

Antibacterial Activities of Medicinal Plant Leaves Extract Against Isolated *Escherichia coli* Bacteria from Different Water Sources

Santhanam Selvaraj¹, Chellasamy Prashanth², Gurusamy Ponraj³, Kamatchi Rameshkumar^{4,*}

Abstract

Objective: *Escherichia coli* are the most common pathogenic bacteria to accountable for food and waterborne illness. Therefore, in this way the study was conducted to resolve the occurrence of *E. coli* bacteria from different water sources in around Tamil Nadu along with to determine their antibiotic resistance profiles. **Methods:** Water samples were analysed for the presence of *E. coli* bacteria using specific agar media Eosin Methylene Blue and McConkeys. Colony morphology, Gram staining, various biochemical tests and antibacterial tests performed by disc diffusion method were used to confirm *E. coli* bacteria. **Results:** The isolated bacteria were susceptible to Gentamycin (Mullai & Dindigul 18mm) and Tetracycline (Mullai 16mm & Vaigai 18mm) at 37°C for 24 hours. The isolate was susceptible to acetone leaves extract of *Abutilon indicum*, *Calotropis gigantean* and *Solanum trilobatum* in various concentrations (50µl, 75µl, & 100µl) and of *A. indicum* was most effective inhibition of zone formed in Mullai 24mm, Vaigai 18mm and Dindigul 23mm, triplicate value respectively. **Conclusions:** In this study revealed that *E. coli* bacteria were resistant to some antibiotics drugs. Compared to chemical antibiotic, the acetone leaves extract of *A. indicum* showed excellent inhibition effect (Mullai (24mm), and Dindigul drinking water (23mm) in 100µl concentrations only. But *S. trilobatum* was showed equal inhibition effect against Vaigai river water (18mm). Therefore, *E. coli* bacterium was controlled by natural antibiotics of *A. indicum* and it may reduce the resistance power of food and waterborne illness.

Keywords: *Escherichia coli*, Zone of inhibition, *Abutilon indicum*, Gram staining, Water borne illness.

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INTRODUCTION

Pathogenic bacteria cause may be illness and death. Although different pharmaceutical companies have introduced a number of new anti-bacterial in the last years, but resistance to these agents has also increased and has now become a worldwide problem [1]. World Health Organization has recognized herbal medicine as an important building block for primary health care of vast countries like India [2]. The leaves of the plant have been used as an herbal remedy for their medicinal properties since ancient times. Some bioactive compounds (alkaloids, tannin, flavonoid and phenolic etc.) of these plants are responsible for their medicinal value [3]. Drugs are found in every plant part such as leaves, stem, bark, roots, flowers, seeds, etc. Medicinal plants are important sources of therapeutic help in reducing human illness [4]. The

leaves and bark of the guava plant are well documented in the treatment of diarrhea, stomach upset, toothache, fever, and inflammation [5].

In addition, neem leaves are able to preventing hepatitis and controlling diabetes and marigold leaf is known to be highly effective in healing of burns and bruises [6]. Herbal extracts were used to treat various ailments based on the Indian medical systems [7]. Leaves and rhizomes of *S. hyacinthoides* has source of natural antioxidant and antimicrobial agent against both Gram-positive and Gram-negative bacteria suggested by Aliero *et al* [8]. Methanol, ethanol and acetone from different plant parts (leaves, roots, bark and stem rhizome), of *Indigo feradaleoides*, *Punica granatum*, *Syzygium cordatum*, *Gymnosporia senegalensis*, *Ozoroa insignis*, etc., showed notable antibacterial activity against *Vibrio cholera*, *Escherichia coli* and *Staphylococcus aureus*, *Shigella* species and *Salmonella typhi* [9]. Population rises, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs [10] and antibiotic resistance has become a common problem in developing countries, both in hospitals and the community, causing high mortality in each year [11]. Therefore, in the present work the antibacterial activity of medicinal plants against isolated bacteria and compare with chemical antibiotics using well diffusions assay to evaluate the Minimum Inhibitory Concentration (MIC) of the leaves extract against chosen bacteria.

