

HERBAL EXTRACTION AND PLASMID CURING (CURING OF MULTIPLE DRUG RESISTANCE IN BACTERIA USING HERBAL EXTRACTS)

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SUMMARY

Due to overuse of antibiotics to treat microbial infections, microorganisms are becoming highly resistant to available antibiotics. So Multiple Drug Resistance (MDR) is a serious, global and future challenge to the chemotherapy or drug discovery programs. Genes responsible for resistance to antibiotics are present in plasmid DNA. Medicinal plants contain wide variety of active compounds such as terpenoids, alkaloids, sulphur compounds, phenols, esters, aldehydes, alcohols, glycosides, thymol are responsible not only for antibacterial, antifungal, anti-helminth, antioxidants, anti-inflammatory activities but also have R plasmid elimination property. So use of herbal extracts is effective and efficient method as herbal compounds are safer to use and not toxic like that of the chemical agents such as acridine orange,

ethidium bromide.

KEYWORDS: terpenoids, alkaloids, sulphur compounds, phenols, esters, aldehydes, alcohols, glycosides, thymol.

INTRODUCTION

The discovery of antibiotics in 1928 and effective and efficient use of antibiotics to control infectious agents begin in 1940.^[13] Due to overuse of antibiotics to treat microbial infections, microorganisms are becoming highly resistant to available antibiotics. Bacteria have evolved numerous defenses against the antibiotics, susceptibility to antibiotics get reduced now a days and drug resistant pathogens are on the rise.^[40] Immense clinical problems arise in immune

compromised and cancer patients due to MDR bacteria.^[34] So Multiple Drug Resistance (MDR) is a serious, global and future challenge to the chemotherapy or drug discovery programs.^[68]

Medical plants are used in the preparation of herbal medicines since ancient times, as they have antiviral, antifungal and antibacterial activity.^[3] With the development of new technologies, study of plants, isolation of active components, their role and pharmacological effects become easier. Medicinal plants contain wide variety of active compounds such as terpenoids, alkaloids, sulphur compounds, phenols, esters, aldehydes, alcohols, glycosides, thymol are responsible for antibacterial, antifungal, anti-helminth, antioxidants, anti-inflammatory activities.^[14]

Why plasmid curing is essential in drug resistance pattern

Phytochemicals have a novel anti-bacterial actions like inhibition of MDR efflux pump, anti-antibiotic resistance properties, R plasmid elimination. So, elimination of R plasmid containing genes are responsible for MDR is useful method.^[34] Genes responsible for resistance to antibiotics are present in plasmid DNA. Plasmids are self-replicating, extrachromosomal DNA molecules found in Gram positive, Gram negative bacteria, in some yeast with fungi. R plasmids contains variety of genes encoding resistance to a broad spectrum antimicrobial agents like antibiotics, heavy metals, disinfectant like formaldehyde, mutagenic agents like ethidium bromide. Resistance to antibiotics by R plasmid are due to conjugation.^[40] Gram negative microorganisms have developed coping mechanisms in order to survive in environments with high toxic levels of metals. Therefore they can be used as bio-indicators to detect heavy metal pollution in the environment. The survival of these organisms relies on the intrinsic biochemical, structural, and physiological properties and genetic adaptations.

There are generally four basic mechanisms used in heavy metal resistance and include. (i) exclusion of toxic heavy metal ions from the cell by the alteration of membrane transport systems involved in initial cellular accumulation, (ii) extra- and intra-cellular sequestration of metal binding components similar to metallothioneins. (iii) cation/anion efflux systems that are encoded by resistance genes, and (iv) enzymatic detoxification of heavy metals from toxic to less toxic forms.^[19] Resistance genes play important role in acquisition and dissemination by the exchanges between plasmid(s) and the bacterial chromosome and the integration of resistance genes into specialized genetic elements, called integrons.^[18]

Plasmid curing

Plasmid curing is a process of completely removing plasmids from bacteria by means of chemical agents or now a days by means of herbal extracts or phytochemicals. Synthetic plasmid curing agents like acridine orange, ethidium bromide, sodium dodecyl sulfate are toxic and mutagenic in nature. So, they are unsuitable for therapeutic application. But herbal Extracts are more effective in use, as they do not have toxicity and mutagenic effects.^[39]

Plasmids carry resistance genes which are mobile. During plasmid borne antibiotic resistance, the dosage and treatment is also important. Plasmid which have resistance genes are in competition with other plasmids that do not carry the resistance genes.^[74]

The mechanisms of microbial^[24]

The genetic material of one organism can be transferred to the other via conjugation, transduction and transformation.

