



## Antibacterial Activity of Leaf and Root of *M. pudica* L. against Selected Human Pathogenic Microorganisms

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### Abstract

*Mimosa pudica* L. is an important medicinal plant and it possess traditional medicinal value. The present study was aimed to investigate the antibacterial activity of leaf and root of *M. pudica* against selected bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptococcus pyogenes* by disc diffusion method. The solvents acetone, aqueous, benzene, diethyl ether and ethanol were used for the preparation of extracts from leaf and root of *M. pudica*. In the present study, various concentrations of extracts 2.5 mg/50  $\mu$ l, 3.75 mg/75  $\mu$ l and 5 mg/100  $\mu$ l were used and prepared discs individually for the determination of antibacterial activity against selected bacterial species. The prepared discs were placed on each petriplate with respective bacterial species along with control dimethyl sulfoxide (DMSO) and standard nitrofurantoin discs and then the plates were incubated at 37°C for 24 hrs. After incubation period, the diameters of zones formed around the discs were measured. The antibacterial activity of leaf and root of *M. pudica* was in the range between 08  $\pm$  0.2 mm and 26  $\pm$  0.5 mm. Among the tested concentrations, 5 mg of both leaf and root extracts showed maximum antibacterial activity than other concentrations 2.5 and 3.75 mg. The maximum levels of zone of inhibition were observed in benzene leaf extract against *S. pyogenes* 26  $\pm$  0.5 mm, *E. coli* 25  $\pm$  1.2 mm and *K. pneumoniae* 25  $\pm$  0.8 mm. The minimum level of zone of inhibition was observed in acetone root extract against *E. coli* 08  $\pm$  0.2 mm and *P. mirabilis* 08  $\pm$  0.6 and aqueous leaf extract against *K. pneumoniae* 08  $\pm$  0.7 mm. Minimum inhibitory concentration (MIC) of extracts of leaf and root of *M. pudica* against selected bacterial species were also determined at different levels based on the tested microorganisms. The results of this study confirmed that the antibacterial activity of leaf and root of *M. pudica* against selected bacterial species and it may be source for the discovery of novel antimicrobial compounds.

**Keywords:** *Mimosa pudica*; Antibacterial activity; Leaf; Root; Microorganisms; Disc diffusion method

### Introduction

Plants contribute strongly to fulfil necessities of life such as food, medicine, clothing, and construction. The World Health Organization has catalogued 20,000 plant species studied for medicinal purposes [1]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and source of many potent and powerful drugs [2]. Plants synthesize secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins and volatile oils. The therapeutic efficacy of plants is because of these secondary metabolites for curing many diseases. Phytochemicals are pharmacologically active compounds such as alkaloids have antispasmodic, antimalarial, analgesic and diuretic activities, terpenoids possess antiviral, anthelmintic, antibacterial, anticancer, antimalarial and anti-inflammatory properties, glycosides have antifungal and antibacterial properties, phenols and flavonoids have antioxidant, antiallergic and antibacterial properties and saponins possess anti-inflammatory, antiviral and plant defence activities [3,4]. For a long period in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs [5]. The World Health Organization (WHO) recommends world-wide development of research on medicinal plants for therapeutic purposes, in order to obtain new possibilities for the treatment of diseases, especially in developing countries [6]. Bacterial and fungal infections are one of the serious global health problems in recent years [7]. The prevalence of microbial infectious diseases and their complications are continuously increasing throughout the world mainly due to microbial drug resistance towards commonly used antimicrobials [8]. Contrary to the synthetic drugs, antimicrobial substances of plant origin are not associated with many side effects and have an enormous therapeutic

potential to heal many infectious diseases [9]. Therefore, novel types of effective and healthy antimicrobial compounds against infection are highly demanded. Plant derived antimicrobials are also considered to be safer compared with synthetic compounds because of their natural origin. The place of plant-based pharmaceuticals in global economy and also as a component of healthcare delivery system is critical and it makes research on medicinal plants crucial [10]. Medicinal plants are used as medicine and source for many potent drugs. Antimicrobials from plant source would be an excellent choice due to no side effects. Bacteria cause serious infection in human as well as other animals. The rapid spread of bacteria expressing multidrug resistance (MDR) has necessitated the discovery of new antibacterial and resistance modifying agents. So there is need to develop new alternative plant based antimicrobial drug instead of synthetic drug. Plants are used medicinally in different countries and as sources of many potent and powerful drugs [11]. Medicinal plants constitute an effective source of antimicrobial natural products. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs [12]. Medicinal plants are abundant source of antimicrobial agents. A wide range of medicinal plant extracts are used to treat several infections caused by

