

Research Article

Study of Efflux Pumps in Multi-Drug Resistant Bacteria and the Investigation of Plant Extracts as Potential Inhibitors

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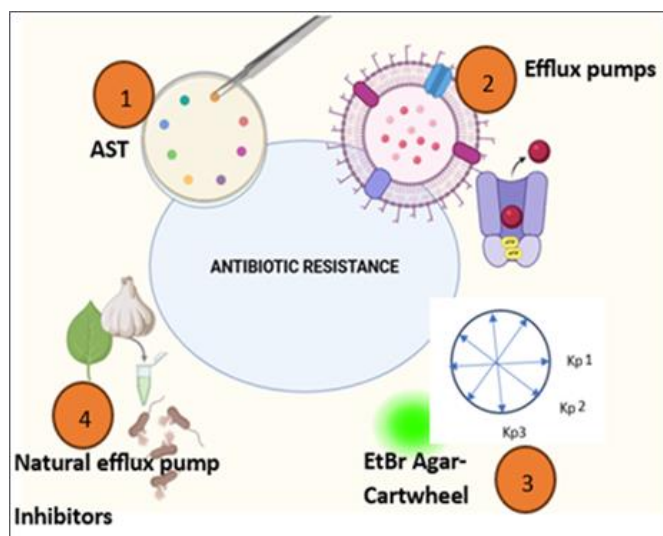


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Abstract— Antimicrobial resistance (AMR) is a rising global health concern, as bacteria continue to develop resistance to antibiotics through time. A very common resistance process in multidrug-resistant (MDR) bacteria is the efflux pump system that pumps antibiotics and other toxic substances out of the bacterial cell. Both gram-positive and gram-negative bacteria have efflux pumps, with gram-negative organisms having all types of efflux pumps. The chemical efflux pump inhibitors (EPIs) exist, but toxicity at higher doses restricts their utilization, emphasizing the search for plant-based alternatives. The objective of the study was to determine the presence of efflux pumps in MDR strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* using the Ethidium Bromide (EtBr)-agar cartwheel assay and investigate the role of certain Indian plants in inhibiting the efflux pump activity. The extracts obtained from the plants such as *Tagetes erecta* (Marigold), *Mangifera indica* (Mango) and *Allium sativum* (Garlic) were screened for their potential as a source of natural EPIs. Antibacterial action of these extracts was evaluated using agar cup and broth dilution assays to determine the Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of the potential extracts. The study illustrated that Garlic and Marigold plant extracts have the potential to be employed as MDR bacterial efflux pump inhibitors.

Keywords— Antimicrobial resistance, *Tagetes erecta*, *Mangifera indica*, *Allium sativum*, Multidrug resistant, Efflux pump, Efflux pump inhibitors

Graphical Abstract-



- EtBr-Agar cartwheel assay is a simplest method for the detection of efflux pumps phenotypically
- The most common gram-negative pathogenic bacteria associated with humans such as *Klebsiella pneumoniae*, *Enterobacter cloacae* possess efflux pumps and have emerged as multi-drug resistant (MDR) organisms
- The easily accessible Indian plants such as garlic, marigold have proven to be excellent source of efflux pump inhibitors

1. Introduction

Antimicrobial resistance (AMR) arises when microorganisms survive and multiply despite the use of drugs—such as antibiotics, antifungals, antivirals and antiparasitics—that are intended to eliminate or suppress them [1]. The trouble of AMR is very critical in bacteria [2]. According to Lancet reports published in 2019, bacterial antimicrobial resistance (AMR) was responsible for 1.27 million deaths across the globe. The overuse of many drugs happens as their sale is unregulated in many countries and they are available over the

counter without a prescription. This promotes the misuse of antibiotics as they are cheap, readily available, and abundant. By promoting genetic alterations like mutagenesis, horizontal gene transfer (HGT) and gene expression changes, the subinhibitory and subtherapeutic drug levels facilitate the development of antibiotic-resistant bacteria [3].

The widespread utilization of antibiotics also impacts the environmental microbial niches. Around 90% of antibiotics administered to livestock are excreted through urine and feces, eventually spreading widely into the environment via fertilizers, groundwater, and surface runoff. The antibacterial products utilized in hygiene or cleansing activities also form part of the AMR issue since they constrain the generation of immunities against environmental antigens in both kids and adults. This compromises the immune system versatility in individuals, increasing morbidity and mortality due to infections that might normally not be virulent [3].

