Review Article

A Review on Efflux Pump Inhibitors of Medically Important Bacteria from Plant Sources

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ABSTRACT

Antibiotic resistance has become a major clinical problem today. Multidrug resistance efflux pumps are an important cause of antibiotic resistance in bacteria. Large number of efflux pumps has been characterized so far. These pumps are responsible for the intrinsic resistance of bacteria to antibiotics. Lots of putative EPIs, both chemically and plant derived have been discovered till date against many important bacteria. Initially discovered EPIs have a drawback that they are toxic at the concentrations required for their EPI activity. Some plant extracts also are found to have the potential for containing such compounds based on their very significant synergistic activity with the antibiotics. Many of these 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 still continues in this direction with an endeavour to hunt a novel, potent, broad-spectrum and promising inhibitor in every way.

Keywords: Antibiotic resistance, Efflux pumps, EPIs, Bacteria, Broad-spectrum.

INTRODUCTION

Antibiotic resistance mechanisms reported in bacteria are causing a worldwide health problem. In MDR bacteria, the over-expression of efflux pumps contributes to the reduced susceptibility by decreasing the intracellular concentration of antibiotics.[1] Efflux pumps are transport proteins involved in the extrusion of toxic compounds like antibiotics and present in both type of bacteria i.e., Gram- positive and Gram-negative and even in some eukaryotic cells.[2] Active efflux is a wide-spread mechanism for bacterial resistance to antibiotics, which contributes to poor intrinsic susceptibility and cross resistance to structurally diverse classes of drugs.[3]

Efflux is the mechanism in which bacteria transport compounds outside the cell wall which are potentially toxic, such as drugs or chemicals.[4] Most of the efflux systems in bacteria are non-drug-specific proteins and can recognize and pump out a broad range of chemically and structurally unrelated compounds from bacteria in an energy-dependent manner.[5] Because of their overwhelming presence in pathogenic bacteria, these active multi-drug efflux mechanisms are a major area of intense study, so that measures may be discovered to inhibit these active multi-drug efflux pumps.[6]

Efflux pumps express their strongest intrinsic and acquired antibiotic resistance in Gram-negative bacteria, as this is attributed to the combined effect of the trans-envelope efflux and reduced uptake across the outer membrane.[7] To fight with drug resistance three methods can be employed: 1) the first approach to meet this situation is the development of new antibiotics. 2) An alternative therapy to treat antibiotic resistant microorganisms is the use of plant extracts. 3) As high level acquired resistance to conventional antibiotics is frequent, it is reasonable to use combination therapy in order to achieve bactericidal synergism. Plants derived antimicrobials have been found to be synergistic enhancers. Though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug.[8]

Efflux pump inhibitors (EPIs) are particularly the substances that give most promising approach in blocking the efflux pumps. They are the molecules which interfere with the process of removing toxic substances and antibiotics from the bacterial cell. Efflux pump inhibitors act as adjuvant to potentiate the activities of conventional antibiotics by inhibiting them either competitively or non-competitively.[9] Therefore an attempt has been made in this review to enlist the synthetic and plant derived EPIs discovered till date against Gram negative and Gram positive bacteria of human pathogenesis to the best of our knowledge.

Efflux Pumps in Bacteria

The first incident of resistance due to efflux was observed in *E. coli* against tetracycline.[10, 11] In bacteria there are five major families of efflux transporters: 1. MF (major facilitator), 2. MATE (multidrug and toxic efflux), 3.RND (resistance-nodulation-division), 4. SMR (small multidrug resistance), and 5.ABC (ATP binding cassette). All these families utilize the proton motive force as an energy source, apart from the ABC family, which utilizes ATP hydrolysis for the export of substrates.[12] MFS and RND are the most abundant pumps. MFS is found in both Gram positive and Gram negative bacteria while RND is found only in Gram negative bacteria.[13]

In Gram-negative bacteria, most of the efflux pumps that contribute to resistance to most antibiotics are three component structures that traverse both inner membrane & outer membrane. This structural organization allows extrusion of substrates directly into the external medium bypassing the periplasmic space and makes efflux pumps more efficient.[14, 15]

There are several modes of action of efflux pumps: a) The EPI may bind directly to the pump in a competitive or non competitive manner with the substrate, causing the blocking of the efflux pump; b) EPI may also cause a depletion of energy, through the inhibition of the binding of ATP or the disturbance of the proton gradient across the membrane; c) EPI may have affinity for substrates, and bind them, forming a complex that facilitates the entry of the drug in the cell and prevents its efflux.[16,17]

Efflux Pump Inhibitors: The use of efflux pump inhibitors can facilitate the re-introduction of therapeutically ineffective antibiotics back into clinical use and might even suppress the emergence of MDR strains.[18]

Synthetic EPIs against different bacteria: Synthetic compounds remain to be the major approach in finding bacterial efflux pump inhibitors, because little is known about substrate- pump binding interaction.

