

## Evaluation of the Antibacterial Activity of *Aegle marmelos* and *Cassia siamea* Extracts Against Biofilm and Extended Spectrum $\beta$ -Lactamase Producing Uropathogenic *Escherichia coli*

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**Abstract:** The aim of this study was to evaluate the antibacterial efficacy of *Aegle marmelos* and *Cassia siamea* extracts against biofilm and extended spectrum  $\beta$ -lactamase (ESBL) producing uropathogenic *Escherichia coli* (UPEC). *A. marmelos* and *C. siamea* are widespread medicinal plants traditionally used to treat infectious diseases. Acetone, chloroform, petroleum ether and aqueous extracts of leaves and flowers of *A. marmelos* and *C. siamea*, respectively were tested for antimicrobial activity *in vitro* by the minimum inhibitory concentration (MIC) method. In MIC studies of *A. marmelos* at 50 and 100  $\mu\text{g ml}^{-1}$  of all the four extracts recorded no inhibition of growth in all the 20 UPEC strains. At 150  $\mu\text{g ml}^{-1}$  acetone extract inhibited 40% UPEC strains followed by petroleum ether (35%), chloroform (30%) and aqueous (25%) extract. At 200  $\mu\text{g ml}^{-1}$  all the four extracts inhibited the growth of all the UPEC strains and was considered as the MIC to our UPEC strains. In MIC studies of *C. siamea*, at 50 and 100  $\mu\text{g ml}^{-1}$  of all the four extracts recorded no inhibition of growth in all the 20 UPEC strains. In 150  $\mu\text{g ml}^{-1}$  chloroform extract inhibited 50% UPEC strains followed by acetone (30%), petroleum ether (25%) and aqueous (10%) extract. At 200  $\mu\text{g ml}^{-1}$  all the four extracts inhibited the growth of all the UPEC strains and was considered as the MIC to our UPEC strains. The present study shows that crude extracts of *A. marmelos* and *C. siamea* especially the acetone and chloroform extracts exhibited significant activity against biofilm and ESBL producing Uropathogenic *E. coli* strains.

**Key words:** Biofilm • ESBL • *Escherichia coli* • *Aegle marmelos* And *Cassia siamea* • Minimum Inhibitory Concentration (MIC)

### INTRODUCTION

The medicinal value of plants lies in some chemical substances present in them. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds. The expanding bacterial resistance to antibiotics has become a growing concern worldwide. Intensive care physicians consider bacterial antibiotic resistance a significant problem in the treatment of patients. Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains. A vast number of medicinal

plants have been recognized as valuable resources of natural antimicrobial compounds. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [1].

In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms has developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects. *Aegle marmelos* (L.) belongs to the family *Rutaceae* and is popularly known as vilvam tree

in Tamil language. Traditional Indian medicinemen regard the unripe or half ripe fruit as astringent, digestive, cure to urinary problems and stomachic medicine and prescribe it for diarrhoea and dysentery. The fresh juice of the leaves is taken with honey as UTIs, laxative and febrifuge; it is used in asthmatic complaints [2].

*Cassia siamea* belongs to the family *Leguminosae: caesalpinoideae*. The leaf, stem bark and root of the plant are used for medicinal purposes. A combination of the root, leaf and flower extracts is taken for indigestion and as expectorant. The root of the plant is used for treating conjunctivitis. The leaf is used for heartburn and as antipyretic. Leaf and flower extracts are combined to heal blotches on skin due to menstrual disorder. Microorganisms such as *E. coli*, *S. typhi* and *S. aureus* are reported to cause diseases like diarrhoea, typhoid etc, therefore, there is a need to study plants which may have effect on these organisms and this will improve the effective use of plants against diseases caused by the pathogens. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of herbal extracts [3].

Urinary tract infections (UTIs) are among the most common bacterial infectious diseases encountered in clinical practice and account for significant morbidity and high medical costs. *Escherichia coli* is the most predominant pathogen causing 80-90% of community-acquired UTIs and 30-50% of nosocomially-acquired UTIs. Recurrent UTIs (RUTIs) are reported in 25% of women within 6 months of an acute UTI episode and pose a major problem [4].

