

**Original Research Article**

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IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *MIMOSA PUDICA* L.S. Ramesh^{1*}, P. Muruganatham², C. Karpagasundari¹, K. Karthikeyan³, S. Madhavan⁴, Chandran.C¹

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ABSTRACT: *Mimosa pudica* L is highly medicinal plants. It commonly known as sensitive plant, shame plant, prayer plant, touch me not. Antibacterial activity against gram positive and gram negative pathogenic bacteria were procured collection from Microbial Type Culture Collection (MTCC), Chandigarh, Punjab, India. *Escherichia coli*, (MTCC 433) *Bacillus subtilis*, (MTCC109), *Streptococcus pyogenes*, (MTCC 899) and *Klebsilla pneumonia* (MTCC 424) was determined by an agar well diffusion method. The root and leaf extract of the plants were extracted with four different solvents extracts, as ethyl acetate, ethanol, methanol and n- butanol. The results of this antibacterial screening showed that root and leaf of ethyl acetate extract (EtOAc) was displayed good activity for pathogenic bacteria. The antibacterial activity results were obtained for roots EtOAc MIC value between 18 ± 0.67 , concentration 100 μ l / MIC. These results may help to improve these natural antibacterial substances that could identified as bacterial disease. Antioxidant activity was analysed by the DPPH radical scavenging activity mechanism. The results showed that ethanol, ethyl acetate, n- butanol and methanol extract of *M. pudica* root and leaf. The most antioxidant activities against DPPH were displayed by the ethyl acetate extracts of *M. pudica* roots exhibiting 81.79 ± 4.77 and inhibition 81% respectively. The present results potential medicinal plants used by antioxidant, antibacterial and various properties were also present.

KEYWORDS: *Mimosa pudica*, Antibacterial activity, Phytochemical, Antioxidant activity, Medicinal uses.

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1. INTRODUCTION

Natural products principally medicinal plants have been long prescribed in traditional medicine, for centuries as treating different diseases. The significance of herb in the management of human ailments cannot be overemphasizing. The repetitive investigation into the secondary metabolites for anti-infective agents has gained consequence because of alarming increase in the rate of antibiotics resistance of pathogenic microorganism to existing antibiotics [8]. Natural products are defined as natural sources derived substance having biological activities. Natural products have long been implemented as alternative health care treatment and in discovery of modern drugs [8]. *Mimosa pudica* L. (Mimosaceae) is a creeping and perennial herb [6]. It commonly known as humble plant, shame plant, touch me not, sensitive plant, sleeping grass (tropical biological association) and prayer plant [1]. The whole plant are highly medicinal properties and important used in several preparations of siddha, ayurvedic and folk medicine. It has been previous reported that the phytochemical compounds revealed the presence of most highly in toxic alkaloids as mimosine and orientin, isoorientin, D-pinitol, norepinepherin mucilage, tannins, non-protein amino acid, tannins, flavonoids, C-glycosides, steroids, terpenoids, fatty acids, saponins, coumarin, major and minor chemical constituents are also present [2]. Phytomedicines, due to their potential benefits, have remained in practice in all traditional systems of therapies, including Greco-Arab (Unani-Tibb), Ayurveda, and Chinese [24, 26]. *M. pudica* is known and valued for its analgesic, anti-inflammatory [25] hypoglycemic [27], Since it has been whole plant well known used as ayurvedic and homeopathic medicine. The root has bitter, acrid, cooling vulnerary, alexipharmic and treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leukoderma and fatigue and blood disease etc., NPGS/GRIN [5]. The unani system of medicine root has alternative treatment, blood impurities, bilious fevers, piles, jaundice and leprosy, diarrhoea, amoebic, dysentery, bleeding piles, gynaecological disorder to cure as traditional medicine healthcare system [3,4, 7]. This plant objective of the study to presents of phytochemical, antibacterial and antioxidant activity to evaluate the medicinal plant. It has been reported that the antibacterial activity of plants is related with the defense mechanism against bacteria [21]. The *Mimosa Pudica*, invites attention of the researchers worldwide for its pharmacological activities such as anti diabetic, antitoxin, antihepatotoxin, antioxidant and wound healing [22, 23].