MATERIALS AND METHODS

Collection of water samples

Water samples collected from three different places such as Mullai River water from Theni, Vaigai river water from Madurai and Drinking water from Dindigul.

Isolation and identification of Bacteria

The collected samples (100ml) were immediately passed through the Whatmann no.1 filter paper and remove the large particles. Filtrates were transferred to the specific agar medium like Eosin Methylene Blue (EMB) for the isolation of bacteria. A serial dilution technique was used to get Colony Forming Units (CFUs) from water samples. Individual colonies were separated using a streak plate method and the process was repeated three times for purification. Bacteria are stored in nutrient agar at 37°C for 24 hours. Pure colonies are stored at -20 °C in agar slants and used for further experiment [12]. After 24 hours of incubation, isolated bacteria were identified based colony morphology, Gram staining, motility and biochemical characters.

Antibiotic sensitivity assay for isolated bacteria

The isolated bacterial strains were assayed following Seidler *et al.* [13] procedure to find the most effective inhibitory antibiotic among the selected common antibiotics such as Chloramphenicol (CHL), Ampicillin (AMP), Tetracycline (TET), Gentamycin (GEN), Kanamycin (KAN), Co-Trimoxazole (CTZ), Amikacin (AMI) and Streptomycin (STR) using standard antibiotic discs (HiMedia Laboratories Pvt. Ltd. India).

Collection and processing of medicinal plants leaves

Fresh medicinal plant leaves of *Abutilon indicum*, *Calotropis procera* and *Solanum trilobatum* were collected nearby Vivekananda College, Madurai district, Tamil Nadu. These plant samples were washed with tap water in order to remove the dust. Then, collected plants were dried under the shade for a week. Dried leaves were ground into a powder and shared airtight container [14].

Preparation of leaves extract

Fine powder of each leaf was used to prepare solvent extractions with polar solvent of acetone. In this method, Soxhlet extraction apparatus was used to extract the 30g powdered material of each leaf through 300ml of acetone solvent. The collected extract was kept in separate petri plate for evaporation. The extract was stored in an airtight container and stored in refrigerator. The dried extracts were mixed with both solvent and used for further experiment [15].

Antibacterial susceptibility test

Antibacterial activity of plant leaves extract of acetone solvent was carried out by disc diffusion method [16]. Pre prepared agar plates were inoculated with isolated bacterial cultures (12 hours). 10 mm wide wells were punched with cork borer in the agar plates. Three different concentrations (50 μ l, 75 μ l & 100 μ l) of extracts were added in the respective well. The bacteria inoculated plates were incubated 37°C for 24 hours in incubator. The included plates are tested for growth disturbances over the inoculums. The diameter of the zones formed around the well were measured in mm and recorded.

RESULTS AND DISCUSSION

Isolation of bacteria

The pure culture of three water sources bacteria (Brown colonies appeared) were obtained by streaking methods using specific agar medium like Eosin Methylene Blue (EMB). A total of 296 *E. coli* bacteria isolated from pooled samples with specific agar medium like Eosin Methylene Blue and MacConkeys [17]. The growth curve (38h) was measured to all isolates with nutrient broth medium. Though, the Colony Forming Unit (CFU) is calculated to Mullai (1.6X10⁷CFU/ml), Vaigai (1.5X10⁶CFU/ml) and Dindigul drinking water (1.6X10⁵CFU/ml) and it's used for enumerate the zone of inhibition. Sixty-four samples were dispersed on MacConkey agar plates and two to three colonies per plate were selected from each plate and tested using biochemical experiments to confirm the presence of *E. coli* [6].

