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REVIEW ARTICLE **OPEN ACCESS**

Phytoconstituents from Plant as an Efflux Pump Inhibiter to Reverse Multidrug Resistance in Bacteria: A Review

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ABSTRACT
The emergence of resistance against antibiotics is a serious problem faced nowadays. Universal health is under constant risk, due to use of antibiotics in larger amount. Bacteria are getting resistant to prevailing antibacterial agents. There is a rapid rise in bacterial strains with a massive rate of multidrug resistance in bacteria. Each antibiotic class can become susceptible to more than one single mechanism of resistance. The infected bacterium could develop non-susceptibility to multiple antimicrobial agents, leading to multidrug resistance (MDR). Certain plant extracts found to have the potential for containing such compounds based on their very significant synergistic activity with the antibiotics. Many of these Efflux pump inhibiters (EPIs) are well studied about their action on the specific pumps and the drug so effluxes. Some of the EPIs are broad spectrum while some narrow-spectrum acting only against one drug/EP family. Research is still ongoing in this direction to discover a novel, potent, broad-spectrum and a promising inhibitor. This review emphasizes on role of phytochemicals derived from plants to inhibit efflux Pumps.
Keywords: *MDR; efflux pump inhibitors; plant secondary metabolites*

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INTRODUCTION

In the today's scenario, treatment options for acquired infections by multi-drug resistant (MDR) bacteria are decreasing day by day, causing the concern for human health. The uncontrolled use of Antibiotics leads to development of MDR. Anew study shows that to limit antibiotic resistance rationalizing of antibiotic is necessary [2]. Due to antibiotic resistance, there will be challenges in managing the diseases at the grass root level and also public health care services will get hampered. Various bacteria adopt different mechanisms that help them show resistance towards antibiotics; these include modification of drug target, and inactivation of drug and eliminating antimicrobial agents from the cell interior to exterior environment using efflux pumps. Bacteria which shows Multi-drug resistance their efflux pumps are encoded chromosomally by relevant bacteria. Based on amino acid sequence similarity efflux pump transporters can be classified into five major families [3]. These are the ATP-binding cassette(ABC) superfamily, the resistance nodulation division superfamily, the major facilitator superfamily, the small multidrug resistance(SMR) superfamily, and the multidrug and toxic compound extrusion(MATE)family. All these families utilize the proton motive force as an energy source, apart from the ABC family which utilizes hydrolysis of ATP to exclude drug [3]. The consequences of inhibiting the efflux pumps of *P. aeruginosa* was determine by genetic approach undertaken by Lomovskaya *et al*. [4] inhibition markedly decreased MICs for both antibiotic-susceptible and resistant bacteria, reversed acquired resistance. *P. aeruginosa* mutant strains which are highly resistant to fluoroquinolones was markedly decreased. Hence it is very important to identify efflux pump inhibitors so that concentration of antibiotic can be increased inside the cell by doing so it will help to increase the life of existing antibiotics.

LITERATURE STUDY

EPI derived from plant source:

Living organisms such as plants produce certain Bioactive substances that provide protection against bacterial infections caused by various species, such as cytotoxic phytonutrients that facilitates vitality [5]. Synergism between these substances with antibiotics can be useful for rejuvenating the antibiotics

treatment of MDR bacteria. So many EPI's can be obtained from the phytoconstituents derived from plants.

Plant alkaloids:

Reserpine is isolated from the dried roots of *Rauwolfia vomitoria* and *Rauwolfia serpentina* and is most common known EPI of BMR pump of Gram-positive bacteria *Bacillus subtilis*. It is mainly involved in fluoroquinolone efflux [6]. In MRSA containing two isolates which have Tet(K) pumps reserpine shows a 4-fold decrease in the Minimum Inhibitory Concentration (MIC) for tetracycline and can remove resistance of Nor A efflux transporter in S.aureus, which resembles to BMR about 44% of sequence. The expression of BMR protein pump of B. subtilis auguments susceptibility to fluoroquinolones and other structurally diverse molecules after the administration of reserpine [7]. The minimum concentration required for inhibition of NorA Pump is neurotoxic so it is not possible to use it at higherconcentrations which may be proven toxic at Pre-clinical study level[8]. Another example of Nor A alkaloid inhibitor is piperine obtained from plant piper nigrum (piperaceae) can reverse ciprofloxacin resistant strains of MRSA [9].

Phenolic metabolites:

A) Flavolignans:

The Barbeery plant has phytoconstituents flavolignan 5'-methoxy-hydnocarpin (5'-MHC) which is successful inhibitor of Nor A efflux pump. It results into accumulation and restoring the activity of the berberine antibiotic. The berberine shows minimum antibiotic activity when the MIC is high (256mg/L). In a synergistic study it is observed that MIC get decrease upto 16 mg/L when used in combination of norfloxacin and 5'-MHC [10]. At present, silibininis only the one which is used as an anticancer agent [11].