2. MATERIALS AND METHODS

2.1. Plant materials and chemicals

M. pudica root and leaf which was collected from authenticated by the Department of Botany and Microbiology A. V. V. M. Sri Pushpam College, (Botanical Garden) Poondi, Thanjavur, (District) Tamil Nadu, India, in June middle 2017. The plant materials were shade dried room temperature for 20 days. The powdered plant leaf and root material was extracted by maceration with different solvents for 72 h at room temperature. The extracts was maintained at low temperature (5°C) and

before used. The extracts was filtered before drying using What man No. 2 filter paper, the excess of solvent removed by vacuum distillation units in a rotary evaporator at 40°C (8). DPPH (1, 1 – diphenyl 1-2 picrylhrazyl) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMSO (dimethyl sulfoxide), sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and peptone were purchased from Merck (Dramtadt, Germany). Ascorbic acid, 1-naphthol and bismuth (III) nitrate were obtained from Sigma (St. Louis, MO, USA). Nutrient broth was obtained from (Detroit, M1 USA).

2.2. Extraction

Plant root and leaf 50g powder in each solvents were extracted successively, with as ethyl acetate, ethanol, methanol, and n-butanol solvent at room temperature 32°C with continuous shaking for 48 h. This process was then repeated. Following filtration of the suspension through what man No.1 filter paper, the crude alcohol extracts were rot evaporated at 35°C and put in a vacuum oven to near dryness to yield the plant extract. The extracts was filtered before drying using what man No. 2 filter paper, the excess of solvent removed by vacuum distillation units in a rotary evaporator at 40°C. [12].

2.3. Antibacterial assay

Antibacterial assays were conducted using the agar well disk diffusion method as previously reported with slight modification [13]. *Esherichia coli*, (MTCC 433), *Bacillus subtilis*, (MTCC 109) *Klebsilla pneumonia* (MTCC 108) and *Streptococcus pyogenis* (MTCC 899) were used in all experiments. Nutrient agar (Himedia) was used as 20 ml of sterile media were transferred to Petri dishes and allowed to solidify. The media plates were cut using a sterile cork borer. Streptomycin (15 µg/disc) antibiotics were used as positive control. The plates were incubated in the dark at room temperature 32° C for 24 h. The bacterial colony Zones of inhibition around the well were measured in mm; activity index was calculated as the mean of inhibition, zone for the standard drugs [9].

2.4. Antioxidant assay

The sample was first dissolved in (DMSO) and used at a 30 times dilution for the experiment. The DPPH radical scavenging method was performed as previously described [13]. UV absorption was measured on a Shimadzu UV-VIS 1240 Spectrophotometer (Shimadzu Crop., Kyoto, Japan).

2.4.1 DPPH radical – scavenging activity

The mixture of 2 ml aliquot DPPH methanol (25µl/ml) and 0.5 ml different concentration sample it was well thoroughly shaken, the absorbance was measured at (517 nm) in spectrophotometer.

$$\text{Radical scavenging activity (\%)} = 100 - \frac{AC-AS}{AC} \times 100$$

Where Ac = control is the absorbance and As = sample is absorbance of reaction mixture (in the presence of sample).

2.4.2 Determination of Total Antioxidant activity

The Total Antioxidant activity was calculated by phosphor molybdenum method. The reduction of Mo (VI) – Mo (V) by the extract and subsequent formation a green phosphate /Mo (V) complex at acid pH 0.3 ml was combined with 3ml of reagent 0.6M H₂SO₄, 28 mM Sodium phosphate and 4 mM Ammonium molybdate show the reaction were incubated at 95° C for 90 min the absorbance solution was measured 695nm.

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

2.4.3 Nitric oxide scavenging activity

Nitric acid radical scavenging activity was determined according to report. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions (2 ml Sodium nitroprusside, 0.5 ml phosphate buffer, pH 7.4 was mixed 0.5 ml extract at various concentrations) and added 33% sulfanilic reagent, 20% gallic acetic acid incubated at room temperature 30 min. The absorbance at 540 nm was analyzed by a spectrophotometer.

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ control and A₁ absorbance

2.4.4. Fe²⁺ chelating activity

The chelating activity of the extracts for ferrous ions was measured. To 0.5 ml extracts, 1.6 ml of deionized water 0.05 ml of FeCl₂ was added, after 30 s, 0.1 ml ferrozine (5mm) was added and 10 min room temperature, absorbance was measured at 562 nm.