1. **L-phenylalanyl-L-arginyl-b-naphthylamide (PAβN)**:

It is a dipeptide amide and had potentiated the activity of levofloxacin by 8 fold at 10 µg/mL against *Pseudomonas aeruginosa.*[19] It also increases the susceptibility of erythromycin 8-32 fold and & rifampicin 8-64 folds against *Campylobacter jejuni* & *Campylobacter coli.*[20] It also inhibited the efflux pump in *E.coli* and reduced the susceptibility of rifaximin.[21] PAβN convertes ciprofloxacin resistant strains of *Pseudomonas aeruginosa, Acinetobacter baumannii* & *E.coli* to susceptible ones.[22] In combination with fluoroquinolones, it seems to have inhibitory activity against the MexCD-OprJ and MexEF-OprN pumps of *P. aeruginosa*, and against the AcrAB-TolC efflux pump of Gram-negative bacteria, including *K. pneumoniae*, *E. coli*, *S. thyphimurium* and *E. aerogenes.*[23-27]

2. **Arylpiperidines and arypiperazines:**

Some of the members of Arylpiperazines family are Capable of reversing multidrug resistance in *Escherichia coli* over-expressing RND Efflux Pumps, eg: 1-(1-Naphthylmethyl)-piperazine enhanced the susceptibility of *Escherichia coli* to fluoroquinolones and levofloxacin.[28]

3. **Quinoline derivatives:**

They have been proved as promising inhibitors of antibiotic efflux pump in multidrug resistant *Enterobacter aerogenes* isolates. Various quinoline derivatives significantly increased the intracellular concentration of chloramphenicol & thereby inhibit the transport of drug by AcrAB-TolC pump.[29]

4. **Alkoxyquinolone derivatives:**

Alkoxyquinolone derivatives such as 2,8-dimethyl-4-(2'-pyrrolidinoethyl)-oxyquinoline, inhibit efflux pumps in *E. aerogenes* and *K. pneumoniae.* This EPI increased the efficacy of chloramphenicol, norfloxacin, tetracycline and cefepime by up to 8-fold.[30]

5. **Carbonyl cyanide m-chlorophenylhydrazone (CCCP):**

CCCP is a protonophore. It considerably affects the energy level of the membrane and cell viability by causing a dissipation of the proton motive force of the membrane, affecting the transporters that depend on this mechanism. Besides its high toxicity for the cell, it is described as a substrate of bacterial efflux pumps.[31, 32] It has shown inhibitory activity in *Mycobacterium smegmatis* [33] and in *Mycobacterium fortuitum* by inhibition of the MFS efflux pump.[34]

6. **Phenothiazines:**

Thioridazine an EPI belongs to neuroleptic drugs, phenothiazines. Thioridazine is known due to its inhibitory effect on multidrug efflux pumps.[35] It inhibits efflux pumps in *M. tuberculosis.*[36] Phenothizines also act as EPIs against *S. aureus*, *B. pseudomallei, E.coli, P. aeruginosa* and *S. typhimurium.*[37, 38]

7. **Sodium Orthovanadate:**

Sodium orthhovanadate (Na_3VO_4) is an inhibitor of ABC efflux pumps of *Streptococcus pneumoniae*. It also abolishes both the efflux and resistance to ciprofloxacin & Ethidium bromide.[39]

8. **Amide derivatives:**

Two compounds of this family named 5,9-dimethyl-deca-2,4,8-trienoic acid amides and 9-Formyl-5-methyl-deca-2,4,8-trienoic acid enhance the activity of ciprofloxacin against *Staphylococcus aureus.*[40]

9. **Substituted Polyamines:**

N-benzylated polyazaalkanes and N-benzylated polyaminoalkanes have ability to behave as EPIs against *Haemophilus influenza.*[40]

10. **Nocardamines:**

They are iron chelators and acts as EPIs against TetB and TetK efflux pump of *Staphylococcus aureus.*[41]

11. **Arylated benzothiophenes and tiophenes:**

These compounds act as EPIs against NorA efflux pump of *Staphylococcus aureus* by restoring the activity of ciprofloxacin against a resistant *Staphylococcus aureus* strain in which this efflux pump is overexpressed.[42]

