Review Article

NATURAL PRODUCTS AS EFFLUX PUMP INHIBITORS ENHANCING THE ANTIBACTERIAL EFFICACY OF QUINOLONES

Mohan Arya[1], A.R. Nisha*[2] and V. Keerthika[3]
Department of Veterinary Pharmacology and Toxicology
College of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University
Mannuthy, Thrissur, Kerala, India

ABSTRACT

Antimicrobial resistance is a global pandemic and one of the biggest public health challenges. Efflux pumps are one of several antimicrobial resistance mechanisms. Efflux pumps are proteins that act by efflux or pumping substances out of cells. The major efflux pumps in bacteria include the ATP-binding cassette (ABC) Superfamily, Small Multidrug Resistance Family (SMR), Major Facilitator Superfamily (MFS), Multidrug and Toxic Compound Extrusion Family (MATE), and Resistance Nodulation Cell Division (RND). Quinolones are antimicrobials that inhibit DNA synthesis by inhibiting type IV topoisomerase and DNA gyrase of bacteria. Efflux-mediated quinolone resistance can happen by the overexpression of some efflux pump genes in the prokaryotes. The substrate for most of the efflux pumps is fluoroquinolones like ciprofloxacin and Norfloxacin. A novel strategy to combat antimicrobial resistance is by the use of efflux pump inhibitors which can effectively block the efflux pumps. Plant-derived phytochemicals are promising lead molecules in developing an efflux pump inhibiting drug and thereby combat quinolone mediated efflux resistance. This review discusses plant-derived EPIs that can be used to combat quinolone-resistant microorganisms.

Key words: Antimicrobial resistance, Quinolones, DNA gyrase,Topoisomerase, Efflux pump inhibitors

INTRODUCTION

Efflux pumps are proteins that act by efflux or pumping substances out of cells. Efflux pump systems are present in all organisms. Molecular genetic studies back up the idea that efflux pumps are ancient tools found in organisms especially in bacteria for dealing with the difficulty of living in toxic environments (Du *et al*., 2018). Efflux pumps are a type of membrane-bound transporter protein found in all living organisms that contribute to resistance to drugs used to treat various diseases (Pages *et al*., 2005; Zechini and Versace, 2009; Pasqua *et al*., 2019). Efflux

*Corresponding author, email: draryavet@gmail.com
[1]Postgraduate scholar
[2]Assistant Professor and Head
[3]Postgraduate scholar

pumps present in bacteria can pump out a wide variety of antibiotics and thus lead to the emergence of multidrug-resistant bacteria.

Thus, multidrug-resistant (MDR) efflux pumps are one of the most obvious examples of determinants conferring cross-resistance to various antibiotics in bacteria (Blanco *et al*., 2016). Bacterial resistance to antibiotics is caused by three main mechanisms: alteration of the target, enzymatic inactivation of the antibiotic, and reduced entry of antibiotic into the cell (Marquez, 2005). Some efflux pumps are selective in extruding specific antibiotics, but some efflux pumps can cause extrusion non-selectively leading to the emergence of multidrug-resistant strains. Same bacterial cells may possess multiple efflux transporters thereby conferring resistance to wide spectra of antibiotics (Markham and Neyfakh, 2001; Webber and Piddock, 2003). These pumps are coded by genes and most of the genes conferring antibiotic resistance are constitutively expressed and are present in the chromosome or plasmid of the bacteria (Li and Nikaido, 2004). The overexpression of the efflux pump protein or an amino acid substitution in the protein makes it more effective in causing antimicrobial resistance in efflux mutants (Zechini and Versace, 2009). The location or position of genes and their horizontal or vertical distribution determine the spread of efflux-mediated resistance. The transfer of these genes whether on the chromosome or mobile genetic elements such as plasmids, transposons, or integrons, or gene cassettes occur through horizontal gene transfer mainly via transduction, transformation, and conjugation (Butaye *et al*., 2003).

