

Antibacterial Activity of Plants Extracts against Methicillin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant *Enterococcus faecalis*

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Abstract

The antibiotics resistant micro-organism has increased in past three decades. The Methicillin Resistant *Staphylococcus aureus* (M.R.S.A) and Vancomycin resistant *Enterococcus faecalis* (VRE) is a problematic in the field of medical science. This organism tends to cause serious infection in the burnt patients and mortality. MRSA is a major nosocomial pathogen. Thereby our study aims to control these micro-organisms using various plants extracts. Four plant species *Ageratum conyzoides*, *Phyllanthus emblica*, *Camellia sinensis* and *Mentha longifolia* were collected and subjected to ethanolic extraction. The ethanolic extracts were tested against clinically isolated multidrug resistance *Enterococcus faecalis* and *Staphylococcus aureus*. The ethanolic plants extracts were prepared at variable concentrations for *Staphylococcus aureus* and *Enterococcus faecalis*. In view of MRSA against clinically isolated *Staphylococcus aureus* and *Enterococcus faecalis* the concentration based analysis showed the zone of inhibition against the selected micro-organism in which *Camellia sinensis* was found potent against *Staphylococcus aureus* and *Mentha longifolia* against *Enterococcus faecalis*. In future after the detailed study it can be used as a potent biochemical drugs to overcome the infections caused by these organisms.

Keywords: MRSA (methicillin resistant *Staphylococcus aureus*); VRE (vancomycin resistant *Enterococcus faecalis*); MDR (multidrug resistant)

Introduction

The emergence and spread of multidrug resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy [1]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [2]. 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 [3]. The most problematic bacteria include, but are not limited to, extended-spectrum β -lactamase-producing *Escherichia coli* (ESBL-EC) and *Klebsiella pneumoniae* (ESBL-KP), carbapenem-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE) [4,5]. Thus, Infectious Diseases Society of America has recognized MRSA, VRE, ESBL-EP, ESBL-KP and *Pseudomonas aeruginosa* as notorious pathogens among the six major pathogens to which therapies with effective newer antimicrobials are urgently required [1,5]. Incidences of epidemics due to drug resistant microorganisms are now a common global problem posing enormous public health concerns [6]. The global emergence of multi drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections [7]. Antimicrobial drug resistance is also of economic concern with impact on doctors, patients, health-care administrators, pharmaceutical companies and the public [8]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [9]. Therefore, there is a need to look for substances from other sources with proven antimicrobial

activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs [10,11]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds [12]. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [6]. A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens [13-15]. Many plant species have been used by the indigenous people of India as traditional medicines, including as treatments for infectious diseases. Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds [16]. Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts [17]. The aim of this study is to investigate the antibacterial properties of these extracts by exploring the activities against a collection of clinical isolates of MRSA and VRE.

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Materials and Methods

Plant material

Four plant samples were collected on the basis of traditional medicinal history of India from garden, local markets were studied. All the plant materials were further identified in the Department of Botany, D. N PG College Meerut, UP, India. Table 1 shows the botanical name, family, parts used and ethno-medicinal use of the plants under this study.

Preparation of plant extracts

The plant extracts were prepared with some modifications in the guidelines of [18]. Plant material was freeze-dried and milled to a coarse powder to extract sequentially with hot water and 80% ethanol. Each solvent was replaced three times with fresh solvents and was allowed to remain in contact with the plant materials for 48 hrs. The concentrated extract was centrifuged at 10,000 × g for 20 min at 20°C. The supernatant was recovered, filter sterilized evaporated to dryness in vacuum and stored at -70°C.

Microbial strains

The MRSA strains used in this study were clinical isolates from patients presenting with symptoms of *S. aureus*-associated diseases. The isolates were identified as *S. aureus* according to colonial and microscopic morphology, positive catalysis, hemolysis and coagulase production. Strains of *Enterococcus faecalis* were isolated from patients suffering from nosocomial infections and identified on the basis of glucose fermentation without gas production, catalysis reaction, gamma hemolysis, reduction of litmus milk. Standard strains of *Enterococcus faecalis* (EFS) and *Staphylococcus aureus* (SAS) were done in the Department of Zoology, D. N PG College Meerut, UP, India. Standard strains and clinical isolates of MRSA and VRE.