12. **Indole derivatives:**

Indole derivatives like INF-55 and INF-271 act as EPIs against NorA efflux pump of *Staphylococcus aureus.*[43] Indole derivatives like 3-amino-6-carboxyl- indole and 3-nitro-6-amino-indole had potentiated antibacterial effects

of chloramphenicol, tetracycline, erythromycin and ciprofloxacin against *E.coli* YD2 and FJ307 overexpressing AcrAB-TolC efflux pump and also decreased MIC at 2-64 folds.[44]

13. **GG918, biricodar (VX-710) and timcodar (VX-853):**

These are the compounds which show synergism with fluoroquinolones against *S. aureus, S. pneumoniae* and *E. faecalis* and also reduced the MIC of EtBr upto 2 to 31 fold against these three pathogens.[45]

14. **Verapamil:**

It is a drug used in the treatment of hypertension, cardiac arrhythmia & cluster headaches. It acts as an EPI against *Mycobacterium tuberculosis* and also enhances the activity of isoniazid, rifampin and pyrazinamide.[46] It also acts as an EPI of LmrA efflux pump of *Lactococcus lactis.*[47]

15. **Phenylpiperidine selective serotonin reuptake inhibitors:**

P-SSRIs are inhibitors of MFS and RND efflux pumps of different Gram positive and Gram negative bacteria.[48] P-SSRIs particularly inhibited the NorA efflux pump of *Staphylococcus aureus.*[49]

16. **Valinomycin & Dinitrophenol (DNP):**

They are used to abolish completely the efflux of different molecules. DNP dissipate the proton-motive force of the membrane by modifying the trans-membrane potential. Whereas valinomycin dissipates the electrochemical gradients generated by K^+.[50, 31, 51]

Out of the EPIs discussed above, only few like PAβN and CCCP are found to be of some use and are the most common synthetic EPIs. PAβN is routinely used in the laboratories to indicate efflux-mediated antibiotic resistance in Gram-negative bacteria. However, it is not in clinical use due to toxicity and bioavailability issues.[52] It can be considered as a broad spectrum efflux pump inhibitor because it can restore the activity of unrelated antibiotics such as chloramphenicol and macrolides.[53, 54] CCCP is another important EPI which is an inhibitor of proton motive force in bacteria. Conventionally, 0.1 mM or 1mM CCCP with incubation time less than 10min is used to detect the bacterial efflux system.[55]

Table 1: Efflux pumps of some important pathogens[2, 115-117]

Efflux pump family	Nature of substrate	Antibiotics used	Bactria containing efflux pump
SMR	Lipophilic, multicationic Substrates	Tetracycline, erythromycin, sulfadiazine	*Staphylococcus aureus* & *Acinetobacter baumannii*
RND	Aphiphilic, charged substrates	Tetracycline, fluoroquinolone, erythromycin, rifampicin, β- lactam, fusidic acid, chloramphenicol, aminoglycosides	*Escherichia coli* & *Pseudomonas aeruginosa*
MFS	Amphiphilic, mono or dicationic substrates	Tetracycline, fluoroquinolone, erythromycin, lincosamides, rifampicin, pristinamycin, chloramphenicol, aminoglycosides	*Staphylococcus aureus* & *Escherichia coli*
ABC	Amphiphilic neutral or cationic substrates	Teracycline, fluoroquinolone, macrolids, lincosamides, rifanpicin, chloramphenicol, aminoglycosides	*Staphylococcus aureus* & *Lactococcus lactis*
MATE	Low molecular weight Cationic substrates	Norfloxacin, Fluoroquinolone, amioglycosides	*Staphylococcus aureus, Escherichia coli* & *Vibrio parahaemolyticus*

➢ **Plant Derived EPIs against different bacteria:**

Plants produce many cytotoxic compounds which protect them from pathogenic microbes that is the reason why very less infective diseases are seen in wild plants.[18]

Gram-positive bacteria

Multidrug resistant gram positive bacteria represent a major public health problem.[56] Gram positive cocci are a major cause of nosocomial and community acquired infections. They frequently show a high natural, intrinsic resistance to antimicrobials.[57]