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microorganisms as they have potential antimicrobial activity. *Mimosa pudica* L. (Family Mimosaceae) is locally known as lajwanti or chuimui in Hindi and is native of Central America, Tanzania, South Asia, East Asia and many Pacific Islands [13]. It is a common plant in moist waste ground, lawns, open plantations and weedy thickets [14]. The *Mimosa pudica* is distributed throughout India in moist locality [15]. *M. pudica* is a famous ornamental plant commonly known as sleeping grass, sensitive plant, humble plant, shy plant and touch-menot among other names. Its ornamental use can be attributed to its thigmonastic and seismonastic movements in which closure of leaves and hanging down of petioles takes place in response to certain stimuli like light, vibration, wounds, wind, touch, heat, and cold [16,17]. Besides its ornamental use, *M. pudica* is a popular plant among folk healers to treat several diseases. Traditionally *M. pudica* is used in the treatment of headache, migraine, insomnia, diarrhoea, dysentery, fever, piles and fistula. Roots in the form of decoction are used to treat urinary complaints and in diseases arising from corrupt blood and bile. The paste of the leaves is applied to glandular swelling and dressing for sinus [18-21]. *M. pudica* is known and valued for its analgesic, anti-inflammatory [22], hypoglycaemic [23], diuretic, astringent, antispasmodic, and blood-purifying activities [24]. It has been used to treat high blood pressure [25], menorrhagia, and leucorrhoea [26,27]. Many studies on pharmacological activities of *M. pudica*, but there are a few studies on antimicrobial activity. However there is no study on antibacterial activity of acetone, aqueous, benzene, diethyl ether and ethanol extracts of leaf and root of *M. pudica* against selected bacterial species *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptococcus pyogenes*. So, the present study was designed to investigate the antibacterial activity of leaf and root of *M. pudica* against selected human pathogenic microorganisms.

## Materials and Methods

### Collection and preparation of plant material

The fresh plants of *M. pudica* L. were collected from natural habitats of Thirupanipet Village, Thanjavur District, Tamilnadu, India. Thanjavur is geographically located in between 10.8° N and 79.15° E in Tamil Nadu is the state of India. Thanjavur has a tropical climatic condition, the average temperature 36.6°C in summer season and the average temperature 22°C in the winter season. The selected plant *M. pudica* was collected from January to March, which is between winter and summer seasons. The collected plant was identified by Rev. Dr. S. John Britto, Director, Rabinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and deposited in the herbarium (Voucher specimen number: KV 001). The collected plants were brought into the laboratory and washed thoroughly in running tap water to remove the soil particles and adhered debris and then finally washed with sterile distilled water. The leaf and root of *M. pudica* were separated and dried under shade for 10 days at room temperature. Then the plant materials were pulverized into powder. The powdered materials were stored in air tight containers until the time of use.

### Preparation of plant extract

Fifty gram of leaf and root powder of *M. pudica* were soaked in 500 ml of acetone, aqueous, benzene, diethyl ether and ethanol individually and then kept in orbital shaker for 48 h at room temperature. After 48 h, the mixture was filtered through a clean muslin cloth. Then the filtrate again filtered by using a Whatman No. 1 filter paper and the extracts were concentrated and dried in a rotary evaporator at 37°C [28]

till a sticky mass was obtained. After evaporation of solvents, the dried extracts were stored at 4°C until further use.

### Disc preparation

The 6 mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. The different solvent extracts of leaf and root of *M. pudica* were prepared in dimethyl sulfoxide (DMSO, 5% w/v). Then various solvent extract discs at different concentrations (2.5 mg, 3.75 mg and 5 mg) and control discs were prepared.

### Microorganisms

The microorganisms were selected for this study obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The bacterial species *Bacillus subtilis* (MTCC 2057), *Escherichia coli* (MTCC 1089), *Klebsiella pneumoniae* (MTCC 4030), *Pseudomonas fluorescens* (MTCC 2421) and *Streptococcus pyogenes* (MTCC 1926) were used in this study. The bacterial cultures were maintained in nutrient agar (NA) slants at 4°C.