Efflux pump systems in bacteria

The drug efflux by bacteria was first described by McMurry *et al*. (1980), who found out the resistance in *E. coli* towards tetracycline were by extruding the antibiotic through plasmid-encoded proteins (Butaye *et al*., 2003; Blanco *et al*., 2016). Five distinct families of efflux pumps have been discovered in bacteria till now. They include the ATP-binding cassette (ABC) Superfamily, Small Multidrug Resistance Family (SMR) of the Drug Metabolite Transporter (DMT) superfamily, Major Facilitator Superfamily (MFS), Multidrug and Toxic Compound Extrusion Family (MATE) of the Multidrug Oligosaccharidyl-lipid Polysaccharide Flippase (MOP) superfamily and Resistance Nodulation Cell Division (RND) Superfamily (Lomovskaya and Watkins, 2001; Poole, 2002; Lynch, 2006). Among the five, the ABC transporter is a primary transporter and uses ATP as its energy source and the remaining four are secondary transporters and they derive energy from the exchange of protons (RND, SMR, MFS) or sodium (MATE) ions, the so-called proton motive force (PMF) (Askoura *et al*., 2011; Prasch and Bucar, 2015).

Among the two types of transporters, the secondary transporters play a role in efflux mediated resistance in prokaryotes, especially those involving the proton motive force have been extensively studied (Moreira *et al*., 2004). The SMR, RND, and MATE family efflux pumps are unique to prokaryotes whereas the remaining two are seen in both prokaryotes and eukaryotes (Lynch, 2006). The salient features of the five efflux systems are depicted in table-1. In Gram-positive bacteria, the

efflux mediated resistance is mainly conferred by the MFS superfamily of efflux pumps, and the most extensively studied one is the NorA efflux pump conferring resistance to fluoroquinolones, biocides, and dyes found in *Staphylococcus aureus*. In Gram- negative bacteria, the major contributor of efflux-mediated resistance is the RND family- the main efflux pumps in this family includes AcrAB-TolC in *E. coli* and MexAB-OprM in *Pseudomonas aeruginosa* (Marquez, 2005). Though efflux systems are associated with multidrug resistance, they function as host defense proteins in bacteria, conferring self-immunity to antibiotic-producing bacteria (Lynch, 2006).

Table 1. Efflux families in bacteria

Efflux pump family	Substrates	Examples	Organism
ABC Superfamily	Transport amino acids, drugs, sugars, ions, polysaccharides, and proteins	LmrA	*Lactococcus lactis*
MFS	Efflux of sugars, intermediate metabolites, anions, drugs, and bile salts	NorA, NorB	*Staphylococcus aureus*
SMR	Transport of cationic drugs or hydrophobic molecules	EmeR	*E. coli*
MATE	Efflux of cationic dyes, fluoroquinolones	MepA	*Staphylococcus aureus*
RND	Efflux of antibiotics, detergents, dyes, heavy metals, solvents, and many other substrates	AcrAB-TolC	*E. coli*

Quinolones

Quinolones are considered ideal antibacterials since their introduction in 1960. This may be because they conceivably offer significant attributes like high potency, the wide spectrum of activity, oral and intravenous formulations, high activity, strong bioavailability, high serum levels, wide distribution volume, and a high concentration in tissues (Andersson and MacGowan, 2003). They have got a basic chemical structure of the bicyclic ring and various manipulations and substitutions in the ring structure yielded various antibiotic agents derived from quinolones like fluoroquinolones (Kocsis *et al*., 2016). Nalidixic acid is the first quinolone to be discovered and was found to be an impurity in the processing of quinine, its parent molecule (Andersson and MacGowan, 2003). With the development of fluoroquinolones by substitution of fluorine group the antibacterial spectrum of quinolones got widened and the drug could be used against a wide spectrum of aerobic and anaerobic bacteria (Heeb *et al*., 2011). The positions in the bicycle ring-like C2, C3, and C4 cannot be changed since any substitutions in these positions may result in

the loss of activity. A cyclopropyl group at C1 and either a methoxy or methyl group at C5 and C8 are optimal. The plantar configuration of the compound is affected by any substitutions at C5 and C8. The substitution of fluorine at C6 may result in fluoroquinolones. Instead of fluorine, hydrogen and amino groups may also be attempted at C6 resulting in useful compounds (Peterson, 2001).

