

## Original Research Article

Effect of *Nymphaea alba* Petal Extract on Multidrug Resistant BacteriaAmrita Basak<sup>1</sup>, Bhaskar Narayan Chaudhuri<sup>2</sup>, Sayan Naskar<sup>3</sup>, Partha Guchhait<sup>2</sup>, Satadal Das<sup>2\*</sup><sup>1</sup>Department of Biotechnology & Dr. B.C. Guha Centre for Genetic Engineering & Biotechnology, University of Calcutta, Kolkata, India<sup>2</sup>Department of Microbiology, Peerless Hospitex Hospital and Research Center Limited, Kolkata, India<sup>3</sup>Department of Microbiology, Vijaygarh Jyotish Ray College, University of Calcutta, Kolkata, India

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**Abstract:** This study aimed to determine the antibacterial effect of the extract of *Nymphaea alba* petals, and if antibacterial activities are present then the Minimum Inhibitory Concentration (MIC) of the extract is determined. Currently multidrug resistant bacteria are a major concern for clinicians as most conventional antibiotics do not work against MDR strains and infections with MDR strains are increasing day by day, with many resulting in death. This study focused mainly on the antibacterial activity of ethanol extract. Our study showed that the crude extract is significantly effective against MDR *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with MIC value <0.78 mg/ml. This confirms the effectiveness of this extract against MDR bacteria.

**Keywords:** *Nymphaea Alba*, MDR Bacteria, Antibacterial, Medicinal Plants, Minimum Inhibitory Concentration.

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## INTRODUCTION

*Nymphaea alba* (Fig.1) an aquatic flowering plant belonging to family Nymphaeaceae. It is also known as European White Water Lily, Nenuphar, Shandh shaluka (in Bengali). It is widely distributed throughout the world, occurring in pond, lakes, and marshy regions. Its various components have been used historically to treat a variety of ailments in a variety of medicinal systems, such as those of Arabic medicine, Unani medicine, Ayurveda medicine, and Chinese medicine. Utilizing modern pharmacological techniques, the traditional use of *Nymphaea alba* has been demonstrated to be effective in the treatment of various ailments. Comprehensive research has provided an estimation of the pharmacological properties of various extracts or isolated compounds derived from *Nymphaea alba* [1].

Plants have long been a major source of pharmaceutical drugs for human health. According to the World Health Organisation (WHO), medicinal plants will be the best way to get a wide range of drugs. Approximately 65% to 80% of people in developed countries take traditional medicine that contains compounds from medicinal plants. [2]. In recent years, the prevalence of multiple resistant microorganisms in human pathogens has been on the rise, primarily due to

the widespread use of commercial antimicrobial drugs used to treat infectious diseases. This has necessitated a search for novel antimicrobial compounds from a variety of sources, such as medicinal plants [3]. This is why aquatic plants have caught the attention of scientists and have shown promising antimicrobial properties [4].

In Indian folk medicinal products *N. alba* is used as antiseptic, astringent, radical scavenger, anti-inflammatory, antioxidant while the rhizomes are applied as rubefacient externally [2, 3].

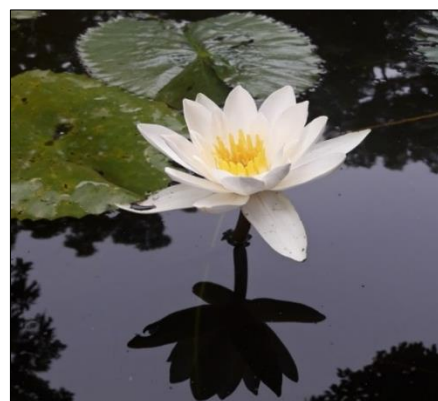


Figure 1: Picture of a *Nymphaea Alba* plant in pond

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## MATERIALS AND METHODS

### Plant Materials

The flower of the aquatic plant *Nymphaea alba* was collected from local pond and was identified by a Botanist. The flower was cleaned of extraneous matter, and necrotic parts were removed and washed with fresh water. The flower was transported to the laboratory in polythene bags. In the laboratory, the flowers were washed thoroughly three times with running water and once with distilled water.

