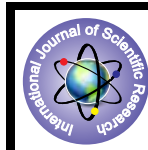


Piper Longum, Syzygium Aromaticum Extract as Supplementary Drug for Reversal of Plasmid Generated Multiple Drug Resistance.



Microbiology

KEYWORDS : Plasmid curing, Maceration, Piper longum, Syzygium aromaticum, MIC, MDR

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ABSTRACT

Multiple Drug Resistance (MDR) in microbial strains has become a severe health threat to human kind and one of the biggest challenges to global drug discoverer programs. MDR genes are usually located on R-plasmid. Present investigation is aimed to evaluate the activity of alcohol and aqueous extract of Piper longum and Syzygium aromaticum as a plasmid curing agent by supplementing it with drugs. study was carried out on Ampicillin resistant Escherichia coli which encodes resistance with plasmid encoded gene. Within this extracts, Piper longum shows 12% plasmid curing and Syzygium aromaticum shows 09% plasmid curing.

INTRODUCTION

Microbial resistance to antimicrobial agents is usually mediated by resistant gene-coded bacterial plasmids. These plasmids, called R-plasmids, harbor a variety of genes encoding resistance to a wide spectrum of antimicrobial compounds. Those microbes which are able to grow almost anywhere especially in a pressurized environment are resistant. With concern to this it may threaten the success of antimicrobial chemotherapy as antibiotic resistance causes great therapeutic and economic burden in the treatment of infectious diseases. It is estimated that antibiotic resistance increase the hospital stay and morbidity rate two-fold (Schelz et al., 2010).

The most important and problematic thing is that the MDR is directly transferred from one cell to another by horizontal gene transfer and it is related with the emergence of drug (Shriram et al, 2010). Because of this many clinical isolates now harbor the drug resistance for e.g. Clinical isolates of *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Shigella sonnei*, as well as some strains of *Escherichia coli* (RP4) and *Bacillus subtilis* (pUB110) harbor the plasmid originated drug resistance.(Patil,S.B et al). If we concentrate at this problem it seems to be a very huge problem and that's the reason why the scientific community is in search of new antimicrobial compounds .An alternative method to solve this problem is by plasmid curing. Plasmid curing is nothing but a process in which plasmid is removed from the bacterial cell by interfering in its replication, because of this some scientist are searching and using plasmid curing agents. Synthetically originated plasmid curing agents like Acridine dyes, Ethidium bromide and Sodium dodecyl sulfate exactly have the ability to cure the plasmid but are unsuitable for therapeutic application because they may be toxic or can cause mutations due to their toxicity or mutagenic nature. Thus, there is a constant need of identifying novel curing agents that which may have the ability to overcome these problems.

Piper longum and *Syzygium aromaticum* are such spices which contain some aromatic and multiple chain compounds (Manoj P et al 2004) and these aromatic compounds are integrated into the super coiled structure of the plasmid and inhibit their replication

without affecting chromosomal replication of host cell and thus show promising activity against MDR.(Manuel Viuda-Martos et al 2007) bacteria and cause reversal of antibiotic resistance. Indian spices are always stable into the GIT because of this if such content is supplemented with the drugs it will reverse the drug resistance.

Piper longum is one of the best spices, famously known as long pepper. It is found in various parts of India including evergreen forests from Konkan to Travancore regions of Western Ghats. The fruits of this plant are source of famous traditional drug Pip-pali .besides being used as a spices it is also used in the manufacture of pickles. It is known for curing cough, leprosy, diabetes, piles, cardiac diseases, chronic fever and to improve appetite as the plant has tremendous medicinal values. Various pharmacological activities including anti-allergy, antibacterial, anti-hepatitis and anti-tubercular have been reported from long pepper. However, we are trying it to add with the antibiotics and used to reverse the drug resistance by adding it with drugs.

MATERIAL AND METHODOLOGY:

Plant Materials:

Piper longum and *Syzygium aromaticum* material was collected from a local market at Kankavli and samples were authenticated by Dr. Balkrishna Ganpat Gawade from the Department of Botany at Kankavli College, Kankavli, India

Extraction of Plant Material:

The fruits brought from respective place were already traditionally dried, so the fruits were finely powdered with auto-mix blender which was followed by Maceration Technique in which 100 gm. dry powder of fruits was soaked in 250 ml Ethanol. The crude extract was prepared by cold percolation for 24 hours at room temperature (28 ± 2°C). The filtrate was concentrated by using traditional method of extraction in which filtration was done by muslin cloth and the extract collected alcohol was placed for evaporation for 24 hrs at the place where it may not be contaminated by other content. Such cycle was repeated for three times and the extract was collected into the clean and dry bottles for further use.

