IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 9, Issue 1 Version. I (January 2019), PP. 01-05

Antimicrobial potential of *Mimosa pudica* Linn against multi-drug resistant bacteria species.

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Abstract: Therapeutic applications of herbal drugs have been crucial in Indian system of medicine. A plethora of modern drugs have their roots originated from nature's basket. In our current work, we also tried to explore the antimicrobial potential of one such medicinal plant, *Mimosa pudica* Linn. against resistant bacterial species. The aqueous extract was tested against gentamycin resistant MRSA, which showed significant bactericidal activity. The effectiveness of the *Mimosa pudica* Linn. against microbial infection has been stated in the medical text of Indian system of medicine.

Keywords: Antimicrobial, Resistant, Mimosa, MDR, Ayurveda,

Date of Submission: 20-12-2018 Date of acceptance: 03-01-2019

I. INTRODUCTION

Natural product has been the main source of drugs used in traditional system of medicine [1]. Other than that, a plenty of modern drug molecules are also originated from natural products [2]. These herbal products are relatively safe in contrast to the synthetic molecules, which carry a lot of side effects with them. Before the establishment of modern medicine, traditional practices like Ayurveda, Yunani, Shidda and Homeopathy have been mostly relying on plant products, as a source for drugs [3]. Over three-quarters of the world population in modern world relies mainly on plants and plant extracts for health care. Developed countries such as United States, use as much as 25% plant drugs of their entire lot. On contrary, developing countries such as China and India use plenty of drugs from natural origin having significant stake within the total drugs consumed [4]. As a whole, rural population of developing countries mostly rely on indigenous system of medicine due to many reasons like inadequate supply of modern drugs, low cost of treatments and side effects when compared with allopathic drugs. Hence, we can see an increased use of plant materials as a source of medicines for a wide variety of human ailments [5]. Approximately, 4 billion populations in the world cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material [6].

Mimosa pudica Linn. is a common herb which is grows all over in India. The herb stems are branched, coverd with bristly hairs. *Mimosa pudica* Linn. is used in Indian system of medicine as a traditional medicine for the treatment of various diseases. This is a short lived evergreen, annual sub shrub. A perennial plant, it grows to a height of 0.5m with a spread of 0.3m. The herb stem is erect, slender and branching and the leaves are bi-pinnate, fern like pale green and it use to close when disturbed [7]. Due to its ability to fix nitrogen from the air the herb does well on poor soils [8]. The herb is called as "Sensitive Plant" which folds up its leaves when touched or exposed to a flame. This plant requires a medium light exposure, moist soil, and temperatures between 60-85 degrees. The plant dislikes root disturbance, so caution should be taken when handling seedlings. Mimosa may be sometime difficult to grow and it is sensitive to over watering.

Biology of the Mimosa

Taxonomy - Kingdom: Plantae, Division: Spermatophyta, Class: Magnoliopsida, Order: Fabales, Family: Fabaceae, Genus: Mimosa, Species: *M. pudica* [7, 9]

II. METHODOLOGY

2.1 Plant material Collection and Authentication

The entire plant *Mimosa pudica* Linn. were collected from Panikhaiti hills near Narengi, Guwahati of Assam. They were thoroughly washed in running water, segregated from the grass and other extraneous material and the field data of the plant like its height, flower colour and soil condition were noted in the note book. Then

the leaves were taken and dried in shade for 30 days. The shade-dried leaves were made into coarse granules and were used for further investigation .

2.2 Chemicals

The chemical agents such as Sulphuric acid, Nitric acid, Potassium hudroxide, Methanol, Hydrochloric acid, Sodium hydroxide, Picric acid, Chloroform, Glacial acetic acid, Iodine, Ferric chloride, NB agar media, YPD broth media of analytical grade were obtained from Himedia, Mumbai**.**

2.3 Preparation plant extract

The plant were collected and washed thoroughly with water to remove any unwanted matter. This was further dried in shade. After complete drying it was powdered and passed through sieve no 60 and stored in an air tight container. Later, the dry powder of the plant specimen was extracted using deionized water in shaking incubator at room temperature for 24 hours. The extract was collected and lyophilized for further experimental purpose [10].