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

2.4.5 Reducing power assay

The extracts (0.75 ml) at various concentrations with 0.75 ml of phosphate buffer (0.2M, pH 6.6) and 0.75 ml potassium hexacyanoferrate, incubating at 50° C in water bath for 20 min, 0.75ml of trichloroacetic acid (TCA) and centrifuged 800g for 10 min, 1.5 ml of distilled water, 0.1 ml ferric chloride solution (0.1 %) for min. The absorbance at 700nm was measured.

2.4.6 Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC₅₀ was graphically estimated using a nonlinear regression algorithm.

3. RESULTS AND DISCUSSION

3.1 Plant extract

The plants root from were macerated by different solvent was ethanol, methanol, ethyl acetate, and n- butanol at room temperature, successively.

3.2 Antibacterial assay

The development of bacterial resistance to presently available root and leaf, such an antibacterial activity [10]. The bacterial resistance active compounds isolated from plant species *M. pudica* L. commonly used as traditional medicine. These plant having a new compounds present as antimicrobial activities [11]. In the assay antibacterial against *E. coli*, *K. pneumonia*, *B. subtilis*, *S. pyogenisis*, and 4 different root and leaf extract i.e ethyl acetate, ethanol, methanol, and n- butanol, the ethanol and ethyl acetate extracts of good resistance activities of at 25-100µg / well. The ethanolic extract is high efficient and control of gram negative and gram positive pathogenic bacteria to causing human, animal and plant disease, such as inflammation [11, 12]. *S. pyogenisis* has also been reported to causing human and animal disease. The results were obtained in the screening as antibacterial activity of different extracts from roots and leaf against the studied bacteria are shown in, Table 3. Antibiotics inhibit the growth of pathogenic microorganisms. However, several microorganisms have developed resistance against these synthetic drugs, which are also expensive and have side effect [29]. Furthermore, fungi destroy food by producing mycotoxins and cause diseases [28 30]. Consequently, researchers are focusing This is first report on the antibacterial activity of *M. pudica* L. root and leaf extract, plants have been ability and accumulate phytochemical compounds and substantial amounts of alkaloids and phenolic compounds to as well known antimicrobial activity, alkaloids and other major and minor compounds play in role of antibacterial agents against the pathogenic bacteria *E. coli*, *B. subtilis*, *K. pneumonia*, *S. pyogenisis* and *P. aeruginosa* [21]. The root and leaf extract 25µg to 100µg. The measurement zone of inhibition highest percentage MIC value ranged from 18 ± 0.67 in leaves and root 13 ± 0.67 referring to antibacterial activities shown against *E. coli*, *K. pneumonia*, *B. subtilis*, *P. aureoginosa*, and *S. pyogenisis* at concentration of 25µl / ml to 100µl / ml ,Table. 3.

3.3 Antioxidant activity

The DPPH radical scavenging activity of *M. pudica* roots and leaf screen in methanolic extracts showed strong radical scavenging activity 80 µg/ml (78.89 ± 4.77) the half inhibition concentration (IC 50) of plant extracts was $55.06 \mu\text{g/ml}^{-1}$ respectively (Table1,2). The 11-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was measured by the method developed [33, 34]. The ascorbic acid potential to scavenging DPPH radical is directly proportional to as concentration, so as DPPH activity was very near to standard as ascorbic acid. Phenolic compounds are important plant constituents because DPPH scavenging as total phenolic used as screening of antioxidant activity [14]. Flavonoids were highly effective scavenging of antioxidant

