Research Article

# Antimicrobial Activity Studies of *Mussaenda erythrophylla*

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**ABSTRACT**

The main aim of this research work is to study the anti-microbial activity of *Mussaenda erythrophylla*. The *Mussaenda erythrophylla* flower extracts is obtained by cold and hot extraction process. Cold extraction is carried out by using maceration and hot extraction is carried out by using soxhlet extractor. In this, polar solvents (water and methanol) non-polar solvent (n-hexane) and semi polar solvents (acetone and ethyl acetate) are used. Antimicrobial activity of *Mussaenda erythrophylla* flower extracts was studied on the gram positive (Bacillus subtilis, streptococcus aureus) and gram negative (Escherichia coli) bacteria by using cup plate method. In this, Amikacin sulphate is used as standard. The extracts of the *Mussaenda erythrophylla* flowerare compared with standard. The zone of inhibition of Amikacin sulphate and flower extracts was determined. From the results, *Mussaenda erythrophylla* shows the mild antimicrobial activity.

**Keywords:** *Mussaenda erythrophylla*, flower extracts, soxhlet extractor, cup plate method, Amikacin sulphate.

## INTRODUCTION

### Extraction process

The freshly-collected flowers were shade dried and powdered. The powdered material was then subjected to cold extraction and hot extraction.

### Antimicrobial activity

The main aim of the work was to carry out the Phytochemicals and antimicrobial activity studies of *Mussaenda erythrophylla* flowers belong to the family, Rubiaceae. The extracts of the flowers are collected by cold extraction (maceration) and hot extraction (soxhlet extraction).The antibacterial activity of thiadiazoles derivatives was studied comparatively with that of standard antibiotics amikacin (100 µg /ml) by cup plate method using gram positive organisms *Bacillus subtilis, Streptococcus aureus* and gram negative organism namely *Escherichia coli.*

### Materials and Methods

- Hexane, Ethyl acetate, Acetone, Methanol, Water.
- Soxhlet extractor (hot extraction apparatus).
- Thimbles.
- Heaters.
- Distillation apparatus.
- Aluminium foil.
- Sterilized Petri dishes, pipettes, boiling tubes and beakers.
- 8 to 10 hours old growth cultures in nutrient broth.
- Sterilized test tubes.
- Sterile 6mm cork borer.
- Sterile inoculation loop.
- Sterilized fine pointed forceps.
- Nutrient agar.
- Tuberculin syringes.

### Cold extraction

In the cold extraction process, the powdered material of the plant was initially macerated with hexane using maceration tank for 3 days. The hexane extract was concentrated by distillation, dried completely and weighed. The residue of the plant material thus obtained after acetone extraction was again macerated with ethyl acetate for 3 days. The ethyl acetate extract was then concentrated by distillation, dried completely and weighed. The residue of the plant material thus obtained after ethyl acetate extraction was again macerated acetone for 3 days. The acetone extract was then concentrated by distillation, dried completely and weighed.

The residue of the plant material thus obtained after hexane extraction was again macerated with methanol for 3 days. The methanol extract was then concentrated by distillation, dried completely and weighed. The residue of the plant material thus obtained after methanol extraction was again macerated with water for 3 days. The water extract was then concentrated by distillation, dried completely and weighed.

**Hot extraction**

Hot extraction is carried out using soxhelate apparatus. It consists of a flask to hold the menstruum, extractor to pack drug and condenser to condense vapours. The extractor has a vapour tube to carry vapours from flask to the condenser and siphon tube which siphons over the percolate from extractor to the flask.

The powdered material of the plant is placed in a thimble made from filter paper and it is placed into the extractor. The menstruum is placed in flask and boiled. The vapours arising from flask do not directly come in contact with the drug but enter into the condenser through the vapour tube. The condensate falls on to the drug as fresh menstruum and percolates. When the extractor gets filled with the percolate, the level of siphon tube also arises up to its top. The percolate in siphon tube siphons over and empties the percolates into flask. The alternate filling and emptying of the body of extractor goes on continuously until the drug is exhausted. It requires only constant boiling solvents. In this process vapours do not directly heat the drug but the condensate having temperature less than vaporisation temperature comes in contact with the drug.

By using distillation apparatus the extracts are separated from the solvents such as hexane, ethyl acetate, methanol, acetone and water.

**Preparation of Media**

The organism used in the present study for evaluating antibacterial activity of test compounds were obtained from the laboratory stock .On the day of testing, the organisms were sub cultured in to sterile nutrient broth . After incubating the same for three hours, the growth thus obtained was used inoculums for the test.

