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# Antibacterial activity of *Plectranthus Amboinicus* against multiple drug-resistant isolates obtained from samples of urinary tract infection

**Shinu Yadav[1], Darshana Raut[2], Shubhada Nayak[3*]**

[1] Post graduate Student (MScII), Department of Microbiology, Rayat Shikhan Sanstha's Karmaveer Bhaurao Patil College (Autonomous), Vashi, Navi Mumbai, Maharashtra, India

[2] Assistant Professor, Department of Microbiology, Rayat Shikhan Sanstha's Karmaveer Bhaurao Patil College (Autonomous), Vashi, Navi Mumbai, Maharashtra, India

[3] Principal, Department of Microbiology, Rayat Shikhan Sanstha's Karmaveer Bhaurao Patil College (Autonomous), Vashi, Navi Mumbai, Maharashtra, India

**Abstract**
The emergence of multi drug resistance has critically challenged the treatment of infectious diseases. It has thus created an urgent need for development of new antimicrobial agents as alternatives or adjuvants to the currently available antimicrobials. Phytochemicals are one such compound that are actively sought to be a safe alternative to antimicrobials. Hence, the current study was carried out to evaluate the antibacterial activity of *Plectranthus amboinicus* (Indian borage) against 30 multidrug resistant pathogens obtained from samples of urinary tract infections. For this purpose, the bioactive components were extracted from *P. amboinicus* using methanol, butanol, hexane, chloroform and petroleum ether. Qualitatively, methanol extract showed maximum antibacterial activity as compared to other solvents. The MBC of methanol extract against clinical isolates was determined to be in the range of 15-20mg/mL. The potential of methanol extract to inhibit the biofilm formation was also checked by tube method, which showed strong anti-biofilm activity against all 26 isolates that formed biofilms in test tubes. The phytochemical analysis of the aqueous extract showed presence of tannins, alkaloids, phenolic compound, terpenoids, carbohydrates, glycoside, flavonoids, triterpenoids and steroids indicating biochemically rich profile of the plant. Altogether, the observations of the present study suggested the potential use of *P. amboinicus* as an antibacterial agent or an adjuvant in similar preparations, for treatment of infections caused by multidrug resistant pathogens.

**Keywords:** *plectranthus amboinicus*, multidrug resistance, phytochemicals, biofilm, adjuvant

## Introduction

Micro-organisms have the ability to overcome the obstacles in their cell cycle due to environmental stress or physical agents. This adaptability enables them to survive in unfavourable conditions. In view of this fact, the emergence of antimicrobial resistance among pathogens is certainly inevitable. However, the lack of consciousness regarding the long-term exposure of antibiotics on the bacterial community has posed a serious health threat to the common population. This is especially true for the ignorant society lacking awareness regarding the harmful consequences of antibiotic abuse [1]. The unnecessary use of antibiotics in sub-minimal doses has led to the emergence, and alarming spread, of multi-drug resistance (MDR) strains globally.

Urinary tract infections (UTIs) are one of the most common bacterial infections among men and women, affecting 150 million people each year worldwide [2]. The main etiological agent of UTI i.e., *E. coli* and *K. pneumoniae*, which are also a common flora of the intestines, is the main reason for its widespread prevalence. The development of drug resistance among these pathogens increases the occurrence and further spread of resistant determinants in the microbial population through horizontal gene- and plasmid transfer [3]. Although overcoming the bacterial defense mechanism may be difficult, certain strategies like the use of combination therapy have worked effectively in the past in treating infectious diseases. However, the treatment protocols may present themselves with serious antibiotic overdose and dependence leading to decreased immunity, in highly infectious cases [4]. More recently, attempts are made towards the use of herbal remedies in treating infections caused by drug-resistant strains of pathogens [5, 6].

Due to the high incidences of extreme drug resistance, natural compounds from plants are considered as potential candidates for treating infectious diseases. Plants are rich in a wide variety of secondary metabolites like tannins, terpenoids and alkaloids that have been found to possess antibacterial properties *in vitro* [7]. Many plant-derived compounds have shown promising activity against drug susceptible pathogens [8]. Even the World Health Organization has estimated that about 80% of people around the globe depend on traditional medications of plant origin due to their low cost, easy accessibility and negligible or low side effects in comparison to allopathic medicines [9]. However, very few studies have been published on the efficacy of herbal medicines against drug resistant pathogens.

*Plectranthus amboinicus*, commonly known as Indian borage, is one such example of a herbal source that is explored for its activity against infectious agents. It belongs to the family of *Lamiaceae* and consists of more than 300 species. They are known for their rich ethnobotanical diversity and unique medicinal properties which are highly valuable for the development of new drugs [10]. The plant is

a large, succulent, aromatic, perennial herb distributed throughout India and Sri Lanka [11]. Many pharmacological properties of *P. amboinicus* including urolithiasis, antiepileptic, anti-tumorigenic, antimutagenic, radio protective, anti-viral, antifungal and neuro pharmacological, have also been reported [12]. A decoction of its leaves is used in treatment of chronic cough, asthma, spasm, stomach ache due to dyspepsia, fever and epilepsy [13]. It is also used in the treatment of skin ulcerations and urinary diseases as well as to alleviate inflammation, kidney troubles, and nervous disorders, and in conditions of congestive heart failure [11].
Considering the increasing antibiotic resistance, and the search for potential alternative to antibiotics, the objective of our study was to investigate the efficacy of leaf extracts of *P. amboinicus* as an antibacterial and antibiofilm agent against MDR pathogens of UTI origin.

## Material and Methods

### Sample collection

A total of 30 MDR pathogens of UTI origin were obtained from Mahatma Gandhi Memorial Hospital, Kamothe, Navi Mumbai, Maharashtra. All the isolates were checked for their viability and re-isolated on Nutrient Agar (NA) plates to confirm the purity of the cultures. The isolates were maintained on NA slants in duplicates and preserved at 8°C until further use.