Mechanism of action of quinolones

Quinolones inhibit DNA synthesis efficiently by facilitating bacterial DNA cleavage in the DNA replication enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in bacterial death. The inhibition of DNA gyrase by quinolones is more pronounced in Gram-negative bacteria and type IV topoisomerase is more pronounced in Gram-positive bacteria (Oliphant and Green, 2002). DNA gyrase is an enzyme that introduces supercoiling into DNA using energy by hydrolysis of ATP and this enzyme is only present in bacteria (Fabrega *et al*., 2009). In contrast to gyrase, topoisomerase IV untangle knots that form in the bacterial chromosome as a result of simple cellular processes and decatenate daughter chromosomes after replication. Quinolones bind to the enzyme–DNA interface in the cleavage–ligation active site in a non-covalent manner. They increase the steady-state concentration of cleavage complexes by serving as a physical block to ligation as a result of this bond formation. When drug-stabilized gyrase or topoisomerase IV DNA cleavage complexes collide with replication forks, transcription complexes, or other DNA tracking systems, the complexes are transformed into permanent chromosomal cuts. Thus they inhibit DNA ligation. Since quinolones stabilize cleavage complexes by inhibiting DNA ligation, they additionally impede the overall catalytic functions of gyrase and topoisomerase IV, acting as catalytic inhibitors (Aldred *et al*., 2014).

Efflux mediated quinolone resistance

Quinolone resistance happens through a variety of mechanisms and clinical implications. During fluoroquinolone therapy, mutations can occur quickly, and this may be the most significant factor restricting the use of these antibiotics (Oliphant and Green, 2002). The main mechanisms that confer resistances in quinolones include the mutations altering the drug target and drug accumulation and acquisition of resistance conferring genes (Jacoby, 2005; Hooper and Jacoby, 2015). Since the target enzymes of the drug are present within the bacterial cell, the drug has to transverse across the bacterial cell. Any mutations or alterations may result in reduced intracellular drug accumulation or increased drug efflux (Correia *et al*., 2017). Efflux mediated quinolone resistance is alarmingly increasing day by day notably in *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections. The substrate for most of the efflux pumps are fluoroquinolones like ciprofloxacin and norfloxacin. Rarely moxifloxacin and sparfloxacin can be substrates (Hooper and Jacoby, 2016). The efflux determinants of fluoroquinolone resistance are mediated by the chromosomally encoded multidrug transporters (Poole, 2005). Gram negative bacteria that are shown to have fluoroquinolone resistance due to efflux include *Burkholderia cepacia*, *Campylobacter*

jejuni, *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Typhimurium, *Shigella dysentariae*, *Stenotrophomonas maltophilia*, *Vibrio parahaemolyticus*, and the anaerobe *Bacteroides fragile*. The efflux pump families like RND and MFS family are contributing more towards the fluoroquinolone resistance. The MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM and MexVW-OprM efflux systems belonging to the RND efflux family present in *P. aeruginosa* can attribute to intrinsic resistance to quinolones (Poole, 2000). A study by Taneja *et al*. (2015) in clinical isolates of *Shigella* strains found out that the overexpression of two genes *acrA* and *acrB* coding for the pump AcrA and AcrB are associated with fluoroquinolone resistance especially that of Ciprofloxacin. The RND efflux pump AcrAB-TolC is contributing to fluoroquinolone resistance among the bacteria of Enterobacteriaceae family including *E. coli, Klebsiella, Enterobacter and Salmonella, Cambylobacter* and *Acinetobacter* (Poole, 2000[a]). Among the Gram positive bacteria, the efflux mediated quinolone resistance is marked in *Staphylococcus aureus*. Besides, *S. aureus*, the efflux mediated resistance to fluoroquinolones are also found in *Streptococcus pneumonia*, *Enterococcus* and *Bacillus subtilis* (Poole, 2000[b]). In *S. aureus*, the efflux pump responsible for fluoroquinolone resistance belonging to MFS family is the Nor family efflux pump which includes the NorA, NorB and NorC (Costa *et al*., 2013). PmrA is the MFS efflux pump which is associated with fluoroquinolone resistance in *Streptococcus pneumonia* and BmrA is in the case of *Bacillus sp.* Besides, MFS, some ABC superfamily transporters are also mediating the fluoroquinolone resistance. The important ones are the efflux pumps of *Enterococcus faecalis* EfrAB and the LmrA pump of *Lactococcus lactis*. In *Mycobacterium sp.* LfrA efflux pump belonging to MFS is responsible for fluoroquinolone resistance (Poole, 2007).