### Mueller Hinton Broth

The broth powder was procured from Himedia, and 4.2gm of broth powder was added to 200ml of distilled water, mixed well, and autoclaved. After autoclaving, the sterile medium was stocked in the laboratory for the study.

### Microbial Cultures

*Staphylococcus aureus* ATCC 25923, Methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* ATCC 25922, *Escherichia coli* MDR, *Klebsiella pneumoniae* MDR, *Pseudomonas aeruginosa* MDR were isolated from some infectious sources of human.

### Preparation of Plant Extracts

Petals (Fig.2) of the flower were first separated then all the petals were cut into very small pieces using clean blade. Then 2 gram of the cut pieces were measured and added to 10ml of 70% alcohol and kept in dark place for about 72 hours (Fig.3). All of this procedure was done in aseptic conditions.



Figure 2: Petals of *Nymphaea Alba*



Figure 3: *N. Alba* extract

## METHODOLOGY

At first, the bacterial strains were subcultured on respective solid mediums to obtain pure strains. All the wells of a microtiter plate were filled up with 100µl of Mueller Hinton broth. Then 100 µl of the extract was added to the first well, and thoroughly mixed and the 100 µl of this was transferred to the next well in a horizontal row, mixed again, and 100 µl of it again transferred to the next well. In this way, serial double dilution of the extract was performed up to the eighth well of the row. Thus, the concentration of the extract in the first well was 100 mg/ml, and this was diluted to 50mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.56 mg/ml, and 0.78 mg/ml. Then test bacterial suspensions in sterile normal saline (0.5 MacFarland standard) was added in each well in 10 µl amounts.

In the next horizontal row of eight wells, the vehicle of the extracted ethanol was similarly diluted, and the test bacteria was similarly added in each well. This row was done as a control to compare the results. Other bacterial strains were similarly tested in each separate row with one control row. After completing the procedure, the microtitre plate was gently rotated to mix the bacteria uniformly in each well. Then an initial

reading at 0 hour was taken at 620nm in a Microscan reader to find out the baseline optical densities.

After 24 hours another reading at a similar wavelength was also taken. Then the baseline reading of each well was deducted from the final reading of each well.

### Synergy Method

A pan drug resistant *Pseudomonas aeruginosa* was selected for the study. A lawn culture was prepared on Mueller Hinton (MH) plate with 0.5 MacFarland standard concentration of the bacteria. After that one antibiotic disc (Ceftazidime/Amikacin/Levofloxacin/Imipenem) and one disc soaked with the ethanolic extract of *N. alba*. (2gm in 10 ml petal extract) were placed on

the lawn culture. Then one antibiotic disc with one extract disc were combined together Thus, in each set one antibiotic disc, one extract disc and one combined disc were placed over the lawn culture to observe if there is any sensitive zone after overnight incubation at 37°C.

## RESULTS

Our study showed that the crude extract is significantly effective against MDR *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with MIC value <0.78 mg/ml. Some antimicrobial action was also present against *Staphylococcus aureus* with MIC values varied from 25-50 mg/ml. Against *E. coli* the action was extremely variable and it was unpredictable (Fig. 4-9).