### Presumptive characterization of uropathogens

Hi Chrome UTI Agar (HiMedia M1353R) was used as a differential medium which is recommended for presumptive characterization of pathogens causing UTI [14]. A loopful of suspension of each isolate was streaked on above plates and the cultural characteristics were observed after incubation at 37°C for 24 h.

### Antimicrobial susceptibility testing of clinical isolates

To study the drug resistance pattern of clinical isolates, the antimicrobial susceptibility testing (AST) was performed using the Kirby Bauer disc diffusion test [15, 16]. The susceptibility of the clinical isolates was determined against 12 antibiotics, which are the commonly used first-line drugs for the treatment of UTI. The antibiotics included cephalothin (CEP, 30 µg), ceftizoxime (CZX, 30 µg), cefuroxime (CXM, 30 µg), cefotaxime (CTX, 30 µg), amoxyclav (AMC, 20/10 µg), norfloxacin (NX, 10 µg), ofloxacin (OF, 2 µg), levofloxacin (LE, 5 µg), nitrofurantoin (NIT, 300 µg), trimethoprim (TR, 5 µg), amikacin (AK, 30 µg), tobramycin (TOB, 10 µg), in the form of Dodeca discs (HiMedia Dodeca UTI 13-DE739).
The isolates were inoculated in sterile peptone water and incubated at 37°C for 4h till the density reached 0.3 O.D at 530nm. Then, 0.5mL of culture was spread on the sterile Mueller Hinton (MH) agar plate with the help of a sterile cotton swab. The antibiotic discs (Dodeca UTI 13-DE739) were placed on the plate using alcohol sterilized forceps under aseptic conditions, and incubated at 37°C for 24h. After incubation, the zones of inhibition were measured (in mm), and the results were compared with the standard Kirby Bauer interpretation chart (Table 1).

**Table 1:** Kirby Bauer interpretation chart

| Sr. No. | Antibiotics | Abbreviation | Disc content in µg | Zones of inhibition in mm | | |
|---|---|---|---|---|---|---|
| | | | | Sensitive | Intermediate | Resistant |
| 1 | Cephalothin | CEP | 30 | ≥18 | 15-17 | ≤14 |
| 2 | Ceftizoxime | CZX | 30 | ≥25 | 22-24 | ≤21 |
| 3 | Cefuroxime | CXM | 30 | ≥23 | 15-22 | ≤14 |
| 4 | Cefotaxime | CTX | 30 | ≥26 | 23-25 | ≤22 |
| 5 | Amoxyclav | AMC | 20 | ≥18 | 14-17 | ≤13 |
| 6 | Norfloxacin | NX | 10 | ≥17 | 13-16 | ≤12 |
| 7 | Ofloxacin | OF | 2 | ≥16 | 13-15 | ≤12 |
| 8 | Levofloxacin | LE | 5 | ≥17 | 14-16 | ≤13 |
| 9 | Nitrofurantoin | NIT | 300 | ≥17 | 15-16 | ≤14 |
| 10 | Trimethoprim | TR | 5 | ≥16 | 11-15 | ≤10 |
| 11 | Amikacin | AK | 30 | ≥17 | 15-16 | ≤14 |
| 12 | Tobramycin | TOB | 10 | ≥15 | 13-14 | ≤12 |

### Identification of plant and preparation of leaf extract

The plant under study, i.e., Indian borage (*Plectranthus amboinicus*), was collected from a local garden and authenticated by Blatter Herbarium of St. Xaviers College, Mumbai. The leaves of *Plectranthus amboinicus* plant were thoroughly cleaned with distilled water, dried in shade for 10 days and powdered using a grinder before commencing the extraction procedure. The bioactive components were extracted from 15g of powdered leaves in 200mL of organic solvents *viz*, n-hexane, petroleum ether, methanol and chloroform for the duration of 8 h with the help of Soxhlet apparatus. The obtained extracts were concentrated by allowing the solvents to evaporate at Room temperature (RT, ~28°C). These concentrates were prepared in large volumes and stored at 4°C until further use to avoid batch to batch variation [17].

### Sterility testing of solvent extracts

The sterility of extracts obtained using four different solvents was tested by streaking a loopful of each extract on Sterile Nutrient Agar (NA) and Sterile Sabouraud dextrose agar (SAB) plates for checking bacterial and fungal contamination respectively. The NA plates were incubated for 24 h at 37ºC and the SAB plates for 48 h at RT [18, 19].

### Detection of antimicrobial activity of *Plectranthus amboinicus* leaf extract

The leaf extracts obtained with four different solvents were tested for their antimicrobial activity by agar well diffusion and disc diffusion method. To carry out the agar well diffusion method, 20 mL of sterile and molten NA butt was cooled to around 40°C, seeded with 0.5 mL test cultures (0.3 O.D at 540nm) and poured into sterile Petri plates [20]. Triphenyl tetrazolium chloride (TTC) was also added to the plates for detection of viable growth. After solidification of

the agar, wells were punched in each plate using a sterile Cork borer and 50 µL of the extract was added to it. To carry out the disc diffusion method, plates were prepared as described above. The sterile Whatman filter paper (No.1) discs of size 4mm were impregnated with the extracts and placed onto these plates using sterile forceps. The controls of different solvents were also maintained. The plates were then incubated at 37°C for 24 h and zones of inhibition (in mm) were measured after incubation [21]. All experiments were performed in triplicates.

