

Short Communication

ANTIMICROBIAL ACTIVITY OF NATURAL CHLOROPHYLLIN FROM ENDANGERED MEDICINAL PLANT *MIMOSA PUDICA* L.

RAJALAKSHMI K.¹, N. BANU²

^{1,2}Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India
Email: rajalakshmik06@gmail.com

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ABSTRACT

Objective: Chlorophyllin (CHL) belongs to a group of compounds, porphyrins that contain a chelated metal ion in the center of the molecule. The objective of this present study was to extract the Chlorophyllin from *Mimosa pudica* and to study its antimicrobial activity.

Methods: The antimicrobial activity of chlorophyllin from the leaf extract of *Mimosa pudica* L., was determined *in vitro*, using well diffusion method against human pathogenic bacteria and fungi, viz., two Gram-negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*, and two Gram-positive bacteria: *Staphylococcus aureus* and *Klebsiella pneumoniae*, and one fungal pathogen, *Candida albicans*.

Results: Chlorophyllin from the leaf extract of *Mimosa pudica* recorded potential antimicrobial activity against the tested microorganism with the range of 9 mm-18 mm at 25-100 µg/ml.

Conclusion: Natural Chlorophyllin from *Mimosa pudica* has significant activity against the five pathogenic test microorganisms.

Keywords: Medicinal plants, Chlorophyllin, *Mimosa pudica*, Pathogenic bacteria, Pathogenic fungi

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Many of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by a majority of cultures around the world for example, Chinese medicine and Indian medicine. *Mimosa pudica* Linn. (Mimosaceae) commonly known as the 'sensitive plant' is diffuse under a shrub. It is native to America but has naturalized throughout India [1]. The plant is well-known for its medicinal properties particularly for the treatment of piles [2]. It has been traditionally used in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections [3-5]. The present study was done to determine the antimicrobial activity of Chlorophyllin from *Mimosa pudica*. Chlorophyllin is semi-synthetic water soluble sodium copper salt of chlorophyll. During the synthesis of chlorophyllin the magnesium atom at the center of the ring is replaced with copper and the phytol tail is lost. Unlike natural chlorophyll, chlorophyllin is water-soluble [6]. The antioxidant property of CHL from *Mimosa pudica* was proved by the spectrophotometric method by the formation of Phospho-molybdenum complex [7]. We designed the present work to determine the antimicrobial activity of chlorophyllin extracted from *Mimosa pudica*. *Mimosa pudica* plant leaves were collected from Vels University campus, Pallavaram, Chennai.

The plant was authenticated by Prof. P. Jayaraman, National Institute of Herbal Sciences. Ten grams of fresh leaves were taken and 1 gm of sodium carbonate was added to neutralize the acidity. The plant material was ground with 50-100 ml acetone and filtered using filter paper. This procedure is repeated until the residue becomes colorless. It was then washed with 50-150 ml of diethyl ether to wash off acetone. The mixture was poured into a separating funnel and acetone was washed off using distilled water. This was repeated until yellow color separates off which consists of flavones. The solution was poured into a bottle and 10-25 ml of methanol saturated with

potassium hydroxide pellets was added. The solution was shaken thoroughly and kept in the icebox for overnight. The alkaline solution of chlorophyllin was poured into a separating funnel, and 100 ml diethyl ether was added and left for 30 min. Chlorophyllin separates off greenish layer which was removed. The ether layer was washed off with dilute potassium hydroxide and distilled water, to remove traces of chlorophyllin salts. The filtrate was evaporated to dryness in a rotary evaporator, and the extract was stored in ice box [8].

Two Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and two Gram-positive *Staphylococcus aureus* and *Klebsiella pneumoniae* bacterial pathogens and one fungal pathogen *Candida albicans* were used for *in vitro* antimicrobial activity. They were obtained from MTCC (IMTECH, Chandigarh, India). The antibacterial activity was determined by well diffusion methods [9]. About 25 ml of molten Mueller Hinton agar and potato dextrose agar were poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify after which 18 h grown (OD adjusted to 0.6) 100 µl of above said pathogenic cultures were transferred onto a plate and made culture lawn by using sterile L-rod spreader. After five minutes a sterile cork borer was used to make 5 mm well on the agar. The test samples were dissolved in methanol and loaded in to wells with various concentrations such as 25, 50, 75 and 100 µg/ml. Triplicates were maintained and the experiment was repeated thrice for each replicates the readings were taken in three different fixed directions and the average values were recorded. The streptomycin and Nystati (10µg/ml) added well served as positive control. The plates were incubated at 37 °C in a 40 W fluorescent light source (~400 nm) for 24 h for bacterial pathogen and 48 h for the fungal pathogen. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

Table 1: Zone of the Inhibition of Standard Chlorophyllin for antimicrobial activity

Name of the organisms	Zone of the Inhibition (mm)				Streptomycin Sulphate	Nystatin
	25	50	75	100		
Concentration(µg/ml)	25	50	75	100	10	10
<i>Staphylococcus aureus</i>	11±0.33	12±0.66	13±0.57	14±0.57	16±0.33	19±0.33
<i>Pseudomonas aeruginosa</i>	10±1.20	12±0.16	14±0.66	16±0.57	18±0.33	16±0.33
<i>Klebsiella pneumoniae</i>	12±0.57	14±0.23	16±0.57	17±0.51	14±0.33	14±0.57
<i>Escherichia coli</i>	14±0.23	15±0.66	16±0.42	18±0.88	15±0.33	16±0.33
<i>Candida albicans</i>	11±0.57	13±0.79	14±0.33	16±0.57	16±0.33	18±0.57

