's history since plants are sources of many life-sustaining metabolites. A major part of the world's population depends on traditional medicine. The greater part of traditional therapy involves the use of plant extracts or their active principles[11].

In India, medicinal plants have long been used to treat various diseases. There is an evidence of the use of medicinal plants in 'Rigveda', which is believed to have been written between 3500 to 1800 B.C. Also, a detailed information about herbs has been recorded in 'Atharveda' and 'Ayurveda'.

Numerous wild plants growing in Indian forests have been used as medicines to prevent and cure several diseases[12]. The roots of *Aristolochia indica* have been shown to be a good antidote to snake bite, insects and scorpions, whereas *Andricus fecundator* has been recommended in constipation and its powder is given as a purgative, either alone or in combination with other drugs[13]. The fruits of *Trapa bispinosa* are considered useful in some parts of Northern India in bilious affectious with diarrhea[14]. Similarly, the bark of *Rheum emodi* is given for irritation of the bowels, common among children when they are teething, in chronic dysentery and in duodenal catarrh of the biliary ducts with jaundice[13].

When the medicinal activities of organic and aqueous extracts of fifteen Palestinian medicinal plants were explored against eight different species of bacteria, it was reported that *Thymus vulgaris* was the most active antibacterial plant extracts against both gram positive and gram-negative bacterial strains including *Enterococci*[15]. Cycloartane type triterpenes (16α-hydroxymollic) exhibited moderate antimicrobial activity against VRE[16]. Yet, in another study, naphthazabins from *Arnebia euchroma* were also active against VRE[17].

Hence, one of the measures to combat the increasing rate of resistance in the long run is to have a continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute the non-effective ones. Natural products as anti VRE agents, would therefore be one of the promising fields on the way of preventing VRE infections.

EXPERIMENTAL

Plant material

Four plants having medicinal properties and importance in the control of urinary tract infections (UTI) as reported in the ayurvedic literature were used for the study (TABLE 1). The plant material was collected and its identity was established with the help of Department of Botany, Panjab University, Chandigarh. The plants were shade dried, powdered and used for the preparation of extracts.

Preparation of plant extracts

Two types of extracts (aqueous and methanolic) were prepared by stirring the plant material overnight at room temperature with a seven-fold amount of water/methanol. The suspension was cold centrifuged

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TABLE 1: Plants and their parts used

S.NO.	Botanical name	Common name	Part used
1	Aristolochia indica	Isarmula	Root
2	Andricus fecundator	Muphal	Galls
3	Trapa bispinosa	Singhara	Dry fruits
4	Rheum emodi	Revand chini	Stem

at 3000rpm for 15-20 minutes and the supernatant collected. The suspension was filtered through a Whatman filter paper No. 1, concentrated to half of its original volume at room temperature, filter sterilized with 0.45µm millipore filters and stored in screw capped vials at -20°C.

Microorganisms used

Clinical isolates of *enterococci* from patients with UTI were obtained from the post graduate Institute of Medical Education and Research (PGIMER), Chandigarh. Screening for vancomycin resistance was done by agar screen method on both Muellar-Hinton agar and brain heart infusion agar(Difco Laboratories, Detroit, USA)[18]. In addition, a standard strain of *Enterococcus faecalis* (MTCC439) was purchased from the Institute of Microbial Technology, Chandigarh.

Evaluation of antimicrobial activity of extracts

Mueller-Hinton agar was prepared and inoculated with 0.5 MacFarland standard of the bacterial culture. 100µl of the suspension was spread on the test plate. Sterile discs (6mm diameter) impregnated with 20µl of the plant extract (aqueous/methanolic) were placed on the inoculated plate, incubated at 37^{0}C for 24 hrs and the zone of inhibition measured. The experiment was repeated thrice and the mean observations were recorded.

Determination of minimum inhibitory concentration (MIC)

The microdilution broth method[19] was used to determine the MIC. A stock solution of 25.6mg/ml of the methanolic plant extract was prepared in Mueller-Hinton broth. Further, serial double dilutions ranging from 25.6mg/ml to 0.005mg/ml were made. A 100µl aliquot of each dilution was put in individual wells of a micro titer plate. Positive and negative controls were set up simultaneously. 5ml of bacterial suspension of 0.5 McFarland standard was added to each well and the inoculated plate was incubated at 37^{0}C for 24 hrs. After examining turbidity visually, 40µl of 0.02mg/ml 2,3,5-triphenyl tetrazolium chloride (TTC) was added to each well and incubated at 37^{0}C for 30min. The MIC value was measured as the lowest concentration of the extract that prevented the growth. All samples were examined in duplicate in two separate experiments.