Identification of bacteria

All the isolated bacteria were documented their characters such as rod shaped, motile and Gram negative in nature [18]. This report was useful for identification of *E. coli* bacteria in selected water sources. Besides, the results of biochemical identification such as Citrate utilization (-), Lysine utilization (-), Ornithine utilization (-), Urease (-), Phenylalanine Deamination (+), Nitrate reduction (-), H₂S production (-), Glucose (-), Adonitol (+), Lactose (+), Arabinose (+, +, -) and Sorbitol (+). Based on biochemical analysis the test pathogens were confirmed Gram negative bacteria.

Accordingly, these isolated bacterial characters may consider as *E.coli* bacteria. Odonkor *et al.*, suggested, the water samples were transferred to specific media such as MacConkey, Eosin Methylene Blue for the isolation of *E.coli* bacteria and identified based on the Colony characters, staining and biochemical test [17].

Antibiotic sensitivity assay with chemical antibiotics

Antibiotic sensitivity assay results for the isolated bacterial strains are shown in Figure 1. The zone size interpreted as per "The Clinical & Laboratory Standards Institute" (CLSI) and "The European Committee on Antimicrobial Susceptibility Testing" (EUCAST). The triplicate value of isolated three strains have showed high degree of antibiotic resistance against Streptomycin, Co-trimoxazole, Azithromycin, kanamycin, Chloramphenicol and Amikacin. Although intermediate resistance showed against Tetracycline (Dindigul 14mm) and Gentamycin (Vaigai 12mm), while the susceptible to Gentamycin (Mullai & Dindigul 18mm) and Tetracycline (Mullai 16mm & Vaigai 18mm). A total of 56 water samples of *E.coli* isolated were tested from nine different antibiotics. Among them, 37.90% were resistant, 12.90% were intermediate and 49.20% were susceptible [18].

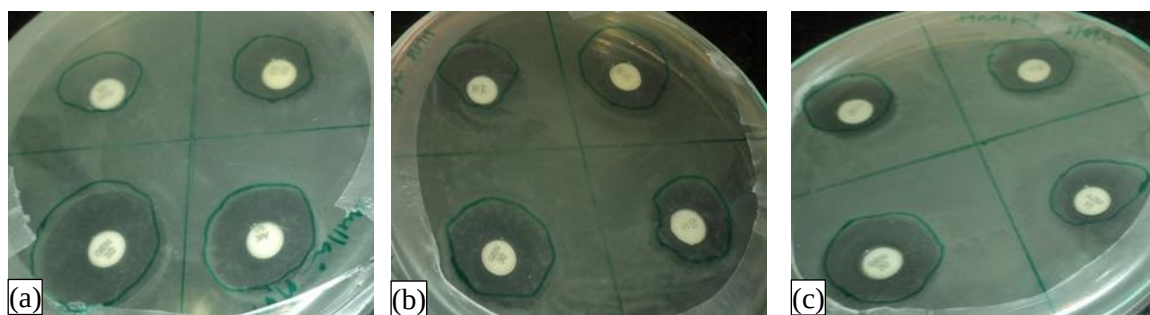


Figure 1. Antibacterial activity of isolated bacteria (a) Mullai, (b) Vaigai, (c) Dindigul drinking water.

Therefore, the most effective lytic activity was exposed against isolated bacteria such as Mullai (Gentamicin 18 mm), Vaigai (Tetracycline 18 mm) and Dindigul drinking water (Gentamicin 18 mm) (Figure 2). Whereas the isolation of coliform especially *E. coli* from water sources is caused by contamination of human and animal origin and this is important for health as these insects are often the agent of gastroenteritis in humans [19].