Although there are different mechanisms in bacteria responsible for AMR, the efflux system is the most common mechanism in Multidrug-resistant (MDR) bacteria for the elimination of drugs. Due to this mechanism, the bacteria become resistant to multiple categories of drugs. In bacteria, these efflux pump genes can be either plasmid-encoded or chromosomally encoded. Efflux pumps in bacteria play a wide-ranging role and are crucial for pathogenicity, as well as for maintaining bacterial physiology and metabolic functions [4].

In antimicrobial resistance, efflux pumps can either target a specific antibiotic or expel multiple therapeutic agents from the bacterial cell [5]. These efflux pump genes are found in both antibiotic-sensitive and antibiotic-resistant bacterial strains. The increased efflux of drugs allows the bacterium to survive for a longer duration of time. This facilitates the buildup of bacteria with mutations in other genes, such as those encoding target proteins like topoisomerases, which are the binding sites for fluoroquinolones [6], [7].

There are five major families of chromosomally encoded efflux pump proteins. The ATP-binding cassette (ABC) transporters are active systems that utilize energy from adenosine triphosphate (ATP) to move substrates across cellular membranes. For example, the YbhFSR system in *Escherichia coli* is involved in tetracycline efflux and $\text{Na}^+(\text{Li}^+)/\text{H}^+$ exchange. The major facilitator superfamily (MFS) includes pumps such as NorA in *Staphylococcus aureus* and PmrA in *Streptococcus pneumoniae*. Other important families include the small multidrug resistance (SMR) system, the resistance-nodulation-cell division (RND) family—of which the AcrAB-TolC complex in *E. coli* is a well-known example found predominantly in gram-negative bacteria—and the multidrug and toxic compound extrusion (MATE) system, which is responsible for expelling toxic substances from the cell [8], [9].

Efflux pump inhibitors (EPIs) are substances that block the action of efflux pumps and are considered promising therapeutic agents for restoring the effectiveness of

antibiotics that have become ineffective against bacteria [7]. However, the chemical-based EPIs above certain concentrations are harmful to human cells, which limits their use. Hence, obtaining natural EPI alternatives such as plant-based EPIs has garnered the most attention. The phytochemicals are excellent alternatives as resistance-altering agents. The plants belonging to the families of *Apocynaceae*, *Cucurbitaceae*, *Fabaceae*, *Berberidaceae*, *Convolvulaceae*, *Lamiaceae* and *Zingiberaceae* are known to be an excellent source of EPIs [9].

Certain Indian medicinal plants are also known to be excellent sources of EPIs. The secondary metabolites produced by these plants include alkaloids, polyphenols, flavonoids, etc that act as EPIs [9],[10].

The purpose of this study was to detect the presence of efflux pumps in multidrug-resistant bacteria using the EtBr agar cartwheel assay. EtBr-Agar cartwheel assay is a simple method for phenotypic detection of the efflux pumps in both gram-positive and gram-negative bacteria. The fluorescent dye EtBr acts as substrate for the efflux pumps. The absence of efflux pumps leads to the retention of EtBr and thus the fluorescence of the bacterial cells when exposed to ultraviolet light. On the other hand, bacteria with efflux pumps extrude EtBr, leading to no fluorescence under Ultraviolet light [11].

The further experimentation involved the evaluation of the therapeutic potential of the crude extracts of the easily accessible Indian plants to study their activity as efflux pump inhibitors of MDR bacteria so that they can be used as therapeutic agents to treat infections.

2. Related work

Numerous studies have focused on understanding the genetic and functional aspects of efflux systems in MDR pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Among the most studied efflux pump families are the Resistance-Nodulation-Division (RND), Major Facilitator Superfamily (MFS), ATP-Binding Cassette (ABC), and Multidrug and Toxic Compound Extrusion (MATE) transporters, all of which contribute to bacterial survival in hostile antimicrobial environments [12].

The Ethidium Bromide (EtBr) cartwheel assay has been widely used for presumptive detection of over-expressed efflux pumps even in the clinical isolates, due to its simplicity and reliability [11].

Recent studies have increasingly explored natural products, especially plant extracts, as sources of novel EPIs. For instance, Akinpelu *et al.*, investigated the inhibitory effect of plant extracts on efflux pumps in *Klebsiella* species, a pathogen frequently involved in both community-acquired and hospital-acquired infections [13]. However, no studies have been reported on marigold, mango and garlic extracts as efflux pump inhibitors.