The mutations occurs by interactions between the chromosomal DNA and the antibacterial agents.

Mechanism of Plasmid Curing

Metabolic function get regulated by bacterial plasmids. Genes responsible for antibiotic resistance could be readily removed by heterocyclic compounds; that bind to plasmid DNA and eliminate the plasmid DNA. Elimination of bacterial plasmids from bacterial species grown as pure or mixed bacterial is achieved in the presence of sub-inhibitory concentration of non-mutagenic heterocyclic compounds. Amphiphilic compounds having a planar ring system act as antiplasmid compounds with substitution in the L-molecular region. Antiplasmid effect achieved by a symmetrical π electron conjugation at the highest occupied molecular orbitals. Superhelical structure of plasmid DNA binds more to the heterocyclic compound than linear or open circular form; So it exhibit more antiplasmid activity and helpful to reduce the spread of antibiotic resistance plasmid in the ecosystem.^[71]

Table 1.1 Plasmid Curing using Different Plant Extracts

Sr No.	Name of microorganisms	Name of Plant	Plant part used for extraction	Type of Extract	Phytocompounds present	Reference
1.	<i>Staphylococcus aureus</i>	<i>Nigella sativa</i>	Leaves, flowers, fruits, seeds	Crude alkaloid, Distilled water	Thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene, α -pinene and thymol	Aftab <i>et al.</i> (2013)
2.	<i>Proteus mirabilis</i> , <i>Staphylococcus aureus</i>	<i>Cardamom (Elettaria cardamom)</i>	Seeds	Absolute ethanol, methanol, distilled water	Terpinyl acetate, cinole, monoterpenes including alcohols and esters	Akrayiet <i>al.</i> (2011)
3.	<i>Bacillus cereus</i> , <i>Escherichia coli</i> (ATCC25938)	<i>Coccinia grandis L.</i>	Leaves	Peroleum ether	Polyterpene hydrocarbons, tetraterpene carotenoid, isoprenoids	Bhattacharya <i>et al.</i> (2013)
4.	<i>E.coli</i> , <i>K.pneumoniae</i> , <i>Citrobacter spp.</i> , <i>P.aeruginosa</i> and <i>Proteus spp.</i>	<i>Trachyspermum ammi</i>	Seeds	Ethanol	Tannins, Terpenoids, alkaloids	Tariq <i>etal.</i> (2014)
5.	<i>Enterococcus faecalis</i> , <i>Escherichia coli</i> , <i>Bacillus cereus</i> and <i>E.coli</i> (RP4)	<i>Helicteres isora L.</i>	Fruits	Acetone	Quinolones	Shriram <i>et al.</i> (2010)
6.	<i>Escherichia coli</i> (ATCC10536), <i>Staphylococcus aureus</i> ATCC25923, <i>Pseudomonas aeruginosa</i> ATCC15442, <i>Candida tropicalis</i> ATCC40042, <i>Candida krusei</i> ATCC2538	<i>Richardia brasiliensis</i>	Leaves	Ehanol, Hexane	Coumarins, Flavonoids, Steroids, Alkaloids and Resin as secondary metabolite	Morais <i>et al.</i> (2013)
7.	<i>S.aureus</i>	<i>Rhus coriaria</i>	Dried plant	Distilled water	Sulphur compounds, Terpenes, Terpene derivatives, Phenols,esters,	Akrayiet <i>al.</i> (2013)

