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Antimicrobial Sensitivity Profile of *Mimosa pudica* Leaf Extract and its Combination Treatment with Potassium Aluminum Sulphate on Some Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mimosa pudica is a famous ornamental plant commonly known as sleeping grass, sensitive plant, humble plant, shy plant and touch-me not, among other names. The study was aimed at determining the antibacterial activity of *Mimosa pudica* extract in combination with alum. The plant was obtained, identified, prepared and extracted using both aqueous and methanolic medium. The extracts were reconstituted to final concentration of 250g/ml, 125g/ml, 62.5g/ml (w/v). The bacterial isolates identified were; *Pseudomonas aeruginosa NC002516, Staphylococcus aureus FR821779* and *Escherichia coli CP031892.1* obtained from wound source at the University of Port Harcourt Teaching Hospital and maintained in stock culture and were further confirmed using biochemical and molecular methods. In-vitro bioactivity of various concentrations of the extracts and in

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combination with alum were evaluated by measuring diameter of inhibition zones. From the bioassay, the combinations of the aqueous extract and alum demonstrated the highest inhibitory potential; (26.00±0.00mm) at 250g/ml for *Staphylococcus aureus FR821779* and the least antibacterial effect was recorded in the consortium extract for *Staphylococcus aureus FR821779*; (13.00±0.00mm) at same concentration compared with other bacteria (*E. coli* and *P. aeruginosa*). The result obtained showed that all the treatments and combination treatments recorded MIC at 0.50g/ml for all the bacteria studied. The composition in percentage of the various phytochemicals were as follows: Flavanoid, 7.96±1.17%, Alkaloid, 9.85±0.21%, Saponin, 7.34±0.35% and Total-phenol, 1.63±0.00%. Alkaloids (9.85±0.21%) had the highest concentrations in the extracts while Total-phenol (1.63±0.00%) was the least in concentration. This study has been able to ascertain the sensitivity profiles of *Mimosa pudica* leaf extracts and its combination treatment with potassium aluminium sulphate (Alum) on some microbes specifically *S. aureus*, *E. coli, P. aeruginosa.*

Keywords: Mimosa pudica leaf extract; microorganisms; alum; phytochemistry; antibacterial activity.

1. INTRODUCTION

Plants are used medicinally in different countries and they are a good source of many potent and powerful drugs [1]. Herbal medicines are gaining growing interest because of several reasons ranging from their cost effectiveness, eco-friendly attributes and true relief from disease conditions [2]. The World Health Organization has catalogued twenty thousand (20,000) plant species studied for medicinal purposes [3]. Medicinal plants represent a rich source of antimicrobial agents.

Mimosa pudica is a famous ornamental plant commonly known as sleeping grass, sensitive plant, humble plant, shy plant and touch-me not, among other names (Ahmad et al., 2012). Its ornamental use can be attributed to its thigmonastic and semimonastic 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 (Ahmad *et al*, 2012). The mechanism behind antibacterial activity of the *Mimosa pudica* plant extracts includes the disruption of bacterial membrane and leakage of the cellular contents [4].

Antibiotics known to be effective on some bacteria species in the past now have little or no effects on these organisms making it difficult to treat infections arising from these organisms (Fair & Tor, 2014). In developing countries, the recent emergence of strains reducing susceptibility to antibiotics demand search for new therapeutic agents [5]. Historically, plant has been a source of medicinal agents used to treat chronic as well as acute infectious diseases based on the premises that they contain natural substances [6]. The antimicrobials of some plants have proven to be effective against some infectious diseases [7]. There is growing need to develop alternative antimicrobial drugs to treat infectious diseases using medicinal plants because they have been shown to have enormous therapeutic potential even in the face of increasing prevalence of multidrug resistant strains of bacteria [8].