3. Materials and Methodology

3.1 Bacterial Isolates:

For this study, a total of seven bacterial isolates were used. Two clinical isolates, *CRE Pseudomonas aeruginosa* (CRE S from the Department of Microbiology, Nanavati Max Super Speciality Hospital, Mumbai. Three clinical isolates and one reference strain were obtained from the Department of Microbiology and Pathology, Breach Candy Hospital Trust, Mumbai. Among these were three strains of *Klebsiella pneumoniae* (n=3) and *Escherichia coli* ATCC 25922 respectively. These *Klebsiella* strains were named Kp1, Kp2 and Kp3. One bacterium initially named as the soil isolate, later identified as *Enterobacter cloacae/asburiae* using Matrix-assisted laser desorption ionization spectrometry time-of-flight (MALDI-TOF) from SRL Diagnostics-Dr. Phadke Labs, Mahim was isolated from the soil sample collected from the ground nearby to the college.

3.1.1 Reference Strain:

Susceptible to most antibiotics and efflux pump negative strain: *Escherichia coli* ATCC 25922.

3.1.2 Isolation of bacteria from the soil:

The soil was collected from a place nearby to the college. After consecutive isolations on the Sterile Nutrient Agar medium, a pure culture was obtained.

3.2 Antimicrobial susceptibility testing using Kirby Bauer Disc Diffusion method:

As per the Clinical and Laboratory Standards Institute (CLSI) Guidelines, sterile Mueller-Hinton agar plates were used for the AST determination of the organisms. 24 hours old cultures were used for performing the AST. During the time of AST, the culture suspensions were made using sterile saline and the cell density equivalent to 0.5 McFarland tube was adjusted. These inoculums were then swabbed onto the respective sterile Mueller-Hinton agar plates by dipping the sterile cotton swabs in the respective culture suspension. After swabbing the cultures, the commercially available sterile antibiotic discs from HiMedia, Mumbai of the selected antibiotics—Ampicillin (AMP)- 10mcg, Azithromycin (AZM)- 15mcg, Cefuroxime (CXM)- 30mcg, Ceftazidime (CAZ)- 30mcg, Cefoxitin (CX)- 30mcg, Colistin (CS)- 10mcg, Kanamycin (K)- 30mcg, Meropenem (MRP)- 10mcg, Nalidixic acid (NA)- 30mcg, Polymyxin-B (PB)- 300mcg, Streptomycin (S)- 10mcg, Tetracycline (TE)- 30mcg, were placed on the agar as per the organism and the plates were incubated at 37°C for 24 hours.

The diameters of the zones of inhibition of the antibiotics were recorded after 24 hours of incubation and then compared with the standard CLSI chart to find whether the organism showed sensitivity, intermediate or resistance against a particular antibiotic [14].

3.3 Efflux pump detection using EtBr-agar Cartwheel assay:

For this, the cultures were inoculated into Luria Bertani (LB) Broth for 24 hours. LB broth is rich in nutrients and induces

the expression of efflux pump genes if present in an organism. The Tryptic Soy Agar (TSA) media was prepared on the same day as the experiment. After autoclaving the media, the Ethidium Bromide (EtBr) was incorporated into the media at concentrations ranging from 0.5mg/L to 2.5mg/L. The plates were covered with aluminium foil, as EtBr is light-sensitive.

The 24-hour-old culture suspensions were adjusted to a cell density equivalent to the 0.5 McFarland standard. The respective cultures were swabbed onto the EtBr containing media plates from the centre and extending to the edges in the form of a cartwheel. The cultures were also swabbed on a plate containing only TSA, which was considered the control plate [11], [15].

The plates were covered with aluminium foil and were incubated at 37°C for 16 hours. After the incubation period was over, the fluorescence of the cultures was observed under an Ultra-Violet (UV) transilluminator. The minimum concentration of EtBr at which fluorescent growth was seen for each culture was noted. Fluorescent growth was not exhibited by some of the cultures under UV up to a certain concentration of EtBr. This might be due to the presence of efflux pumps. Accordingly, the results were noted.

3.4 Plant extracts preparation:

The plants used for the study were *Tagetes erecta* (marigold) flowers, *Mangifera indica* (mango) leaves and *Allium sativum* (garlic) cloves.