### Preparation of microbial suspension culture

One ml of a 24 h broth culture of selected bacteria was aseptically distributed onto nutrient agar slopes and incubated for 24 h at 37°C. The bacterial growth was collected and washed with 100 ml of sterile normal saline to produce a suspension containing about 10<sup>6</sup>-10<sup>9</sup> colony forming units per millilitre (CFU/ml). The suspension was stored in the refrigerator at 4°C till the time of further use. The average number of viable organisms per millilitre of the stock suspension was determined. Serial dilution of the stock suspensions were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette to the surface of solidified nutrient agar plates. The plates were allowed to stand for 2 h at room temperature for dry, and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each 0.02 ml was counted. The average number of the colonies per 0.02 ml was multiplied by 50 and by the dilution factor to give the viable count of stock suspension cultures, expressed as the number of colony forming units per millilitre (CFU/ml) of suspension. Each time a fresh stock suspension culture was prepared for all the bacterial species were selected in this study.

### Assay of antibacterial activity

Antibacterial activity test was carried out by disc diffusion method originally described by Bauer et al. (1966) with slight modification. Muller Hinton Agar (MHA) media was prepared and autoclaved at 15 lbs pressure and 121°C for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The prepared discs with difference concentration (2.5 mg, 3.75 mg and 5 mg) of solvent extracts were placed separately on each petriplate along with control dimethyl sulfoxide (DMSO) and standard nitrofurantoin (100 µg) discs. The plates were incubated at 37°C for 24 hrs. After incubation period, the diameter of zone of inhibition formed around the paper discs were measured and expressed in millimetre (mm). Three replicates were performed and results were recorded.

### Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) for the leaf and root extracts of *M. pudica* against selected bacterial strains was determined

according to the method of Clinical and Laboratory Standards Institute [29]. The selected bacterial species (1%) of  $10^8$  CFU/ml of fresh culture was inoculated individually in 10 ml of nutrient broth containing various concentrations (1 mg, 2 mg and 3 mg in 20  $\mu$ l, 40  $\mu$ l and 60  $\mu$ l) of leaf and root extracts of *M. pudica* and then incubated aerobically at 37°C for 24 h. After 24 h of incubation, the MIC of plant extract against selected bacterial species was determined. The MIC was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation. The MIC measurement was done in triplicate to confirm the value of MIC for each tested bacteria.

### Statistical analysis

The results of this study were subjected to statistical analysis and expressed as mean  $\pm$  standard deviation of three replicates.

## Results

### Antibacterial activity

The antibacterial activity of different solvent extracts (5% w/v) of leaf and root of *M. pudica* at different concentrations 2.5, 3.75 and 5 mg were analyzed against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptococcus pyogenes* by disc diffusion method. The observed results were measured as diameter of zone of inhibition (mm). The antibacterial activity of various solvent extract at different concentrations was compared with the bactericidal activity of positive control. The positive control nitrofurantoin showed zone of inhibition ranges between  $24 \pm 0.5$  mm and  $25 \pm 1.8$  mm against all selected bacterial species.

### Antibacterial activity of leaf extracts

The antibacterial activity of acetone, aqueous, benzene, diethyl ether and ethanol extracts of leaf of *M. pudica* was carried out and the results were presented in Table 1. The benzene extract of leaf of *M. pudica* showed highest zone of inhibition against *Streptococcus pyogenes*  $26 \pm 0.5$  mm and *Escherichia coli*  $25 \pm 1.2$  mm at the concentration of 5 mg (100  $\mu$ l). The acetone extract of leaf showed highest zone of inhibition

against *Streptococcus pyogenes*  $25 \pm 0.6$  mm and *Klebsiella pneumoniae*  $24 \pm 0.4$  mm. The aqueous extract of leaf showed antibacterial activity against *Streptococcus pyogenes*  $24 \pm 0.6$  mm and *Bacillus subtilis*  $23 \pm 1.8$  mm. The diethyl ether extract showed zone of inhibition against *Streptococcus pyogenes*  $24 \pm 0.9$  mm and *Bacillus subtilis*  $24 \pm 0.5$  mm. The ethanol extract showed antibacterial activity against *Pseudomonas fluorescens*  $23 \pm 0.5$  mm and *Streptococcus pyogenes*  $22 \pm 1.1$  mm. The benzene, diethyl ether and ethanol extracts of leaf were also showed antibacterial activity against selected microorganisms in all tested concentrations.

