

# ANTIMICROBIAL POTENTIAL OF FIVE MEDICINAL PLANT EXTRACTS AGAINST THREE PATHOGENIC BACTERIA

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## ABSTRACT

Medicinal plants are nature's gift to cure a number of diseases in human beings and animals. Herbal medicine represents one of the most important fields of traditional medicine all over the world because of its ecofriendly nature. To promote the proper use of the herbal medicines and to determine their potential as a source for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way. The assessment of antimicrobial potential of five selected medicinal plant extracts dominant in Dharwad region, namely *Catharanthus roseus*, *Azadirachta indica*, *Eucalyptus globulus*, *Mimosa pudica* and *Costus igneus* against three pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were carried out. Antimicrobial activity of various plant extracts were tested by Agar well diffusion method. In extraction of antimicrobial compounds from the medicinal plant materials, the solvents alcohol, acetone and water were used. The plant extracts tested exhibited different degree of antimicrobial activity against gram +ve and gram -ve pathogenic bacteria. The *Catharanthus roseus*, *Eucalyptus globulus*, *Mimosa pudica*, *Costus igneus* plant extracts showed very effective degree of inhibition and *Azadirachta indica* showed moderate degree of inhibition against three pathogens in alcohol and acetone. The aqueous extracts of the plants were not showed degree of inhibition except *Azadirachta indica*, *Eucalyptus globulus* against *Staphylococcus aureus*.

## INTRODUCTION

In recent years the interest of natural extracts as an alternative for microbial pathogens has been increased. The use of medicinal herb in the treatment of diseases is attracting the attention of scientist worldwide. The plants kingdom has long serve as a prolific source of useful drugs, food, additives, flavoring agents, colorants, binders, and lubricants. Medicinal plants are nature's best gift to cure a number of diseases of men and animals. There are about 121 clinical useful prescription drugs that are worldwide derived from higher plants. About 70% of them came to the attention of pharmaceutical houses because of the use in traditional medicine (Abelson, 1990). The drugs used in indigenous systems of medicine like Ayurveda in India has about 18000 species of Angiosperms of which about 3000 species are considered as important sources of medicinal and aromatic chemical compounds (Rajasekaran, 2001; Hemashenpagam *et al.*, 2010).

Over the past 20 years there has been an increased interest in the investigation of natural materials of sources of new antimicrobial agents. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result, some natural products have been approved as new antimicrobial drugs, but there is an urgent need to identify novel substances that are active towards pathogens, with high resistance (Recio, 1989). Recently multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs

commonly used in the treatment of infectious diseases (Service, 1995) making it a new source antimicrobial agents with a possibly novel mechanism of action (Barbour *et al.*, 2004). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al.*, 1999). The isolation of microbial agents less susceptible to regular antibiotics and recovery of the increasing resistance isolates during antibacterial therapy is rising throughout the world, which highlights the need for new principles (Kandukuri and Singara, 2010).

In the present study the antimicrobial activity of five medicinal plants namely *Catharanthus roseus*, *Costus igneus*, *Eucalyptus globulus*, *Mimosa pudica* and *Azadirachta indica* around Dharwad region were tested against three pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* because of their higher antimicrobial activity and ecofriendly nature.

## MATERIALS AND METHODS

### Collection of plant materials

Five medicinal plants *Catharanthus roseus*, *Costus igneus*, *Eucalyptus globulus*, *Mimosa pudica* and *Azadirachta indica* were collected in Dharwad district. The leaf, stick, flowers, were plucked and washed under running tap water, dried in hot air oven and powdered.

### Collection of test organisms

The test organisms namely one gram negative *Escherichia coli* and two gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis* were obtained from Microbial Type Culture Collection Institute of Microbial Technology (MTCCIMT) Chandigarh.

#### Culture media and chemicals

The culture media consist of Nutrient Agar (NA), Nutrient Broth (NB) and for the growth of microbes were purchased from Hi-media. All other chemicals, including organic solvents, used for the extraction of the plant metabolites were of analytical grade, purchased from Hi-media.