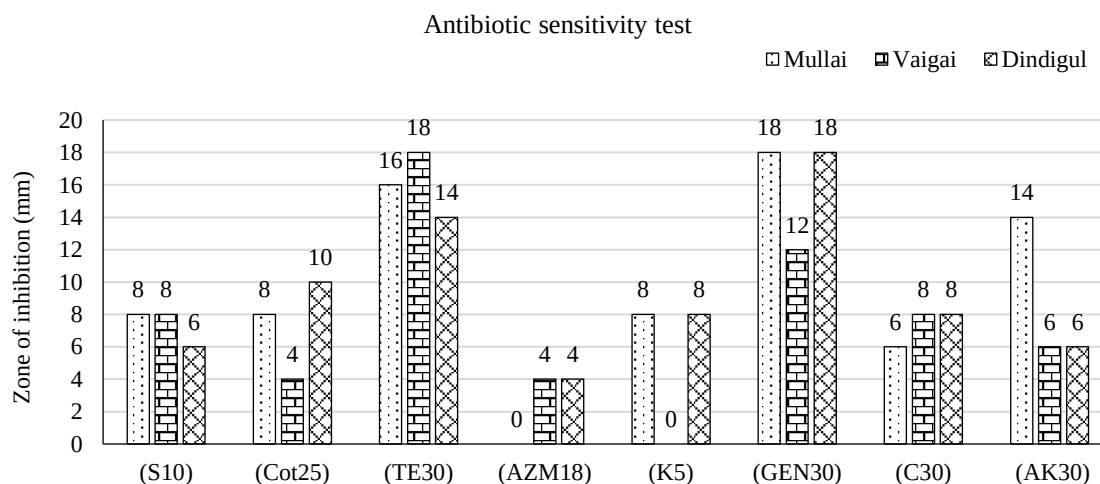


Figure 2. Comparison of the antibiotic sensitivity test with isolates.

Antibacterial susceptibility assay with leaf extracts

The leaf extracts were obtained using polar solvent such as acetone was assayed for their antibacterial activity using well diffusion method. The triplicate value of zone on inhibition around the well represented the antibacterial activity of the above-mentioned solvent extracts. The acetone leaves extract with isolated bacteria shown in Figure 3.

Antibacterial activity of *Abutilon indicum*

Abutilon indicum extracts of fruits, root and leaf recorded antimicrobial resistance against *Escherichia coli* [20]. The acetone leaf extract (100 μ l) of *A. indicum* showed exceptional activity against Mullai (24 mm), Dindigul drinking water (23 mm), Vaigai (18 mm). Then, the acetone leaf extract (75 μ l) of *A. indicum* showed excellent activity against Dindigul drinking water (16 mm), Mullai (15 mm) and Vaigai (15 mm). Subsequently, the acetone leaf extract (50 μ l) of *A. indicum* showed good activity against Dindigul drinking water (12 mm), Vaigai (10 mm) and Mullai (8 mm). Beta-sitosterol were isolated from petroleum ether leaf extract of *Abutilon indicum* revealed mosquito larvicidal activity [21]. Methanolic extract and aqueous extract have important anti-diarrheal activity in Castor oil [22]. Ethanolic extract of *Abutilon indicum* at a dose of 400mg / kg exhibit the wound healing activity [23]. The different solvent extract of *Abutilon indicum* investigated its anti-arthritis activity in *in vitro* studies in albino mice [24].

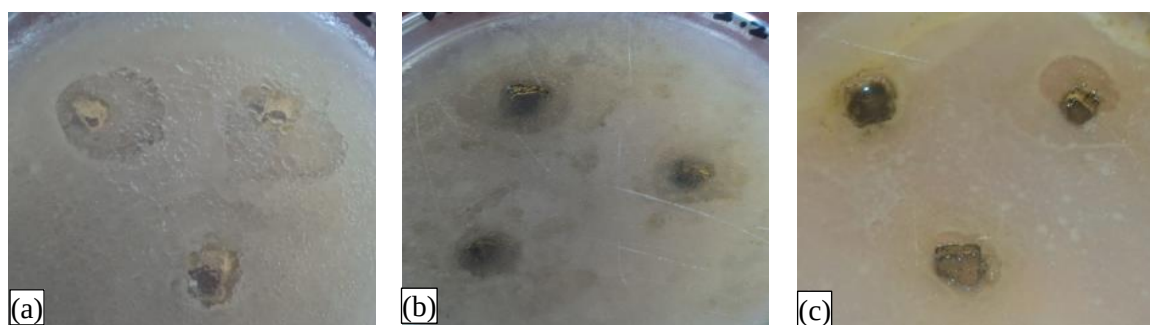


Figure 3. Antibacterial susceptibility assay with acetone leaf extracts against isolated bacteria (a) Mullai, (b) Dindigul, (c) Vaigai.