Assay for antibacterial activity

After identification on selective medium all bacteria were grown on Nutrient Agar (Hi-Media M001A) and in Nutrient Broth (Hi-Media M002) at 37°C. Antimicrobial activity of the plant extracts was determined by the agar well diffusion method (Holder et al 1994), with modifications. 200 µL of overnight NB culture were added to 15 ml of molten MUELLER-HINTON Agar (Hi-Media M-173), mixed well, poured into a sterile PETRI dish and allowed to set. A sterile cork-borer (5 mm diameter) was used to make wells in the set agar. 25 µL of plant extract, diluted 1:200 in sterile water, were added to triplicate wells and the plates were incubated overnight at 37°C. Antibacterial activity was recorded as a zone of growth inhibition of greater than 5 mm around the well. All *S. aureus* isolates were tested for methicillin resistance. The disk diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS) was used with a 1 µg oxacillin disk (Oxoid). Zone sizes were read after incubation at 35°C for 24 h. Isolates with zone sizes 10 mm were considered methicillin resistant.

Results and Discussion

The plant extracts were prepared in different concentrations ranging from 0.2 mg/ml to 2.0 mg/ml using four different plants, the best results were obtained by enlisted concentration of plants- *Ageratum conyzoides* (Family- Asteraceae) 0.42 mg/ml and 1.52 mg/ml, *Phyllanthus emblica* (Family- Phyllanthaceae) 0.75 mg/ml and 0.85 mg/ml, *Camellia sinensis* (Family- Theaceae) 0.85mg/ml and 0.70 mg/ml and *Mentha longifolia* (Family- Lamiaceae) 1.32 mg/ml and 0.55 mg/ml for MRSA and VRE, respectively. As the bioactivity of plant

extracts depends on the water and ethanol concentration used in the extraction process. Since ethanol is safe to ingest thereby it is most commonly used organic solvent to extract plants extracts which is used as potential therapeutic agent by human society. These extracts were tested for antimicrobial activity against gram positive bacteria Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from burnt patients and gram positive bacteria Vancomycin *Enterococcus faecalis* (VRE) isolated from patients suffering from nosocomial infections. These micro-organisms are responsible for causing serious infection in the human body thus need to be treated. The antimicrobial activity was studied using zone of inhibition and zone >5mm was considered as positive result as shown in Table 2 and Figure 1 for MRSA and Table 3 and Figure 2 for VRE. It was observed that *Camellia sinensis* shows greater zone of inhibition against Methicillin resistant *Staphylococcus aureus* (MRSA), *Mentha longifolia* against Vancomycin *Enterococcus faecalis* (VRE), as compared to other plant's extracts.

Conclusion

Our studies have suggested that the plant *Camellia sinensis* is found potential antimicrobial agent against gram positive Methicillin resistant *Staphylococcus aureus* (MRSA) at 0.85 mg/ml concentration and plant *Mentha longifolia* against gram positive Vancomycin *Enterococcus faecalis* (VRE) at 0.55 mg/ml concentration. The activity of the *Camellia sinensis* and *Mentha longifolia* extracts provides preliminary scientific validation for the traditional medicinal use of this plant. Thus

Botanical name	Family name	Common Name	Part Used	Ethnomedicinal Uses
<i>Ageratum conyzoides</i>	Asteraceae	Billygoat-weed	Leaves	Pneumonia [19], bactericide, antidiarrhetic [20], fever, rheumatism, headache and colic [21,22]
<i>Phyllanthus emblica</i>	Phyllanthaceae	Amla	Arial Part	Antimicrobial [23], antioxidant [24,25], anti-inflammatory [26], analgesic and antipyretic [27,28], Adaptogenic [29], hepatoprotective [30].
<i>Camellia sinensis</i>	Theaceae	Green Tea	Leaves	Anticancer activity [31], Cardiovascular Diseases [32].
<i>Mentha longifolia</i>	Lamiaceae	Horse Mint	Arial Part	Throat irritation, mouth and sore throat [33].

Table 1: Plant used in antimicrobial assay with their Ethno-medicinal uses.

Plant Species	Antibacterial activity against*				
	SA 1	SA 2	SA 3	SA 4	SAS
<i>Ageratum conyzoides</i>	[6]	[6]	[6]	[6]	6
<i>Phyllanthus emblica</i>	6	5	6	6	6
<i>Camellia sinensis</i>	6	7	8	8	10
<i>Mentha longifolia</i>	[6]	[6]	[6]	[6]	7
<i>Penicillin G</i> (Reference Drug Control)	12	12	12	12	12

* Activity of plants extracts was determined from agar well diffusion assay. Numbers indicates zone of inhibition of test cultures around the wells. Each numbers was the average of quadruplets Zone of inhibition >5mm was considered as positive result. Numbers in the square brackets indicates incomplete inhibition (hampred growth but not complete abolition of growth) was observed within the specified zone. Concentrations of extracts were 0.425, 0.75, 0.85 and 1.32 mg/ml for *Ageratum conyzoides*, *Phyllanthus emblica*, *Camellia sinensis*, *Mentha longifolia*, respectively, *Penicillin G* was used as a reference control drug

Table 2: Activity of plant extracts against MRSA isolates.