**Determination of minimum bactericidal concentration of *Plectranthus amboinicus* leaf extract**

The agar dilution method was used to determine the Minimum Bactericidal Concentration (MBC) of *P. amboinicus* leaf extracts. Different concentrations of various solvent extracts (1-20 mg/mL with an interval of 5 mg/mL) were supplemented into sterile molten NA butts cooled to around 40°C [22]. After solidification of the medium, 5 µL of 30 clinical isolates was spot-inoculated and incubated at 37°C for 24 h. MBC was defined as the lowest concentration of plant extract that completely inhibits the growth of test cultures [13]. All experiments were performed in triplicates.

**Determination of biofilm formation by isolates**

The biofilm-producing ability of all the clinical isolates was checked qualitatively by modified Congo Red Agar method (CRA). It helps in distinguishing between the biofilm producers and non-producers based on color change of the medium. The modified CRA medium was prepared by adding 5% of sucrose to 100 mL of Brain heart infusion (BHI) agar. After 24 h of incubation period at 37°C, the intensity of colour formed by the colonies were checked. They were classified as grade 0 (biofilm non-producers), grade 1 (weak biofilm producers) and grade 2 (strong biofilm producers) based on observation of red, orange/brownish and black colonies respectively [23].

**Antibiofilm assay of plant extract**

Antibiofilm assay was carried out by tube method. To carry out the technique, BHI medium was supplemented with 2% glucose and inoculated with a loopful of organisms from overnight cultures in a polystyrene test tube (27). To this, 0.35% of methanolic *P. amboinicus* extract was added, to achieve sub-MBC concentration. Positive and negative controls were set up in BHI broth with and without test inoculum respectively. Methanol solvent control was also prepared. The tubes were incubated for 48 h at 37°C. After incubation, the media was decanted and the tubes were washed with phosphate-buffered saline (pH 7.2), dried and stained with 0.1% crystal violet for 20mins. The stain was removed by rinsing the tubes with PBS and dried. To these tubes, 5 mL of 95% ethanol was added to elute the crystal violet stain binding to the biofilm. The concentration of crystal violet eluted is directly proportional to the concentration of biofilms, and was measured by checking its absorbance at 620 nm [23, 10].

**Phytochemical analysis of plant extract**

Aqueous extracts were used for phytochemical screening of the plant. It was prepared by adding 10 g of dried and powdered leaves into 100 mL of distilled water and boiled for 15 mins in a boiling water bath. The mixture was then cooled and filtered using Whatman filter paper no.1 and stored at 4°C until use [9, 22, 24].

Following tests were performed for phytochemical screening of the extract

**Test for terpenoids:** 2 mL of the extract was taken in a test tube to which 2 mL of chloroform and 3 mL of concentrated sulphuric acid was added. Formation of reddish-brown colour was indicative of a positive test.

**Test for carbohydrates:** 2 mL of the extract was taken in a test tube to which 1 mL of Benedict's solution was added and the mixture was heated for 5 mins. Formation of green colour was considered as a positive test.

**Test for glycoside:** 25 mL of the extract was taken in a test tube to which 12.5 mL of concentrated sulphuric acid was added and heated for 1-2 mins. The mixture was then neutralized using 10% NaOH and 3-4 drops of Fehling's solution were added. Formation of the brick red precipitate indicated a positive test.

**Test for reducing sugars:** 2 mL of the extract was taken in a test tube to which 2 mL of distilled water was added. After adding 5-8 drops of Fehling's solution the tube was warmed over a flame. Formation of a brick red precipitate at the bottom of the test tube was indicative of a positive test.

**Test for phenolic compound:** 2 mL of the extract was taken in a test tube to which 3-4 drops of 10% ferric chloride solution was added. Formation of black colour confirmed the presence of phenolic compounds.

**Test for alkaloids:** 2 mL of the extract was taken in a test tube to which 1 mL of 1% HCl and 1 mL of 0.1% picric acid were added. Formation of a turbid precipitate indicated a positive test.

**Test for triterpenoids:** 2.5 mL of the extract was taken in a test tube to which 1 mL of chloroform and 1.5 mL of concentrated sulphuric acid was added. Formation of reddish-brown colour indicated a positive test.

**Test for saponins:** 2 mL of the extract was taken in a test tube, shaken vigorously and warmed for 2 mins. Formation of foam at the top of the tube indicated the presence of saponins.

**Test for phlobatannins:** 2 mL of the extract was taken in a test tube to which 2 mL of 1% HCl was added and the mixture was warmed for 5 mins. Formation of brick red precipitate at the bottom of the test tube is indicated a positive test.

**Test for flavonoids:** 2 mL of the extract was taken in a test tube to which 2 mL of 10% lead acetate was added. Formation of a yellow precipitate indicated presence of flavonoids.

**Test for steroids:** 2 mL of the extract was taken in a test tube and then evaporated to dryness. The residue was dissolved in 1 mL acetic anhydride and 1 mL of chloroform was added from the sides of the test tube. The formation of the brown ring at the interphase of 2 liquids indicated presence of steroids.

**Test for quinones:** 2 mL of the extract was taken in a test tube to which 2 mL of concentrated HCl was added. Formation of a yellow precipitate indicated the presence of quinones.

**Test for tannin:** 2 mL of the extract was taken in a test tube to which 3 drops of 10% ferric chloride were added. Formation of green or black blue colouration indicated a positive test.

## Results and Discussion

The characteristic observations of Hi Chrome agar streaked with isolates obtained from samples of UTI is indicated in Table 2. Thirty isolates were used in our study and their susceptibility was tested against *P. amboinicus*. Among the 30 MDR pathogens, 14 isolates were identified as *Escherichia coli*, 6 isolates as *Klebsiella pneumoniae*, 3 isolates as *Proteus mirabilis*, 1 isolate as *Pseudomonas aeruginosa*, 1 isolate as *Staphylococcus aureus*, 3 isolates belonged to the genus *Citrobacter*, 1 isolate as *Acinetobacter* sp., and 1 other isolate as *Enterococcus* sp.