All the above plant samples except for garlic were collected and shade-dried for a week. Following drying, the samples were collected and processed into a fine powder using a mixer grinder. Approximately 10 grams of the powder were taken and subjected to alcoholic extract preparation using the Soxhlet extractor. Methanol was used as a solvent for obtaining the marigold extract and ethanol was used as a solvent for obtaining the mango leaves extract. Five extraction cycles were carried out for obtaining the extracts. The extracts were collected into sterile petri plates (weight measured) and covered with aluminium foil. For the evaporation of the alcohol small holes were made on the aluminium foil cover and kept at 37°C to obtain the semi-solid extracts. The dried extracts were measured to know the concentrations obtained. The obtained powders were then dissolved in a minimum amount of 10% DMSO and used for further tests. The extracts were then refrigerated at 4°C [9],[10],[17].

A day prior to the experiment, garlic aqueous extract was prepared by crushing chopped garlic and filtering the juice through a muslin cloth. The filtrate was collected in a sterile screw-cap bottle and stored at 4°C. The sterility testing of the extracts was performed.

These obtained extracts were considered as their 100% concentration.

3.5 Screening the antibacterial activity of the plant extracts using Agar cup method:

The antibacterial activity of the above mentioned plant extracts (at 100% concentrations) was determined against all the MDR cultures irrespective of the presence or absence of efflux pumps. 24 hours old cultures were used to perform the agar cup method. Prior to the experiment, culture suspensions were prepared using sterile saline, and the cell density was adjusted to the 0.5 McFarland standard. The sterile Mueller-Hinton agar butts were bulk seeded with 0.3 ml of the respective cultures and poured into the sterile petri plates [17],[18].

Following solidification of the media, wells were aseptically punched with a sterile cork borer and marked according to the extract. 50 µl of the prepared extracts were pipetted into the respective wells using a micropipette. 10% DMSO control was kept in the centre. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition around the wells containing the respective extracts were measured for each organism.

From the Agar cup method, the extracts showing antibacterial zones of inhibition against each culture were measured and the potential plant extracts i.e. garlic and marigold were selected for performing the MIC.

3.6 MIC determination of the potential plant extracts:

The dilutions of the garlic and marigold extracts were performed using sterile Nutrient broth (NB) as the diluent. A total of 2 ml volume of the system was decided and the dilutions of the garlic extract were prepared as 0.25%, 0.5%, 1% and 2%. Similarly, dilutions for the marigold extracts were 5%, 10%, 15% and 20%.

The suspensions of 24 hours old cultures were prepared using sterile saline. The cell density was adjusted to the 0.5 McFarland standard, and a loopful of each culture was inoculated into tubes containing the respective dilutions, followed by incubation at 37°C for 24 hours. Since the plant extracts themselves contributed to the turbidity of the medium, MBC determination was necessary [18], [19].

3.7 MBC determination of the potential plant extracts:

For this experiment, sterile Nutrient Agar plates were prepared and divided into grids and labelled as per the concentration and name of the extracts used to perform MIC. A loopful of each culture from the respective MIC tubes was inoculated into the designated columns, followed by incubation at 37°C for 24 hours.

After incubation, the concentrations of extracts at which no microbial growth occurred were noted for each organism, indicating the Minimum Bactericidal Concentration (MBC). [19].

3.8 Determination of the phytochemicals in the extracts:

3.8.1 Flavonoids test:

Few drops of dil. NaOH were added to 1ml of the respective extracts in test tube. Few drops of dil. HCl were added and the colour change was observed [20],[21].

3.8.2 Saponins test:

2ml of distilled water was added to 2ml of the respective extracts and the mixture was shaken. The appearance of foam was observed [21].

3.8.3 Steroid test:

10ml of chloroform was added to 1µl of the respective extracts in a test tube and mixed till dissolution. 10ml of conc. H₂SO₄ was added to the tubes from sides and the appearance of red coloration in the upper layer and yellow coloration with green fluorescence in sulphuric acid layer was observed [20],[21].

4. Results

After performing the AST, it was confirmed that all the organisms were MDR. The quality control strain *Escherichia coli* ATCC 25922 exhibited susceptibility to all tested antibiotics except it was resistant to Ampicillin (10 mcg).