Values are mean of three independent replicates ± standard deviation

Table 2: Zone of the Inhibition of *Mimosa pudica* Chlorophyllin for antimicrobial activity

Name of the organisms	Zone of the Inhibition (mm)				Streptomycin Sulphate	Nystatin
	25	50	75	100		
Concentration($\mu\text{g/ml}$)	25	50	75	100	10	10
<i>Staphylococcus aureus</i>	12 \pm 0.89	14 \pm 0.33	16 \pm 0.33	18 \pm 0.88	14 \pm 0.33	16 \pm 0.33
<i>Pseudomonas aeruginosa</i>	8 \pm 0.33	11 \pm 0.66	12 \pm 1.20	13 \pm 0.57	12 \pm 0.33	12 \pm 0.11
<i>Klebsiella pneumoniae</i>	14 \pm 0.57	16 \pm 0.33	18 \pm 0.33	19 \pm 0.79	11 \pm 0.33	14 \pm 0.57
<i>Escherichia coli</i>	13 \pm 0.57	14 \pm 0.57	16 \pm 0.33	18 \pm 0.57	13 \pm 0.33	16 \pm 0.33
<i>Candida albicans</i>	9 \pm 0.33	10 \pm 0.33	12 \pm 0.66	13 \pm 0.65	14 \pm 0.33	14 \pm 0.89

Values are mean of three independent replicates \pm standard deviations

The result obtained in the present study revealed that chlorophyllin from *Mimosa pudica* possesses potential antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*. The test material was effectively inhibited all test pathogens at all the tested concentrations. The lowest concentration of 25 $\mu\text{g/ml}$ showed a zone of inhibition against all the tested pathogen were ranged between 6 mm and 15 mm whilst, the highest concentration of 100 $\mu\text{g/ml}$ showed zone of inhibition ranged between 13 mm and 23 mm (table 1&2).

Chlorophyllin from *Mimosa pudica* showed almost similar antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* is (12 mm-18 mm) and (14 mm-19 mm) at the concentration of 25-100 $\mu\text{g/ml}$. It shows almost similar or higher antibacterial activity in *Pseudomonas aeruginosa* followed by *E. coli*. Chlorophyllin standard showed almost similar or higher zone of inhibition in the entire five test organism (table 1). Antibacterial activity in standard chlorophyllin and chlorophyllin from *Mimosa pudica* were almost similar. The least activity was observed in *Pseudomonas aeruginosa* (10 mm) and the highest inhibitory activity was observed in *Klebsiella pneumonia* (18 mm). Zone of inhibition was almost similar or lower when compared with the streptomycin sulphate. Standard Chlorophyllin recorded better antifungal activity than Chlorophyllin from *Mimosa pudica* (table 2). Both showed less antifungal activity when compared with Nystatin.

Medicinal plants are an important source for the development of new chemotherapeutic agents [10]. Many reports are available on the antibacterial, antifungal, antiviral and anti-inflammatory properties of medicinal plants [11-14], and there are many reports on antimicrobial activity of *M. pudica* whole plant but this is the first report on antimicrobial activity of Chlorophyllin extracted from the leaf of *Mimosa pudica* L. There were many reports available on antimicrobial activity from leaf extract, aqueous extract of stem bark, methanolic extract of leaves, and seed of *Mimosa pudica* (15-17). The uses of the whole plant of *Mimosa pudica* was proven for various pharmacological and biological activities. Mostly Root and leaves of *Mimosa pudica* are showed maximum pharmacological activity [18]. In the present study antimicrobial activity of *Mimosa* was studied using well diffusion method at the concentration of 25-100 $\mu\text{g/ml}$ against the microbe.

The result of this study revealed that the *M. pudica* leaf extract and standard chlorophyllin possesses inhibitory activity against the test pathogen used for screening, similar study was reported by Pawaskar against whole plant extract of *Mimosa pudica* against *E. coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Proteus vulgaris*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Shigella flexneri*, *Klebsiella pneumonia*, and *pseudomonas aeruginosa*. The *Mimosa pudica* whole plant extract possesses good antimicrobial activity between 7 mm-18 mm [19]. The present study reveals that both CHL and whole plant extract [19, 20] from *Mimosa pudica* have antimicrobial property. Mukesh Chandra Sharma et al. reported the antimicrobial activity against the crude extracts of *Mimosa pudica* Linn using six different solvents by cold maceration process. Inhibitory activity of aqueous extracts of *Mimosa pudica* plants were investigated by agar disc well diffusion method against bacterial pathogens *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia Coli*, *K. Pneumonia*, *M. luteus* and *C. Albicans* and the result showed good antimicrobial activity (28 mm) in *E. Coli* at 100% concentration of sample, in others it was found more or less similar to our present investigation. So the data indicated that gram

negative was the sensitive strain, CHL from *Mimosa pudica* have been effectively proven for their utilization as a source for antimicrobial compound [20].

The natural Chlorophyllin from *Mimosa pudica* has potential antimicrobial activity, and it may be useful for the development of pharmaceutical industries as a therapy against various diseases. Further studies required to understand the mechanism and the actual efficacy of the chlorophyllin.

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CONFLICT OF INTERESTS

Conflict of interest declared none

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