2.4 Pharmacognostical and Phytochemical studies

The macroscopical observations were carried out and the microscopical investigations, histochemical tests, stomatal index, vein islet number, fluorescence analysis were performed as described by *Kokate et al* [10]. The powdered drugs were analysed for physical constants and the phytochemical analysis were carried out. The data were reported for the conclusions.

2.5 Fluorescence analysis

The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". The powdered drug was examined under U.V. and ordinary light with different reagents. About 10 gms of the powdered drug was taken in a petridish and treated with different reagents. These were observed under different wavelengths i.e., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted were observed and noted [10].

2.6 Behaviour of powder of *Mimosa pudica* leaves with different reagents / solvents

Powder of the leaves of Mimosa pudica was examined by mixing with different solvents or reagents as per the procedure and the colour changes was observed in naked eyes under sufficient light [10].

2.7 Antimicrobial Assay

Antibacterial assays were carried out by spread plate method for assessment cidal activity of the plant *Mimosa pudica* Linn against candidate microbial species. Briefly, *Staphylococcus aureus* NCTC 8530, *Escherichia coli* MG1655 and Gentamicin resistant MRSA (ATCC 33592) were cultured in 1.3% (w/v) nutrient broth (NB) at 37°C overnight. Mid log phase bacterial species of a second subculture was prepared for the antimicrobial assay. The cells were washed and re-suspended in sodium phosphate buffer (10 mM, pH 7.4). The resulting suspension was diluted to 2×10^6 CFU/ mL. The inoculums (50 µL) the bacterial suspension was treated with required concentrations of extracts, and incubated for 2 hours at 37±2℃. 20 µL of the 10 fold diluted incubation broth was plated while keeping untreated bacterial suspension of similar dilution as negative control [11]. *Candida albicans* MTCC 1637 were cultured for 24 hours at 30°C in 10 ml of YPD broth, then diluted to 10 ml YPD broth to OD_{600}=0.2 and grown for an additional 4 hrs. with shaking till the OD_{600}=0.8. The candicidal activities of the the extract was tested using above mentioned standard assay techniques [12].

III. RESULTS AND DISCUSSION

In the present study, we explored the possibilities of evaluating antimicrobial activity of the plant *M. pudica* against multidrug resistant bacteria. As per the traditional texts of Indian System of Medicine, these plants have been predominantly used to treat multiple ailments related to microbial infection [7]. The plant specimen was collected and processed as per the standard practices involved in herbal drug testing protocols. The plant specimen was shade dried and powdered prior extraction. The authentication was carried out by the help of **Dr. A. A. Mao (Scientist-in-charge)**, **Botanical Survey of India (BSI)**, Shillong Eastern Regional Centre, Shillong- 793003. We carried out a series of phytochemical studies for the identification of the phytochemicals found in the collected plant specimen (Table 1).

Table 1: Phyto-chemical screening

Test	Aqueous Extract
Alkaloids	+
Carbohydrates	-
Glycosides	+
Phytosterols	-
Fixed oil and fats	-
Phenolic compound andTannins	-
Saponins	-
Proteins & Amino acids	-
Gums and Mucilage	-

'+' = test for positive, '_' = test for negative

There was an indicative presence of alkaloids and glycosides,that may be reflected in the following study of bactericidal activity of plant material. Fluorescence analysis was also carried out of our plant material. This is an indicative analysis where the presence of various chemical constituents can be qualitatively hypothesize (Table 2).

Table 2: Fluorescence analysis of the powdered drug

Treatment of powder of *Mimosa pudica* leaves	Visible rays	Ultra -violet light	
		short wave (254 nm)	long wave (365 nm)
Powder as such	Light brown	Deep brown	Black
Powder+50% H_2SO_4	Brown	Dark brown	Black
Powder+50% HNO_3	Brown	Dark brown	Black
Powder+5% KOH	Brown	Deep brown	Black
Powder+Methanol	Brown	Deep brown	Black
Powder+1N HCl	Light brown	Deep brown	Black
Powder+1NMethanolic NaOH	Deep brown	Dark brown	Black
Powder+Cold water	Light brown	Brown	Black
Powder+Hot water	Light brown	Brown	Black
Powder+Picric acid	Deep brown	Dark brown	Black
Powder+Ammonia solution	Brown	Deep brown	Black
Powder+Chloroform	Deep brown	Dark brown	Black
Powder+Glacial acetic acid	Deep brown	Dark brown	Black
Powder+5% Iodine solution	Deep brown	Dark brown	Black
Powder+$FeCl_3$	Brown	Dark brown	Black