**Table 2:** Presumptive characterization of clinical isolates on Hi Chrome UTI Agar

| Sr. No. | Name of Clinical Isolate | Number of isolates | Colour of colony on Hi Crome UTI agar |
|---|---|---|---|
| 1 | *Escherichia coli* | 14 | Magenta |
| 2 | *Enterobacter spp.* | 1 | Green |
| 3 | *Klebsiella pneumoniae* | 6 | Dark blue-purple (mucoid) |
| 4 | *Proteus mirabilis* | 3 | Colourless |
| 5 | *Pseudomonas aeruginosa* | 1 | Greenish pigment |
| 6 | *Staphylococcus aureus* | 1 | Golden yellow |
| 7 | *Citrobacter spp.* | 3 | Prussian blue-Magenta |
| 8 | *Acinetobacter spp.* | 1 | Dark blue |

**Antibacterial susceptibility testing of clinical isolates**

The antibiotic resistance profile of above isolates is indicated in Table 3. All the isolates used in this study showed resistance towards amoxyclav and cefuroxime antibiotics. Twelve isolates were resistant to all antibiotics used in the present study indicating widespread resistance to first line antibiotics among pathogens causing urinary tract infections. Resistance to cefotaxime, tobramycin, norfloxacin, nitrofurantoin, trimethoprim and ceftizoxime were also common among all isolates. The observed resistance for levofloxacin, amikacin and ofloxacin was also high, but comparatively less prevalent and observed in 16, 20 and 22 isolates respectively. Urinary tract infections by bacterial pathogens are one of the common causes for seeking medical attention in the community. The resulting action of treating UTI infections with extensive use of antibiotics have contributed considerably to the emergence of multidrug-resistance in bacterial pathogens [25]. The resistance to antibiotics among pathogens develop rapidly over a short period of time. It is especially true for developing countries where the practise of self-Prescription is common based on previous infections, and the dose of antibiotic therapy is rarely completed [12, 13, 26]. Resistance to the above antibiotics has also been reported by other researchers [27, 28]. The high degree of resistance among pathogens to commonly prescribed drugs, as observed in this study, leaves very few alternative options for treatment of infections. Consequently, a large number of plants are explored for evaluating their antibacterial property and developing effective alternatives and safer drugs for treating infectious diseases in humans. However, very few studies have reported antibacterial and anti-biofilm forming potential of plants against multi drug resistant pathogens.

**Table 3:** Antibiotic resistance profile of the clinical isolates

| Sr. No. | Clinical isolates | Interpretation based on Kirby Bauer chart | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| 1 | *K. pneumoniae*- p969 | - | - | CTX,TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 2 | *K. pneumoniae*- p969 | OF | LE, NX,TR | CTX, AK, CXM, CZX, NIT, CEP, AMC, TOB |
| 3 | *E. coli*- p94 | OF,NX,TR | LE | CTX, AK, CXM, CZX, NIT, CEP, AMC,TOB |
| 4 | *E. coli*- p244 | CTX, OF, LE, NX | TR,CEP | AK, CXM, CZX, NIT, AMC, TOB |
| 5 | *E. coli*- p04 | - | AK | CTX, TR, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 6 | *E. coli*- p15 | AK | TOB | CTX, TR, CXM, CZX, NIT, OF, CEP, LE, AMC, NX |
| 7 | *E. coli*- p756 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 8 | *E. coli*- p227 | - | OF, LE | CTX, TR, AK, CXM, CZX, NIT, CEP, AMC, TOB, NX |
| 9 | *E. coli*- p779 | - | - | CTX,TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 10 | *E. coli*- p608 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 11 | *K. pneumoniae*- p988 | - | - | CTX,TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 12 | *K. pneumoniae*- p936 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 13 | *E. coli*- p152 | CTX, LE | AK, CZX,OF, TOB | TR, CXM, NIT, CEP, AMC, NX |
| 14 | *Citrobacter* sp.- p154 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC,TOB, NX |
| 15 | *E. coli*- p860 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 16 | *Citrobacter sp.* -p624 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 17 | *Enterobacter*- p325 | NIT, OF, LE | NX | CTX, TR, AK, CXM, CZX, CEP, AMC, TOB |
| 18 | *E. coli*- p52 | AK,TR | - | CTX, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 19 | *Citrobacter* sp.- p244 | AK, OF, LE, NX | CZX | CTX, TR, CXM, NIT, CEP, AMC, TOB |
| 20 | *S. aureus*- p926 | OF, LE | AK,CZX, NX | CTX, TR, CXM, NIT, CEP, AMC,TOB |
| 21 | *P. mirabilis*- p121 | CEP | - | CTX,TR, AK, CXM, CZX, NIT, OF, LE, AMC, TOB, NX |
| 22 | *P. mirabilis*- p154 | TR, AK, OF, LE, NX | - | CTX, CXM, CZX, NIT, CEP, AMC, TOB |
| 23 | *Acinetobacter sp.*- p154 | TR | - | CTX, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 24 | *K. pneumoniae*- p201 | - | TR,AK, LE, NX | CTX, CXM, CZX, NIT, OF, CEP, AMC, TOB |
| 25 | *P. aeruginosa*-p931 | AK, OF, LE, NX | - | CTX, TR, CXM, CZX, NIT, CEP, AMC, TOB |
| 26 | *E. coli*- p770 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |
| 27 | *P. mirabilis*- p35 | LE | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, AMC, TOB, NX |
| 28 | *E. coli*- p103 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |

| | | | | |
|---|---|---|---|---|
| 29 | *K. pneumoniae*- p35 | - | TR,AK,LE, NX | CTX, CXM, CZX, NIT, OF, CEP, AMC, TOB |
| 30 | *E. coli*- p51 | - | - | CTX, TR, AK, CXM, CZX, NIT, OF, CEP, LE, AMC, TOB, NX |