From the EtBr Agar-cartwheel assay, it was observed that the cultures *CRE Klebsiella pneumoniae* (CRE Kp), *Klebsiella pneumoniae* 2 (Kp2) and the soil isolate exhibited minimal or no fluorescence under UV light at a concentration of 0.5 mg/L of EtBr whereas the other cultures showed the same fluorescence as that of the control strain *Escherichia coli* ATCC 25922. Further, it was observed that Kp2 and CRE Kp didn't show fluorescence even at a concentration of 1.0mg/L of EtBr. At 2.5mg/L concentration of EtBr, all the cultures showed fluorescence under UV.

From the agar cup method, it was found that the garlic clove extract inhibited the growth of almost all the MDR organisms, also those showing the presence of efflux pumps. The marigold flower extract was only effective against in inhibiting the growth of five strains, i.e., CRE Pa, CRE Kp, Kp2, Kp3 and *Escherichia coli* ATCC 25922. The mango leaf extract was ineffective in inhibiting the growth of any of the MDR organisms. Thus, garlic was further selected for determining their MIC and MBC of all the MDR organisms irrespective of the existence/absence of efflux pumps in few organisms. The MIC and MBC of the marigold extract was also determined based on agar-cup results against CRE Kp, CRE Pa, Kp2, Kp3 and *Escherichia coli* ATCC 25922.

From the MBC tubes containing the garlic and the marigold extracts, it was unclear to determine the bactericidal concentration by visualization. Hence, loopful of the media from every MIC tube containing garlic extract was taken and placed in the respective column. All the cultures showed growth at 0.25% and 0.5% of the concentration of the garlic extract. At 1% concentration of garlic extract, the growth of *Escherichia coli* and *CRE Klebsiella pneumoniae* (CRE Kp) was not observed. The growth of all the cultures was inhibited at 2% concentration.

In the case of the marigold extract, the growth of Kp3 and *Escherichia coli* ATCC 25922 was seen upto 5% of the concentration. The growth of both these organisms was inhibited at 10% of the extract. Thus, the MIC value of the

extract against the above cultures was determined to be 10%. For all the other cultures, the MIC value of the marigold extract was found to be 5% concentration of the garlic extract.

With the help of phytochemical tests, the presence of flavonoids, steroids and saponins was determined, all of which are known for their antibacterial activities.

5. Discussion

This information adds to the body of knowledge already available about bacterial efflux pumps, a major global problem right now. Although efflux pumps are the most common mechanism of antibiotic resistance, very few studies are carried out on this topic. Most of the time, routine analysis of efflux pumps is very difficult. The study regarding efflux pumps up to a genetic level, such as creating knock-out bacterial models, is a tedious task. Therefore, the EtBr-agar cartwheel method has so far proven to be the most effective phenotypic approach for detecting efflux pump activity.

A related study was conducted by Akinpelu *et al.*, focusing on the inhibition of efflux pumps in *Klebsiella*, a major cause of both community-acquired and nosocomial infections. The above study carried out in the Lagos University Teaching Hospital shows that the *Klebsiella* strains isolated were MDR, having efflux pumps that were also associated with the biofilm activity, which is yet another threat all around the globe. The current results obtained through the above study followed the same pattern in which it was found that the *Klebsiella* strains tested positive for the efflux pump activity. Thus, we can conclude that *Klebsiella* strains acquired from hospital samples have efflux pump activity, which contributes to the problem of MDR [13].

The Whole genome sequencing of extensive-drug resistant *Enterobacter* by Ferreira *et al.*, shows the presence of efflux pump genes [22]. Hence, efflux pumps are major contributors to the MDR *Enterobacter* strains.

Our study aligns with the study conducted by Sharma on the inhibitory effect of vegetable juices on MDR *Klebsiella*, whereby we observed the inhibition of MDR bacteria by easily available Indian plants i.e., garlic and marigold [23]. Similar inhibitory activity of pot marigold was observed against the gram-negative pathogen *Pseudomonas aeruginosa* in a study conducted by Pal [24].

The current study contradicts a study that was conducted by Osei-Djarbeng *et al.*, on the ethanolic mango plant part extracts in which different parts of the mango plant were used. The study showed that all the mango plant part extracts had antibacterial activity against most of the MDR strains of *Klebsiella pneumoniae*, *Escherichia coli*, etc., with the leaf and bark extracts showing the highest activity [25].

The search for efflux pump inhibitory molecules using plant extracts has been done widely. Some studies indicate that the most commonly found terrestrial plants are an excellent source of EPI. A study Brown *et al.*, shows the inhibitory

activity of the active molecules in the terrestrial plants against the *Staphylococcus aureus* efflux pump. The efflux pump inhibitory activity of the garlic extracts has been studied by Bruns *et al.* where they found that the main bioactive compound, allyl sulfide, was responsible for the inhibition of Emr-3 efflux in *Vibrio cholerae* [26],[27].