**Sterilization of Media and Glassware**

The media used in the present study, nutrient agar and nutrient broth, were sterilized in the conical flasks of suitable capacity by autoclaving at 15lbs pressure for about 20 minutes. The cork borer, petridish, test tubes and pipettes were sterilized in hot air oven at $160^0$c for one hour.

**Preparation of Solutions**

***Amikacin***

5mg of amikacin was dissolved in 100 ml of sterile water to get a concentration of 50 µg /1 ml.

***Compound***

10 mg of each test compound was dissolved in 10 ml of hexane, ethyl acetate, and acetone, and methanol, water in serial and suitably labelled sterile test tubes, thus given a final concentration of 100µg/1 ml.

**Method used to determine the Anti-microbial activity**

***Cup Plate method***

This method depends on the diffusion of an antibiotic form activity through the solidified agar layer in a petri dish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity. A previously liquefied medium was inoculated appropriated to the assay with the requisite quantity of the suspension of the microorganisms between 40-50°C and the inoculated medium was poured in to petridish to give a depth of 3-4mm.It was ensured that the layers of medium were uniform in thickness by placing the dishes on a leveled surface .The dishes thus prepared was stored in a manner so as to ensure that no significant growth or death of the test organisms occurs, before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, two cups of each 6 mm diameter was punched and scooped out of the set agar in each petridish. Using injection the standards blank and the test sample solutions (1 ml) of known concentration were fed in to the borer cups. The order of the solutions was as follows.

- Cup-1: standard (Amikacin).
- Cup-2: blank solution.
- Cup-3: test compound -1
- Cup-4:test compound-2

The dishes were left standing for one to four hours at room temperature at a period of pre incubation diffusion to minimise the effects of variation in time among the application of different solutions. These were then incubated for 24hrs for 37°C. The zone of inhibition developed, if any, was then accurately recorded. Each zone of inhibition recorded was average of four measurements.

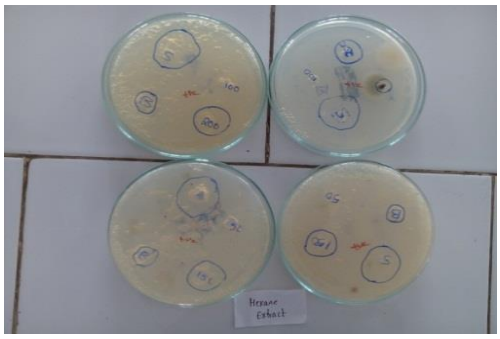
## RESULTS AND DISCUSSION

**Table 1:** Weight of the extract obtained by extraction

| Plant material | Solvent used | Volume of the solvent | Weight of the extract |
|---|---|---|---|
| Flowers 1 kg | Hexane | 3x 3L | 30.13gm |
| | Ethyl acetate | 3x 3L | 10.24gm |
| | Acetone | 3x 3L | 10.20gm |
| | Methanol | 3x 3L | 10.52gm |
| | Water | 3x 3L | 20.84gm |

**Table 2:** Inhibition Zones produced by the test sample on both gram positive and gram negative organisms

| S. No | Name of the Bacteria | Test sample (5mg/ml) | Inhibition zone (mm) | Standard (1mg/ml) | Inhibition zone (mm) |
|---|---|---|---|---|---|
| 1 | *Streptococcus aureus* | Hexane | 12 | Amikacin Sulphate | 25 |
| 2 | *Streptolococcus aureus* | Ethyl acetate | 7 | Amikacin Sulphate | 24 |
| 3 | *Streptococcus aureus* | Acetone | 11 | Amikacin Sulphate | 25 |
| 4 | *Streptococcus aureus* | Methanol | 12 | Amikacin Sulphate | 26 |
| 5 | *Streptococcus aureus* | Water | 4 | Amikacin Sulphate | 26 |
| 6 | *Bacillus subtilis* | Hexane | 14 | Amikacin Sulphate | 28 |
| 7 | *Bacillus subtilis* | Ethyl acetate | 8 | Amikacin Sulphate | 27 |
| 8 | *Bacillus subtilis* | Acetone | 7 | Amikacin Sulphate | 28 |
| 9 | *Bacillus subtilis* | Methanol | 11 | Amikacin Sulphate | 29 |
| 10 | *Bacillus subtilis* | Water | 4 | Amikacin Sulphate | 30 |
| 11 | *Escherichia coli* | Hexane | 15 | Amikacin Sulphate | 31 |
| 12 | *Escherichia coli* | Ethyl acetate | 10 | Amikacin Sulphate | 30 |
| 13 | *Escherichia coli* | Acetone | 9 | Amikacin Sulphate | 31 |
| 14 | *Escherichia coli* | Methanol | 12 | Amikacin Sulphate | 33 |
| 15 | *Escherichia coli* | Water | 3 | Amikacin Sulphate | 31 |