An alum is a type of chemical compound, usually a hydrated double sulfate salt of aluminium with the general formula X Al(SO. 4) 2·12 H. 2O, where X is a monovalent cation such as potassium or ammonium. By itself, "alum" often refers to potassium alum, with the formula KAl (SO 4)2·12 H2O. Other alums are named after the monovalent ion, such as sodium alum and ammonium alum. Potassium aluminum sulphate (PAS), has lately attracted the attention of the scientific community as a cost-effective, efficient, and environmentally benign inorganic chemical [9]. It has historically and scientifically showed a strong proclivity for antibacterial action in a range of systems (Invitro and invivo studies). Invivo investigations show that alum, alone or in combination, has antibacterial effects against a wide range of microorganisms. Alum has potent antibacterial properties against Gram positive and Gram negative bacteria, as well as yeasts and molds. This action is dosage and incubation period dependent, and it is greatly enhanced when used in combination with plant extracts, inorganic compounds, or antibiotics [9]. Oneda *et al*. [10] showed that use of Aluminium potassium sulphate (Alum) at concentration of 1.0, 2.5, 5.0 and 10.0% (w/w) did not exert tumorigenic or any other toxic actions in mice. It is therefore likely that the concentration of Alum (4.0%) used in this study is safe.

1.1 Aim and Objectives of the Study

The aim of the study was to determine the sensitivity profiles of *Mimosa pudica* leaf extracts and its combination treatment with alum on some microbes.

The objectives of the study were to determine the phytochemical properties of *Mimosa pudica* leaf extract, confirmation of stock culture by conventional method and molecularly, susceptibility of test microorganisms to various concentrations of *Mimosa pudica* leaf extracts and to determine the Minimum Inhibitory Concentration of extracts on isolates.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification of Plant Material

Mimosa pudica leaf was obtained from okehi in Etche Local Government in Rivers State, Nigeria. The samples were then transported to the Department of Microbiology Laboratory in Rivers State University for preparation and subsequent extraction. The samples were identified by Dr. (Mrs) Mercy G. Ajuru in Department of Plant Taxonomy and Biosytematics. Stock cultures of bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) were isolated from wound sources from the medical laboratory of the University of Port Harcourt Teaching Hospital which were further characterized and identified using biochemical and molecular identification methods.

2.2 Identification of the Obtained Isolates

The obtained bacterial isolates were further characterized and identified based on their microscopic and biochemical characteristics with reference to the Bergey's Manual of Determinative Bacteriology. The isolates were characterize\ed genotypically by cloning and sequencing the 16S rRNA. The genomic DNA from a pure culture of each isolate was extracted and purified for PCR amplification of the 16S rRNA sequence using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

2.3 Preparation of *Mimosa pudica* Leaf Extract

Crude extraction and Methanol extraction was adopted for this study. Methanol extraction was carried out as described by Hakam *et al*. (2020). In this method, all the leaves were washed with clean running water to remove sand particles and debris and subsequently sun dried. Dried leaves were then milled into coarse powder using a mechanical blender. Twenty gram (20.0g) of the blended sample was weighed and extracted with 100ml sterile distilled water and 100ml of 99.5% methanol respectively, then soaked in respective solvents for 24hrs and filtered with Whatman No. 1 filter paper. The filtrates were concentrated by evaporation in water bath at 60°C for 12-15hrs and 24hrs for methanolic and aqueous extracts respectively. The resultant filtrates were reconstituted in 70% methanol and sterile distilled water to obtain final concentrations of methanolic mimosa leaf extract and aqueous mimosa leaf extract as 250g/ml, 125g/ml, 62.5g/ml (w/v) and stored at 4°C for subsequent experiment.

2.4 Phytochemical Screening

Qualitative phytochemical screening of *Mimosa pudica* leaf extracts was done by making use of the standard method [11]. The phytochemicals screened were flavonoids, saponins, alkaloids and total-phenol.

2.5 Preparation of Combination of *Mimosa pudica* Leaf Extracts

Different concentration of aqueous *mimosa pudica* extract and methanol *mimosa pudica* crude extract were used in combination with alum at a ratio of 1:1 to make a final concentration of 250g/ml, 125g/ml, 62.5g/ml respectively.