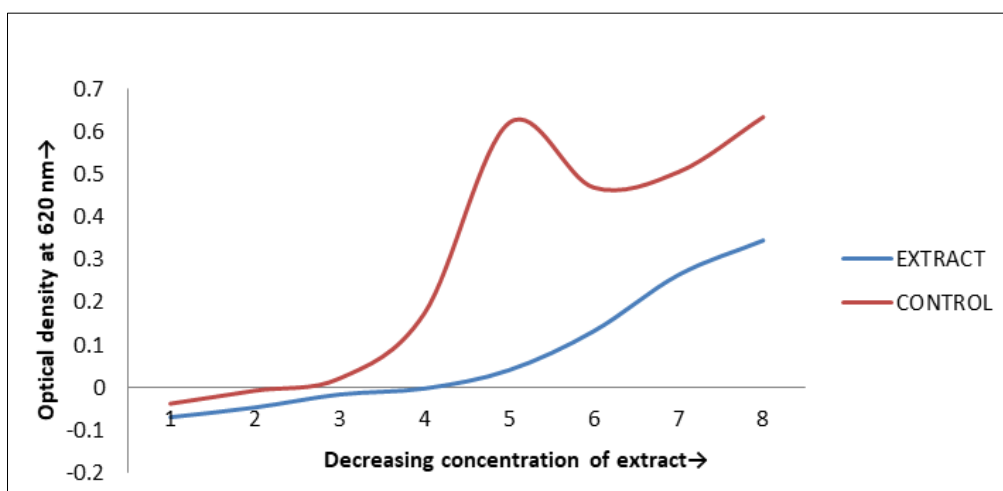


Fig. 4: Showing MIC value (< 0.78mg/ml) of extract against *Pseudomonas aeruginosa* MDR; Concentration of extract: 1= 100mg/ml;2= 50mg/ml; 3= 25mg/ml ;4= 12.5mg/ml; 5= 6.25mg/ml; 6= 3.12mg/ml; 7= 1.56mg/ml; 8= 0.78mg/ml

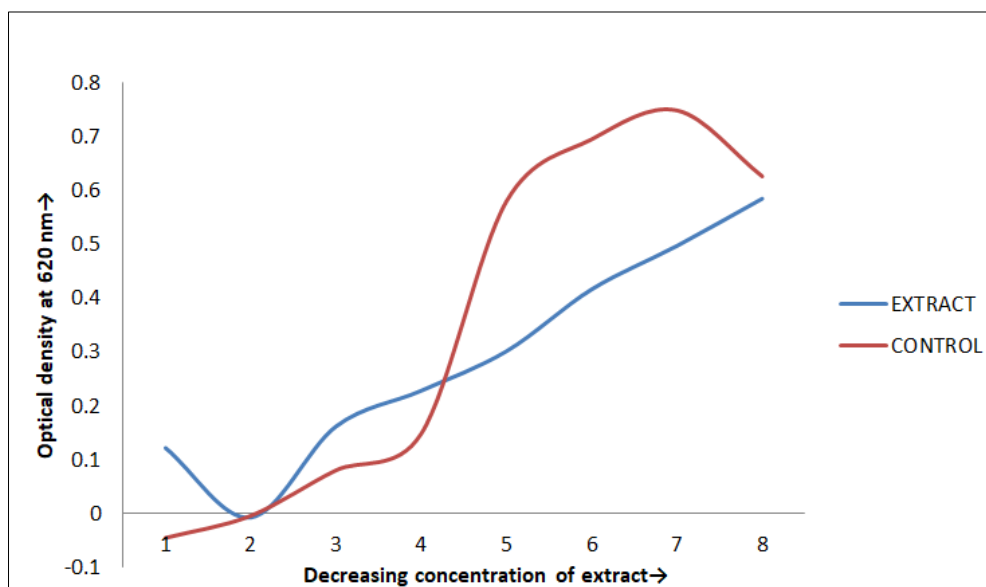
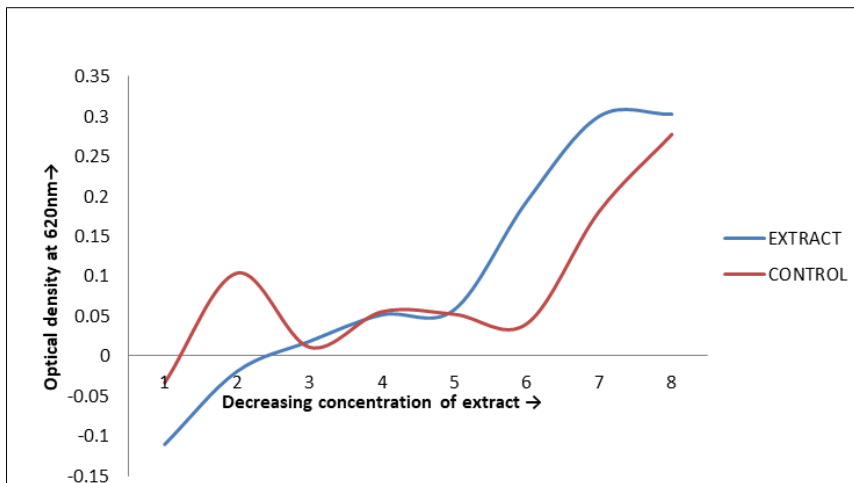
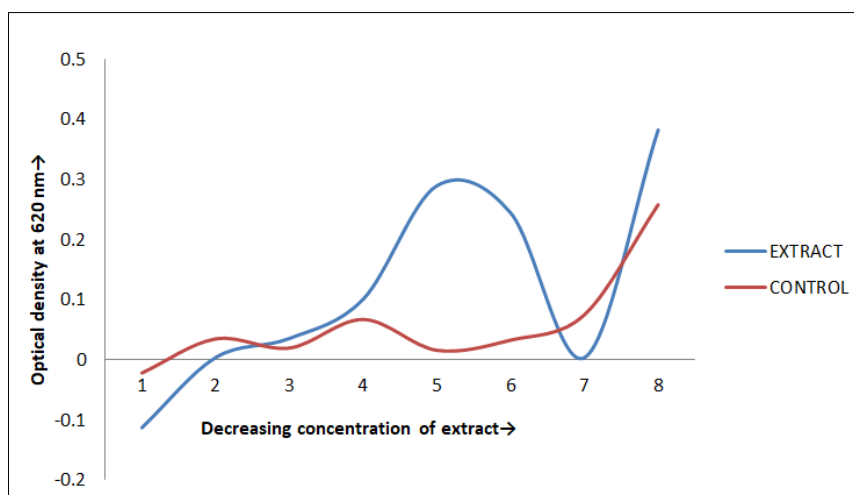