B) Methoxylated flavones or isoflavones:

Baicalein is anantibacterial flavone which is a trihydroxy compound which is extracted from leaves of thyme plant. Baicalein when combined synergistically with tetracycline or the β-lactam antibiotics increases the vulnerability of some isolates of MRSA. There arises complexity for obtaining assay results of antibiotic activity of MDR bacteria containing many inhibitors of efflux pump proteins [12]. The plants such as *Lupinus argenteus* and *Dalea spinosa* containing isoflavones boost the activity of antibiotic berberine, reducing MIC upto 16-fold via NorA [13].

C) Catechin gallates:

Catechin gallates which are basically polyphenols extracted from green tea, has been known reverse MRSA resistance [14].Gibbons and Kaatz see that when epicatechin gallate and epigallocatechin gallate combined with other each at 20 mg/L, the MIC of norfloxacin was decreased by 4-folds in the1199B isolate of *S. aureus* and S. epidermis. Itwas determined that both epicatechin gallate and epigallocatechin gallate shows diminished inhibitory activity against the NorA transporter while epicatechin gallate shows slightly higher potency than epigallocatechin [15]. Epicatechin gallate and epigallocatechin gallate at minute concentrations shown to inhibit the high binding affinity sites, showing improved efflux activity [15]. Epigallocatechin gallate show moderate increased in tetracyline activity by blocking Tet(K) pump in staphylococci species [16].

1) Phenolic diterpenes:

The phytoconstituents extracted from herb rosemary *Rosmarinus officinalis* are carnosol and carnosic acid, which are abietane diterpenes and can be used as EPI. Both enhances the activity of tetracycline and erythromycin when used against S. aureus having Tet(K) and Msr(A) pumps. The addition of carnosic acid and carnosol about 10 mg/Lof tetracycline boosted activity of antibiotic containing Tet(K) pump in an *S. aureus* strain. The MIC of erythromycin has been decreased from 256 to 32mg/L in strains which express the Msr(A) efflux pump transporter such as RN4220 [17]. Apart from that other active phenolic diterpene is totarol which is an EPI against MDR strains of *S.aureus*. Smith *et al*. study shows prominent decrease in ethidium bromide efflux activity with totarol when used synergistically with totarol-resistant mutant strain which overexpresses Nor A. Totarol not only reduces the MIC of ethidium bromide but also moderately acts as an active antibiotic [18].

2) Polyacylated Neohesperidosides

The Polyacylated Neohesperidosides is the presumed inhibitors of Nor A pump extracted from Geranium caespitosum. The compound Polyacylated Neohesperidosides which can strengthen the activities of ciprofloxacin and norfloxacin as well cassic acid an antibacterial constituent found in rhubarb Still, these pump inhibitors do not have appropriate inhibitory action when used in combination with berberine towards the 8325-4 wild-type strain of *S. aureus* [19].

Essentials oil as EPI:

The bacteria can efflux a wide variety of different compounds which includes synthetic antibiotics. The essential oils can be effectively use to inhibit the multidrug resistant bacterial efflux pump of several

bacteria [20]. Lorenzi *et al*. (2009) estimated that phytoconstituent in the essential oil of *Helichrysum italicum* diminishes chloramphenicol resistance to the multi-drug resistant *Enterobacter aerogens* which not only overexpresses efflux pumps but also modifies the intrinsic resistance gram-negative bacteria [21].The essential oil present in *Rosmarinus officinalis* and ciprofloxacin when used in combination against gram-positive bacteria show less activity while *Rosmarinus officinalis*/ciprofloxacin when used against gram-negative bacteria shows a promising synergistic activity [22].

FUTURE ASPECTS

MDR due to the mechanism of efflux pumps adopted by bacterial strains is a growing clinical problem, causing many antibiotics to be nearly ineffective. The solution is to go for research for the antibiotics with alternative mechanisms to overpower the spread of MDR bacteria. It is better to have a different approach to search for molecules have the capacity to inhibit the efflux process. There is no such EPI's or any combination of EPI/antibiotics currently available in the market. The research to recognize potential EPIs is going on both in academic organizations and in the pharmaceutical industry [23, 24]. EPI act by increasing the concentration of antibiotics inside the cell which can help in tackling antimicrobial resistance. The molecular mechanisms of antimicrobial resistance can be studied by these compounds which can behave as the chemical tool, mainly in Gram-negative bacterial strains.

CONCLUSION

- Antibacterial drug-resistant Bacteria are posing a major hindrance in successful therapeutic treatment required for bacterial diseases. Hence, detail study of secondary metabolites with antibacterial activity from plants resources urges immediate attention.
- Phytoconstituents from plants having antibacterial and antifungal properties is best approach for developing of bioactive products.
- Plants are rich in a plethora of Phytochemicals, including alkaloids, flavonoids, terpenoids tannins which have been found in vitro to have anti-microbial properties.
- These studies characterize to select a molecule having maximum inhibitory efflux pump activity belonging to the indicated categories and will become useful curative tools in the near future.

In conclusion now days, People are showing more awareness towards herbal – based medicines due to presence of antibacterial properties. Still numerous studies still need to be done in order to know the actual mechanism and the safety of antimicrobial Phytoconstituents.

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