2.6 Antimicrobial Activity

The identified pathogenic bacteria *Staphylococcus aureus* (FR821779), *Escherichia coli* (CP031892.1), and *Pseudomonas aeruginosa* (NC002516), were used for antimicrobial activity adopting the method of Amadi et al. [12]. The identified bacterial isolates were standardized using 0.5 McFarland standard. The standardized bacteria were swabbed on freshly prepared Mueller Hinton agar and a sterile cup-borer was used to bore the wells on the inoculated medium. The extract and the combinations of different concentration prepared were aseptically transferred into the wells, 10µg of commercially prepared antibiotic, ciprofloxacin was also used as a positive control and all the plates were incubated at 37°C for 24

hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) was determined for alum and plant extracts as described by Ogbonna et al., (2013). Each bacterium was pre-cultured and further diluted with media to a concentration of around 10^6 CFU/ml. The presence or absence of colonies was noted after 10µL of diluted cell culture was injected into the medium using a micro-planter and incubated at 30^0C for 72 hours. The minimal inhibitory concentration was determined to be the lowest extract concentration that inhibited the bacteria's growth.

2.8 Data Analysis

Statistical Package for Social Sciences (SPSS) version 25 and Microsoft Office Excel 2010 was used to analyse the data obtained from the measurement of the zones of inhibition of the extracts. Descriptive statistics was used to summarise all data obtained. ANOVA was carried out to test for significant difference in the MIC obtained for the extracts.

3. RESULTS AND DISCUSSION

The result of the morphological and biochemical reaction of the bacterial isolates displayed in Table 1 revealed the following probable bacteria*; Escherichia. Coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The results of the phytochemical composition of the *Mimosa pudica* leaf extracts in this study is shown on Table 2. The composition in percentage of the various phytochemicals were as follows: Flavanoid, 7.96±1.17%, Alkaloid, 9.85±0.21%, Saponin, 7.34±0.35% and Total-phenol, 1.63±0.00%. Alkaloids (9.85±0.21 %) had the highest concentrations in the extracts while Total-phenol (1.63±0.00%) was the least in concentration. In a study of the Analysis of Phytochemical Constituents and Anthelmintic Activity of Leaf Extracts of *Mimosa pudica* leaf, the phytochemical screening of the crude extract showed the presence of, alkaloids, saponins, flavonoids and phenols, Pratap et al [13], Gandhiraja et al. [14]. Alkaloids play an essential role in both human medicine and in an organism's natural defence and it makes up approximately 20% of the known secondary metabolites founds in plants [15]. In plants, alkaloids protect plants from predators and regulate their growth [16]. Therapeutically, alkaloids are known for their anaesthetics, cardioprotective, and anti-inflammatory functions. Well-known alkaloids used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine [17].

The antibacterial activity of *Mimosa pudica* leaf extracts on *Pseudomonas aeruginosa* is shown on Table 3. Comparatively, the highest activity on *Pseudomonas aeruginosa* was recorded by Aqueous extract in combination with alum with zone of inhibition (mm), 16.00±0.00mm, 20.00±0.00mm and 22.00±0.00mm for the concentration of 62.5g/ml, 125g/ml and 250g/ml respectively. This was followed by the combination of methanol extract with alum recording zone of inhibition (mm) of 11.00±0.00 mm, 16.00±0.00 mm and 21.00±0.00 mm for concentration of 62.5g/ml, 125g/ml and 250g/ml respectively while the least inhibitory activity was recorded by the aqueous extract alone recording zone of inhibition of 10.00±0.00 mm, 13.00±0.00 mm and 15.00±0.00 mm respectively for concentration of 62.5g/ml, 125g/ml and 250g/ml respectively. The positive control (ciprofloxacin) were proven to be more effective in the inhibition of *Pseudomonas aeruginosa* by producing the highest zone of inhibition; 45±0.02mm. The highest inhibitory antibacterial activity was seen and recorded in the combination of Aqeous extract + alum and can be attributed to the antibacterial and synergistic effect of alum (Birnim-Yauri & Aliyu, 2014).