#### Preparation of plant extracts (cold extraction method)

The plant extracts were prepared by cold extraction method. The three solvents alcohol, acetone, and aqueous were used in the present study. Two grams of air dried fine powder of plant was placed in 20 mL of water and solvents in different flasks to prepare plant extracts. The flasks were plugged with cotton and then kept on a rotary shaker for 3 days. After three days the contents were centrifuged at 2500 rpm for 15 min, the supernatant was collected and the solvent was evaporated to make higher concentration and it was stored at 4°C in air tight bottles for further studies.

#### Antimicrobial activity (Agar well diffusion method)

The extracts from leaf, stem, sticks and flowers were studied for antimicrobial activity. A loop full of standard strains were inoculated in 30mL nutrient broth in a conical flask and incubated for 24hr to activate the strain. In agar well diffusion method (Perez *et al.*, 1990) the media and the test bacteria cultures were poured into petridishes. The test strain 0.25mL was inoculated into the media care was taken to ensure the proper homogenization. The experiment was performed under strict aseptic condition. After the medium was solidified, a well was made in the plates with sterile borer (6mm). The extracted compound (0.05 and 0.1mL) was introduced into the well and the plates were inoculated at 37°C for 24hr. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of the zone of inhibition controls without test compounds were used and the control and activity was deducted from the test and the results were observed as zone of inhibition in mm.

## RESULTS AND DISCUSSION

The antimicrobial activity of five medicinal plants namely *Catharanthus roseus*, *Azadirachta indica*, *Eucalyptus globulus*, *Mimosa pudica* and *Costus igneus* were tested against three pathogenic bacteria namely *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. All the plant extracts were tested, exhibited different degrees of antibacterial activity against three pathogenic bacteria (Table 1 and 2). The aqueous extracts of various parts of five medicinal plants did not showed any antimicrobial activity except *Azadirachta indica* (Leaf Stick) and *Eucalyptus globulus* at 0.1mL concentration were found to be effective against *Staphylococcus aureus*. The alcohol extract of leaf and flower of *Mimosa pudica*, *Costus igneus*, *Catharanthus roseus*, *Eucalyptus globulus* were found to be very effective against all the pathogenic bacteria at 0.1mL with maximum zone of



Figure 1: Zone inhibition photos of five medicinal plants against three pathogenic bacteria *Mimosa pudica* leaf

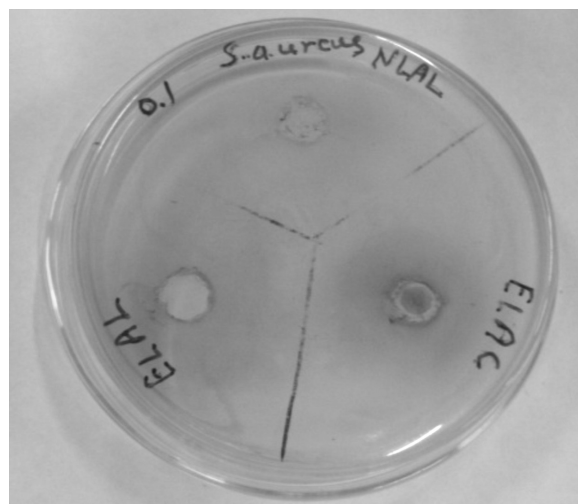


Figure 2: Zone inhibition photos of five medicinal plants against three pathogenic bacteria *E.globulus* and *A. indica* leaf



Figure 3: Zone inhibition photos of five medicinal plants against three pathogenic bacteria *E.globulus* and *A. indica* leaf

**Table 1: Antibacterial activity of five medicinal plants in 0.05mL solvents extract against three pathogenic bacteria**