					aldehydes, alcohols, glycosides	
8.	<i>Enterococci</i>	<i>Eugenia jambolana</i>	Seeds	Methanol, acetone, hexane	Alkaloids, flavonoids, terpenoids, proteins	Jasmine <i>et al.</i> (2011)
9.	<i>Shigella sonnei</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhi</i>	<i>Piper longum</i>	Fruit pulp	Methanol, distilled water	Oxazolidinone linezolid	Kumar <i>et al.</i> (2013)
10.	<i>E. faecalis</i> , <i>S. aureus</i> , <i>S. sonnei</i> , <i>S. typhi</i>	<i>Alpinia galangal</i>	Rhizome	Methonal. Distilled water	-	Shriram <i>et al.</i> (2013)
11.	<i>Bacillus cereus</i> , <i>Escherichia coli</i> (ATCC 25938)	<i>Coccinia grandis L.</i>	Leaves	Petroleum ether	Isoprenoids, Tetraterpene carotenoid pigments	Bhattacharya <i>et al.</i> (2013)
12.	<i>E. coli</i>	<i>Quercus infectoria</i> , <i>Linum usitatissimum</i> , <i>Cinnamomum zeylanicum</i>	Sedds, Plant bark	Absolute ethanol	-	Khder <i>et al.</i> (2010)
13.	<i>Streptococcus pneumonia</i> , <i>Staphylococcus aureus</i>	<i>Thyme</i> , <i>Cinnamon</i> , <i>Clove</i>	Dried plant		Cinnamaldehyde, ethyl cinnamate, eugenol, β -caryophyllene, linalool, methyl chavicol, furfural, α pinene, 1,8- cineole, β - bisabolene, camphor, β -caryophyllene, methyl cinnamate	Zeid <i>et al.</i> (2010)

Methanol, Ethanol, Acetone, Petroleum ether, Hot and cold water extracts are used for testing their ability to cure antibiotic resistance by eliminating the plasmid DNA. To fractionate the extract, column chromatography was performed. Antimicrobial activity of the extract was determined by Kirby-bauer disc diffusion method, well diffusion method and also MIC, MBC and SIC were calculated for extracts by broth dilution method. Curing of plasmid DNA was performed. Phytochemical analysis of plant extracts were carried out according to standard methods to tests alkaloids, steroids, proteins, and terpenoids. For visualization and chemical composition determination NMR technique was used. Finally the physical loss of plasmid confirmed by agarose gel electrophoresis.^[14]

Applications of Herbal Extracts

Medicinal plants as a plasmid curing agents

Medicinal plants contain wide variety of active compounds such as terpenoids, alkaloids, sulphur compounds, phenols, esters, aldehydes, alcohols, glycosides, thymol are not only responsible for antibacterial, antifungal, anti-helminth, antioxidants, anti-inflammatory activities but also have plasmid curing activity against different multiple drug resistant organisms such as *Pseudomonas aeruginosa*, *Proteus spp.* and *E.coli* etc.^[14]

Medicinal plants as anti-ulcer agents

Peptic ulcer disease (PUD) is the common disease in the world-wide and its treatment with synthetic drugs contain active chemical constituents which have great importance. Peptic ulcer is a gastro intestinal disorder due to an imbalance between the acid, pepsin, bicarbonate secretion, gastric mucus. So, to maintain the balance between these factors, the herbal medicines are considered as treatment of peptic ulcer.^[72]

The antioxidant and DNA protection potential of Indian tribal medicinal plants

Medicinal plants have antioxidant property which play important role in inhibiting and scavenging free radicals and provide protection to human against infectious and degenerative diseases. Medicinal plants also have DNA protection potential that means phytochemicals present in plant extracts prevent DNA damage (Oxidative stress).^[30]

Plant profile, phytochemistry and pharmacology of Avartani (*Helicteres isora* Linn.):

Avartani (*Helicteres isora* Linn.) have hypolipidaemic, antibacterial, antiplasmid activity, cardiac antioxidant, antiperoxidative potency, brain-antioxidation potency, anticancer activities, antinociceptive activity, hepatoprotective activity, anti-diarrheal activity and wormicidal activity. Fruits, leaves, stem bark, seeds, roots contain different phytochemicals which are responsible for different pharmacological activities.^[40]

In-vitro antiviral activity of *Solanum nigrum* against Hepatitis C Virus-

Hepatitis C is a major health problem that causes liver cirrhosis, hepato cellular carcinoma and death. So there is a need for development of antiviral agents. Methanolic and chloroform extracts of *Solanum nigrum* seeds have efficient antiviral agents.^[75]

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