Efflux pump inhibitors

In the current scenario of efflux-mediated resistance, a novel strategy to combat antimicrobial resistance by hindering efflux pumps becomes relevant. Efflux pump inhibitors (EPI) are substances that can inhibit or block efflux pumps via various mechanisms. The efflux pump action may be eliminated in a multitude of ways- (i) interfering with genetic regulation to down-regulate efflux pump gene expression, (ii) antibiotics that are no longer accepted as substrates are being revamped, (iii) hindering the assembly of functional efflux pumps, (iv) preventing substrate binding to the active site of the pump by blocking the pump and (v) collapsing the energy mechanism that powers these pumps (Sharma *et al*., 2019). Efflux pump inhibitors are molecules that block the efflux pumps by one of the above mechanisms, preventing drug transfer out of the cell and resulting in drug accumulation within the cell, potentially contributing to drug or antibiotic activity (Mohan *et al*., 2020). Even though the mechanism of action by which the EPI function is not clearly understood but it is proposed that the inhibitor molecule work by inhibiting energy dissipation and preventing direct binding. The EPIs can be classified based on their origin as plant-derived EPI,

EPIs from microbes, and Synthetic EPIs. This review mainly focuses on plant-derived EPIs.

Plant derived EPIs

Plants are being used for medicinal purposes since time immemorial. Now, it is the era of ethnopharmacology which deals with the use of plants and plant products as a potential drug candidate. Secondary metabolites or phytochemicals are active principles found in plants that have a range of activities such as antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties (Hussein and El-Anssary, 2019). Results of these studies suggest that the plant-derived phytochemicals are promising lead molecules in developing an efflux pump inhibiting drug. The efflux pump inhibitors function by inhibiting the efflux pumps to avoid substrate binding to the active site or collapsing the energy mechanisms responsible for powering the pumps (Sharma *et al*., 2019). Many researchers have developed novel plant compounds that are enhancing or combating the efflux-mediated resistance to fluoroquinolones. Handzlik *et al*. (2013) reviewed that the molecules like chalcones, piperine-like compounds, citral amide derivatives, N-cinnamoylphenalkylamides, dihydronaphthyl-, Indole-, 2-chloro-5-bromo-phenyl- or piperidine can be good candidates of plant-derived EPI targeting the NorA efflux overexpression in *S. aureus.* Studies conducted by Schmitz *et al*. (1998) revealed that reserpine, an alkaloid from the roots of *Rauwolfia serpentine* could inhibit the efflux pump and lower the MIC of quinolones like ciprofloxacin, sparfloxacin, and moxifloxacin up to four-fold. The study also envisaged that the effect of reserpine was more pronounced with hydrophilic drug-like ciprofloxacin than hydrophobic agents like sparfloxacin and moxifloxacin. Reserpine along with ciprofloxacin could potentiate the effect of antibiotics and combat the resistance against fluoroquinolone (Markham, 1999). Kumar *et al*. (2008) highlighted the efflux pump inhibiting the activity of the alkaloid piperine which could lower the minimum effective concentration of ciprofloxacin up to two to fourfold in NorA overexpressed strains of *S. aureus*. A study conducted by Vanishree *et al.* (2021) revealed that piperine could also lower the MIC of enrofloxacin and ciprofloxacin and could potentially inhibit AcrAB-TolC efflux pump in *E. coli*. Chan *et al*. (2011) established that at 16 µg/ml, baicalein could restore the antibacterial effects of ciprofloxacin against the overexpressed NorA efflux pump. The study conducted by Nisha *et al*. (2020) suggested that capsaicin is a promising molecule in combating the efflux mediated resistance conferred by the AcrAB-TolC efflux pump in *E. coli*. Lorenzi *et al*. (2009) reported that geraniol an essential oil could significantly restore the antibacterial activity of quinolones by inhibiting the AcrAB efflux pump and thereby lowering the MIC. Shiu *et al*. (2013) investigated the antibacterial activity of a product isolated from *Hypericum olympicum* and established that this product could inhibit the overexpressed NorA efflux pump in *S. aureus,* later the product was found to be Olympicin A. Piddock *et al*. (2010) studied the efflux pump inhibiting the activity of various compounds against the AcrAB-TolC efflux pump in Gram-negative clinical isolates and found that among the compounds tested Cathinone and theobromine could effectively lower the MIC of ciprofloxacin. A hypothesis was made by Musumeci *et al*.