Antibacterial activity of *Calotropis procera*

Recently, acetone leaf extracts from nine plants found only in the Myrtaceae family with excellent activity against pathogenic *E. coli* bacteria [25]. The acetone leaf extract (100µl) of *C. procera* recorded good activity against Vaigai, Mullai (15 mm) and no activity against Dindigul drinking water. The acetone leaf extract (75µl) of *C. procera* demonstrated moderate activity against Mullai (8mm), Vaigai (6mm) and no activity against Dindigul drinking water. The acetone leaf extract (50µl) of *C. procera* observed moderate activity against Mullai, Vaigai (4mm) and no activity against Dindigul drinking water. Similarly, the petroleum ether and ethyl acetate *A. lebbbeck* bark extract showed moderate zone of inhibition against both Gram positive and Gram-negative bacteria. Both extracts of *A. lebbbeck* showed maximum and moderate activity against *S. typhi* (13 and 14mm, respectively), *S. dysenteriae* (14 and 13mm) and *S. aureus* respectively [26].

Antibacterial activity of *Solanum trilobatum*

The acetone and aqueous crude extracts of *S. birrea* for the concentrations (50 & 100mg / mL) were tested against *S. pyogenes* and *P. shigelloides*, were the most suitable (21–38 mm) organisms to both acetone and aqueous, while *Salmonella* was the least susceptible with partial zone of inhibition [27]. The acetone leaf extract (100µl) of *S. trilobatum* exhibited good activity against Dindigul drinking water (16mm), moderate activity of against Mullai (10mm) and no activity against Vaigai river water. The acetone leaf extract (75µl) of *S. trilobatum* observed moderate activity against Dindigul drinking water (10mm), Mullai (6mm) and no activity against Vaigai river water. The acetone leaf extract (50µl) of *S. trilobatum* showed moderate activity against Dindigul drinking water (8mm), Mullai (2mm) and no activity against Vaigai river water. Besides, Methanol Quotes *S. trilobatum* leaf were investigated for anti-inflammatory activity by active and chronic models. At 100, 200 and 300 mg / kg doses showed significant ($P < 0.05$) anti-inflammatory activity in all tested models [28]. The antibacterial activity of selected medicinal leaf extracts (50, 75 & 100µl concentration) with three isolates comparative results showed in Figure 4.