Table 4 shows the antibacterial activity of *Mimosa pudica* leaf extracts on *Escherichia coli* isolates. From the results, the positive controls (Ciprofloxacin) had higher zones of inhibition on the isolates than all the extracts tested. The highest antibacterial activity on *Escherichia coli* was recorded by Aqueous extract in combination with alum with zone of inhibition (mm), 15.00±0.00 mm, 20.00±0.00 mm and 22.00±0.00 mm for the concentration of 62.5g/ml, 125g/ml and 250g/ml respectively. This was followed by the combination of methanol extract with alum recording zone of inhibition (mm) of 14.00±0.00 mm, 17.00±0.00 mm and 20.00±0.00 mm for concentration of 62.5g/ml, 125g/ml and 250g/ml respectively followed by the consortium (Aqueous extract + Methanol extract + alum) with the inhibition zone of 13.00±0.00 mm, 17.00±0.00 mm and 18.00±0.00 mm for the

concentration of 62.5g/ml, 125g/ml and 250g/ml respectively and the least inhibitory activity was recorded by the aqueous extract alone recording zone of inhibition of 10.00±0.00mm, 13.00±0.00mm and 15.00±0.00mm respectively for concentration of 62.5g/ml, 125g/ml and 250g/ml. The higher bacteriological activity of Alum+Aqueous extract recorded in this study for both *Pseudomonas* and *Escherichia coli* is in line with the report of Amadi, [9] which reported a synergistic effect of alum with other plant extract resulting in higher beneficial antibacterial effect. Combination of Aqueous extracts + Alum showed higher zones of inhibition on *Escherichia coli* when compared to the other extracts and this result is also similar to the findings of Lakshmibai & Amirtham [11] in their study of the antimicrobial activity of *Mimosa pudica* in which there was a clear indication that the aqueous thorn extracts of *Mimosa pudica* exhibited highest zone of inhibition of 24.2±0.34mm against the bacteria *Escherichia coli* when compared to the ethanolic extracts. In their study, *Escherichia coli* showed maximum zone of clearance in both the ethanolic and aqueous thorns extracts of *Mimosa pudica* at higher concentrations. In a previous antibacterial study, it was reported that aqueous extracts of *Mimosa Pudica* leaf extracts showed a maximum zone of inhibition against *Escherichia coli* [18].

The antibacterial activity of *Mimosa pudica* extract on *Staphylococcus aureus* is shown on Table 5. The highest activity on *Staphylococcus aureus* was recorded by Aqueous extract in combination with alum with the zone of inhibition (mm), 16.00±0.00mm, 17.00±0.00mm and 26.00±0.00mm for the concentration of 62.5g/ml, 125g/ml and 250g/ml respectively. This was followed by the combination of methanol extract with alum recording zone of inhibition (mm) of 14.00±0.00mm, 17.00±0.00mm and 22.00±0.00mm for concentration of 62.5g/ml, 125g/ml and 250g/ml respectively followed by the alum alone with the inhibition zone of 13.00±0.00 mm, 16.00±0.00 mm and 17.00±0.00 mm for the concentration of 62.5g/ml, 125g/ml and 250g/ml respectively and the least inhibitory activity was recorded by the consortium aqueous (Aqueous extract + Methanol extract + alum) with the inhibition (mm) zone of 10.00±0.00 mm, 10.00±0.00mm and 13.00±0.00 mm for the concentration of 62.5g/ml, 125g/ml and 250g/ml respectively. Just like other bacteria analysed, the positive control (ciprofloxacin) there has higher effect in the inhibition of the bacteria, *Staphylococcus aureus* compared to treatment with the extract of *Mimosa pudica* and its combination. The result of *Mimosa pudica* extract against *Staphylococcus* as shown in this study corroborate with the result of Amadi et al. [19], in which alum in combination with extract of turmeric produced an effective bacterial effect on *Staphylococcus* species recording zone of inhibition of 8.8±0.3mm at concentration of 300 mg/ml.

In a study by Balsaraf & Chole [20], the leaf extracts of *Mimosa pudica* showed strong antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis.* The antibiotic used as control also proved more effective than all the extracts. Ilham *et al* [21] also revealed the Antibacterial Activity of Aluminum Potassium Sulfate and *Syzygium Aromaticum* Extract against Pathogenic Microorganisms in which *S. aureus* was one of the organisms tested.

