# SCREENING OF ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF ETHNOMEDICINAL PLANT *Hygrophila schulli* (Buch.-Ham.) M. R. *et*. S. M. Almeida

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## Abstract

Methanolic, ethanolic and aqueous extracts of leaf part of *Hygrophila schulli* (Buch.-Ham.) M.R.*et*.S.M.Almeida (Acanthaceae), a ethnomedicinal plant was screened for antibacterial activity against pathogenic strains of three gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and *Klebseilla pneumoniae* and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Antibacterial activity of all the extracts was performed by disc diffusion method. Alcoholic extract showed pronounced antibacterial activity than aqueous extract. Among all bacterial stains, methanolic extract (400 mg/ml) showed greater antibacterial activity against *S. aureus* (18 mm) and MIC value was 0.156 mg/ml. *P. aeruginosa* and *K. pneumoniae* were more resistant strains followed by *E. coli* and *B. subtilis* against all the extract of *H. schulii*. Thus methanolic leaf extract of *H. schulli* is more effective in treating the pathogenic diseases, which may opens up the possibilities of finding a new clinically antibacterial compound.

**Keywords:** Antibacterial activity, ethnomedicinal plant, *Hygrophila schulli*, MIC

## 1. Introduction

Ethnomedicinal plants have been used by the poor ethnic peoples against different pathogenic diseases [1]. Such plants are quite important due to their therapeutic potentialities, which are widely used in screening antimicrobial properties [2]. Moreover, antibacterial compounds of plant origin are useful in killing the growth of pathogenic bacteria [3]. In many situations, use of antibiotics was banned [4]. In such cases, use of medicinal plants with potent antimicrobial property could be an alternative source of medicine. Medicinal plants are popularly used in Indian herbal medicinal system treating pathogenic bacterium [5]. More than 50% of all clinical drugs are originated from natural product [6] and medicinal plants are widely used in drug as a source of natural product [7]. Due to indiscriminate use of antibiotics, a number of sensitive bacteria pathogenic to human and plants become resistant. It will be a big problem in future in controlling the pathogenic bacterium treating with antibiotics. Thus medicinal plants rich in antibacterial substances could be use to kill the pathogenic bacteria instead use of synthetic medicine and antibiotics [7]. Antibacterial assay of extract of medicinal plants have been investigated by a number of researchers in different parts of the world [8-15]. *Hygrophila schulli* (Buch.-Ham.) M.R.*et*. S.M.Almeida is used in traditional medicine system and commonly known as Kulekhara in Bengali, belonging to the family Acanthaceae [16]. The plant is popularly used in skin disease, dysentery, urinary affection, sleepless, stomachic, anaemia problems etc. The present investigation was carried out to evaluate the antibacterial activity of *Hygrophila schulli* against selected clinical pathogens [17].

## 2. Materials and Methods

### 2.1 Collection of plant material and Identification

The plant *Hygrophila schulli* (Buch.-Ham.) M.R.*et*.S.M.Almeida. was collected from field as well as from different markets of Arambagh subdivision of Hooghly District of West Bengal, India. Taxonomic identification of the plant was authenticated by Prof. Subrata Mondal of Visva- Bharati, West Bengal. Collected leaf parts were subjected to dry in an oven at about 60°C for 5 min after careful washing under tap water.

### 2.2 Preparation of extracts

100 g of dried plant material were grinded in a mortar and pestle for powder form and 5 g of which was used in solvent extraction with aqueous, ethanol and methanol (200 ml) separately for 24 h at 65°C. The extracts were concentrated in a rotary evaporator till dryness and kept at 40°C for further antibacterial bioassay [18].

### 2.3 Test organisms

Three gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebseilla pneumoniae* and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* were collected as pure culture from the Microbiology Laboratory of the Department of Botany, Visva-Bharati, Santiniketan, West Bengal. Collected test organisms were maintained at 4°C on nutrient agar slant.

### 2.4 Antibacterial assay

#### 2.4.1 Determination of antibacterial activity

Disc diffusion method was employed to determine the antibacterial activity with some modification [19]. 15 ml of molten Mueller Hinton Agar (MHA) were poured in to petridish in aseptic condition and allowed for solidification. Different concentration (50, 100, 200 and 400 mg/ml) of plant extracts were prepared by dissolving in aqueous, ethanol and methanol solvent. 50 µl of plant extract of each of 4 different concentrations of aqueous, ethanol and methanol were soaked individually in sterile circular filter paper disc (Whatman No. 1) with a diameter of 6 mm. The impregnated discs with test solution were allowed to dry before being placed on agar plates previously inoculated with each of test organisms. The inoculum density was adjusted to 0.5 Mc Farland turbidometry [20]. The discs soaked in sterile distilled water, ethanol and methanol served as negative control. The disc soaked in Chloramphenicol 30 mg/disc was used as positive control. Each experiment was performed in triplicate and plates were incubated at 37°C for 24 h. Diameter of inhibition zone was measured in millimeter (mm). Diameter of inhibition zone in between 8-10 mm, 10.1-16 mm and >16 mm were considered as having low, moderate and high antibacterial activity.

#### 2.4.2 Minimum Inhibitory Concentration (MIC)

Broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) of methanolic extract. For this purpose 96 well plates were filled with Mueller Hinton Broth (MHB) and various concentrations of methanol extract and comparing the various concentrations of extract which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition [21]. Antibiotic solvent of chloramphenicol was used as control. All the plates were inoculated with 0.5 µl test organism ($10^8$ cfu/ml) and incubated at 37°C for 24 h. All the experiments were performed in triplicate. Minimum bactericidal activity (MBA) was determined through plating the treated broth culture from well which showing no visible growth in MIC assay on sterile MHA plate. The

lowest concentration of the methanolic extract which inhibits the colony formation on solid agar medium after incubation at 37° for 24 h was considered as MBC (Minimum bactericidal activity) [22].