A study conducted by Rana *et al.*, exhibited synergistic activity of the methanolic extract of the Indian plant *Terminalia chebula* along with antibiotics against MDR *Klebsiella pneumoniae* strain possessing AcrAB-TolC efflux pump [28].

As per our study, it can be said that land plants are an excellent source of EPIs, as both garlic and marigold extract showed activity against the efflux-positive strains of *Klebsiella* and the soil isolate *Enterobacter cloacae/asburiae*.

Thus, these studies show that phytochemicals have proved to be a great alternative to the available drugs against MDR organisms and the efflux pumps.

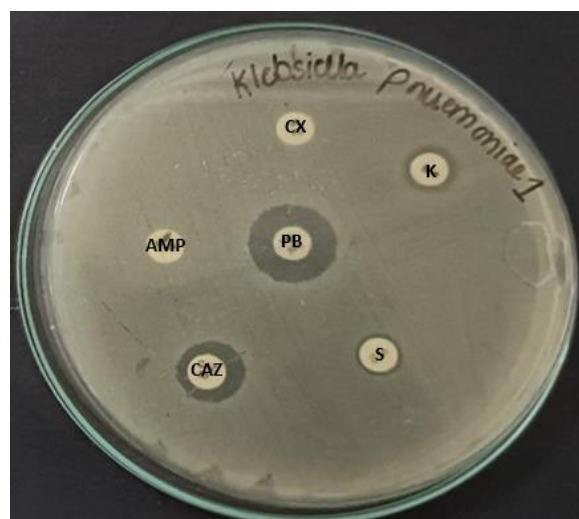


Figure 1: Antibiogram pattern of *Klebsiella pneumoniae* 1 (Kp1) (Kp1 shows resistance against all the test antibiotics except for Polymyxin B)

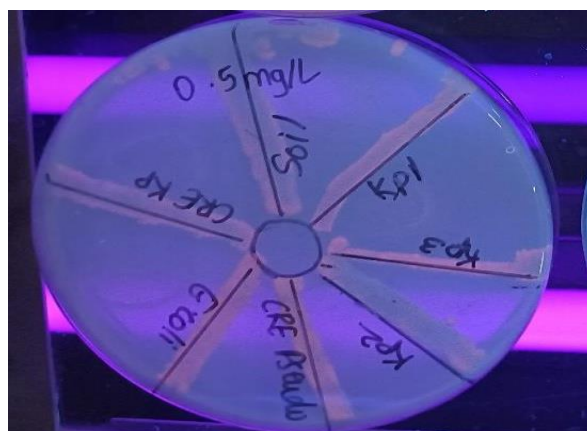


Figure 2: EtBr-agar Cartwheel Assay (0.5mg/L EtBr in TSA- the cells mass of CRE Kp, Kp2 and the Soil isolate *Enterobacter cloacae/asburiae* showing little or no fluorescent growth under UV light, rest all the cultures show fluorescence at this concentration of EtBr. This indicates that CRE Kp, Kp2 and the Soil isolate possess efflux pump)

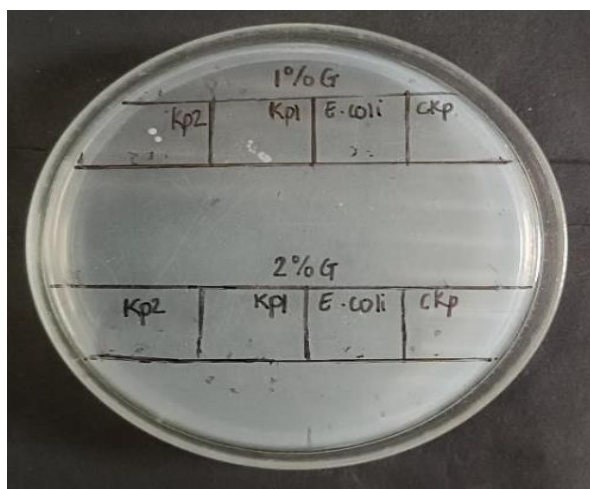


Figure 3: MBC determination of the garlic extracts against all the organisms (A loopful of the cultures was placed from the MIC tubes containing different concentrations of the garlic extract was placed on the respective grids sterile NA plate, at 1% concentration Kp1 and Kp2 showed growth. At 2% concentration of the garlic extract, the growth of all the cultures was inhibited)