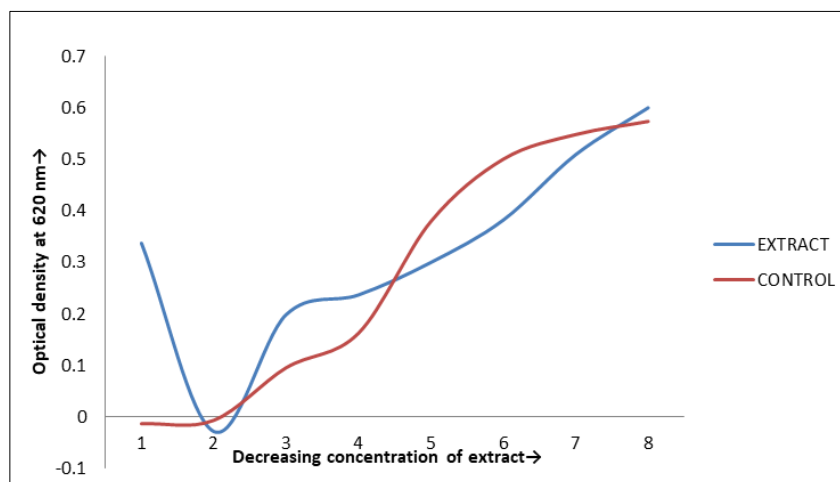
Fig. 5: Showing MIC value (<0.78 mg/ml) of extract against *Klebsiella pneumoniae* MDR; Concentration of extract: 1= 100mg/ml;2= 50mg/ml; 3= 25mg/ml ;4= 12.5mg/ml; 5= 6.25mg/ml; 6= 3.12mg/ml; 7= 1.56mg/ml; 8= 0.78mg/ml