Uropathogenic *E. coli* form intracellular bacterial communities with biofilm-like properties within the bladder epithelium [5]. According to National Institutes of Health, "more than 60 % of all microbial infections are caused by biofilm". A biofilm is a population of cells growing on a surface and enclosed within an exopolymer matrix that can restrict the diffusion of substances and bind antimicrobials [6]. It is well documented that biofilms are notoriously difficult to eradicate and are often resistant to systemic antibiotic therapy [7]. The mechanism of resistance of biofilm bacteria is not conclusively established, but it has been suggested that the resistance may be related to  $\beta$ -lactamase production by the biofilm bacteria [8]. Since no previous attempts have been made to examine the antimicrobial effects of *A. marmelos* and *C. siamea* against uropathogenic *E. coli* strains, we focus on these plants. The aim of this paper was to substantiate the antimicrobial sensitivity of different extracts of

*Aegle marmelos* (Leaves) and *Cassia siamea* (Flowers) against uropathogenic Biofilm and ESBL producing *E. coli* isolates to lengthen the queue of antimicrobial herbs.

## MATERIALS AND METHODS

**Collection of Plant Materials:** Leaves of *Aegle marmelos* and flowers of *Cassia siamea* were collected from villages in and around Chidambaram, Tamilnadu, India. Plant leaves and flowers were shade-dried. The dried leaves and flowers were fine-powdered and stored in polythene bags at room temperature ( $30\pm 2^\circ\text{C}$ ) until use.

**Extract Preparations:** To obtain the solvent extracts, the dried and finely powdered leaves of *A. marmelos* was weighed in 10 gram units and homogenized using 100 ml of 70% acetone. They were added to Soxhlet apparatus and the boiling point of acetone was set at  $56.6^\circ\text{C}$ . The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted. The extract was then transferred to a sterile petri dish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in a refrigerator. The same procedure was followed to obtain the solvent extracts from dried and finely powdered flowers of *C. siamea*.

An identical procedure was followed to obtain the following: Petroleum ether ( $60^\circ\text{C}$ ), chloroform ( $61.2^\circ\text{C}$ ) and aqueous ( $100^\circ\text{C}$ ) extracts.

**Determination of Minimum Inhibitory Concentration (MIC):** Minimum Inhibitory Concentration (MIC) was determined using "Tube technique" as described in Vollekova *et al.* [9] and Usman *et al.* [10].

## RESULTS AND DISCUSSION

**Determination of Minimum Inhibitory Concentration (MIC) of *Aegle marmelos* (Leaves) and *Cassia siamea* (Flowers):** In traditional medicine, aqueous decoctions are used to treat patients, so we first prepared the extract from an aqueous decoction. Because of the presence of tannins, an extract was prepared with acetone, petroleum ether, chloroform 70% and aqueous 100 ml, a better solvent for tannin. These extracts were tested on uropathogenic *E. coli*. The MICs of acetone, petroleum ether, chloroform and aqueous extracts of leaves of *A. marmelos* and flowers of *C. siamea* are presented in Table 1 and 2.

Table 1: Minimum inhibitory concentration of (MIC) *Aegle marmelos* against biofilm and ESBL producing uropathogenic *E. coli*