(2003) that pheophorbide extracted from the plant *Berberis aetnensis* could potentially inhibit the MexAB-OprM efflux pump in *P. aeruginosa*.

CONCLUSION

Several pieces of research have been going on in identifying a potential plant molecule as EPI against Gram-positive bacteria. However, fewer compounds have been reported as effective against multidrug-resistant Gram-negative bacteria, possibly due to the complex cell wall structure that prevents the entry of plant molecules. There is a dire need in identifying potential molecules for combating the multidrug resistance in Gram-negative bacteria against the fluoroquinolones in this antibiotic era. More thorough studies are needed, including high-throughput screening aided by in silico methods for discovering the most potent plant EPIs and their related targets. Although the use of EPIs as therapeutic agents is fraught with difficulties, this should in no way diminish their usefulness or benefits. When assessing the potential of EPIs, it appears that, while using EPIs is an appealing method, it is still a long way off. Numerous holes must be filled, as well as a significant amount of ground to be covered. The EPIs' technical flaws and limitations require immediate attention.

REFERENCES

Aldred, K.J., Kerns, R.J. and Osheroff, N. (2014). Mechanism of quinolone action and resistance. *Biochemistry,* **53**: 1565-1574.

Andersson, M.I. and MacGowan, A.P. (2003). Development of the quinolones. *Journal of Antimicrobial Chemotherapy,* **51**: 1-11.

Askoura, M., Mottawea, W., Abujamel, T. and Taher, I. (2011). Efflux pump inhibitors (EPIs) as new antimicrobial agents against *Pseudomonas aeruginosa*. *Libyan Journal of Medicine,* **6**: 1-8.

Blanco, P., Hernando-Amado, S., Reales-Calderon, J.A., Corona, F., Lira, F., Alcalde-Rico, M., Bernardini, A., Sanchez, M.B. and Martinez, J.L. (2016). Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. *Microorganisms*, **4**: 1-19.

Butaye, P., Cloeckaert, A. and Schwarz, S. (2003). Mobile genes coding for efflux-mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. *International Journal of Antimicrobial Agents*, **22**: 205-210.

Chan, B.C., Ip, M., Lau, C.B., Lui, S.L., Jolivalt, C., Ganem-Elbaz, C., Litaudon, M., Reiner, N.E., Gong, H., See, R.H. and Fung, K.P. (2011). Synergistic effects of baicalein with ciprofloxacin against NorA over-expressed methicillin-resistant *Staphylococcus aureus* (MRSA) and inhibition of MRSA pyruvate kinase. *Journal of Ethnopharmacology,* **137**:767-773.

Correia, S., Poeta, P., Hebraud, M., Capelo, J.L. and Igrejas, G. (2017). Mechanisms of quinolone action and resistance: where

do we stand?. *Journal of Medical Microbiology,* **66**: 551-559.

Costa, S.S., Viveiros, M., Amaral, L. and Couto, I. (2013). Multidrug efflux pumps in *Staphylococcus aureus*: an update. *Open Microbiology Journal,* **7**: 59-71.

Du, D., Wang-Kan, X., Neuberger, A., van Veen, H.W., Pos, K.M., Piddock, L.J. and Luisi, B.F. (2018). Multidrug efflux pumps: structure, function, and regulation. *Nature Reviews Microbiology,* **16**: 523-539.