properties and oxidizing molecules, including singlet oxygen and various other compounds implicated several diseases [15, 16]. Flavonoids and other phenolic compounds of these plants *M. pudica* was reported as scavengers and inhibitors. In the present study, revealed the presence of phenolic compounds scavenged DPPH radicals significantly. [17,18]. The Total antioxidant activity of the plant extract was showed minimum concentration 20 $\mu\text{g/ml}$ (17.59 ± 0.87) and maximum concentration of 80 $\mu\text{g/ml}$ (82.89 ± 3.77) as inhibition was IC 50 were $42.41 \mu\text{g/ml}^{-1}$ and $62.24 \mu\text{g/ml}^{-1}$ (Fig 1,2). *M. pudica* act as reducing agents exhibiting antioxidant property. These mechanisms providing protection to human against pathogen and degenerative disease [19]. Our synthesized AgNPs showed higher antimicrobial activity against *E.coli* and *B. subtilis* as compared to *P. aeruginosa*. [36, 36]. Nitric acid radical scavenging activity of the plant extract was showed minimum concentration 20 $\mu\text{g/ml}$ (18.03 ± 0.77) (and maximum concentration of 80 $\mu\text{g/ml}$ (80.51 ± 2.66) as inhibition the root was IC 50 value were $52.98 \mu\text{g/ml}^{-1}$ and leaf was $51.98 \mu\text{g/ml}^{-1}$ ascorbic acid standard value 46.98 and leaf 45.95. (Fig1, 2). The ferrous ion chelating activity the formation Fe^{2+} complex, interrupted by value was (15.89 ± 5.77 and maximum value 69.24 ± 5.85) ascorbic acid $32.41 \mu\text{g/ml}^{-1}$ and $56.24 \mu\text{g/ml}^{-1}$ respectively. Reducing power activity 20 $\mu\text{g/ml}$ (0.23 ± 0.01) and maximum value was 0.79 ± 0.01) (Table 1, 2). The presents as phytochemical were potential antioxidant compounds, and prevent against free radical disease, protection DNA damage, carcinogenesis and pharmacological activities [20].

Table 1: In vitro antioxidant activity (%) of *M. pudica* L. roots extract

<i>M. pudica</i> L. Root extract	DPPH scavenging assay radical	Fe^{2+} - Iron Chelating Activity	Total antioxidant Assay	Reducing Power assay	Nitric oxide Scavenging Activity
20 $\mu\text{g/ml}$	21.87 ± 1.82	15.49 ± 1.38	17.50 ± 0.97	0.23 ± 0.01	18.03 ± 0.77
40 $\mu\text{g/ml}$	30.97 ± 2.76	30.87 ± 2.25	31.25 ± 2.28	0.43 ± 0.03	38.57 ± 1.33
60 $\mu\text{g/ml}$	50.00 ± 3.87	42.45 ± 4.99	66.87 ± 2.85	0.65 ± 0.03	55.33 ± 1.88
80 $\mu\text{g/ml}$	78.16 ± 4.98	69.24 ± 5.85	82.60 ± 3.40	0.79 ± 0.02	80.51 ± 2.66
IC50	56.09	50.28	63.24	00 \pm 00	52.98
Standard as ascorbic acid	34.99	31.96	42.98	00 \pm 00	46.98