***Bacillus*:** *Bacillus cereus* causes minority of food borne illnesses like severe nausea, vomiting and diarrhoea etc.[58] *B. subtilis* causes disease in severely immunocompromised patients, and can be used as a probiotic in healthy individuals.[59] Drug resistance due to efflux is a common problem in *Bacillus.* Reserpine a compound belonging to a class of rauwolfia alkaloids, first isolated from the roots of *Rauwolfia vomitoria* is used in the treatment of high blood pressure. It is also used to treat the patients in case of severe agitation in mental disorders and works by slowing down the activity of the nervous system, causing the heartbeat to slow and the blood vessels to relax.[60] Bmr efflux pump found in *Bacillus* is inhibited by reserpine. The antihypertensive reserpine was first shown to block Bmr-mediated multidrug resistance in *Bacillus subtilis.*[31,12] Chalcones belonging to flavonoids class of chemicals found mainly in plants like *Bridelia ferruginea Benth* and *Dalea versicolor* as natural

defence mechanisms is an inhibitor of NorA efflux pump.[61] These compounds, along with the stilbene, also increased the activity of tetracycline against *Bacillus cereus.*[62]

***Staphylococcus*:** *Staphylococcus aureus* is one of the most important community and major hospital-acquired pathogen.[63,64] *Staphylococcus aureus* is cause for concern due to its ability to acquire resistance towards the newest antibacterial drugs currently in the market.[65] Reserpine enhances the activity of fluoroquinolones on MDR Gram-positive bacteria, and also decreases the emergence of resistant mutant strains of *Staphylococcus aureus* and *Streptococcus pneumoniae.*[51,66,67,68] Although reserpine has been used to treat hypertension from a long time, it cannot be used in combination with antibiotics for the treatment of staphylococcal infections, as the concentrations required to inhibit NorA efflux pump are neurotoxic.[69] Caffeoylquinic Acids from *Artemisia absinthium* showed efflux pump inhibitory activity against Gram-positive pathogenic bacteria like *Staphylococcus aureus* & *Enterococcus faecalis.*[70] NorA efflux pump of *S. aureus* is inhibited by several natural products, such as the porphyrin pheophorbide and the flavoligan 5′-methoxy-hydnocarpin (5′-MHC), isolated from Berberis plant.[71,72] A study of *Geranium* has led to the isolation of acylated neohesperidosides an inhibitor of *S. aureus* NorA, from *Geranium caespitosum.*[73,74] The carnosic acid and carnosol are isolated from herb Rosemary (*Rosmarinus officinalis*) and potentiate tetracycline and erythromycin against *S. aureus* strains possessing the Tet(K) and Msr(A) efflux pumps, respectively.[75,76] *Dalea versicolor* 'mountain delight' contains phenolic metabolites that enhanced the activity of berberine, erythromycin and tetracycline against *S. aureus.*[77] The catechin gallates are a group of phenolic metabolites that was reported by Hamilton-Miller's group to reverse methicillin resistance in MRSA.[78,79,80] An extract of *Lycopus europaeus* (Lamiaceae) was investigated by Gibbons *et al.,* in 2003. Lipophilic extract of *Lycopus europaeus* caused a potentiation of tetracycline and erythromycin against strains IS-58 and RN4220 of *S. aureus* possessing multidrug efflux pumps Tet(K) and Msr(A), respectively.[81] Baicalein a trihydroxy flavone isolated from the leaves of the thyme (*Thymus vulgaris*), was identified as possessing a strong synergistic activity with tetracycline or the β-lactam antibiotics oxacillin, cefmetazole and ampicillin against MRSA.[82] Marquez *et al.*, in 2005 done a study on extract of *Jatropha elliptica* (Euphorbiaceae) and led to the isolation of the penta-substituted pyridine, 2, 6-dimethyl-4-phenyl-pyridine-3, 5-dicarboxylic acid diethyl ester, which is not antibacterial but does augment ciprofloxacin and norfloxacin activity against *S. aureus* SA-1199B.Study of *Ipomoea violacea* species by Pereda *et al*., in 2006, led to the isolation of three oligosaccharides exerting a potentiation effect of norfloxacin against the NorA overexpressing *S. aureus* strain SA-1199B.[83] Piperine, a major plant alkaloid isolated from the family Piperaceae including black pepper (*Piper nigrum*) and long pepper (*Piper longum*), has recently been reported to increase the accumulation of ciprofloxacin by *S. aureus.*[84] Salicylic acid, a phenolic compound present in many plants like *Salix alba*, has been proved to induce a reduction of both the antibiotic ciprofloxacin and MDR substrate ethidium bromide for *S. aureus.*[85] Several *Berberis* spp such as *Berberis repens, B. aquifolia, and B. Fremontii* produce an inhibitor of the *Staphylococcus aureus* NorA MEP identified as 5'-methoxyhydno-carpin (5'-MHC).[71] *Momordica balsamina* were evaluated for their ability to inhibit the activity of bacterial efflux pumps of Methicillin-resistant *Staphylococcus aureus (*MRSA). Some compounds isolated from *Momordica balsamina* significantly inhibited efflux of EtBr by MRSA.[86] Isopimaric acid isolated from *Pinus nigra* blocks the Nor (A) efflux pump of MRSA and shows synergism with reserpin.[87] According to Schmitz *et al*., 1998, Isoflavones isolated from *Lupinus argenteus* inhibit MDR pump in *S. aureus.*[68] Two flavonols from *Artemisia annua* potentiate the activity of berberine and norfloxacin against a resistant strain of *Staphylococcus aureus*, possessing the MDR pump.[88] Murucoidins from *Ipomoea murucoides* inhibits NorA efflux pump in *S. aureus.*[89]According to Silva *et al.,* 2009, Kaempferol glycoside from *Herissantia tiubae* inhibits norA efflux pump in *Staphylococcus aureus.*[90] A plant named *Persea lingue* also contains a compound Kaempferol-3-0-L-(2,4-bis-E-p-coumaroyl) rhamnoside which inhibits NorA efflux pump in *Staphylococcus aureus.*[91] Nor A efflux pump of *Staphylococcus aureus* is also inhibited by an active polyphenolic amide: N-trans-feruloyl 4'-O-methyldopamine present in *Mirabilis jalapa.*[72] Reserpine shows synergism with norfloxacin against *Staphylococcus aureus.*[92]