| Leaves of <i>Aegle marmelos</i> |           |         |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |                        |           |
|---------------------------------|-----------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|------------------------|-----------|
| Extract Strains                 | Levels    | UPEC 85 | UPEC 105 | UPEC 107 | UPEC 108 | UPEC 112 | UPEC 116 | UPEC 119 | UPEC 125 | UPEC 128 | UPEC 140 | UPEC 152 | UPEC 155 | UPEC 159 | UPEC 162 | UPEC 167 | UPEC 175 | UPEC 178 | UPEC 186 | UPEC 189 | UPEC 190 | No of positive Strains |           |
| Acetone                         | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | 20 (100%)              |           |
|                                 | 100 µg/ml | +       | +        | +        | +        | -        | +        | -        | +        | -        | +        | -        | +        | -        | +        | -        | +        | -        | +        | -        | +        | -                      | 10 (50%)  |
|                                 | 150 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | +        | +        | -        | +        | +        | -        | +        | +        | -        | +        | +        | -        | +                      | 8 (40%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |
| Petroleum ether                 | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 100 µg/ml | +       | +        | -        | +        | -        | -        | +        | -        | -        | +        | +        | -        | -        | +        | +        | +        | +        | +        | +        | +        | +                      | 12 (60%)  |
|                                 | 150 µg/ml | -       | +        | -        | +        | -        | -        | +        | -        | -        | +        | -        | -        | -        | +        | +        | -        | +        | -        | +        | -        | +                      | 7 (35%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |
| Chloroform                      | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 100 µg/ml | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 18 (90%)  |
|                                 | 150 µg/ml | -       | -        | +        | +        | -        | +        | -        | -        | -        | +        | -        | +        | -        | +        | -        | +        | -        | +        | -        | -        | -                      | 6 (30%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |
| Aqueous                         | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 100 µg/ml | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 150 µg/ml | -       | +        | -        | -        | +        | -        | -        | -        | -        | -        | -        | +        | -        | +        | -        | -        | -        | -        | +        | -        | -                      | 5 (25%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |

UPEC: Uropathogenic *Escherichia coli* +: Indicates growth -: Indicates no growth

Table 2: Minimum Inhibitory concentration of (MIC) *Cassia siamea* against biofilm and ESBL producing Uropathogenic *E.coli*

| Flowers of <i>Cassia siamea</i> |           |         |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |                        |           |
|---------------------------------|-----------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|------------------------|-----------|
| Extract Strains                 | Levels    | UPEC 85 | UPEC 105 | UPEC 107 | UPEC 108 | UPEC 112 | UPEC 116 | UPEC 119 | UPEC 125 | UPEC 128 | UPEC 140 | UPEC 152 | UPEC 155 | UPEC 159 | UPEC 162 | UPEC 167 | UPEC 175 | UPEC 178 | UPEC 186 | UPEC 189 | UPEC 190 | No of positive Strains |           |
| Acetone                         | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | 20 (100%)              |           |
|                                 | 100 µg/ml | +       | +        | -        | +        | +        | -        | +        | +        | -        | +        | -        | -        | +        | -        | +        | +        | +        | +        | +        | +        | +                      | 14 (70%)  |
|                                 | 150 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | +        | -        | -        | +        | -        | +        | +        | -        | +        | +        | -        | +                      | 6 (30%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |
| Petroleum ether                 | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 100 µg/ml | -       | -        | +        | -        | +        | +        | +        | +        | -        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 16 (80%)  |
|                                 | 150 µg/ml | -       | -        | -        | +        | -        | -        | +        | -        | +        | -        | -        | -        | -        | -        | +        | -        | -        | -        | -        | -        | +                      | 5 (25%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |
| Chloroform                      | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 100 µg/ml | +       | -        | +        | -        | +        | +        | -        | +        | +        | +        | +        | -        | -        | -        | +        | +        | +        | -        | -        | -        | -                      | 11 (55%)  |
|                                 | 150 µg/ml | +       | -        | +        | -        | +        | -        | -        | +        | +        | +        | +        | -        | -        | -        | +        | +        | +        | -        | -        | -        | -                      | 10 (50%)  |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |
| Aqueous                         | 50 µg/ml  | +       | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +        | +                      | 20 (100%) |
|                                 | 100 µg/ml | +       | +        | +        | +        | +        | +        | +        | -        | +        | +        | +        | +        | +        | -        | +        | +        | +        | +        | +        | +        | +                      | 18 (90%)  |
|                                 | 150 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | +        | -        | -        | -        | -        | -        | -        | -        | -                      | 2 (10%)   |
|                                 | 200 µg/ml | -       | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -                      | 0 (0%)    |

UPEC: Uropathogenic *Escherichia coli* +: Indicates growth -: Indicates no growth

**Minimum Inhibitory Concentration (MIC) of *A. marmelos* Against Biofilm and ESBL Producing Uropathogenic *E. coli*:** *A. marmelos* extract at 50 and 100 µg ml<sup>-1</sup> of all the four extracts recorded no inhibition of growth in all the 20 UPEC strains. The 150 µg ml<sup>-1</sup> acetone extract inhibited 40% UPEC strains followed by petroleum ether (35%), chloroform (30%) and aqueous (25%) extracts. At 200 µg ml<sup>-1</sup> all the four extracts inhibited the growth of UPEC strains and were considered as MIC of the extract to the UPEC strains.