**Antibacterial activity of *Plectranthus amboinicus* leaf extracts**

The solvent extracts showed absence of fungal and bacterial contaminants and hence used for evaluating their antibacterial activities using agar well diffusion and disc diffusion methods. The extract obtained with methanol showed the highest zone of inhibition against all 30 clinical isolates (Table 4). Hence methanol extracts were used for further experiments. The MBC of methanol extract of *P. amboinicus* was estimated by agar dilution method. It extracts was found in the range of 15-20 mg/mL (Table 5). At 20 mg/mL concentration of plant extract complete inhibition of all the 30 test cultures was observed. The increased efficacy of methanol extract, in this study, may be attributed to its polarity that allows extraction of bioactive components of the plant into the solvent [29].

The use of 2, 3, 5-triphenyl tetrazolium chloride (TTC) salt in the medium further improved the contrast between the zone of inhibition and lawn of viable bacteria, and facilitated precise measurement of the zone of inhibition. Similar to our study, Murthy *et al*. [26] reported effective antibacterial and antifungal activities of *P. amboinicus* against drug susceptible isolates. In addition to *E. coli* and *K. pneumoniae*, other study reported the susceptibility of *Shigella flexneri*, *Staphylococcus aureus*, *Bacillus cereus* and *Yersinia enterocolitica* to leaf extracts of *P. amboinicus* [13, 30]. The susceptibility of natural flora of chicken to acetone and ethyl acetate extracts of *P. amboinicus* has been reported by Gupta and Negi [31], who recommended its use in preservation of food products. The essential oils obtained from *P. amboinicus* have shown antibacterial activity against planktonic and biofilm cells of oxacillin- and vancomycin- resistant *S. aureus* [32].

**Table 4:** Qualitative evaluation of antimicrobial activity of leaf extracts of *Plectranthus amboinicus*

| Sr. No. | Clinical isolates | Diameter of zone of inhibition 'in mm' by plant extracts obtained by using different solvents | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Agar well diffusion method | | | | Disc diffusion method | | | |
| | | Methanol | N-hexane | Methanol | N-hexane | Methanol | N-hexane | Methanol | N-hexane |
| 1 | *K. pneumoniae*- p969 | 20 | 12 | 17 | 13 | 12 | 10 | 8 | 7 |
| 2 | *K. pneumoniae*- p1101 | 20 | 10 | 18 | 14 | 11 | 9 | 8 | 6 |
| 3 | *E. coli*- p94 | 28 | 13 | 22 | 18 | 15 | 11 | 6 | 9 |
| 4 | *E. coli*- p244 | 16 | 15 | 10 | 12 | 8 | 6.5 | 7 | 7.5 |
| 5 | *E. coli*- p04 | 28 | 15 | 23 | 18 | 15 | 9.5 | 10 | 8 |
| 6 | *E. coli*- p15 | 21 | 18 | 20 | 20 | 10.5 | 8.5 | 9 | 7 |
| 7 | *E. coli*- p756 | 28 | 17 | 19 | 24 | 12.5 | 11 | 10.5 | 9 |
| 8 | *E. coli*- p227 | 22 | 14 | 12 | 15 | 10 | 6 | 7 | 7.5 |
| 9 | *E. coli*- p779 | 24 | 17 | 12 | 24 | 10.5 | 9 | 8 | 6.5 |
| 10 | *E. coli*- p608 | 21 | 15 | 13.5 | 11 | 13 | 10 | 9.5 | 6 |
| 11 | *K. pneumoniae*- p988 | 14 | 13 | 15 | 11 | 9 | 6.5 | 8 | 8 |
| 12 | *K. pneumoniae*- p936 | 18.5 | 15.5 | 12 | 14 | 14 | 10 | 11 | 10.5 |
| 13 | *E. coli*- p152 | 12 | 12 | 12 | 14 | 10 | 6.5 | 8 | 7 |
| 14 | *Citrobacter sp.*- p154 | 21 | 16.5 | 15 | 18 | 11.5 | 9 | 7 | 7.5 |
| 15 | *E. coli*- p860 | 26 | 18 | 14.5 | 15 | 13 | 14 | 7.5 | 9.5 |
| 16 | *Citrobacter sp.*- p624 | 20 | 17 | 19 | 13 | 10 | 7 | 8.5 | 9 |
| 17 | *Enterobacter sp.*- p325 | 20 | 13 | 14.5 | 16 | 10 | 6.5 | 9.5 | 8 |
| 18 | *E. coli*- p52 | 20.5 | 14 | 12 | 17 | 11 | 8 | 10 | 9 |
| 19 | *Citrobacter sp.*- p244 | 21 | 18.5 | 15 | 13.5 | 11 | 7.5 | 9 | 8 |
| 20 | *S. aureus*- p926 | 26 | 17.5 | 20 | 22.5 | 11 | 6.5 | 7 | 9 |
| 21 | *P. mirabilis*- p121 | 14 | 12 | 15 | 14 | 8 | 7 | 6.5 | 7 |
| 22 | *P. mirabilis*- p154 | 12 | 12.5 | 14 | 12.5 | 9 | 7 | 7.5 | 8 |
| 23 | *Acinetobacter sp.*- p154 | 18 | 12.5 | 12 | 12 | 9 | 8 | 8 | 9 |
| 24 | *K. pneumoniae*- p201 | 21 | 15 | 16 | 17 | 7 | 6.5 | 6 | 7 |
| 25 | ***P. aeruginosa-p931*** | 20 | 18 | 14.5 | 16 | 12 | 9 | 8 | 9 |
| 26 | *E. coli*- p770 | 22 | 12 | 12.5 | 15 | 11.5 | 8 | 9.5 | 7 |
| 27 | *P. mirabilis*- p35 | 24 | 13.5 | 13 | 15 | 13.5 | 11 | 10 | 9.5 |
| 28 | *E. coli*- p103 | 25 | 16.5 | 12 | 14 | 12 | 9 | 8.5 | 8 |
| 29 | *K. pneumoniae*- p35 | 20 | 15 | 16 | 16.5 | 10 | 7 | 8 | 9 |
| 30 | *E. coli*- p51 | 20 | 17 | 13 | 15 | 10 | 8 | 8.5 | 7.5 |