Some oils also have shown the EPI activity like grapefruit oil contains some of the components that act as potential modulators of efflux pumps in MRSA strains.[93] Except these EPIs discovered so far some plant extracts also have shown EPI like activity eg: Some Kuwaiti plants are known to produce piperidine alkaloids such as julifloridine, juliflorine and juliprosine, their methanol extract was identified to possess resistance-modifying activity by causing a reduction in MIC of norfloxacin against *S. aureus* 1199B.[94] According to the study done by Dickson *et al.,* in 2006 extracts of *Mezoneuron benthamianum* and *Securinega virosa* exerted a potentiation activity against fluoroquinolone, tetracycline- and erythromycin-resistant strains of *S. aureus.*[95] The methanolic extract of *Punica granatum* caused an increase in ethidium bromide uptake in *S. aureus* RN-7044, having an ethidium bromide efflux mechanism.[96] Ethanolic extracts of *Mangifera indica*, *Callistemon citrinus* and *Vernonia adoensis* are a potential source of EPIs against *Staphylococcus aureus, Bacillus cereus and Bacillus subtilis.*[97]From the review of previous literature it can be concluded that a large number of EPIs has been discovered against *Staphylococcus aureus*. Epigallocatechin gallate is the most abundant catechin in tea and is a potent antioxidant and used in the treatment

of cancer also acts as an EPI. It is obtained from *Camellia sinensis* and increases the activity of tetracycline upto four-fold against *Staphylococcus epidermidis.* [18]

***Lactococcus*:** *Lactococcus lactis* is generally considered to be non-pathogenic, but it appears that pathogenicity may be emerged.[98] Two types of efflux pumps are responsible for multidrug resistance in *Lactococcus lactis*, these are LmrA and LmrP. LmrP confers resistance to lincosamides, macrolids, streptogramins and tetracyclines. Verapamil and quinine inhibit the LmrP efflux pump competitively while nicardipin & vinblastin inhibit it non-competitively.[99] Reserpine is also able to inhibit LmrA efflux of *Lactococcus lactis.* [100,101]

Mycobacterium: *Mycobacterium tuberculosis* is one of the oldest and most common causes of infection and death in the World, *Mycobacterium avium* often causes blood infection in AIDS patients, and *Mycobacterium smegmatis* is also an opportunistic pathogen. The active multidrug efflux pump (EP) has been described as one of the mechanisms involved in the natural drug resistance in *Mycobacteria.*[13] Piperine an alkaloid responsible for the pungency of black pepper and long pepper was reported as an inhibitor of Rv1258c efflux pump of *Mycobacterium tuberculosis.*[102] Farnesol a natural 15-carbon organic compound is a colourless liquid extracted from oils of many plants has been reported as inhibitor of mycobacterial efflux pumps.[103]