RESULTS AND DISCUSSION

Medicinal plants may be a new source of antibacterial agents for use in a variety of diseases. In many parts of the world, medicinal plants are used for antibacterial, antifungal and antiviral activities. Their plant extracts are an effective source of medicinal agents to cure urinary tract infections, cervicitis, vaginitis, gastrointestinal disorders[20] and skin infections such as herpes simplex virus type I. Previous studies in our laboratory also, have emphasized their usage against *salmonella typhi*[21, 22]. The focus on plants presently, is due to an increased incidence of (*enterococci*) developing resistance to many antimicrobials drugs that were once the mainstay in the treatment of *enterococci*. These include penicillin's, amino-glycosides, and vancomycin, which are no longer effective in many situations where resistant *enterococci* are encountered.

This research work therefore, was carried out in order to find out the antibacterial activity of some Indian medicinal plants against resistant microorganisms. The present investigation deals with the antibacterial activity of the aqueous and methanolic extracts of *Aristolochia indica*, *Andricus fecundator*, *Trapa bispinosa* and *Rheum emodi* against vancomycin resistant (*enterococci*). Of the eight plant extracts tested (TABLE 2), seven showed activity against VRE strains. Both methanolic and aqueous extracts of *Andricus fecundator* and *Aristolochia indica* showed maximum inhibition against the microorganisms (zone of inhibition=9-15). Moderate antimicrobial activity (zone of inhibition=5-9) was shown by *Trapa bispinosa* and *Rheum emodi*. The aqueous extract of *Trapa bispinosa* did not show any activity. In general, methanolic extracts were more effective than aqueous extracts.

In a different study[23], ethanolic extracts of five traditional Australian medicinal plants were investi-

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TABLE 2: Antimicrobial activity of some medicinal plants by the disc diffusion assay

Microorganism	Size of Zone of inhibition (mm)							
	Andricus fecundator		*Aristolochia indica*		*Trapa bispinosa*		*Rheum emodi*	
	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.
E. faecalis (1)	+++	+++	+	++	-	++	+	-
E. faecalis (2)	+++	+++	+++	++	-	++	+	-
E. faecium (3)	++	+++	+	+++	-	-	-	+
E. faecalis (4)	+++	+++	+	++	-	+	+	++
E. faecalis (5)	++	+++	+	+	-	++	+	+
E. faecium (6)	+++	+++	+	++	-	+	+	-
E. faecalis (MTCC439)	++	++	+	+++	-	-	+	-

- No inhibition
+ Diameter of zone of inhibition ≥ 5-9mm
++ Diameter of zone of inhibition ≥ 9-15mm
+++ Diameter of zone of inhibition ≥ 15mm

TABLE 3: Minimum inhibitory concentration (μg/ml) of plant extracts against *Enterococci* strains

Microorganisms	Plant extracts			
	Andricus fecundator		*Aristolochia indica*	
	Methanolic	Aqueous	Methanolic	Aqueous
E. faecalis (1)	16	32	128	ND
E. faecalis (2)	16	32	128	ND
E. faecium (3)	16	32	128	ND
E. faecalis (4)	16	32	128	ND
E. faecalis (5)	16	32	128	ND
E. faecium (6)	16	32	128	ND
E. faecalis (MTCC439)	16	32	128	ND

ND : Not done

gated for their abilities to inhibit clinical isolates of methicillin-resistant *Staphylococcus aureus* and vancomycin resistant enterococci. Most of the extracts showed bactericidal effects and also reduced the number of viable cells by 4-6 logs within 4 hours, while two extracts exhibited bacteriostatic activity against VRE. Further, a marked synergism between Calozeyloxanthone and vancomycin hydrochloride against VRE was also observed. These findings suggest that a combination therapy may be useful in controlling VRE infections[24]. Similarly, alpha-mangostin, isolated from the stem bark of *Garcinia mangostana* was found to be active against VRE[25]. Antimicrobial activities of hydrophobic 2-arylbenzo-furans and an isoflavone isolated from medicinal plants against VRE and MRSA have also been demonstrated[26].

In our study, the plant extracts showing strong antibacterial activity were further tested to determine their MIC's. The MIC values ranged from 16μg/ml to 128μg/ml (TABLE 3).

In another study[27], the plant *Magnolia officinally* was subjected to bioassay directed fractionation which led to the isolation of some known neolignans from the methanolic extract. These neolignans exhibited antibacterial activities against VRE and MRSA at MIC's in the range of 6.25ug/ml-25ug/ml. Yet another study[28] identified two principal antimicrobial components of an extract derived from *Iostephane heterophylla* which exhibited an MIC of 16ug/ml-32ug/ml against MRSA and VRE.

In general, all the extracts tested by us, exhibited significant inhibitory potential against VRE strains. The present work is an effort to highlight the importance of plants for their possible usage as microbicidal and microbistatic agents in herbal medicines. Both the methanolic and aqueous extracts of these medicinal plants claimed by traditional healers and clinicians to cure urinary tract infections have a strong antimicrobial activity against VRE. Thus, their use in traditional medicine is justified.

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