As shown in this study, *Mimosa pudica* displayed effective antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Staphulococcus aureus* which is in agreement with the report of Lakshmibai & Amirtham, (2019) which reported the effective antibacterial inhibition *of mimosa pudica* on some pathogenic bacterial such as *Staphylococcus, Pseudomonas* and *Bacillus cereus* at 100µg/well.

Previous studies revealed that higher concentrations of some phytochemical compounds in the plant extracts might be the reason for the higher antibacterial activity [22]. Flavonoids were found to exhibit antibacterial activity by the mechanisms like inhibiting nucleic acid synthesis, cytoplasmic membrane functions and energy metabolism [23].

The minimum concentrations of *E.coli, S. aureus* and *P. aeruginosa* shown at Tables 6,7 and 8 respectively shows that the extracts were effective against the bacterial isolates at 1g/ml to 0.50 g/ml concentrations.

Table 1. Morphology and biochemical characteristics of bacterial isolates

	Colony characteristics												
Isolate code	**Morphology**	**Shape**	**Reaction**	**Citrate test**	**Indole**	**Methyl Red**	**VP**	**Motility test**	**Glucose**	**Lactose**	**Mannitol**	**Sucrose**	**Probable Identity**
B2	Small round metallic sheen, flat	Rods	-ve	+	+	+	+	+	A	A	A	A	*Escherichia. coli*
B1	Golden yellow, small round.	Cocci	+ve	+	-	+	+	+	A	A	AG	A	*Staphylococcus aureus*
B3	Bluish-green.	Irregular	-ve	+	-	-	-	+	A	A	A	A	*Pseudomonas aeruginosa*

Keys; A= Acid, G= Gas, MR= Methyl Red, VP= Vogues Proskauer, -ve = negative, +ve = positive

Table 2. Phytochemical composition of *Mimosa pudica* leaf extracts

Phytochemicals	% Composition
Flavonoid	7.96±1.17
Alkaloid	9.85±0.21
Saponin	7.34±0.35
Total-phenol	1.63±0.00

Table 3. Antibacterial activity of plant extract on *Pseudomonas aeruginosa*

Types of Extract	Conc./Diameter of Zones of Inhibition (mm)			Ciprofloxacin
	62.5g/ml	125g/ml	250g/ml	(+control)
Aqueous Extract (-Control)	10.00±0.00	13.00±0.00	15.00±0.00	45±0.02
Methanol Extract	13.00±0.00	14.00±0.00	16.00±0.00	42±0.01
Alum	8.00±0.00	14.00±0.00	16.00±0.00	40±0.02
Aqueous Extract + Alum	16.00±0.00	20.00±0.00	22.00±0.00	38±0.00
Methanol Extract + Alum	11.00±0.00	16.00±0.00	21.00±0.00	45±0.04
Consortium	11.00±0.00	15.00±0.00	16.00±0.00	44±0.02

Table 4. Antibacterial activity of plant extract on *Escherichia coli*

Types of Extract	Conc./Diameter of Zones of Inhibition (mm)			Ciprofloxacin
	62.5g/ml	125g/ml	250g/ml	(+control)
Aqueous Extract (-Control)	8.00±0.00	14.00±0.00	15.00±0.00	42±0.02
Methanol Extract	11.00±0.00	14.00±0.00	18.00±0.00	44±0.02
Alum	10.00±0.00	14.00±0.00	16.00±0.00	43±0.03
Aqueous Extract + Alum	15.00±0.00	20.00±0.00	22.00±0.00	40±0.01
Methanol Extract + Alum	14.00±0.00	17.00±0.00	20.00±0.00	40±0.02
Consortium	13.00±0.00	17.00±0.00	18.00±0.00	43±0.02

Table 5. Antibacterial activity of plant extract on *Staphylococcus aureus*

Types of Extract	Conc./Diameter of Zones of Inhibition (mm)			Ciprofloxacin
	62.5g/ml	125g/ml	250g/ml	(+control)
Aqueous Extract (-Control)	10.00±0.00	13.00±0.00	15.00±0.00	45±0.02
Methanol Extract	10.00±0.00	12.00±0.00	17.00±0.00	45±0.03
Alum	13.00±0.00	16.00±0.00	17.00±0.00	42±0.01
Aqueous Extract + Alum	16.00±0.00	17.00±0.00	26.00±0.00	40±0.02
Methanol Extract + Alum	14.00±0.00	17.00±0.00	22.00±0.00	40±0.03
Consortium	10.00±0.00	10.00±0.00	13.00±0.00	45±0.05