Bacterial strains:

The bacterial strains were obtained from the plasmid preparation kit of GanieprotX. *E.coli* was used as study organism for resistance, which has harbored plasmid, encoding for Ampicillin-resistance. The presence of the plasmid was confirmed by the plasmid extraction followed by electrophoresis.

Determination of minimal inhibitory concentration (MIC) and sub-inhibitory concentration (SIC):

The MIC was determined by nutrient broth supplemented with specified concentration of antibiotic and curing agent. Test bacterial cultures were collected from departmental cultures and incubated at 37°C for 24 h. MIC is the lowest concentration of antibiotic and plasmid curing agent at which the growth of micro-organism was inhibited and SIC was the highest concentration of antibiotic and plasmid curing agent which allowed the growth of micro-organism and thus the ability of the curing agent to cure plasmid was evaluated at SIC. (Per V. Nielsen et al 2000)

Reversal of drug resistance:

Reversal of plasmid-mediated antibiotic resistance was performed by following method. In short, the culture was grown in presence of a curing agent at Sub Inhibitory Concentration (SIC) for 24 h at 37°C and then spot inoculation method was used to obtain isolated colonies. These Isolated colonies were then transferred to the replica plate that of nutrient agar containing antibiotics and also to normal nutrient agar plate to determine the replica efficiency and replica loss. The colonies which grew on normal nutrient agar but failed to grow in presence of antibiotics were considered as putative cured derivative. (Adel Kamal Khder et al,2008)

Results and Discussion:

Recent years there was drastic increase in the number of, multiple drug resistant (MDR) in microbial strain towards the different drugs which is one of the biggest challenge to the human being because it simultaneously increases the need of the novel possible drugs to fulfill requirements against severe health threat to human-kind and one of the biggest topic for study. These problems are due to improper and inappropriate use of the drugs and antibiotics to cure the infections and strains with reduced susceptibility appear (Selim, 2012; WHO, 2012).

The genetic determinants that confer resistance to antibiotics are located in four different locations one chromosomal DNA, transposome, phages and plasmid, within them mostly it located on plasmids those plasmids are known as R-plasmids. These extra-chromosomal DNA sequences are often transferable to other bacteria in the environment and can be responsible for the emergence of resistance to multiple antibiotics and drugs (Schelz et al., 2006).

In present investigation aqueous and alcoholic extracts of *Piper longum* and *Syzygium aromaticum* were used for further experiments. MIC and SIC values were determined for Ampicillin.

TABLE NO – 1
EVALUATION INTERACTION OF THE DRUG WITH MICROBIAL SPECIES OF *E.COLI* K12 STRAIN

| Compound | MIC values (µg/ml) |
|---|--------------------|
| 1 <i>Piper longum</i> | 40 µg/ml |
| 2 <i>Syzygium aromaticum</i> | 50 µg/ml |
| 3 Ethyidium bromide | 20 µg/ml |
| 4 Ampicillin | 12 µg/ml |
| 5 Ampicillin + <i>Piper longum</i> | 12 µg/ml |
| 6 Ampicillin + <i>Syzygium aromaticum</i> | 12 µg/ml |
| 7 Ampicillin + Ethyidium bromide | 12 µg/ml |

TABLE NO-2**EVALUATION OF PLASMID CURING EFFICIENCY ALONG WITH AMPICILLIN RESISTANTE. *COLI* FROM THE GANIE-PROTX**

| Compound | Plasmid Elimination | Colonies examined |
|--|---------------------|-------------------|
| <i>Piper longum</i> (alcoholic extract) | 12% | 3000 |
| <i>Syzygium aromaticum</i> (alcoholic extract) | 09% | 3000 |
| 3 Ethyidium bromide | 60% | 3000 |

The extracts of *Piper longum* and *Syzygium aromaticum* cured the R-plasmids of the *Escherichia coli*. Unlike the other synthetic plasmid curing agents, which may be toxic the results obtained from this experiment may offer a new and safe source of plasmid curing agent which may have effective ability to cure plasmid and cause reversal of antibiotic resistance. The findings of present study hold importance as there are many known antibiotics which are no longer effective owing to resistant strains of bacteria. But the ineffective antibiotics can still be used if used with combination of plasmid curing agents like *Piper longum* and *Syzygium aromaticum* which can effectively cure the R-plasmid-encoded antibiotic resistance in *E. coli*.

CONCLUSION

From the above experiment, it can be concluded that alcoholic and aqueous extracts of the *Piper longum* and *Syzygium aromaticum* successfully reversed the Multiple Drug Resistance in the plasmid cured *E. coli* which made them susceptible to antibiotics/Drugs. Drug resistance reversal was only possible due to curing of plasmid from MDR harbored bacterial strains. These results are a result of the active bimolecular present in spices which have the potential to stop the plasmid-borne Multiple Drug Resistance (MDR).

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