### Antibacterial activity of root extracts

The antibacterial activity of acetone, aqueous, benzene, diethyl ether and ethanol extracts of root of *M. pudica* was also carried out and the results were shown in Table 2. The aqueous extract of root of *M. pudica* showed highest level of zone of inhibition against *Escherichia coli*  $25 \pm 1.8$  mm followed by *Proteus mirabilis*  $24 \pm 0.6$  mm and the minimum zone of inhibition was observed against *Streptococcus pyogenes*  $08 \pm 1.4$  mm. The acetone extract of root showed highest level of zone of inhibition against *Klebsiella pneumoniae*  $21 \pm 0.8$  mm and *Bacillus subtilis*  $20 \pm 0.6$  mm. The benzene extract of root of *M. pudica* showed maximum zone of inhibition against *Klebsiella pneumoniae*  $23 \pm 1.0$  mm and *Proteus mirabilis*  $20 \pm 0.8$  mm. The diethyl ether extract of root of *M. pudica* showed maximum zone of inhibition against *Bacillus subtilis*  $21 \pm 0.1$  mm and *Proteus mirabilis*  $20 \pm 0.7$  mm. The ethanol extract of root showed antibacterial activity against *Klebsiella pneumoniae*  $24 \pm 0.6$  mm, and *Pseudomonas fluorescens*  $21 \pm 0.5$  mm. The acetone and diethyl ether extracts of root were also showed antibacterial activity against selected microorganisms in all tested concentrations.

### Minimum Inhibitory Concentration (MIC) of leaf extracts

The Minimum Inhibitory Concentration (MIC) of the extract (5% w/v) of leaf of *M. pudica* was determined against selected microorganisms using 20  $\mu$ l (1 mg), 40  $\mu$ l (2 mg) and 60  $\mu$ l (3 mg) of extract in 10 ml of nutrient broth (v/v) and the results were presented in Table 3. The inhibition of bacterial growth (no visible growth)

Name of solvent extract	Concentration of plant extract	Diameter of zone of inhibition (mm)					
		Name of microorganism					
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas fluorescens</i>	<i>Streptococcus pyogenes</i>
DMSO	NC	-	-	-	-	-	-
Acetone	50 $\mu$ l (2.5 mg)	$16 \pm 1.2$	$22 \pm 0.6$	-	-	$16 \pm 0.8$	$22 \pm 0.5$
	75 $\mu$ l (3.75 mg)	$18 \pm 0.9$	$22 \pm 0.7$	$21 \pm 0.5$	$14 \pm 1.5$	$18 \pm 0.4$	$22 \pm 0.5$
	100 $\mu$ l (5 mg)	$23 \pm 0.8$	$24 \pm 0.4$	$22 \pm 0.7$	$20 \pm 0.9$	$23 \pm 1.6$	$25 \pm 0.6$
Aqueous	50 $\mu$ l (2.5 mg)	-	-	-	-	$07 \pm 0.4$	-
	75 $\mu$ l (3.75 mg)	$23 \pm 0.6$	$08 \pm 0.7$	-	$20 \pm 0.3$	$18 \pm 0.7$	$22 \pm 0.6$
	100 $\mu$ l (5 mg)	$23 \pm 1.7$	$20 \pm 0.7$	$20 \pm 0.9$	$23 \pm 1.8$	$20 \pm 0.8$	$24 \pm 0.6$
Benzene	50 $\mu$ l (2.5 mg)	$22 \pm 1.4$	$18 \pm 0.7$	$22 \pm 0.8$	$10 \pm 0.4$	$22 \pm 0.6$	$22 \pm 0.3$
	75 $\mu$ l (3.75 mg)	$22 \pm 0.6$	$24 \pm 0.5$	$22 \pm 1.6$	$14 \pm 1.0$	$24 \pm 0.3$	$22 \pm 0.6$
	100 $\mu$ l (5 mg)	$25 \pm 1.2$	$25 \pm 0.8$	$23 \pm 0.1$	$22 \pm 0.7$	$25 \pm 0.6$	$26 \pm 0.5$
Diethyl ether	50 $\mu$ l (2.5 mg)	$18 \pm 0.5$	$12 \pm 1.1$	$10 \pm 0.8$	$20 \pm 0.5$	$18 \pm 0.9$	$20 \pm 0.8$
	75 $\mu$ l (3.75 mg)	$22 \pm 0.6$	$16 \pm 0.6$	$22 \pm 0.7$	$23 \pm 0.4$	$22 \pm 0.8$	$24 \pm 0.3$
	100 $\mu$ l (5 mg)	$23 \pm 0.7$	$16 \pm 0.4$	$22 \pm 0.8$	$24 \pm 0.5$	$22 \pm 1.2$	$24 \pm 0.9$
Ethanol	50 $\mu$ l (2.5 mg)	$14 \pm 1.7$	$10 \pm 0.6$	$18 \pm 0.7$	$12 \pm 0.2$	$22 \pm 0.5$	$19 \pm 0.7$
	75 $\mu$ l (3.75 mg)	$16 \pm 0.7$	$12 \pm 0.3$	$19 \pm 0.5$	$18 \pm 0.6$	$22 \pm 0.8$	$18 \pm 0.9$
	100 $\mu$ l (5 mg)	$20 \pm 0.8$	$18 \pm 1.1$	$21 \pm 0.6$	$21 \pm 0.7$	$23 \pm 0.5$	$22 \pm 1.1$
Nitrofurantoin	PC (100 $\mu$ g)	$25 \pm 1.8$	$25 \pm 1.2$	$24 \pm 0.5$	$24 \pm 0.9$	$24 \pm 1.7$	$25 \pm 1.6$

