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Phytoconstituents as an EPI in antimicrobial resistance

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Abstract
The emergence of multidrug resistance among bacteria is a burning issue nowadays, and demanded for the discovery of the potential chemicals to deal with that resistance problem. Among several mechanisms of acquiring resistance, the over-expression of efflux pumps is very important. Efflux pumps can efflux out a large number of structurally unrelated drugs making them ineffective, which illustrates the importance of efflux pump inhibitors. Here we review the literature on efflux pump inhibitors (EPIs) from the plant sources, which will help to regain the activity of the existing antibiotics. The discovery of the new classes of natural EPIs demands further studies to explore their potential to work in synergy with existing antibiotics.

Keywords: Bacterial efflux pumps, Membrane permeability, MDR, Antibiotic resistance; Plant compounds; Secondary active transporter

Introduction
It is now known that 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 also contributes to the fact that these "ESKAPE" pathogens [1], S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter species, extended here to "ESKAPEE" to include E.coli, are showing increased numbers of multidrugresistantand in some cases pan-drug resistant isolates, making colonisation among patients more rapid and routine operations life-threatening. A recent study shows that the importance of rationalizing use of antibiotic to limit antibiotic resistance is necessary in India [2]. Due to antimicrobial resistance there will be a difficulty in controlling the diseases in the community and delivery of the health care services will be ineffective. Various bacteria adopt mechanisms that help them in resistance to antibiotics; these include target-site modification and antibiotic inactivation and efflux pumps. Multi-drug resistance efflux pumps are chromosomally encoded by relevant bacteria. Based on amino acid sequence homology bacterial efflux transporters can be divided into five main families [3]. These are the major facilitator (MF) superfamily, the resistance nodulation-division (RND) family, the small MDR (SMR) family, the ATP binding cassette (ABC) family and the multiple antibiotic and toxin extrusion (MATE) family. The first three families receive the energy required to extrude a drug out of the cell through the proton motive force in aproton-drug antiport system, while the MATE family gets energy by the exchange of either protonor sodium ions. In contrast, the ABC family causes drug extrusion with the hydrolysis of ATP [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, The emergence of P. aeruginosa mutants highly resistant to fluoroquinolones was markedly decreased. It is therefore very important to identify efflux pump inhibitors and, in so doing, increased the life of existing antibiotics.

EPI derived from plant source
Living organisms such as plants produce certain substances that provide protection against

bacterial infections caused by various species, such as cytotoxic phytonutrients that faciliates vitality [5]. Synergism between these substances with antibiotics can be useful for the treatment of MDR bacterial infections. So many EPI's can be obtain from the phytoconstituents derived from plants.

Plant alkaloids

Reserpine is extracted from the roots of Rauwolfia serpentine and R. vomitoria and is an effective EPI of tetracycline efflux via the BMR pump of Gram-positive Bacillus subtilis [6]. Reserpine directly binds to a BMR target site at two phenylalanine residues and one valine at 143, 306 and 286 positions respectively. It has been concluded that reserpine demonstrates a 4-fold decrease in the Minimum Inhibitory Concentration (MIC) for tetracycline in two MRSA isolates containing Tet(K) and can eradicate resistance of Nor A efflux transporter in S.aureus, which resembles to BMR about 44% of sequence. The expression in B. subtilis of the BMR protein pump thus potentiates susceptibility to fluoroquinolones and other structurally diverse molecules after the administration of reserpine [7]. As reserpine is widely used as antihypertensive the role of reserpine as EPI is however limited, due to the nephrotoxic concentrations needed for effective inhibition of Nor A which cannot be achieved [8]. Another example of Nor A alkaloid inhibitor is piperine obtained from plant piper nigrum (piperaceae) can reverse ciprofloxacin resistance in mutant strains of MRSA [9].

Phenolic metabolites

- Flavolignans
- The Berberis plant has phytoconstituents flavolignan 5'-methoxy-hydnocarpin (5'-MHC) which is a successful efflux inhibitor of the NorA protein pump. It results into accumulationand restoring the activity of the antibiotic berberine, which is produced by the same species of plant. The berberine shows weak antibiotic activity even when the MIC is high (256mg/L). In a synergistic study it is reduced to 16 mg/L when used in combination of norfloxacin and 5'-MHC [10]. Another combination effect of two diastereoisomers of the flavolignan silibinin, isolated from the Mediterranean milk thistle species, Silybummarianum which are inhibitors acting against NorA in wild-type S. aureus. Currently, silibinin has only been used as an anticancer agent [11].

- **Methoxylated flavones or isoflavones:**

- Baicalein is an example of a weak antibacterial flavone (MIC 100mg/L) which is a trihydroxy compound that is extracted from thyme leaves. Baicalein when administered in combination with tetracycline or several of the β-lactam antibiotics enhances the susceptibility of certain MRSA isolates. There arises certain complications when obtaining assay results of antibiotic activity containing many inhibitors of efflux proteins in MDR bacteria [12]. The plants such as Lupinusargenteus and Dalea spinosa containing isoflavones boost the activity of antibiotic berberine, reducing MIC upto 16-fold via NorA [13].

- **Catechin gallates**

Catechin gallates which are basically polyphenols extracted from green tea, known to proven to reverse MRSA resistance [14]. Kaatz and Gibbons showed that when epicatechin gallate and epigallocatechin gallate incorporated each at 20 mg/L, the MIC of norfloxacin was decreased by 4-folds in the1199B isolate of S. aureus as well as in S. epidermis. Itwas concluded both epicatechin gallate and epigallocatechin gallate have a weak inhibitory action towards the Nor A transporter, with epicatechin gallate acting with a slightly higher potency than epigallocatechin [15]. Epicatechin gallate and epigallocatechin gallate at low concentrations inhabit the high affinity sites, leading to improved efflux activity [15]. Epigallocatechin gallate show moderate increased in tetracyline activity by blocking Tet(K) pump in staphylococci species [16].