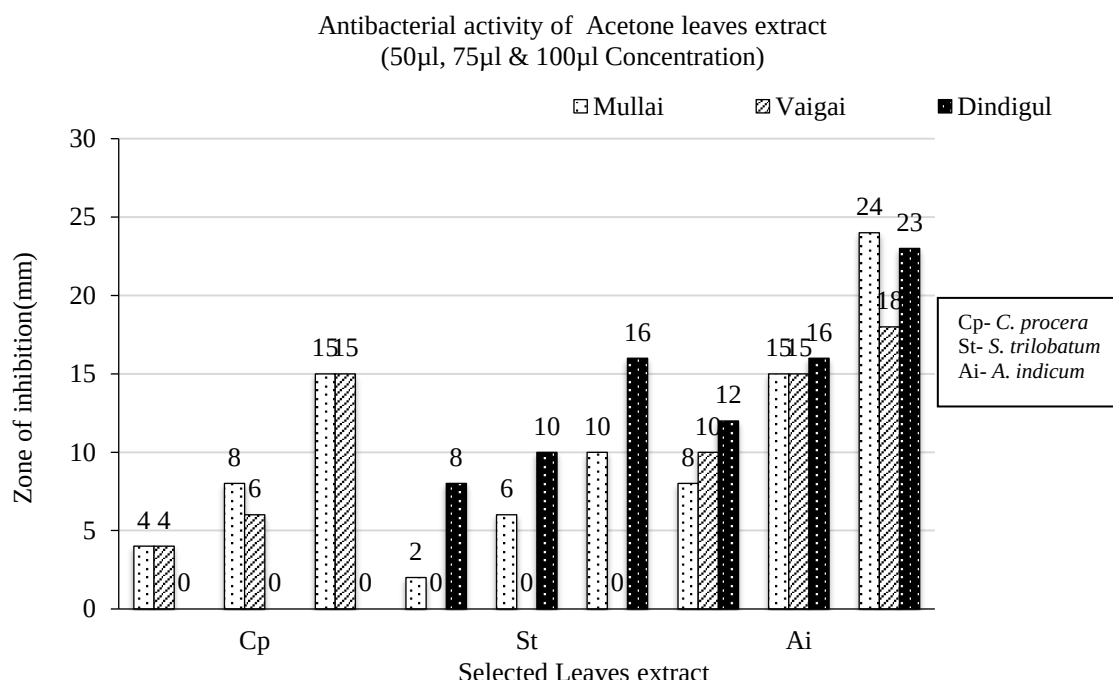


Figure 4. Antibacterial activity of selected acetone leaf extracts with three isolates.

Compare the efficacy of selected leaves with antibiotic discs

Comparative study of the acetone leaf extracts of *A. indicum*, *C. procera* and *S. trilobatum* with standard antibiotic discs were carried out by disc diffusion method [16]. In the present study the

chemical antibiotic exhibited most effective lytic activity against isolated bacteria such as Mullai (Gentamicin 18mm), Vaigai (Tetracycline 18mm) and Dindigul drinking water (Gentamicin 18mm). Moreover, the acetone leaf extract 100 μ l concentration of *A. indicum* was showed excellent lytic activity compared to chemical antibiotics against the isolated bacteria like Mullai (24mm), and Dindigul drinking water (23mm). But acetone leaves extract (100 μ l) of *S. trilobatum* recorded equal antibiotic effect compared to standard antibiotics against Vaigai river water (18mm). The extract *S. trilobatum* observed significant effect than the standard antibiotics test against Gram negative bacteria *P. aeruginosa*. *P. aeruginosa* was more susceptible rather than the other bacteria species tested by the extract of *S. trilobatum* [29]. In Netherland, antimicrobial resistance (AMR) *E. coli* isolated from river and contaminated water reported as pathogenic. The isolated strains identified 84% of resistance against three standard drugs of beta-lactams, tetracyclines, and aminoglycosides [30]. Overall lytic activity comparison of selected leaves extract with effective chemical antibiotics against isolated bacteria showed in Table 1.

Table 1. Lytic activity comparison of selected leaves extract with effective Chemical antibiotics against isolated bacteria.

Isolated bacteria	Acetone leaves extract (mm)									Chemical antibiotics (mm)
	50 μ l			75 μ l			100 μ l			30 μ l
	Ai	Cp	St	Ai	Cp	St	Ai	Cp	St	
Mullai	8	4	2	15	8	6	24	15	10	18 (GEN)
Vaigai	10	4	0	0	6	0	18	15	0	18 (TE)
Dindigul	12	0	8	16	0	10	23	0	16	18 (GEN)

CONCLUSIONS

The present study revealed that the leaf extracts of *A. indicum* recorded as a good antibacterial agent against all three isolated bacteria. *C. procera* and *S. trilobatum* is a less effective than the chemical antibiotics. This study opens the way for further attention as well as research to identify functional compounds responsible for plant biological function. Recent research has suggested that the extracts of plant that have been shown to be effectively used as natural antibiotics to control water-borne diseases, food borne diseases from gram-negative bacteria.

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