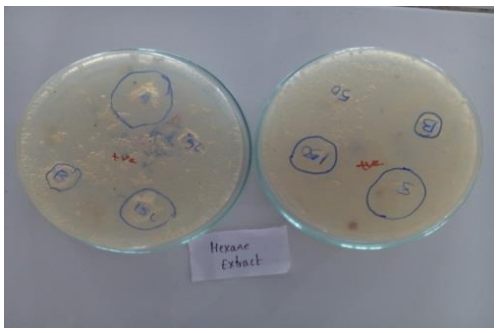




**Figure 1:** Inhibition Zone produced by Hexane extract

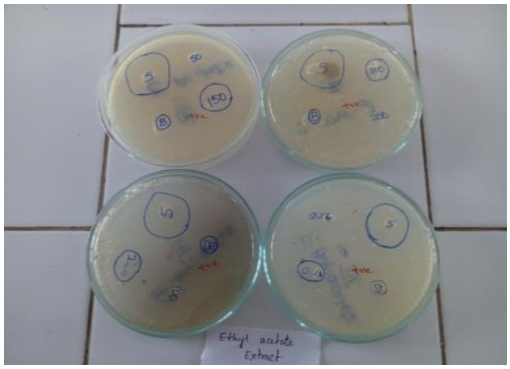
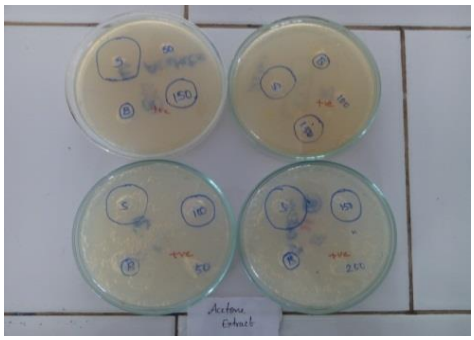