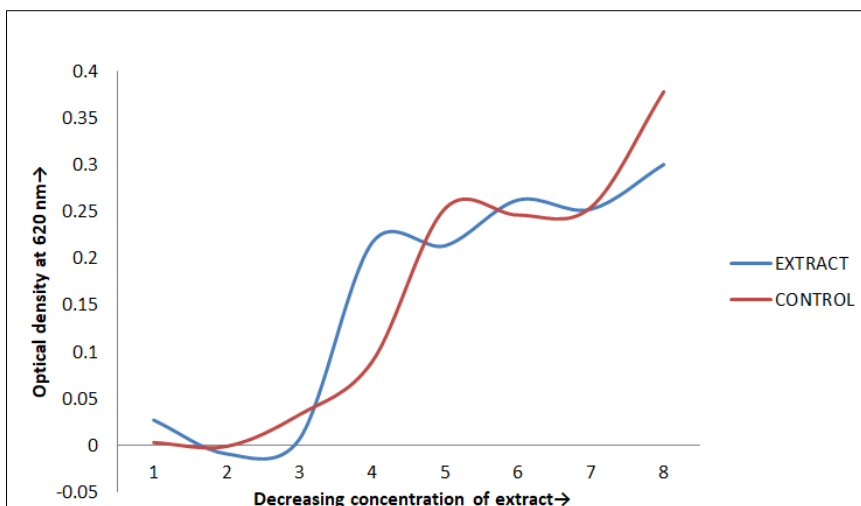
**Fig. 6:** Showing MIC value (25 mg/ml) of extract against *Staphylococcus aureus* ATCC 25923; Concentration of extract: 1= 100mg/ml;2= 50mg/ml; 3= 25mg/ml ;4= 12.5mg/ml; 5= 6.25mg/ml; 6= 3.12mg/ml; 7= 1.56mg/ml; 8= 0.78mg/ml



**Fig. 7:** Showing MIC value (50 mg/ml) of extract against Methicillin resistant *Staphylococcus aureus* (MRSA); Concentration of extract: 1= 100mg/ml;2= 50mg/ml; 3= 25mg/ml;4= 12.5mg/ml; 5= 6.25mg/ml; 6= 3.12mg/ml; 7= 1.56mg/ml; 8= 0.78mg/ml



**Fig. 8:** Showing action of extract against *Escherichia coli* ATCC 25922; Here, the inhibition was extremely variable and not significant; Concentration of extract: 1= 100mg/ml;2= 50mg/ml; 3= 25mg/ml ;4= 12.5mg/ml; 5= 6.25mg/ml; 6= 3.12mg/ml; 7= 1.56mg/ml; 8= 0.78mg/ml