NC: Negative control, PC: Positive control (Nitrofurantoin) Values are expressed as mean  $\pm$  standard deviation of three replicates

**Table 1:** Antibacterial activity of acetone, aqueous, benzene, diethyl ether and ethanol extracts of leaf of *M. pudica*

Name of solvent extract	Concentration of plant extract	Diameter of zone of inhibition (mm)					
		Name of microorganism					
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas fluorescens</i>	<i>Streptococcus pyogenes</i>
DMSO	NC	-	-	-	-	-	-
Acetone	50 µl (2.5 mg)	08 ± 0.2	16 ± 0.6	08 ± 0.6	14 ± 1.0	16 ± 0.2	16 ± 0.4
	75 µl (3.75 mg)	14 ± 0.8	17 ± 0.6	10 ± 0.3	16 ± 0.4	18 ± 0.8	16 ± 0.9
	100 µl (5 mg)	16 ± 0.3	21 ± 0.8	14 ± 0.9	20 ± 0.6	20 ± 0.2	20 ± 0.4
Aqueous	50 µl (2.5 mg)	16 ± 0.2	22 ± 0.5	20 ± 0.9	18 ± 0.5	10 ± 0.3	-
	75 µl (3.75 mg)	23 ± 0.3	22 ± 0.6	20 ± 0.6	18 ± 0.7	12 ± 0.5	08 ± 1.2
	100 µl (5 mg)	25 ± 1.2	24 ± 0.3	24 ± 0.6	20 ± 0.2	12 ± 0.6	08 ± 1.4
Benzene	50 µl (2.5 mg)	-	22 ± 0.8	-	14 ± 1.1	-	10 ± 0.5
	75 µl (3.75 mg)	18 ± 0.5	22 ± 1.2	18 ± 0.9	16 ± 0.5	18 ± 0.2	12 ± 1.2
	100 µl (5 mg)	18 ± 1.1	23 ± 1.0	20 ± 0.8	17 ± 0.6	20 ± 0.4	18 ± 0.9
Diethyl ether	50 µl (2.5 mg)	10 ± 0.2	16 ± 0.7	16 ± 1.4	17 ± 0.7	14 ± 0.4	16 ± 0.7
	75 µl (3.75 mg)	14 ± 0.6	18 ± 0.3	16 ± 0.9	18 ± 0.4	16 ± 0.8	17 ± 0.2
	100 µl (5 mg)	18 ± 0.5	18 ± 0.5	20 ± 0.7	21 ± 1.1	20 ± 0.6	18 ± 0.8
Ethanol	50 µl (2.5 mg)	16 ± 0.8	22 ± 1.4	16 ± 0.7	18 ± 0.8	-	-
	75 µl (3.75 mg)	16 ± 0.7	23 ± 0.8	18 ± 0.4	18 ± 1.0	18 ± 0.8	18 ± 0.9
	100 µl (5 mg)	20 ± 1.2	24 ± 0.6	20 ± 1.1	20 ± 0.9	21 ± 0.5	20 ± 0.7
Nitrofurantoin	PC (100 µg)	25 ± 1.8	25 ± 1.2	24 ± 0.5	24 ± 0.9	24 ± 1.7	25 ± 1.6