***Enterococcus*:** Enterococci are gram –positive commensals that inhabit the gastrointestinal tracts of almost all animals. It can cause diseases like endocarditis, UTI (urinary tract infection) and surgical wound infections. EfrAB, an ABC multidrug efflux pump in *Enterococcus faecalis* is inhibited by reserpine.[104] Karavilagenin C a triterpenoid isolated from *Momordica balsamina* significantly inhibited efflux of *Enterococcus faecalis* ATCC 29212 (86). 4', 5'-O-dicaffeoylquinic acid (4', 5'-ODCQA), a caffeoylquinic acid from *Artemisia absinthium* is a pump inhibitor with a potential of targeting efflux systems in a wide panel of Gram-positive human pathogenic bacteria including *Enterococcus faecal.*[70]

Gram-negative bacteria

Multidrug-resistance phenotype is a very common problem in gram negative bacteria. As per the literature studied not so much of EPIs have been discovered against Gram negative bacteria as they contain, efflux pump complexes consisting of an inner-membrane pump, a periplasmic adaptor protein and outer-membrane channel, providing them an efficient means for the export of structurally unrelated drugs.[105] With a decrease in the number of new agents and in antibiotic development, there is a need to search the compounds that will restore the activity of previous antibiotics against gram negative bacteria.[106]

Very few compounds given below had so far proved to be working for the given Gram-negative bacteria.

Baicalein, a flavone is an efflux pump inhibitor isolated against efflux pumps of *E. coli* from *Thymus vulgaris.*[82] Isopimarane derivatives obtained from *Lycopus europaeus* act as efflux pump inhibitors against efflux pumps of *Enterobacter aerogenes.*[81] The obromine a bitter alkaloid isolated from *Theobroma cacao* plant had shown synergism with ciprofloxacin against RND efflux pump family of different Gram- negative bacteria like *Klebsiella pneumoniae, Salmonella* Typhimurium, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. Cathinone, a monoamine alkaloid isolated from *Catha edulis* shows synergism with ciprofloxacin against *Salmonella* Typhimurium.[107]

Some of the plant extracts has also shown EPI like activity against Gram negative bacteria eg: extracts of *Helichrysum italicum*, *Thymus maroccanus*, *Thymus broussonetii* and *Callistemon citrinus* showed synergistic activity when combined with different antibiotics and they contain some EPI-like compounds that inhibit the efflux pumps of *Pseudomonas aeruginosa*. [108,109] Extracts of *Commiphora molmol, Centella asiatica, Daucus carota,Citrus aurantium* and *Glycyrrhiza glabra* showed good activity against three strains of *Salmonella enteric* serovar Typhimurium that overexpress the AcrAB-TolC efflux protein.[110]

The chloroform extract of *Berberis aetnensis* had shown EPI activity against *E. coli* in combination with ciprofloxacin. Extracts of *Mellisa officinalis* & *Levisticum officinale* had shown activity against strains of *Salmonella* that overproduced AcrAB efflux pump. These extracts had also shown synergistic activity with ciprofloxacin.[106] Ethanolic extracts of *Mangifera indica*, *Callistemon citrinus* and *Vernonia adoensis* are a potential source of EPIs against *Pseudomonas aeruginosa* and *E. coli*.[97] According to the study done by Starvy *et al.*, 2007, chloroform extract of the leaves of *Berberis aetnensis* had shown synergistic activity with the ciprofloxacin against *E. coli* and *Pseudomonas aeruginosa*.

Methanol plant extracts of some Cameroonian spices like *Aframomum citratum*, *Dorsentia psilurus* and *cinnamomum zeylanicum* have shown synergistic activity with aminoglycosides against MDR phenotypes of *Enterobacter aerogenes* and *Klebsiella pneumoniae*.[111]

As evident from table no. 2 majority of compounds reported so far were discovered for *S. aureus*. Some of the given EPIs could be claimed as broad spectrum. Examples are: Reserpine, Chalcone, Totarol, Ferruginol etc.

Table 2: Efflux pumps, antibiotics involved and EPIs derived from plants for various different bacteria.