Values were expressed as Mean \pm SD for triplicates

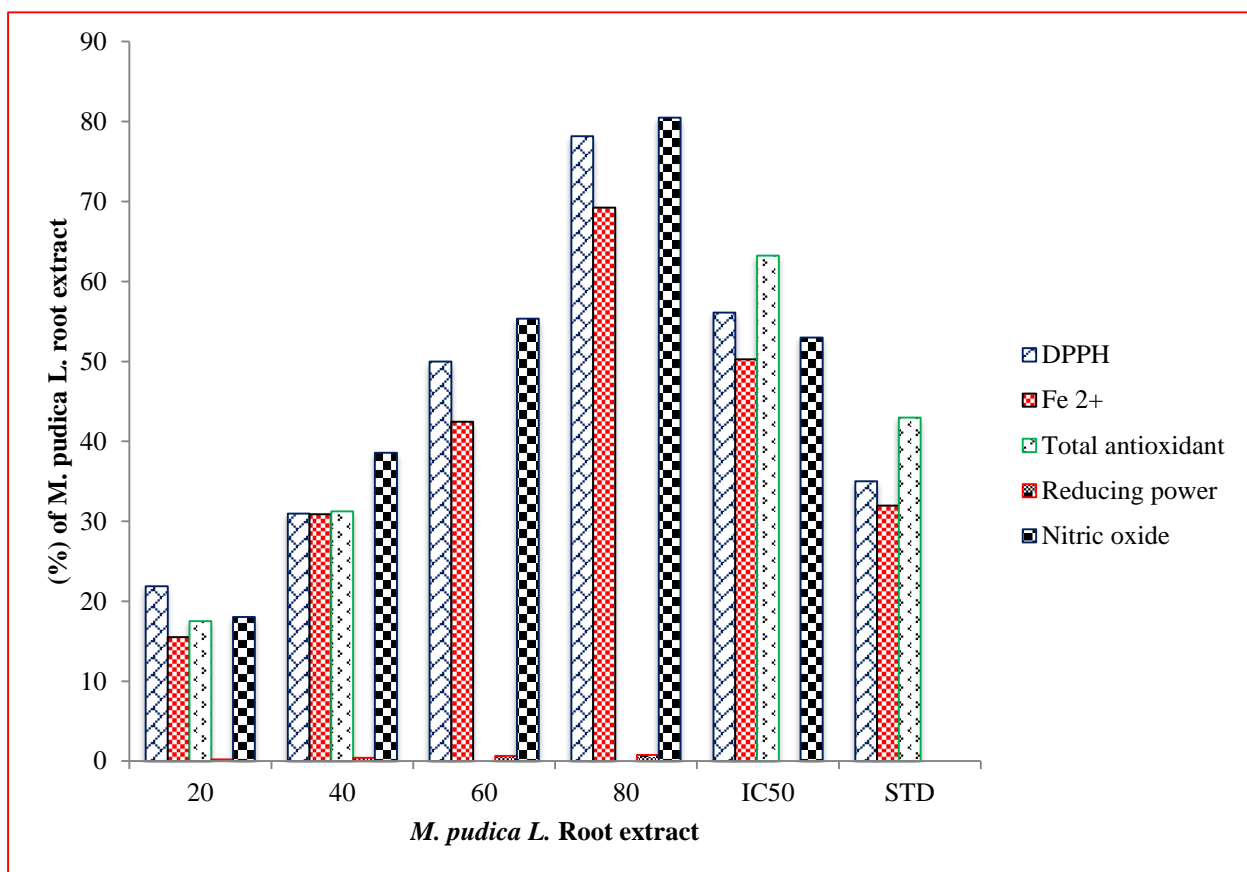


Fig 1: In vitro antioxidant activity (%) of *M. pudica L.* root extract

Table 2: In vitro antioxidant activity (%) of *M. pudica L.* Leaf extract

<i>M. pudica L.</i> Leaf extract	DPPH scavenging assay radical	Fe ²⁺ - Iron Chelating Activity	Total antioxidant Assay	Reducing Power assay	Nitric oxide Scavenging Activity
20µ g /ml	22.87 ± 1.82	16.49± 1.38	16.50 ± 0.97	0.22 ± 0.01	19.03 ± 0.77
40 µ g / ml	29.97 ± 2.76	30.87 ± 2.25	33.25 ± 2.28	0.45 ± 0.03	39.57 ± 1.33
60 µ g / ml	49.00 ± 3.87	43.45 ± 4.99	66.87 ± 2.85	0.65 ± 0.03	55.33 ± 1.88
80 µ g / ml	78.16 ± 4.98	65.24± 5.85	80.60 ± 3.40	0.69 ± 0.02	81.51 ± 2.66
IC50	54.09	50.28	63.24	00.00	53.98
Standard ascorbic acid	33.99	29.96	42.98	12.65	45.98