**Minimum Inhibitory Concentration (MIC) of *C. siamea* Against Biofilm and ESBL Producing Uropathogenic *E. coli*:** *C. siamea* extract at 50 µg and 100 µg ml<sup>-1</sup> of all the four extracts recorded no inhibition of growth in all the 20 UPEC strains. The 150 µg ml<sup>-1</sup> chloroform extract inhibited 50% UPEC strains followed by acetone (30%),

petroleum ether (25%) and aqueous (10%) extracts. At 200 µg ml<sup>-1</sup> all the four extracts inhibited the growth of UPEC strains and were considered as MIC of the extract to the UPEC strains.

The organic solvents are better suited for consistent extraction of antimicrobial substances in medicinal plants [11]. The antibacterial activity of the methanol, chloroform and aqueous extracts from the leaves, bark and fruit of *A. marmelos* was studied using disc diffusion method against *Bacillus subtilis* and *Staphylococcus aureus* (Gram Positive), *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella paratyphi* A and *S. paratyphi* B (Gram Negative). The results suggest that the methanolic extract has significant antibacterial activity against tested bacteria [12]. In the present study, leaves of *A. marmelos* were used in 4 different solvents against 20 biofilm and ESBL producing UPEC strains.

Abhishek and Harpreet [13] have reported that, MIC was 1.429 mg/ml in acetone extract of *Ajuga bracteosa* and inhibitory to *E.coli*. In the present study, acetone extracts of *Aegle marmelos* recorded MIC value of 150 µg/ml against UPEC strains. Myuri *et al.* [14] have reported that, MIC value of 250µg/ml in petroleum ether extract of *A. marmelos* against *E.coli* (MTCC-40). Similarly, in the present study MIC value of petroleum ether extract of *A. marmelos* was found to be 250 µg/ml to all UPEC strains.

In the present study, the MIC value was 200µg/ml in chloroform extracts of *Aegle marmelos* against UPEC strains. Saroj *et al.* [15] have reported that the MIC value of chloroform extract of *A. marmelos* was 500 µg/ml and inhibitory to the tested *E.coli*. In the present study, the MIC value of aqueous extract of *A. marmelos* was 200 µg/ml against UPEC strains. Similarly, Sujatha and Rajan [16] have reported that the MIC value of aqueous extract of *A. marmelos* was 216µg/ml against the tested *E. coli*.

In the present study MIC value 150 µg/ml of acetone extract of *C. siamea* was inhibitory to UPEC strains. Sujogya *et al.* [17] have obtained a MIC of 1.250 µg/ml petroleum ether extract of *Cassia fistula* and was inhibitory to the tested *E.coli*. In the present study MIC value 100 µg/ml of petroleum ether extract of *C. siamea* was inhibitory to UPEC strains. Ram *et al.* [18] have reported that, MIC value of chloroform extracts of *C. siamea* >400 µg/ml was inhibitory to *E. coli*. In the present study, MIC value of chloroform extracts of *C. siamea* 200 µg/ml was inhibitory against all UPEC strains. Sujogya *et al.* [17] have studied that, MIC of 1.250 µg/ml aqueous extract of *Cassia fistula* was inhibitory of tested *E. coli*. In the present study, MIC value 150 µg/ml of aqueous extract of *C. siamea* inhibited all UPEC strains.

In conclusion, the results of this study showed that the *A. marmelos* (leaves) and *C. siamea* (Flowers) have exhibited varied antimicrobial activities against the biofilm and ESBL producing uropathogenic *E. coli*. These findings on antibacterial activity support the claim of the traditional healers that *Aegle marmelos* (Leaves) and *Cassia siamea* (Flowers) would be used against uropathogens.

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