Bacteria	EPI	Antibiotic	Efflux Pump	Plant source	References
Bacillus subtilis	Reserpine	Tetracycline	Bmr	*Rauwolfia vomitoria*	31, 12
Bacillus cereus	Chalcone	Berberine, erythromycin and tetracycline	NorA	*Nicotiana tobacum, Dalea versicolor*	62
Streptococcus pneumoniae	Reserpine	Ciprofloxacin	NorA	*Rauwolfia vomitoria*	51, 66-68
Staphylococcus aureus	Reserpine	Norfloxacin, Tetracycline	TetK, NorA	*Rauwolfia vomitoria*	51, 66-68, 92
	Porphyrin, Pheophorbide	Ciprofloxacin, Norfloxacin	NorA	*Berberis aetnensis*	71, 72
	Polyacylated neohesperidosides	Ciprofloxacin, norfloxacin, rhein, berberine	NorA	*Geranium caespitosum*	73,74
	Carnosic acid & Carnosol	Tetracycline and erythromycin	Tet (K) & Msr (A)	*Rosmarius officinalis*	75, 76
	Chalcone	berberine, erythromycin and tetracycline	NorA	*Dalea versicolor*	77
	Epicatechin gallate & Epigallocatechin gallate	Norfloxacin	NorA	*Camellia sinensis*	78,79, 80
	Baicalein	Tetracycline	tetK	*Thymus vulgaris*	82
	Citropten and Furocoumarins	Norfloxacin	NorA, ermA, ermB	*Citrus paradise*	93
	Orizabin	Norfloxacin	Nor A	*Ipomoea violacea*	83
	Piperine	Ciprofloxacin	MdeA & Nor A	*Piper nigrum, Piper longum*	84
	Salicylic acid	Ciprofloxacin, Ethidium bromide	SarA	*Salix alba*	85
	Balsaminol, Balsaminagenin, Karavilagenin	AcrAB-TolC	NorA	*Momordica balsamnia*	86
	Isopimaric acid	-	Nor (A)	*Pinus nigra*	87
	Crysoplenol and Crysoplenetin	Berberine, Fluoroquinolones, Norfloxacin	NorA	*Artemisia annua*	88
	Murucoidins	Norfloxacin	Nor A	*Ipomoea murucoides*	89
	Kaempferol Glycoside, Tiliroside	Ciprofloxacin	Nor A	*Herissantia tiubae*	90
	Genistein, orobol, Biochanin	Norfloxacin, Berberine	-	*Lupinus argenteus*	118
	Galbanic acid	Ciprofloxacin, Ethidium bromide	-	*Ferula szowitsiana*	119
	Chrysosplenol-D	Berberine	-	*Artemisia annua*	88
	Orobol	-	NorA	*Lupinus argenteus*	120
	Biochanin	-	NorA	*Lupinus argenteus*	120
	Bonducillin	Berberine	-	*Caesolpinia digyna*	120
	Acetoxycavicolacetate	Ehidium bromide	-	*Alpinia galangal*	120
	Totarol	Ehidium bromide	-	*Chamaecyparis nootkatensis*	121
	Ferruginol	Norfloxacin, oxacillin	NorA	*Chamaecyparis lawsoniana*	122
	Olaanolic acid, ulvaol	-	-	*Carpobrotus edulis*	123
	Orizabin	Norfloxacin	-	*Ipomoea violacea*	83
	Harmaline	Ethidium Bromide	-	*Peganum harmala*	124
	Ergotamine	Norfloxacin	-	*Claviceps purpurea*	125
	Julifloridine, Juliflorine and Juliprosine	Norfloxacin	-	*Prosopis juliflora*	18
	Indoles, Indirubicin	Ciprofloxacin	-	*Wrightia tinctoria*	126
	Chalcone	Ethidium bromide	Nor A	*Nicotiana tobacum*	91
	Pterocarpan	Berberine	Nor A	*Dalea spinosa*	120
	Reserpine	-	LmrA	*Rauwolfia vomitoria*	100, 101
	Caffeoylquinic acids	-	NorA	*Artemisia absinthium*	70
Staphylococcus epidermidis	Epigallocatechin Gallate	Tetracycline	Tet(K)	*Camellia sinensis*	17
Mycobacterium spp.	Farnesol	Ethidium bromide	-	*Cymbopogon Citratus, Cyclamen*	103
	Myricetin,	Isoniazid	-	*Allium cepa*	127
	Quercetin	Isoniazid	-	*Allium cepa*	127
	Rutin	Isoniazid	-	*Dimorphandra mollis*	127
	Taxifolin	Isoniazid	-	*Sophora japonica*	127
	Isorhamnetin	Isoniazid	-	*Tagetes lucida*	127
	Kaempferol	Isoniazid	-	*Camellia sinensis*	127
	Baicalein, Biochanin A	Ethidium bromide	-	*Oroxylum indicum*	128
	Epicatechin	Isoniazid	-	*Camellia sinensis*	129
	Genistein	Ethidium bromide	-	*Glycine max*	128
	Resveratrol	Ethidium bromide	-	*Fallopia japonica*	128
	Plumbagin	Isoniazid	-	*Plumbago zeylanica*	130
	Sandaracopimeric acid	Isoniazid	-	*Juniperus procera*	130
	Totarol	Isoniazid	-	*Juiperus procera*	130
	Ferruginol	Isoniazid	-	*Juiperus procera*	130
	Curcumin, Demethoxycurcumin	Isoniazid	-	*Curcuma longa*	120, 129
	Piperine	Ethidium bromide	Rv1258c	*Piper nigrum, Piper longum*	102