Extract	Test organisms	<i>M.pudica</i> leaf Zone of inhibition (mm)	<i>C.roseus</i> flower	<i>C.roseus</i> leaf	<i>C.igneus</i> leaf	<i>E.globulus</i> leaf	<i>A.indica</i> leaf	<i>A.indica</i> stick
Alcohol	<i>E.coli</i>	20	16	16	18	16	11	10
	<i>S.aureus</i>	16	23	15	15	24	12	08
	<i>B.subtilis</i>	24	18	16	20	20	12	11
Acetone	<i>E.coli</i>	18	15	16	18	15	10	09
	<i>S.aureus</i>	16	17	15	16	20	13	09
	<i>B.subtilis</i>	22	18	17	20	18	09	10
Aqueous	<i>E.coli</i>	-	-	-	-	-	-	-
	<i>S.aureus</i>	-	-	-	-	-	-	-
	<i>B.subtilis</i>	-	-	-	-	-	-	-

**Table 2: Antibacterial activity of five medicinal plants in 0.1mL solvents extract against three pathogenic bacteria**

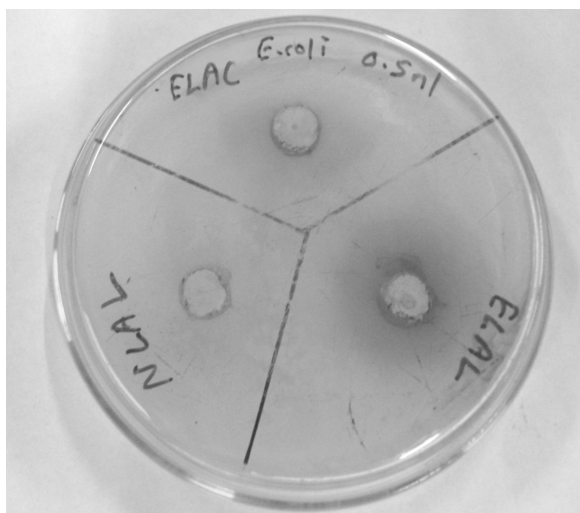
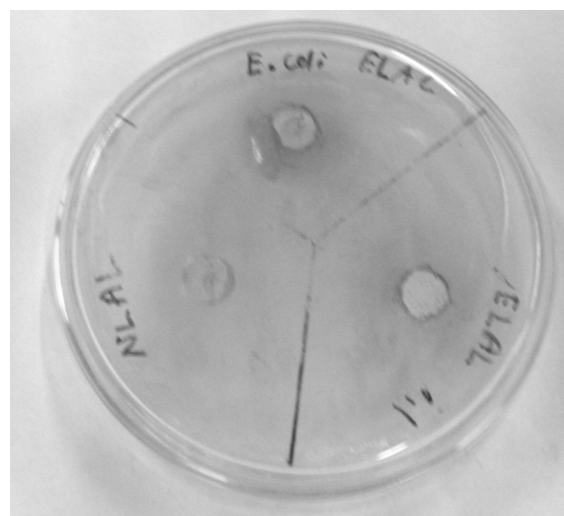
Extract	Test organisms	<i>M.pudica</i> leaf	<i>C.roseus</i> flower	<i>C.roseus</i> leaf	<i>C.igneus</i> leaf	<i>E.globulus</i> leaf	<i>A.indica</i> leaf	<i>A.indica</i> stick
Zone of inhibition (mm)								
Alcohol	<i>E.coli</i>	24	18	22	17	16	12	10
	<i>S.aureus</i>	20	15	16	18	20	11	09
	<i>B.subtilis</i>	20	24	18	20	22	10	08
Acetone	<i>E.coli</i>	16	24	20	16	18	08	10
	<i>S.aureus</i>	18	16	17	20	18	12	08
	<i>B.subtilis</i>	20	20	18	24	20	10	12
Aqueous	<i>E.coli</i>	-	-	-	-	-	-	-
	<i>S.aureus</i>	-	-	-	-	18	-	20
	<i>B.subtilis</i>	-	-	-	-	-	-	-