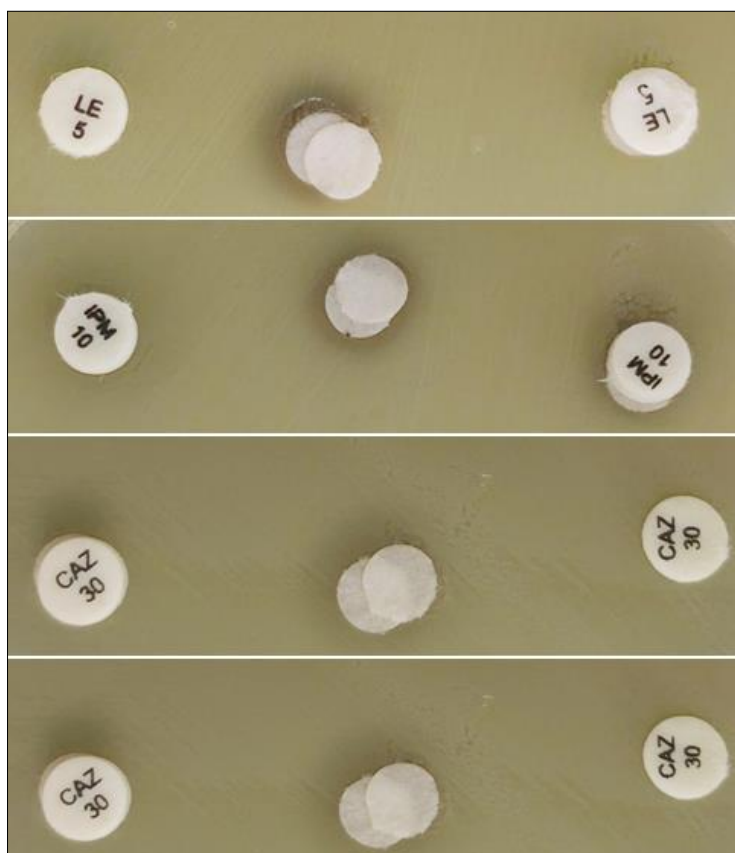


**Fig. 9:** Showing action of extract against *Escherichia coli* MDR; Here, the inhibition was extremely variable and not significant; Concentration of extract: 1= 100mg/ml;2= 50mg/ml; 3= 25mg/ml ;4= 12.5mg/ml; 5= 6.25mg/ml; 6= 3.12mg/ml; 7= 1.56mg/ml; 8= 0.78mg/ml

**Synergy Observation**

It was observed that although there was no sensitive zone in all single disc (either antibiotics or the extract) but in all double disc application (antibiotic with extract) there were sensitive zones.

Further experiments with refined extract and / selective active component of the extract may reveal some novel antimicrobial combination which may be affected in all patients infected with such pan drug resistant *Pseudomons aeruginosa*.



**Fig. 10:** Discs of different antibiotics indicate that there was synergistic action of this extract with different antibiotics



## DISCUSSION

Plants possess a wealth of antibacterial properties that could potentially be utilized in the treatment of human illness. Since ancient times, plants have been a reliable source of sustenance, shelter, and clothing for humans [4, 5]. One of the most significant sources of new chemotherapeutic compounds is derived from traditional medicinal products or folk medicinal products [6]. Prior to the development of antibiotics in the 19th century, human beings relied solely on plant-based treatments for all illnesses and disorders [7]. While some of the fundamental values and uses of certain plants have been identified and published, many of them remain undiscovered. Therefore, there's a need to investigate their uses and conduct wide-ranging and large-scale studies to discover their therapeutic benefits. Flowers and rhizomes of *N. Alba* are renowned for their high levels of phenolic content [8-10]. The phenolic content of the *N. alba* leaves is high, indicating the presence of hydrolytic tannins, predominantly ellagitannin, in addition to flavonoid content and major antioxidant activity. The *N. Alba* leaf was also found to be a source of essential fatty acids with high nutritional value [11].

SaiKoushik *et al.*, [12], evaluated the hydroalcoholic extracts of the flowers from the genus *Nymphaea* for their antibacterial properties against Gram-positive and Gram-negative microorganisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. The results of the study indicated a broad spectrum of activity [12].

## CONCLUSION

This study demonstrates that the flowers of the *Nymphaea alba* possess considerable antibacterial activity particularly against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and can be used as an antimicrobial agent. Peculiarly the extract also showed sensitivity zones against *Pseudomonas aeruginosa* with different antibiotics while they were all resistant when used separately. Further this study encourages the isolation of the attributing compound responsible for the antibacterial activity.

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**Competing Interests:** There is no competing interest of any Author in this manuscript.

## Author's Contribution

A.B and S.N – Experiment, Manuscript writing; B.N.C and P.G – Manuscript editing, Guidance; S.D – Experiment designing, Manuscript correction.

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