Table 1: Interpretation of the EtBr-agar Cartwheel assay

Media	CRE Kp	Kp2	Soil Isolate	CRE Pa
TSA (Control)	-	-	-	+
TSA+ 0.5mg/L EtBr	-	-	-	+
TSA+ 1.0mg/L EtBr	-	-	+	+
TSA+ 1.5mg/L EtBr	+	+	+	+
TSA+ 2.0mg/L EtBr	+	+	+	+
TSA+ 2.5mg/L EtBr	+	+	+	+

***Key:**

‘-’ = No Fluorescence Observed under UV light, indicates the presence of efflux pump in the cell membrane of the organism

‘+’ = Fluorescence under observed UV light, indicates the absence of efflux pump in the cell membrane of the organism

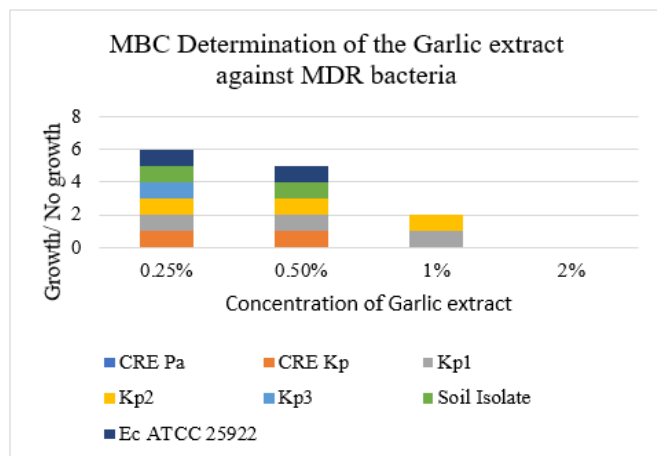


Figure 4: MBC determination of the garlic extracts against all the MDR bacteria (At 2% concentration of the garlic extract, the growth of all the cultures was inhibited)

6. Conclusion and Future Scope

The bacterial isolates obtained from the hospitals, along with the soil isolate, were found to be resistant to multiple antibiotics, indicating that MDR is a serious problem even in environmental niches. With the help of EtBr-agar Cartwheel

method, it was possible to screen for the presence of efflux pumps in the MDR strains.

Three organisms showed efflux pump activity, which includes CRE *Klebsiella pneumoniae* (CRE Kp) *Klebsiella pneumoniae* 2 (Kp2) and the soil isolate *Enterobacter cloacae/ asburiae*. By performing the agar cup method, the aqueous extract of garlic and the alcoholic extract of Marigold were found to be the most potent ones in inhibiting the growth of most of the MDR organisms under study. The MIC of both the potential extracts was determined using the broth dilution method for the efflux pump-positive cultures. From the MIC broth, the MBC of the potential extracts was also noted by spot inoculation on Nutrient Agar plates for the efflux pump-positive cultures.

From the MBC it was determined that even at 2% concentration of the garlic extract, the growth of bacterial strains having efflux pumps, i.e., CRE Kp, Kp2 and soil isolate was inhibited. Although, the marigold extract was not as effective as the garlic extract but at 10% concentration it was able to inhibit the growth of CRE Kp and Kp2.

The potential extracts showed the presence of antibacterial phytochemicals which also have efflux pump inhibitory activities. Thus, the above study suggests that plant extracts can be used as alternatives for antibiotics against the rising threat of MDR. The plant extracts are an excellent source of efflux pump inhibitors. From all the above results it can be concluded that the components of the garlic extract function as EPI against the efflux pumps found in CRE Kp, Kp2 and soil isolate. The components of the marigold extract act as an EPI against the efflux pumps found in CRE Kp and Kp2.

The future research can focus on isolating and characterizing the specific bioactive compounds in plant extracts responsible for efflux pump inhibition. Further performing the structural analysis and molecular docking studies may help identify key functional groups interacting with efflux proteins and thus help in developing new therapeutics against MDR bacteria.

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Authors' Contributions-

Author-1 researched literature, collected the samples, conceived the study and prepared the first draft of the manuscript. Author-2 was involved in data analysis and drafted the results. All authors reviewed, revised and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they do not have a conflict of interest.

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