Table 6. Minimum Inhibitory Concentration of *Mimosa pudica* leaf extract on *Escherichia coli*

	0.062g/ml	0.125g/ml	0.25g/ml	0.50g/ml	0.75g/ml	1g/ml
Methanol	+	+	+	-	-	-
Alum	+	+	+	-	-	-
Aqueous	+	+	+	-	-	-
Methanol + Alum	+	+	+	-	-	-
Alum + Aqueous	+	+	+	-	-	-
Methanol + Alum + Aqueous	+	+	+	-	-	-

Key; + = Presence of microbial growth
- = Absence of microbial growth

Table 7. Minimum Inhibitory Concentration of *Mimosa pudica* leaf extract on *Staphylococcus aureus*

	0.062g/ml	0.125g/ml	0.25g/ml	0.50g/ml	0.75g/ml	1g/ml
Methanol	+	+	+	-	-	-
Alum	+	+	+	-	-	-
Aqueous	+	+	+	-	-	-
Methanol + Alum	+	+	+	-	-	-
Alum + Aqueous	+	+	+	-	-	-
Methanol + Alum + Aqueous	+	+	+	-	-	-

Key; + = Presence of microbial growth
- = Absence of microbial growth

Table 8. Minimum inhibitory concentration of *Mimosa pudica* leaf extract on *Pseudomonas aeruginosa*

	0.062g/ml	0.125g/ml	0.25g/ml	0.50g/ml	0.75g/ml	1g/ml
Methanol	+	+	+	-	-	-
Alum	+	+	+	-	-	-
Aqueous	+	+	+	-	-	-
Methanol + Alum	+	+	+	-	-	-
Alum + Aqueous	+	+	+	-	-	-
Methanol + Alum + Aqueous	+	+	+	-	-	-

Key; + = Presence of microbial growth
- = Absence of microbial growth

4. CONCLUSION AND RECOMMENDATION

This study has been able to ascertain the sensitivity profiles of *Mimosa pudica* leaf extracts and its combination treatment with potassium aluminium sulphate (Alum) on some microbes specifically *S. aureus*, *E. coli, P. aeruginosa.* It has also been able to compare the sensitivity profiles of the leaf extracts to common antibiotic agents. Sensitivity testing has become increasingly important as a result of the constant changes observed in microorganism's response to various drugs and agents. *Mimosa pudica* leaf has also shown to be a promising antimicrobial agents especially in combination with alum. The potential of alum as an antimicrobial agent has been buttressed by this study and its effect when combined with extracts of *Mimosa pudica* leaf has been elaborated, all combination treatment in this study showed high zones of inhibition with increase in concentration against the test organisms. This research further authenticates previous studies and has revealed that the consortium of alum with different concentrations of *Mimosa pudica* leaf extract results in better antibacterial effect on the microrganisms, *S. aureus, P. aeruginosa, E. coli*. The phytochemicals; alkaloids, flavonoids, saponins, and phenols were reported and these are believed to be the bioactive ingredients of *M. pudica.* In the present study, the antimicrobial activity of leaf of *M. pudica* may also be due to the presence of above mentioned bioactive compounds. The results of this study revealed different level of antibacterial activity by leaf extracts of *M. pudica*. The antibacterial activity of *M. pudica* leaf extract was observed at various level of inhibition based on plant extracts, bacterial species and concentrations of extract. The collection of this medicinal plant is easy with low cost. The leaf of *M. pudica* may be an alternative drug for synthetic antimicrobial agents. In near future, the isolation of antimicrobial compounds from the leaf of *M. pudica* and its combination with Alum would be useful to treat infectious diseases caused by microorganisms. So, the extensive research should be carried out on combination of *M. pudica* wit Alum for the development of cost effective drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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