- **Phenolic diterpenes**

The phytoconstituents isolated from herb rosemary *Rosmarinus officinalis* are carnosic acid and carnosol, which are abietane diterpenes and can be used as EPI. They enhances the activity of erythromycin and tetracycline against the respective S. aureus containing Msr(A) and Tet(K)pumps. The combination of carnosic acid and carnosol about 10 mg/Lof tetracycline boosted antibiotic activity in an S.aureus strain containing Tet(K). Only carnosic acid diminished the MIC of erythromycin from 256 to 32mg/L in strains that express the Msr(A) efflux transporter such as RN4220 [17]. Another example of an active phenolic diterpene is totarol which is an EPI against MDR S. aureus. A study by Smith *et al*. revealed that noticeable decreases in ethidium bromide efflux by totarol at 15 μMinside a totarol-resistant mutant the overexpresses Nor A. Totarol not only reduces the MIC of ethidium bromide but also moderately acts as an active antibiotic [18].

- **Polyacylated Neohesperidosides**

The Polyacylated Neohesperidosides is the presumed inhibitors of Nor A protein extracted from Geranium caespitosum. The compound Polyacylated Neohesperidosides containing one additional ester group can strengthen the activities of norfloxacin and ciprofloxacin as well as an antibacterial constituent of rhubarb, named cassic acid. However, these inhibitors does not have direct inhibitory action when in combination with berberine against the 8325-4 wild-type strain of S. aureus [19].

- **Essentials oil as EPI**

The bacteria can efflux a large variety of compounds including synthetic antibiotics. Accordingly, it is believed that bacteria will not simply resist compounds which are natural as compared to the artificial compounds (the later classes of antibacterial agents). The essential oils can be effectively use in inhibiting the multidrug efflux pump and show its potential in inhibiting the pump activity against multi-drug resistant bacterial species [20]. Lorenzi *et al*. (2009) estimated that phytoconstituent in the essential oil of Helichrysum it a licumdiminisheschloramphenicol resistance of the multi-drug resistant Enterobacter aerogens that not only overexpresses efflux pumps but also modifies the intrinsic resistance gram-negative bacteria [21].The essential oil of Rosmarinus officnalis when combined with ciprofloxacin against gram-positive bacteria gave an opposed profile while Rosmarinus officnalis/ciprofloxacin against gram-negative bacteria shows a promising synergistic profile [22].

Future Aspects:

MDR due to the mechanism of efflux pumps adopted by bacterial strains is a growing clinical issue, causing many antibiotics to be nearly inactive. Either to go for research of

the antibiotics with new mechanisms of action to overpower the rise of MDR bacteria. It is better to have an alternative approach to find molecules that can 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]. Efflux pump inhibitors act by stimulating antibiotics to which many bacterial strains have become resistant and play a chief role 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 bacteria. Effluxpump activities can be blocked by interfering with the functional assemblies of efflux pumps' components.

Efflux pump activity may be bypassed or inhibited by employing a plethora of diverse approaches [25] including

i) Modifying the chemical structure of antibiotics to decrease their binding affinity to the transporter cavities; an approach which has been used for tetracycline antibiotics [26]

ii) Using permeabilizers of the bacterial membrane to artificially increase intracellular antibiotic concentration; this approach has been used for Mex AB-Opr M and Mex XY-Opr M efflux pump of P. aeruginosa [27, 28]

iii) Decreasing the number of active efflux complexes in the envelope of bacteria, by down regulating efflux pump gene expression or destabilising the protein component; like what has been applied for OmpF and OmpC [29]

iv) Destroying the drug transporter energy source, using potassium cyanide and carbonyl cyanide m-chlorophenylhydrazone (CCCP) affects the energy level of the bacterial membrane and reduces the efflux of various agents

v) Blocking the functional assembly of the components of efflux systems [30]

vi) Designing inhibitors that bind covalently to the substrate-binding cavities or block the channel of antibiotic transporter pumps; various natural products [31] and nanoparticles like zinc oxide [32] have been shown to inhibit bacterial efflux pumps by blocking them (a "molecular plug").

vii) Applying a decoy substrate as a competitive inhibitor for antibiotic transport inside the pump. Although there have been some encouraging results for EPIs derived from natural sources, there has been very little success in developing an efflux pump inhibitor that can be used clinically as an addition to antimicrobial therapy [33].

Conclusion

- The spread of drug-resistant microorganisms is posing a threat to successful therapy for microbial diseases. Therefore, it is the need of the hour to search for new molecules characterized by diverse chemical structures and mechanisms of action.
- The use of phytoconstituents as antibacterial and antifungal agents is an interesting stratergy for developing bioactive products.
- Plants are rich in a plethora of secondary metabolites, including flavonoids, alkaloids, tannins and terpenoids which have been found *in vitro* to have anti-microbial properties.
- There is a direct relationship between the chemical constituents of extracts derived from plant sources and oils and the antimicrobial activity, even if the comparison between the various literary data may be problematic, owing to the fact that composition of plant extracts and oils varies according to the local climatic and environmental conditions, and to the production of particular chemical compound from different plant species.
- These studies represent a good basis to select a particular molecule belonging to the indicated categories and will become useful therapeutic tools in the near future.

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