## 3. Results and Discussion

Among all the leaf extracts of *H. schulli*, methanolic and ethanolic extract showed remarkable antibacterial activity against all the selected strains of bacteria (Table 1). Methanolic extract possessed promising antibacterial activities than ethanol and aqueous extract. *In vitro* antibacterial screening of chloramphenicol indicated that methanolic extract has significantly high antibacterial activity. Methanolic extract were further evaluated for their MICs and MBCs due to potent antibacterial activity. The MIC and MBC of methanolic extracts and zone of inhibition in MBC were reflected in MIC (Table 2). The zone of inhibition more than 6.2 mm was considered as MIC value. Maximum (18 mm) zone of inhibition was obtained against *Staphylococcus aureus* and MIC of 0.156 mg/ml followed by *Bacillus subtilis* (15.6 mm) and *Escherichia coli* (13 mm) at 400 mg/ml concentration of methanolic extract of *H. schulli*. Ethanolic extract of *H. schulli* showed 12 mm zone of inhibition against *Staphylococcus aureus* and 9 mm and 7.8 mm inhibition zone against *Bacillus subtilis* and *Escherichia coli* at 400 mg/ml concentration. Aqueous extract of *H. schulli* could not inhibit the growth of *Pseudomonas aeruginosa* and *Klebseilla pneumoniae* in all the four different concentrations. No inhibition was observed in all the respective solvent of methanol, ethanol and aqueous as negative control. Thus methanolic extract of *H. schulli* has potential antibacterial activity against the investigated bacterial strains, particularly *S. aureus*, *B. subtilis*, and *E. coli* and high potential antibacterial activity was recorded against *S. aureus*. Similar pathogenic inhibition was also recorded in case of methanolic leaf extract of *H. schulli* by Chandran et al [23,24]. But in the present investigation MIC of 0.156 mg/ml against *Staphylococcus aureus* is more informative in controlling the growth of the bacterium. Moreover, the plant parts are sold in the market for different ethnomedicinal uses and leaf parts are used as vegetable in the different parts of West Bengal of India [1,16]. Traditionally water decoction of leaf extract was used in treating different pathogenic diseases [25].

Table 1: Antibacterial activity of leaf extracts of *H. schulli* against the test organisms. R= No zone of inhibition

| Plant-extract/Bacterial strain | | Concentrations (mg/ml) | Diameter of inhibition zone in mm | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Klebsiella pneumoniae* |
| *Hygrophila schulli* | Methanolic extract | 400 | 15.6 | 18 | 13 | 10 | 10.5 |
| | | 200 | 14 | 16 | 11.7 | 9 | 9 |
| | | 100 | 12.5 | 15 | 10 | 8 | 8.3 |
| | | 50 | 11 | 13 | 9 | 7.2 | 7.5 |
| | Ethanolic extract | 400 | 9 | 12 | 7.8 | 6.8 | 7 |
| | | 200 | 7.8 | 11 | 7 | 6.4 | 6.4 |
| | | 100 | 6.5 | 9.5 | 6.4 | R | R |
| | | 50 | R | 8 | R | R | R |
| | Aqueous extract | 400 | 7.4 | 10 | 7 | R | R |
| | | 200 | 6.5 | 8.5 | 6.4 | R | R |
| | | 100 | R | 7.4 | R | R | R |
| | | 50 | R | 6.3 | R | R | R |
| | Chloram phenicol (Control) | 30 mg/disc | 24.5 | 23.5 | 23 | 31 | 32 |

Table 2: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of *H. schulli* against the test organisms

| Bacterial strain/ Plant extract | Methanolic extract of *H. schulli* (mg/ml) |
|---|---|
| *Bacillus subtilis* | 0.312 |
| *Staphylococcus aureus* | 0.156 |
| *Escherichia coli* | 0.312 |
| *Pseudomonas aeruginosa* | 0.625 |
| *Klebsiella pneumonia* | 0.625 |

## 4. Conclusion

Ethnomedicinally important plants are traditionally used by the tribal community and local ethnic peoples for the treatment of different pathogenic diseases. Such medicinal plants are used in primary health problem due to their comparatively less side effects. Now a days, occurrence of bacterial disease is common due to development of antibacterial drug resistant bacteria and medicinal plant is quite useful in killing such bacteria due to rich in secondary materials. Evaluation of antibacterial potentialities of such ethnomedicinal plant species is important for better treatment of bacterial diseases. The species *Hygrophila schulli* is one of the important medicinal plants of the family Acanthaceae and leaf extract possesses potent antibacterial property. Methanolic extracts are more pronounced in their antibacterial response than that of ethanol and aqueous extract against the investigated bacteria. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are the most resistant bacterial strains followed by *Escherichia coli* and *Bacillus subtilis*. Thus methanolic extract of *Hygrophila schulli* opens the possibility of finding new clinically effective antibacterial substances and therefore, decreasing the drug resistance. Further purification, isolation and identification of such antibacterial compounds are to be needed for the view point of commercialization. Water decoction of *Hygrophila schulli* is traditionally used in household in treating infectious bacterial diseases, but in the present investigation organic solvent particularly methanolic extract is more effective.

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