Fabrega, A., Madurga, S., Giralt, E. and Vila, J. (2009). Mechanism of action of and resistance to quinolones. *Microbial Biotechnology,* **2**: 40-61.

Handzlik, J., Matys, A. and Kiec-Kononowicz, K. (2013). Recent advances in multi-drug resistance (MDR) efflux pump inhibitors of Gram-positive bacteria *S. aureus*. *Antibiotics*, **2**: 28-45.

Heeb, S., Fletcher, M.P., Chhabra, S.R., Diggle, S.P., Williams, P. and Cámara, M. (2011). Quinolones: from antibiotics to autoinducers. *FEMS Microbiology Review,* **35**: 247-274.

Hooper, D.C. and Jacoby, G.A. (2016). Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. *Cold Spring Harbor Perspectives in Medicine*. **6**: 1-21.

Hooper, D.C. and Jacoby, G.A. 2015. Mechanisms of drug resistance: quinolone resistance. *Annals of the New York Academy of Sciences,* **1354**: 12-31.

Hussein, R.A. and El-Anssary, A.A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Journal of Herbal Medicine,* **1**: 11-30.

Jacoby, G.A. (2005). Mechanisms of resistance to quinolones. *Clinical Infectious Diseases,* **41**: 120-126.

Kocsis, B., Domokos, J. and Szabo, D. (2016). Chemical structure and pharmacokinetics of novel quinolone agents represented by avarofloxacin, delafloxacin, finafloxacin, zabofloxacin, and nemonoxacin. *Annals of Clinical Microbiology and Antimicrobials,* **15:** 1-8.

Kumar, A., Khan, I.A., Koul, S., Koul, J.L., Taneja, S.C., Ali, I., Ali, F., Sharma, S., Mirza, Z.M., Kumar, M. and Sangwan, P.L. (2008). Novel structural analogs of piperine as inhibitors of the NorA efflux pump of *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy,* **61**: 1270-1276.

Li, X.Z. and Nikaido, H. (2004). Efflux-mediated drug resistance in bacteria. *Drugs*, **64**: 159-204.

Lomovskaya, O. and Watkins, W.J. (2001). Efflux pumps: their role in antibacterial drug discovery. *Current Medicinal Chemistry***, 8:** 1699-1711.

Lorenzi, V., Muselli, A., Bernardini, A.F., Berti, L., Pagès, J.M., Amaral, L. and Bolla, J.M. (2009). Geraniol restores antibiotic activities against multidrug-resistant isolates from gram-negative species. *Antimicrobial Agents and Chemotherapy,* **53**: 2209-2211.

Lynch, A.S. (2006). Efflux systems in bacterial pathogens: an opportunity for

therapeutic intervention? An industry view. *Biochemical Pharmacology,* **71**: 949-956.

Markham, P.N. (1999). Inhibition of the emergence of ciprofloxacin resistance in *Streptococcuspneumoniae* by the multidrug efflux inhibitor reserpine. *Antimicrobial Agents and Chemotherapy,* **43**: 988-989.

Markham, P.N. and Neyfakh, A.A. (2001). Efflux-mediated drug resistance in Gram-positive bacteria. *Current Opinion in Microbiology,* **4**: 509-514.

Marquez, B. (2005). Bacterial efflux systems and efflux pumps inhibitors. *Biochimie*, **87**: 1137-1147.

McMurry, L., Petrucci, R.E. and Levy, S.B., 1980. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. *Proceedings of the national academy of sciences*, **77**: 3974-3977.

Mohan, A., Nisha, A.R. and Keerthika, V. (2020). A Review on Plant-Derived Efflux Pump Inhibitors Targeting nor An Efflux Pump in *Staphylococcus aureus*. *International Journal of Scientific Research in Science and Technology,***7**: 24-29.

Moreira, M.A.S., Souza, E.C.D. and Moraes, C.A.D. (2004). Multidrug efflux systems in Gram-negative bacteria. *Brazilian Journal of Microbiology,* **35**: 19-28.