Activity of plant extracts against MRSA isolates

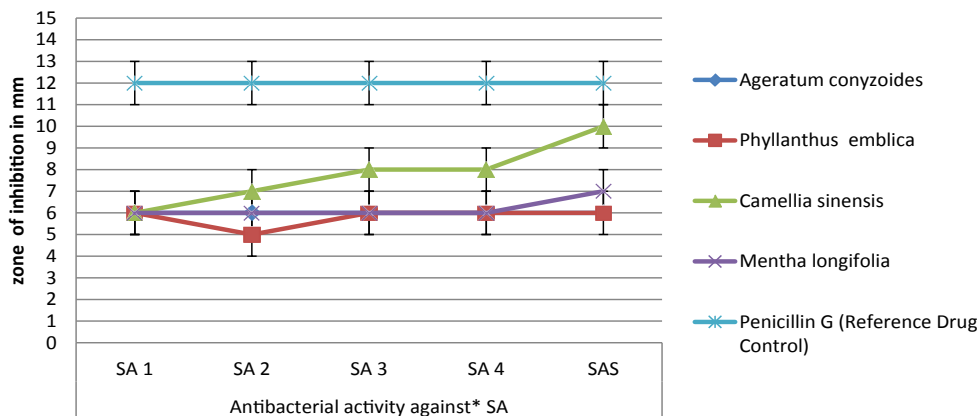


Figure 1: Graph showing the inhibition of MRSA against plant extracts denoted in Table 2.

Plant Species	Antibacterial activity against*				
	EF 1	EF 2	EF 3	EF 4	EFS
<i>Ageratum conyzoides</i>	[7]	[7]	[7]	[7]	7
<i>Phyllanthus emblica</i>	6	-	5	5	6
<i>Camellia sinensis</i>	[6]	[6]	[6]	[6]	7
<i>Mentha longifolia</i>	7	7	9	8	10
Penicillin G (Reference Drug Control)	12	12	12	12	12

*Activity of plants extracts was determined from agar well diffusion assay. Numbers indicates zone of inhibition of test cultures around the wells. Each number was the average of quadruplets Zone of inhibition >5mm was considered as positive result. A negative symbol indicates no inhibition. Numbers in the square brackets indicates incomplete inhibition (hampere growth but not complete abolition of growth) was observed within the specified zone. Concentrations of extracts were 1.52, 0.85, 0.70 and 0.55 mg/ml for *Ageratum conyzoides*, *Phyllanthus emblica*, *Camellia sinensis*, *Mentha longifolia*, respectively, *Penicillin G* was used as a reference control drug

Table 3: Activity of plant extracts against VRE isolates.

Activity of plant extracts against VRE isolates

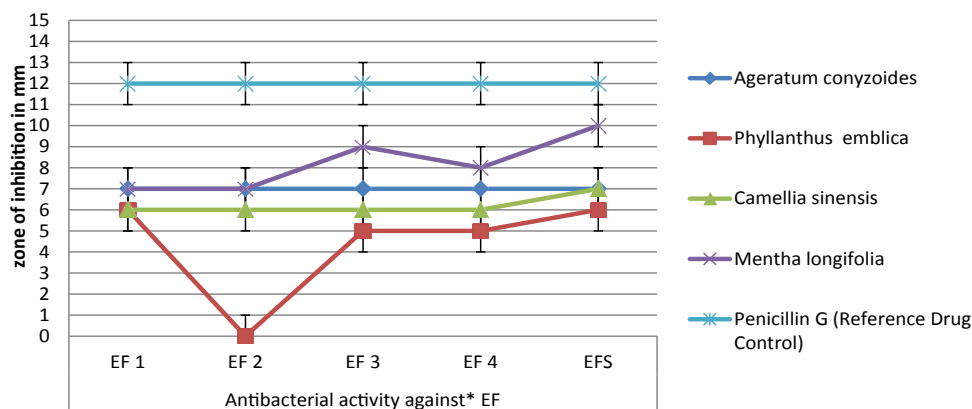


Figure 2: Graph showing the inhibition of VRE against plant extracts denoted in Table 3.

can serve as potential therapeutic agents however, the application of any compounds to medicine will require safety and toxicity issues to be addressed.

Conflict of Interests

The authors have no conflict of interests.

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