**Figure 2:** Inhibition Zone produced by Ethyl acetate extract



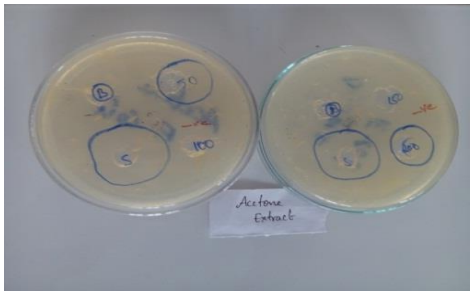




**Figure 3:** Inhibition Zone produced by Acetone extract

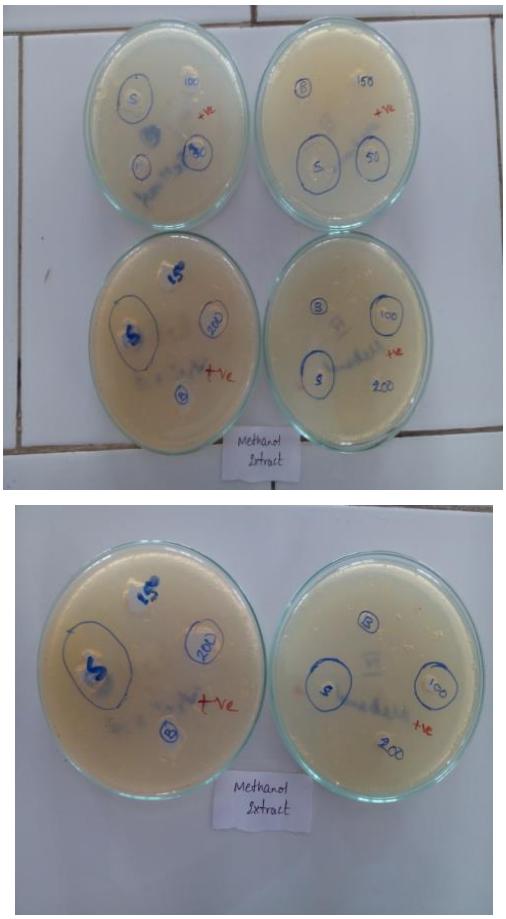



**Figure 4:** Inhibition zone produced by Methanol extract

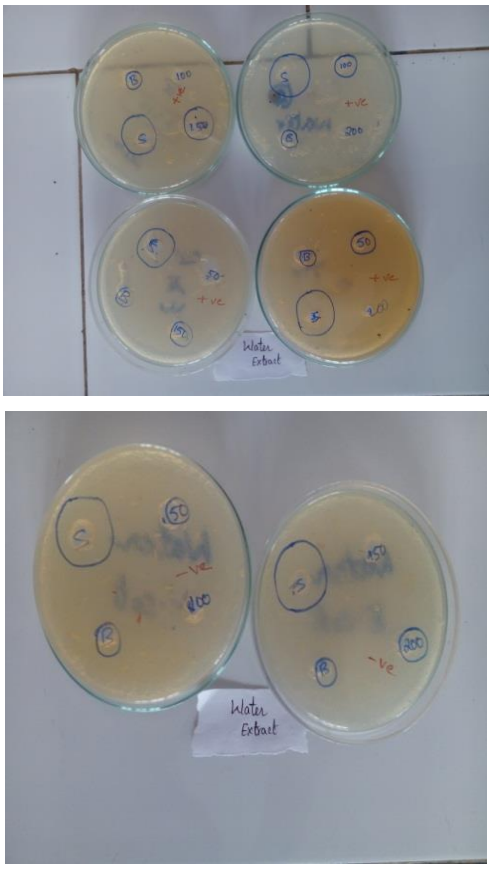
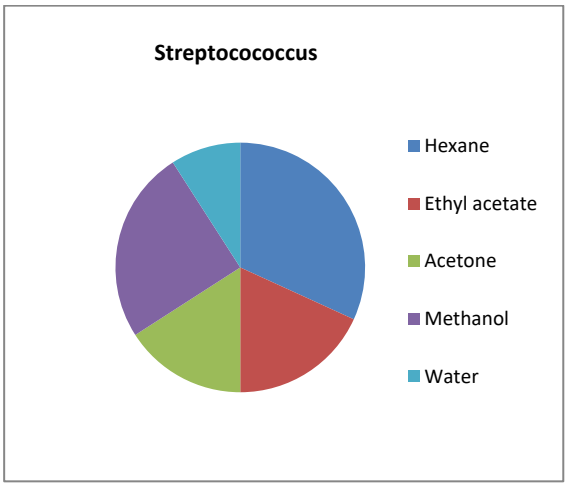
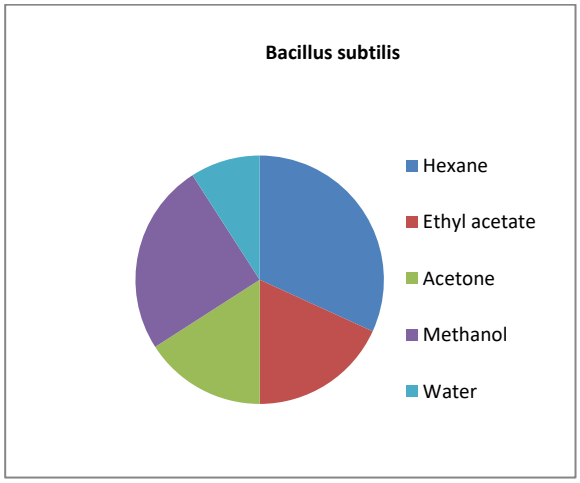
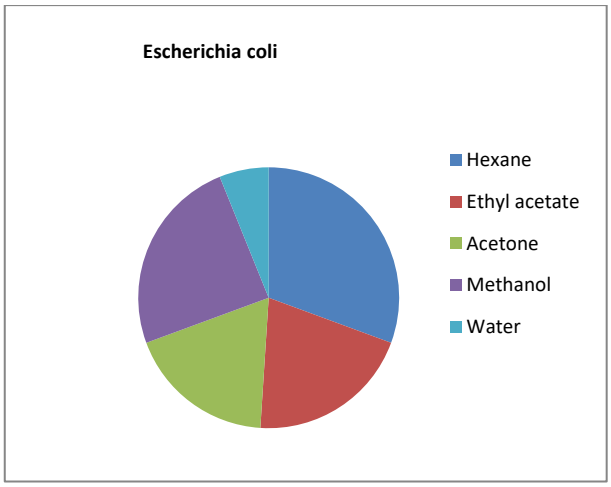



**Figure 5:** Inhibition zone produced by Water extract




Inhibition zone obtained for Gram positive organisms




Inhibition zone obtained for Gram negative organism

**Antimicrobial activity**

The Antimicrobial activity studies of hexane, ethyl acetate, acetone, methanol and water extracts of flowers of the plant *Mussaenda erythrophylla* (Rubiaceae) were performed on gram positive organisms *Streptococcous, Bacillus* and gram negative organisms namely *Escherichia coli* by using cup plate method. The results thus obtained by this method were indicating that the extracts have shown the mild activity on both gram positive and gram negative organisms as shown above.

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