The extractive value of aqueous fraction was found to be 11.12 %. Total ash (8.01%), Acid insoluble ash (2.04%), Water insoluble ash (3.23%), Loss on drying (0.204) and Moisture content (8.92%) were recorded for standardization by *Kokate et al.* We also checked the Behaviour of powder of Mimosa pudica leaves with different reagents as per the routine analysis of herbal drug study (Supplementary Table 1)

Antimicrobial activity

The antimicrobial activity of the aqueous extract was performed against Gentamicin resistant MRSA. The aqueous extract was showing significant activity against the MDR strain (Table 3, Figure 1). The extract showed an approximate killing of 99 percent at 300 mg/ mL extract.

Aqueous extract (mg/ mL)	% Killing efficiency
300	98.79
150	97.64
75	96.75
37.5	96.59
18.8	84.80

Apart from it we also examine the bactericidal potency against *S.aureus*, *E.coli* and *C. albicans* (Supplementary Table 2). The aqueous extract was significantly active against all the bacterial as well as fungal strain

(Supplementary Table 2). Therefore, the antimicrobial property of the plant extract can be stated as broad spectrum and antifungal based on the *in-vitro* analysis.

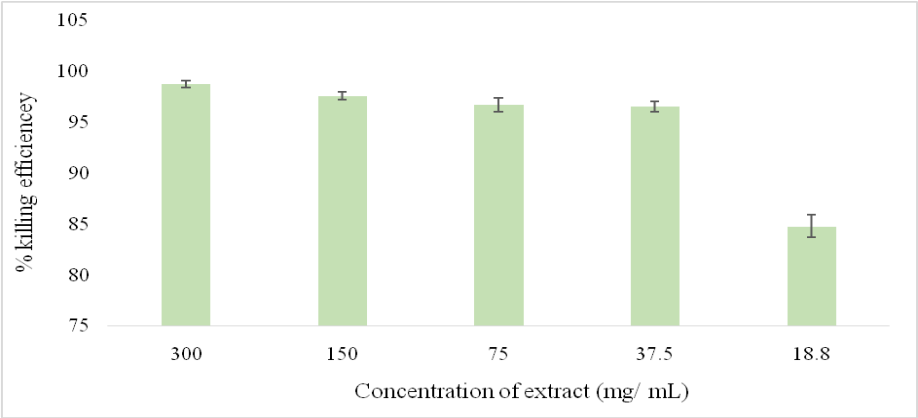

Figure 1: Antibacterial activity of the aqueous extraxt of the plant *M.pudica* against Gentamicin resistant MRSA. The plant extract showed significant antibacterial activity. The erroe bar signifies the standard deviation.

IV. CONCLUSION

Natural products have been the prime source of food as well as drug for the entire civilization. In modern as well as traditional practices, the living beings have been benefited by the natural treasure as per the requirement [13]. Most of the drugs of golden generation and others have been isolated or semi synthesized from natural sources [14]. In the present study, we tried to evaluate the bactericidal potency of the plant *M.pudica* against multidrug resistant *S.aureus*. We could realize the therapeutic potential of the plant, as the aqueous extract depicted significant bactericidal potency against MDR species. In recent years, resistances towards antibiotics have claimed millions of lives across the globe. As per WHO, there would be an average mortality of around 10 million death by 2050 [15]. As per the need, there is an urgent requirement of potential alternative of antibiotics to counter the infections caused by superbugs. Hence, we can rely on alternative medicines, as these drugs have been used by traditional practioners of India System of Medicine with profound activity and negligible side effects.

ACKNOWLEDGEMENTS

I would like to thank the management of NETES Institute of Pharmaceutical Science for providing all the facilities required to carry out my work.

Abbreviations: WHO: World Health Organizations, MDR: Multi Drug Resistant, CFU: Colony Forming Unit, MTCC: The Microbial Type Culture Collection and Gene Bank, NB: Nutrient Broth

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Bhargab Jyoti Sahariah. "Antimicrobial potential of Mimosa pudica Linn against multi-drug resistant bacteria species.". IOSR Journal of Pharmacy (IOSRPHR), vol. 9, no. 1, 2019, pp. 01-05.