**Table 5:** Results of biofilm formation of isolates using Congo red agar method

| Grade -0 | Grade -I | Grade-II |
|---|---|---|
| *P. mirabilis*- p121, *P. mirabilis*- p154, *P. aeruginosa*- p931, *P. mirabilis*- p35 | *K. pneumoniae*- p969, *K. pneumoniae*- p1101, *E. coli*- p608, *Citrobacter* sp.- p624, *Citrobacter* sp.- p244, *S. aureus*- p926, *Acinetobacter* sp.- p154, *K. pneumoniae*- p35 | *E. coli*- p94, *E. coli*- p244, *E. coli*- p04, *E. coli*- p15, *E. coli*- p756, *E. coli*- p227, *E. coli*- p779, *K. pneumoniae*- p988, *K. pneumoniae*- p936, *E. coli*- p152,*Citrobacter* sp.- p152, *E. coli*- p860, *Enterobacter*- p325, *E. coli*- p52, *K. pneumoniae*- p201, *E. coli*- p770, *E. coli*- p103, *E. coli*- p51 |

**Determination of biofilm formation by isolates and evaluation of anti-biofilm activity of *Plectranthus amboinicus* leaf extracts**

The test isolates showed moderate to high biofilm formation in our study. Out of the tested 30 clinical isolates, 18 showed high biofilm formation, 8 showed moderate formation, while 4 isolates did not form biofilms (Figure 1). The methanolic extract of *P. amboinicus* leaves showed strong anti-biofilm activity against all the 26 clinical isolates that formed biofilms in test tubes. The concentration of crystal violet eluted from the test was found to be considerably low as compared to the control (Figure 2).

Biofilm forming bacteria are responsible for recurring infections. They are very difficult to eradicate and exhibit resistance to antibiotics due to various reasons like restricted penetration of antibiotics into biofilms, decreased growth rate and expression of resistance genes [10]. In the present study, 26 out of 30 clinical isolates formed biofilms, indicating their ability to develop complicated infections. The methanolic leaf extract showed anti biofilm activity against all 26 clinical isolates. The observed activity indicates the capability of the extract to inhibit the formation of the exopolysaccharide and suppress the auto-inducers which are responsible for the cell to cell communication which is an important factor for the formation of the biofilms [33, 34].

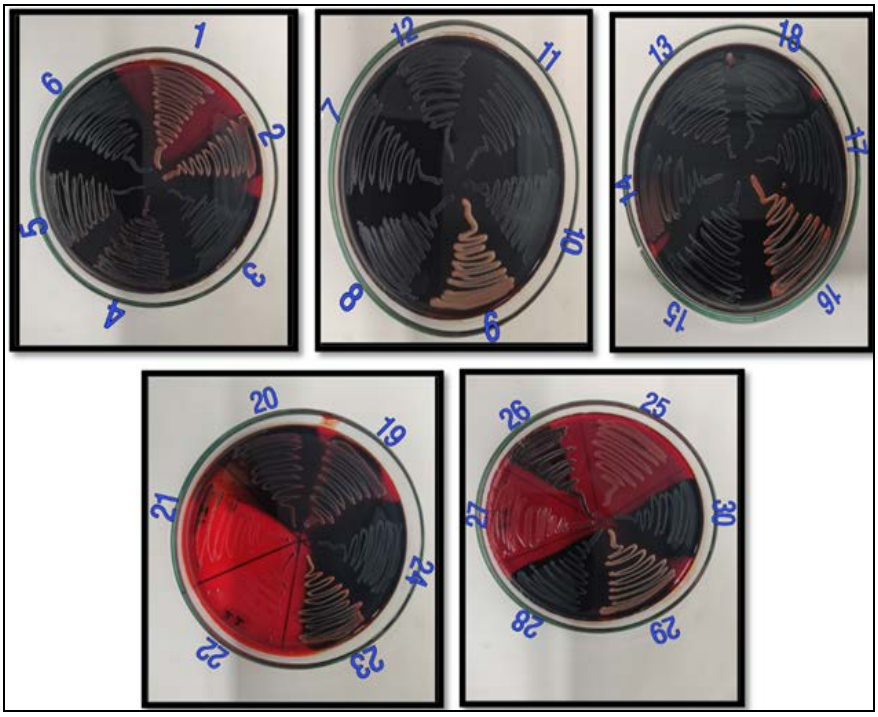

**Fig 1:** Testing of biofilm formation ability of 30 clinical isolates by Congo red agar method
Red colonies indicate non-biofilm producers (grade 0), orange – brownish colonies indicate weak biofilm producers (grade-1), Black colonies indicate biofilm producers (grade-2).