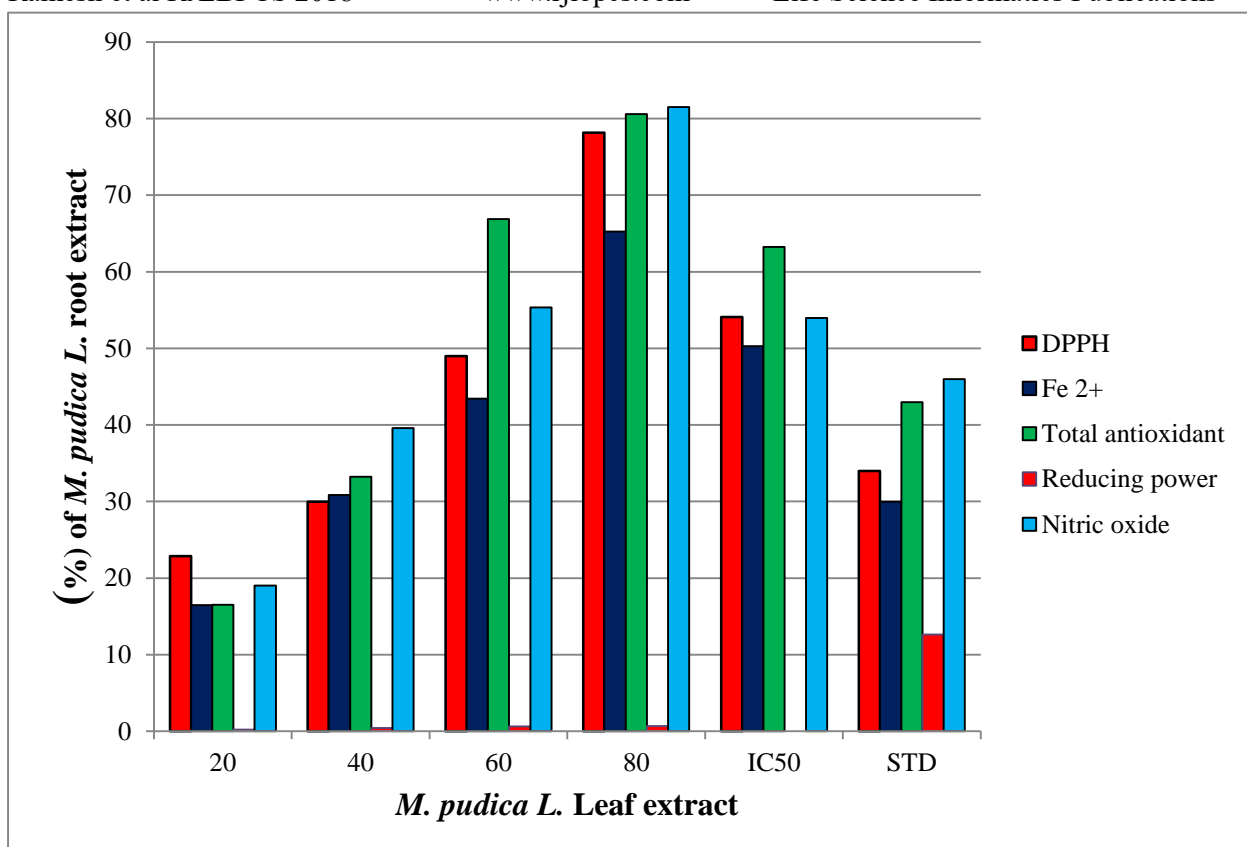


Fig 2: In vitro antioxidant activity (%) of *M. pudica L.* root extract

Table 3: Antibacterial activity of *M. pudica L* of root and leaf against some pathogenic bacteria

Name of Plant	Bacteria	of Plant extracts of parts	Different inhibition zone(mm)($\mu\text{g/well}$)				
			Extraction solvents	Control	25	50	75
<i>E. coli</i>	Root	EtOAc	0	5 ± 0.74	9 ± 0.56	11 ± 0.43	13 ± 0.67
			EtOH	0	0	11 ± 0.76	9 ± 0.76
	<i>B. subtilis</i>	n-Butanol	0	8 ± 0.43	7 ± 0.67	12 ± 0.67	3 ± 0.7
			MtOH	0	3 ± 0	6 ± 0.78	8 ± 0.89
<i>K.pneumoniae</i>	Leaves	EtOAc	0	14 ± 0.45	4 ± 0.57	15 ± 0.78	4 ± 0.78
			EtOH	0	7 ± 0.65	12 ± 0.98	6 ± 0.67
	<i>S.pyogensis</i>	n-Butanol	0	1 ± 0.33	11 ± 0.22	18 ± 0.67	9 ± 0.88
			MtOH	0	6 ± 0.57	13 ± 0.78	11 ± 0.79

4. CONCLUSION

These present study report *M. pudica* showing remarkable with wide spectrum, as various pharmacology activity. The presence of large amount of phytochemical compounds and drugs. It is a potential medicinal plant for treating various dreadful diseases. In conclusions of these present study, antioxidant and antibacterial of root and leaf naturally preservative inhibition and control bacteria and antioxidation compounds. The bioactive compounds, was non-controversial, easy available cheaper drugs. The standard monograms prepared conclude should be useful for medicine.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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