Bacteria	EPI	Antibiotic	Efflux Pump	Plant source	References
Enterococcus faecalis	Karavilagenin C	-	-	*Momordica balsamina*	86
	Caeffeoylquinic acid	Berberine	NorA	*Artemisia absinthium*	70
Enterobacter cloacae	Theobromine	Ciprofloxacin	AcrAB-TolC	*Theobroma cacao*	107
E. coli	Baicalein	Tetracycline	Tet K	*Thymus vulgaris*	82
	Pheophorbide a	Ciprofloxacin	-	*Berberis aetnensis*	131
Salmonella typhimurium	Theobromine	Ciprofloxacin	AcrAB- TolC	*Theobroma cacao*	107
	Cathinone	Ciprofloxacin	AcrAB-TolC	*Catha edulis*	107
Pseudomonas aeruginosa	Pheophorbide a	Ciprofloxacin	MexAB-OprM	*Berberis aetnensis*	131
	Theobromine	Ciprofloxacin	MexAB-OprM	*Theobroma cacao*	107
Klebsiella pneumoniae	Theobromine	Ciprofloxacin	AcrAB-TolC	*Theobroma cacao*	107

Almost all of these compounds have demonstrated EPI activity in vitro and many of them proved to be of doubtful in clinical practice. Therefore they need to be evaluated further for their clinical potential. Here some broad spectrum natural EPIs have been discussed, the most popular one is reserpine. It is active against many different efflux pumps viz. NorA, TetK, Bmr in *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*. A major limitation of combining this EPI with drugs is that this needs to be used at higher concentrations which may be proved toxic at clinical levels.[3] Chalcone is also a well known natural EPI used against NorA efflux pump of *Bacillus cereus* & *Staphylococcus aureus* but it also has many drawbacks like poor efficacy & toxicity. Ferruginol isolated from *Juiperus procera* is used as EPI against *Mycobacterium* spp. It also acts against NorA efflux pump of *Staphylococcus aureus.* Totarol is an EPI which acts against *staphylococcus aureus.* It also acts against *Mycobacterium* spp. It has been reported as an EPI but its therapeutic use is questionable and its purification is laborious and time consuming.[3] The rich chemical diversity in plants promises to be a potential source of antibiotic resistance modifying compounds and has yet to be adequately explored for EPIs which are nontoxic at higher concentrations. Most of these EPIs had only demonstrated their activity in vitro, so further investigations are needed for evaluation of their clinical potential. As large number of synthetic and natural EPIs has been discovered, none have been approved for routine clinical use as a result of doubtful clinical efficacy and high incidence of adverse effects.

CONCLUSION

To date, no efflux pumps inhibitors has been licensed for use in the treatment of bacterial infections in human or veterinary settings, although research continues. In the treatment of bacterial disease cystic fibrosis, one drug development program involving co-administration of an EPI with an antibiotic agent has reached human clinical trials. In this trial, an aerosolized formulation of the EPI compound MC-601, 205 is being combined with ciprofloxacin for the treatment of pulmonary exacerbations in cystic fibrosis patients in a phase II trial being conducted by Mpex Pharmaceuticals.[112] In this disease, the most serious symptoms are observed in lungs, increasing the risk of bacterial infection of the bacteria like *B. Cepacia, P. aeruginosa* and *S. aureus.* [113]

Even if a glance is given on the literature of secondary metabolites of plants they also show activity against Gram positive bacteria and not against Gram negative bacteria because Gram negative bacteria have evolved effective barriers for all amphipathic compounds (cationic, neutral & anionic). In Gram negative bacteria an extra outer membrane is present which inhibits the entry of amphipathic compounds. While in Gram positive bacteria only a single membrane is present. So the entry of amphipathic compounds is easy in Gram positive bacteria.[114] Therefore there is a great need to explore novel plant sources for EPIs against Gram- negative bacteria.

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