NC: Negative control, PC: Positive control (Nitrofurantoin) Values are expressed as mean ± standard deviation of three replicates

**Table 2.** Antibacterial activity of acetone, aqueous, benzene, diethyl ether and ethanol extracts of root of *M. pudica*

Name of solvent extract	Concentration of plant extract in 10ml of broth	Appearance of growth of microorganisms					
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas fluorescens</i>	<i>Streptococcus pyogenes</i>
Control	-	+	+	+	+	+	+
Acetone	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	-	+	+	+	-
	60 µl (3 mg)	-	-	-	-	-	-
Aqueous	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	+	+	+	+	+
	60 µl (3 mg)	-	-	+	-	-	-
Benzene	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	-	-	-	+	-	-
	60 µl (3 mg)	-	-	-	-	-	-
Diethyl ether	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	-	+	-	-	-	-
	60 µl (3 mg)	-	-	-	-	-	-
Ethanol	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	+	+	+	-	+
	60 µl (3 mg)	-	-	-	-	-	-

+ Appearance of visible growth of bacterium (turbid tube) - No appearance of visible growth of bacterium (clear tube)

**Table 3:** Minimum Inhibitory Concentration (MIC) of leaf extract of *M. pudica* against selected microorganisms

was increased with the increased levels of concentration of acetone, aqueous, benzene, diethyl ether and ethanol extracts of leaf of *M. pudica*. The acetone extract exhibited MIC of 2 mg or less but more than 1mg against *Klebsiella pneumoniae* and *Streptococcus pyogenes*, whereas the aqueous extract have no MIC against *Proteus mirabilis* in all tested concentrations 1mg, 2 mg and 3 mg. The benzene extract showed MIC of 2 mg or less but more than 1mg against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas fluorescens* and *Streptococcus pyogenes*. The diethyl ether extract showed MIC of 2 mg or less but more than 1 mg against *Escherichia coli*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptococcus pyogenes*. The ethanol extract exhibited MIC of 2 mg or less against *Pseudomonas fluorescens* between 1 mg and 2 mg. So, the MIC of extract of leaf of *M. pudica* was more than 1 mg against selected bacterial species.

### Minimum Inhibitory Concentration (MIC) of root extracts

The MIC of root extract of *M. pudica* against selected microorganisms (no visible growth) was determined using different concentration such as 20 µl (1 mg), 40 µl (2 mg) and 60 µl (3 mg) of extract in 10 ml of nutrient broth (v/v) and the results were presented in Table 4. The acetone extract showed MIC of 3 mg or less but more than 2 mg against all selected bacterial species. The aqueous extract showed MIC of 2 mg or less against *Escherichia coli* and *Klebsiella pneumoniae*, whereas no MIC was observed against *Streptococcus pyogenes*. The benzene, diethyl ether and ethanol extracts showed MIC of 3 mg or less but more than 2 mg against *Escherichia coli*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptococcus pyogenes*. The benzene and ethanol extract exhibited MIC of 2 mg or less against



Name of solvent extract	Concentration of plant extract in 10ml of broth (mg)	Appearance of growth of microorganisms					
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas fluorescens</i>	<i>Streptococcus pyogenes</i>
Control	-	+	+	+	+	+	+
Acetone	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	+	+	+	+	+
	60 µl (3 mg)	-	-	-	-	-	-
Aqueous	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	-	-	+	+	+	+
	60 µl (3 mg)	-	-	-	-	-	+
Benzene	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	-	+	+	+	+
	60 µl (3 mg)	-	-	-	-	-	-
Diethyl ether	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	+	+	+	+	+
	60 µl (3 mg)	-	-	-	-	-	-
Ethanol	20 µl (1 mg)	+	+	+	+	+	+
	40 µl (2 mg)	+	-	+	+	+	+
	60 µl (3 mg)	-	-	-	-	-	-

+ Appearance of visible growth of bacterium (turbid tube) - No appearance of visible growth of bacterium (clear tube)

**Table 4:** Minimum Inhibitory Concentration (MIC) of root extract of *M. pudica* against selected microorganisms

*Klebsiella pneumoniae*. So, the MIC of extract of root of *M. pudica* was more than 2 mg against selected bacterial species.