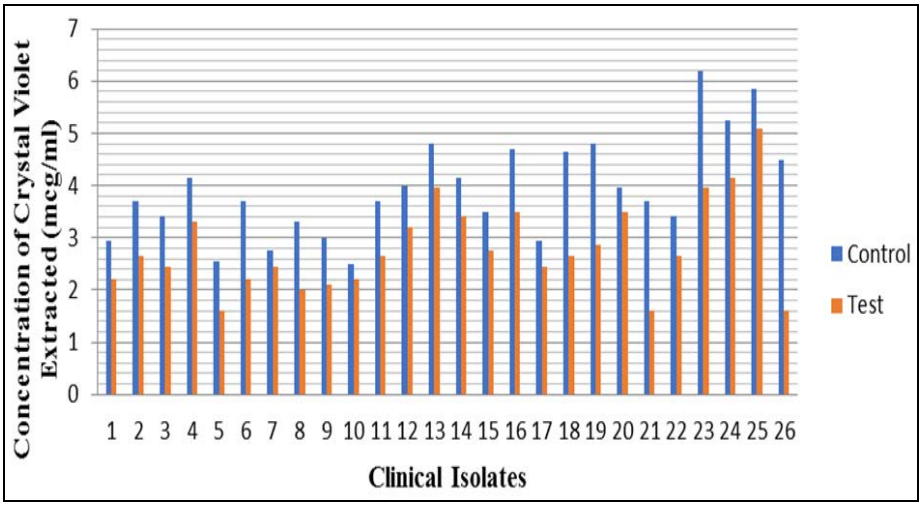


**Fig 2:** Concentration of stain eluted from biofilms on crystal violet assay
(1) *K. pneumoniae*- p969, (2) *K. pneumoniae*- p1101, (3) *E. coli*- p94, (4) *E. coli*- p244, (5) *E. coli*- p04, (6) *E. coli*- p15, (7) *E. coli*- p756, (8) *E. coli*- p227, (9) *E. coli*- p779, (10) *E. coli*- p608, (11) *K. pneumoniae*- p988, (12) *K. pneumoniae*- p936, (13) *E. coli*- p152, (14) *Citrobacter sp.*- p154, (15) *E. coli*- p860, (16) *Citrobacter sp.*- p624, (17) *Enterobacter sp.*- p325, (18) *E. coli*- p52, (19) *Citrobacter sp.*- p244, (20) *S. aureus*- p926, (21) *Acinetobacter* sp.- p154, (22) *K. pneumoniae*- p201, (23) *E. coli*- p770, (24) *E. coli*- p103, (25) *K. pneumoniae*- p35, (26) *E.coli*- p51

**Phytochemical analysis of the extract**

After performing specific tests for the phytochemical analysis the extract showed the presence of tannins, alkaloids, phenolic compound, terpenoids, carbohydrates, glycoside, flavonoids, triterpenoids and steroids. The presence of these components in the leaves of *P. amboinicus* was also indicated by Ismail and Nimila [30] in their study. In addition to antibacterial activity, these bioactive components are also known for its anti-inflammatory, antiseptic, antioxidant as well as antitumor activities. Hence, further studies can be aimed at exploring the above potential for further pharmacological characterization of *P. amboinicus*. Also, apart from leaves, similar studies have reported the antibacterial, antiplatelet aggregation, radical scavenging and anticancer activity of the stem of this plant [35].

**Conclusion**

In conclusion, this study indicates the antibacterial and anti biofilm potential of *P. amboinicus* against MDR strains of pathogens causing UTI. Hence, it can be suitably used in formulations of preparations that can be used for treating infections due to MDR pathogens.

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**Conflict of interest**

Authors do not have any conflict of interests to declare.

**References**

1. Selim SA. Antimicrobial, anti-plasmid and cytotoxicity potentials of marine algae Halimeda opuntia and *Sarconema filiforme* collected from Red Sea Coast. International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering. 2012; 6(1):1154-1159.
2. Karishetti MS, Shaikh HB. Clinico microbial assessment of urinary tract infections in a tertiary care hospital. Indian Journal of Health Sciences and Biomedical Research. 2019; 12(1):69-74.
3. Giske CG, Monnet DL, Cars O, Carmeli Y. on behalf of ReAct-Action on antibiotic resistance, Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrobial Agents and Chemotherapy. 2008; 52(3):813-821.
4. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clinical Microbiology Reviews. 2012; 25(3):450-470.
5. Sharmeen R, Hossain N, Rahman M, Foysal J, Faruque M. In-vitro antibacterial activity of herbal aqueous extract against multi-drug resistant *Klebsiella* sp. isolated from human clinical samples. International Current Pharmaceutical Journal. 2012; 1(6):133-137.
6. Noumedem JA, Mihasan M, Kuiate JR, Stefan M, Cojocaru D, Dzoyem JP *et al. In Vitro* antibacterial and antibiotic-potentiation activities of four edible plants against multidrug-resistant gram-negative species. BMC Complementary and Alternative Medicine. 2013; 13:190.
7. Suchita S, Kamat SD, Kamat DV. Effect of aqueous extract of *Terminalia chebula* on Metallo beta-lactamase. International Journal of Pharmaceutical Sciences Research. 2010; 2(4):172-175.
8. Beg AZ, Ahmad I. Effect of *Plumbago zeylanica* extract and certain curing agents on multidrug resistance bacteria of clinical origin. World Biotechnology. 2004; 16(8):841-844.
9. Ashwini S, Girish K. Phytochemical screening and antibacterial activity of methanolic leaf extract of *Coleus aromaticus* benth. International Journal of Research in Pharmaceutical Sciences. 2014; 5(4):270-274.
10. Manimekalai K, Srinivasan P, Dineshbabu J, Guna G, Priya D, Darshani D *et al*. Anti-biofilm efficacy of *Plectranthus amboinicus* against *Streptococcus pyogenes* isolated from pharyngitis patients. Asian Journal of Pharmaceutical and Clinical Research. 2016; 9(4):348-354.
11. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016; 21(4):369. https://doi.org/10.3390/molecules21040369.
12. Fonseka DLCK, Wickramarachchi WWUI. *Plectranthus ambonicus* and *Plectranthus zeylanicus* as promising medicinal plants. International Journal of Science and Research. 2018; 7(7):1343-1347.
13. Bhatt P, Negi PS. Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus amboinicus*). Food Science & Nutrition. 2012; 3(2):146-152.
14. Kaskar SN, Tendolkar MR, Vaidya SP, Angadi SA, Dalal AR, Karr S *et al*. Comparative evaluation of performance and cost effectiveness of HiCrome UTI agar in detection of urinary tract pathogens. International Journal of Applied Engineering Research. 2018; 4(7):358-366.
15. Chun LY, Dolle-Molle L, Bethel C, Dimitroyannis RC, Williams BL, Schechet SA *et al*. Rapid pathogen identification and antimicrobial susceptibility testing in *in vitro* endophthalmitis with matrix assisted laser desorption-ionization Time-of-Flight Mass Spectrometry and VITEK 2 without prior culture. PLoS ONE. 2019; 14(12):e0227071.
16. Syal K, Mo M, Yu H, Iriya R, Jing W, Guodong S *et al*. Current and emerging techniques for antibiotic susceptibility tests. Theranostics. 2017; 7(7):1795-1805.
17. Anita P, Agnihotri V, Pande V. Selection of solvent and extraction method for determination of antimicrobial potential of *Taxus wallichiana* Zucc. Research in Pharmacy. 2018; 8:1-9.
18. Tariq M, Gore M, Aruna K. Antibacterial and synergistic activity of ethanolic ajwain (*Trachyspermum ammi*) extract on ESBL and MBL producing uropathogens. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(6):278-284.
19. Kalpana T, Tulika M. Study of Antimicrobial activity of betel leaves extracts against ESBL and MBL