Musumeci, R., Speciale, A., Costanzo, R., Annino, A., Ragusa, S., Rapisarda, A., Pappalardo, M.S. and Iauk, L. (2003). *Berberisaetnensis* C. Presl. extracts: antimicrobial properties and interaction with ciprofloxacin. *International Journal of Antimicrobial Agents*, **22**: 48-53.

Nisha, A.R., John, P., Vanishree, Keerthika, V. and Mohan, A. (2020). Effect of capsaicin in inhibiting efflux pump AcrAB-Tol C of tetracycline-resistant *Escherichia coli*. *The Pharma Innovation,***9**: 39-45.

Oliphant, C.M. and Green, G. (2002). Quinolones: a comprehensive review. *American Family Physician*,**65**: 455-465.

Pages, J.M., Masi, M. and Barbe, J. (2005). Inhibitors of efflux pumps in Gram-negative bacteria. *Trends in Molecular Medicine,* **11**: 382-389.

Pasqua, M., Grossi, M., Scinicariello, S. ,Aussel, L.,Barras, F., Colonna, B. and Prosseda, G. (2019). The MFS efflux pump EmrKY contributes to the survival of *Shigella* within macrophages. *Scientific Reports,* **9**: 1-9.

Peterson, L.R. (2001). Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity. *Clinical Infectious Diseases,* **33**: 180-186.

Piddock, L.J., Garvey, M.I., Rahman, M.M. and Gibbons, S. (2010). Natural and synthetic compounds such as trimethoprim behave as

inhibitors of efflux in Gram-negative bacteria. *Journal of Antimicrobial Chemotherapy,* **65**: 1215-1223.

Poole, K. (2000[a]). Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. *Antimicrobial Agents and Chemotherapy,* **44**: 2233-2241.

Poole, K. (2000[b]). Efflux-mediated resistance to fluoroquinolones in gram-positive bacteria and the mycobacteria. *Antimicrobial Agents and Chemotherapy,* **44**: 2595-2599.

Poole, K. (2002). Mechanisms of bacterial biocide and antibiotic resistance. *Journal of Applied Microbiology,* **92**: 55-64.

Poole, K. (2005). Efflux-mediated antimicrobial resistance. *Journal of Antimicrobial Chemotherapy.* **56**: 20-51.

Poole, K. (2007). Efflux pumps as antimicrobial resistance mechanisms. *Annals of medicine*, **39**: 162-176.

Prasch, S. and Bucar, F. (2015). Plant-derived inhibitors of bacterial efflux pumps: an update. *Phytochemistry Reviews,* **14**: 961-974.

Schmitz, F.J., Fluit, A.C., Lückefahr, M., Engler, B., Hofmann, B., Verhoef, J., Heinz, H.P., Hadding, U. and Jones, M.E. (1998). The effect of reserpine, an inhibitor of multidrug efflux pumps, on the in-vitro activities of ciprofloxacin, sparfloxacin, and moxifloxacin against clinical isolates of *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy,* **42**:807-810.

Sharma, A., Gupta, V.K. and Pathania, R. (2019). Efflux pump inhibitors for bacterial pathogens: From bench to bedside. *Indian Journal of Medical Research,* **149**: 129-145.

Shiu, W.K., Malkinson, J.P., Rahman, M.M., Curry, J., Stapleton, P., Gunaratnam, M., Neidle, S., Mushtaq, S., Warner, M., Livermore, D.M. and Evangelopoulos, D. (2013). A new plant-derived antibacterial is an inhibitor of efflux pumps in *Staphylococcus aureus*. *International Journal of Antimicrobial Agents,* **42**: 513-518.

Vanishree, Nisha, A.R., Juliet, S., Suja, R.S., and Thomas, N. (2021). Inhibitory effect of piperine on AcrAB-TolC efflux pump in fluoroquinolone-resistant *Escherichia coli* isolates from bovine mastitis milk samples. *Journal of Pharmacognosy and Phytochemistry,***10**: 319-327.

Webber, M.A. and Piddock, L.J.V. (2003). The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy,* **51**: 9-11.

Zechini, B. and Versace, I. (2009). Inhibitors of multidrug-resistant efflux systems in bacteria. *Recent Patents on Anti-Infective Drug Discovery*, **4**:37-50.