## Discussion

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [30]. The search for antimicrobials from natural sources has received much attention and effort have been put into identify compounds that can act as suitable antimicrobial agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganisms [31,32]. In the present study, different solvent extracts of leaf and root of *M. pudica* showed effective antibacterial activity against Gram positive bacteria *Bacillus subtilis* and *Streptococcus pyogenes* and Gram negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas fluorescens*. The benzene, diethyl ether and ethanol extract of leaf of *M. pudica* exhibited the highest zone of inhibition in all tested concentrations against selected microorganisms. This study revealed that the organic extract particularly exhibited better antibacterial activity and may be due to the antibacterial principles which are either polar or non-polar and effectively extracted only the organic solvent medium [33-35]. The acetone and aqueous extract of leaf showed no activity against *Proteus mirabilis* and *Bacillus subtilis* in the lower concentration of 2.5 mg. The variation in antimicrobial activity of leaf and root of *M. pudica* against selected bacterial species based on concentrations and solvents of extracts. In the present study, the antibacterial effect of different solvent extracts of root of *M. pudica* was exhibited as different level of zone of inhibition against selected microorganisms. The aqueous extract of root showed maximum zone of inhibition against *Escherichia coli* and minimum zone of inhibition against *Streptococcus pyogenes*. The acetone and diethyl ether extract of root of *M. pudica* exhibited the highest zone of inhibition in all tested

concentrations against selected microorganisms. The aqueous and ethanol extracts showed no activity against *Streptococcus pyogenes* in the lower concentration of 2.5 mg. The results of this study showed that the zone of inhibition of all extracts was increased with the increased level of concentrations. The antimicrobial activity of leaf and root of *M. pudica* was observed as concentration dependent, because the higher concentration (5 mg) showed higher level of zone of inhibition and the lower concentration (2.5 mg) showed lower level of inhibition against all selected bacterial species. An important function of plant extracts and their components is hydrophobicity. It is enables to partition the lipids of bacterial cell membrane and mitochondria and disturbing the cell structures and rendering them more permeable, which leads to extensive leakage of intercellular compounds from the bacterial cells or the exit of molecules and ions will lead to bacterial cell death [36]. Similar type of mechanisms may be took place in this study to control the microorganisms by the extracts of leaf and root of *M. pudica*. The present study is also accordance with the previous study, in that the antibacterial activity of leaf, fruits and latex of *Croton bonplandianum* against selected bacterial species was reported [37]. Similarly several reports indicated that the antibacterial activity of medicinal plants such as *Datura metel* [38], *Acalypha indica* [39], *Mangifera indica* [40], *Centella asiatica* [41], *Acacia nilotica* [42] and *Garcinia nigrolineata* [43]. The phytochemicals alkaloids, glycosides, flavonoids, saponins, steroids and tannins were reported and these are believed to be the bioactive ingredients of *M. pudica* responsible for its antimicrobial activity [44]. In the present study, the antimicrobial activity of leaf and root of *M. pudica* may also be due to the presence of above mentioned bioactive compounds. The results of this study revealed that different level of antibacterial activity by leaf and root extracts of *M. pudica*. The antibacterial activity of leaf and root of *M. pudica* was observed at various level of inhibition based on parts of plant, solvents of extract, bacterial species and concentrations of extract.

## Conclusion

From the results of this study, we concluded that the acetone, aqueous, benzene, diethyl ether and ethanol extracts of leaf and root of *M. pudica* possess potential antibacterial activity against human pathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*,

*Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptococcus pyogenes*. The MICs of different concentrations of different solvent extracts of leaf and root of *M. pudica* were also studied. The leaf and root extracts of *M. pudica* can be tested on other human pathogens to elucidate and ascertain their uses. Recent now there are emerging many multidrug resistant human pathogenic bacteria. *M. pudica* is commonly found in waste lands of India at moisture condition. The collection and cultivation of this medicinal plant is easy with low cost. The leaf and root of *M. pudica* may be an alternative drug for synthetic antimicrobial agents. In near future, the isolation of antimicrobial compounds from the leaf and root of *M. pudica* would be useful to treat infectious diseases caused by microorganisms. So, the extensive research should be carried out on phytochemicals of *M. pudica* for the development of cost effective drugs.

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