producing pathogens. World Journal of Pharmaceutical Research. 2017; 6(8):2364-2368.
20. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmacy and Analytics. 2016; 6(2):71-79.
21. Manjamalai A, Berlin G. Volatile constituents and antioxidant property of essential oil from *Plectranthus amboinicus* (Lour). International Journal of Pharmacy and Bio Sciences. 2012; 3(4):445-458.
22. Pillai PG, Suresh P, Aggarwal G, Doshi G, Bhatia V. Pharmacognostical standardization and toxicity profile of the methanolic leaf extract of *Plectranthus amboinicus* (Lour) Spreng. Journal of Applied Pharmaceutical Sciences. 2012; 1(2):76-81.
23. Kaiser TDL, Pereira EM, dos Santos KRN, Maciel ELN, Schuenck RP, Nunes APF *et al*. Modification of the congo red agar method to detect biofilm production by *Staphylococcus epidermidis*. Diagnosis of Microbial Infection and Diseases. 2012; 75(3):235-239.
24. Ruwali P, Negi D. Phytochemical analysis and evaluation of antioxidant activity of Premna Latifolia Roxb. A medicinal plant (Family: Lamiaceae). Journal of Pharmaceutical Innovations. 2019; 8(5):13-20.
25. Preethi SP, Rekha R, Vimal Kumar, Asha PKB, Pratibha B. Uropathogens and their antibiotic susceptibility pattern at a tertiary care teaching hospital in Coastal Karnataka, India. International Journal of Current Microbiology and Applied Sciences. 2016; 5(1):23-31.
26. Murthy PS, Ramalakshmi K, Srinivas P. Fungitoxic activity of Indian borage (*Plectranthus amboinicus*) volatiles. Food Chemistry. 2009; 114(3):1014-1018.
27. Bryce A, Hay AD, Lane IF, Thornton HV, Wootton M. Costelloe. Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by *Escherichia coli* and association with routine use of antibiotics in primary care: systematic review and meta-analysis. British Microbiology Journal. 2016; 352: i939.
28. Vazourasa K, Velalid K, Tassioue I, Anastasiou K, Athanasopoulou K, Barbouni A *et al*. Antibiotic treatment and antimicrobial resistance in children with urinary tract infections. Journal of Global Antimicrobial Resistance. 2019; 4-10.
29. Iloki-Assanga SB, Lewis-Luján LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL *et al*. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. Complementary and Alternative Medicine: BMC Research Notes. 2015; 8(1):396.
30. Ismayil S, Nimila PJ. Antimicrobial activity of *Plectranthus amboinicus* (Lour.) against gram negative bacteria *Klebsiella pneumoniae* and *Shigella flexneri* and their phytochemical tests. International Journal of Health Sciences and Research. 2019; 9(5):304-311.
31. Gupta SK, Negi PS. Antibacterial activity of Indian Borage (*Plectranthus amboinicus* Benth) leaf extracts in food systems and against natural microflora in Chicken Meat. Food Technology and Biotechnology. 2016; 54(1):90-102.
32. Vasconcelos SECB, Melo HM, Cavalcante TTA, Aragao, FE, de Carvalho MG, Menezes FGR, de Sousa OV, Costa RA. *Plectranthus amboinicus* essential oil and carvacrol bioactive against planktonic and biofilm of oxacillin- and vancomycin-resistant *Staphylococcus aureus*. BMC Complementary and Alternative Medicine. 2017; 17:462.
33. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017; 6(4):42.
34. Elmanama AA, Al-Reefi MR. Antimicrobial, anti-biofilm, anti-quorum sensing, antifungal and synergistic effects of some medicinal plants extracts. IUGNES, 2017; 25(2):198-207.
35. Bhatt P, Joseph GS, Negi PS, Varadaraj MC. Chemical composition and nutraceutical potential of Indian Borage (*Plectranthus amboinicus*) stem extract. Journal of Chemistry, 2013. Article ID 320329.