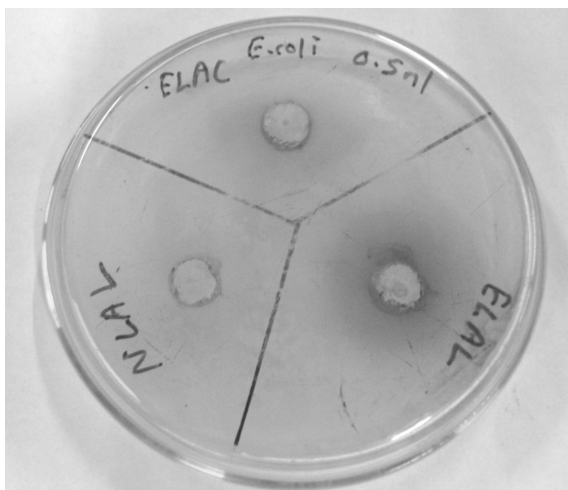
inhibition 15 - 24 mm except alcohol extract of leaf and stick of *Azadirachta indica* was found to be less effective against three pathogenic bacteria for both concentration (0.1 and 0.05 mL) with minimum zone inhibition 8-12mm (Fig. 2). The acetone extract of leaf stick of *Azadirachta indica* was also found to be less effective against three pathogens for 0.1 and 0.05mL concentration with minimum zone inhibition of 8-12mm.

The alcohol extract of leaf of *Mimosa pudica* for 0.1mL concentration showed the very effective antibacterial activity against all three pathogenic bacteria with zone inhibition from 20-24 mm (Fig. 1). The extract of leaf and flower of *Catharanthus roseus* was showed effective antibacterial activity

against all three pathogenic bacteria with zone inhibition ranging from 15-24 mm. The extract of leaf of *Costus igneus* was showed antibacterial activity against all three pathogens with zone inhibition ranging from 17-20mm. The extract of leaf of *Eucalyptus globulus* was showed antibacterial activity against all three pathogens with zone inhibition ranging from 16-22 mm.

The acetone extract of *Mimosa pudica* leaf was showed antibacterial activity against all three pathogens for 0.1mL concentration with zone inhibition ranging from 16-20mm. The extract of leaf and flower of *Catharanthus roseus* was showed effective antibacterial activity with zone inhibition ranging from 16-24mm. The leaf of *Costus igneus* showed

**Figure 4: Zone inhibition photos of five medicinal plants against three pathogenic bacteria *C.roseus* and *C.igneus* leaf****Figure 5: Zone inhibition photos of five medicinal plants against three pathogenic bacteria *A. indica* and *E.globulus* leaf**



**Figure 6: Zone inhibition photos of five medicinal plants against three pathogenic bacteria *E.globulus* and *A indica* leaf**

very effective antibacterial activity against all three pathogens with zone inhibition ranging from 16-24mm. The leaf of *Eucalyptus globulus* showed antibacterial activity with zone inhibition ranging from 18-20mm (Fig. 5). The alcohol extract of leaf of *Mimosa pudica* was showed effective antibacterial activity against all three pathogens for 0.05mL concentration with zone inhibition ranging from 16-24 mm. The leaf and flower of *Catharanthus roseus* was showed effective antibacterial activity with zone inhibition ranging from 15-23mm. The extract of *Costus igneus* was showed effective antibacterial activity with zone inhibition ranging from 15-20mm. The *Eucalyptus globulus* was showed effective antibacterial activity with zone inhibition ranging from 16-24mm (Fig. 6). The extract of leaf and stick of *Azadirachta indica* was showed less antibacterial activity against all three pathogens with zone inhibition ranging from 8-12mm.

The acetone extract of leaf of *Mimosa pudica* was showed effective antibacterial activity against all three pathogens for 0.05mL concentration with zone inhibition ranging from 16-22mm. The extract of leaf and flower of *Catharanthus roseus* was showed effective antibacterial activity with zone inhibition ranging from 15-18mm. The extract of leaf of *Costus igneus* was showed effective antibacterial activity with zone inhibition ranging from 16-20mm. The extract of leaf of *Eucalyptus*

*globulus* was showed effective antibacterial activity with zone inhibition ranging from 16-24mm (Fig. 3). The extract of leaf and stick of *Azadirachta indica* was showed less antibacterial activity with zone inhibition ranging from 9-13mm (Fig. 4). From these results it was observed that the solvents alcohol and acetone extracts have antibacterial compounds from various plant parts.

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