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RUTIN INHIBITS ACRAB EFFLUX PUMP OF *ESCHERICHIA COLI*

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ABSTRACT

The AcrAB efflux pump of *E.coli* is comprises of an RND- type transporter AcrB and a accessory protein AcrA. Efflux pump inhibitors are captivating compounds which helps in reversing multidrug resistance in bacterial pathogens. In the present study we tested the ability of rutin a flavonoid to inhibit AcrAB efflux pump of *E.coli*. A ethidium bromide accumulation assay and berberine assay study suggest that rutin is capable of inhibiting the AcrAB efflux pump. Rutin inhibits the pump at a concentration of 1000µg/ml. only ciprofloxacin and tetracycline MICs were reduced to two fold in presence of rutin at a concentration of 1000µg/ml. Since rutin considerably enhances accumulation of ethidium bromide in ethidium bromide accumulation assay. From the previous reports we suggest that ability of efflux pump inhibitors to restore the susceptibility to different antibiotics is highly substrate specific.

Keywords: AcrAB, Efflux pump, MIC, Rutin

INTRODUCTION:

The discovery of AcrAB efflux pump has completely changed the views that resistance in gram – negative bacteria is completely explained because of the presence of outer membrane permeability barriers **[1]**. The AcrAB efflux pump of *E. coli* comprises of a transporter, a periplasmic protein AcrA and the gene AcrAB formed the operon. The outer membrane protein Tol C worked in combination with AcrAB **[2]**. The Acr B multidrug efflux pump of *Escherichia coli* which is a membrane protein that helps in

identification of different chemically misrelated compounds such as dyes and different antibiotics and throw out them in association with the membrane fusion protein AcrA and TolC which is a outer membrane protein and both of these proteins are require for completing the action. The AcrAB efflux pump existing in *E.coli* belongs to resistance-nodulation- division (RND) family of efflux pumps which is a more usual in Gram – negative bacteria. AcrAB – TolC is specified as one of the best efflux pump in case of *E.coli* **[3-13]**. AcrAB efflux pump is the only single efflux pump of RND family in case of *E.coli* which plays an important role in antibiotic resistance against different antibiotics because of its prenominal expression. The efflux mediated resistance is further more increased due to the occurrence of AcrR OR Mar R gene which leads to the up regulation of efflux pump **[14]**. Due to the large substrate spectrum of AcrAB-TolC, initiation of multidrug resistance because of the efflux of antimicrobial compounds is a actual imminence to treatment options in the hospitals. Plants are vital source of natural products which are rich in secondary metabolites such as flavonoids, tannins, terpenoids and alkaloids. These secondary metabolites possesses antimicrobial properties **[14-24]**. Rutin which is a flavonoid isolated from garlic shows efflux pump inhibitory activity against AcrAB efflux pump of *E.coli.* Rutin shows efflux pump inhibitory activity along with tetracycline. By screening of more psychotropic drugs we discovered that rutin is also a model efflux pump inhibitor.

MATERIAL AND METHODS

Microorganisms used in the study

The strains of *Escherichia coli* used in the present study were procured from Sabine Schuster and Winfried V. Kern, Center for Infectious Diseases and Travel Medicine, University Hospital, and Department of Medicine, *Albert-Ludwigs-University, Freiburg,* Germany and from MTCC and NCTC. The *E.coli* strains used were one knock out strain 1- DC14, wild type strain K-12 AG100 and two standard strains with NCTC number 12923 and MTCC number 1302.

Chemicals and reagents: Nutrient agar, Nutrient broth, DMSO, Methanol, CCCp, Ethidium bromide, Berberine and Mercuric chloride were purchased from Hi- media Pvt. Ltd.

Berberine potentiating assay (Belofsky *et al*., 2006): This assay was used to detect the presence of efflux pump inhibitory activity in the plant extracts. Berberine is a well classified antimicrobial agent and it also acts

as substrate for efflux pumps and it can be effluxed out by resistant bacteria with the help of efflux pumps. When berberine is combined with plant extracts in which efflux pump inhibitors are present, the efflux pump inhibitor presents in the extract inhibits the activity of multidrug resistance bacteria and highly increases the concentration of berberine inside which leads to the death of bacterial cells because of the antimicrobial activity of berberine.

(a) Culture preparation: Cultures of *E.coli* were incubated at 37^0C for 24 hours. Then culture was centrifuged for 2 min at 12,000 rpm, further 0.5 ml, glucose was added in to the culture and was used for the assay.

(b) Berberine Assay: This assay was performed in 96 well micro-titre plate and two different concentrations of plant extracts i.e. 100µg/ml and 1000µg/ml, was taken to perform the assay. 170 µl of nutrient broth and 5 µl of *E.coli* culture was loaded in to each well followed by addition of 20µl berberine (30 µg berberine dissolved in 1 ml Dmso) and 5µl of plant extract(15 µg plant extract dissolved in one ml DMSO). DMSO (5µl) was used as negative control along with the addition of berberine and culture. CCCP was used as a positive control along with berberine. Plates were incubated at 37^0c for 24 hours. Then OD was taken on 595nm in Elisa plate reader (BioTek). An OD less than 0.04 considered to reveal no bacterial growth. No bacterial growth in the presence of berberine indicates the presence of an MDR inhibitor in plant extract.

c) Ethidium Bromide Efflux Inhibition Assay (Kamicker *et al*., 2008):

Ethidium bromide is easily effluxed out by the resistant bacteria and will only accumulate in cells in the presence of an efflux pump inhibitor and emits strong fluorescence. Assay was performed in 96 well ELISA plate in duplicate. In each well of the 96 well plate, 170µl nutrient broth, 5µl of inoculums, 20µl Etbr and 5µl plant extract was added. CCCP (5µl) which is an efflux pump inhibitor was used as a positive control. 5µl of DMSO was used as negative control. Then fluorescence was measured for 30 minutes with 5 minute interval at excitation wavelength of 530 nm and emission wavelength of 600 nm. Readings were taken in the fluorescent ELISA reader.

Minimum inhibitory concentration

Firstly MIC of antibiotics and plant extract was determined. To determine the combined effect of antibiotic & plant extract, combinations of different concentrations ranging from 1/2 x MIC to 8 x MIC of each was used. This assay was performed in 96 well ELISA plate. By this assay a fixed concentration of active compound was

determined which was decreasing the MIC of antibiotic.

RESULTS:

Berberine uptake assay: (Figure 1)

Ethidium bromide accumulation assay: (Figure 2)

Minimum inhibitory concentration of active compound alone and in combination with antibiotics (Table 3).

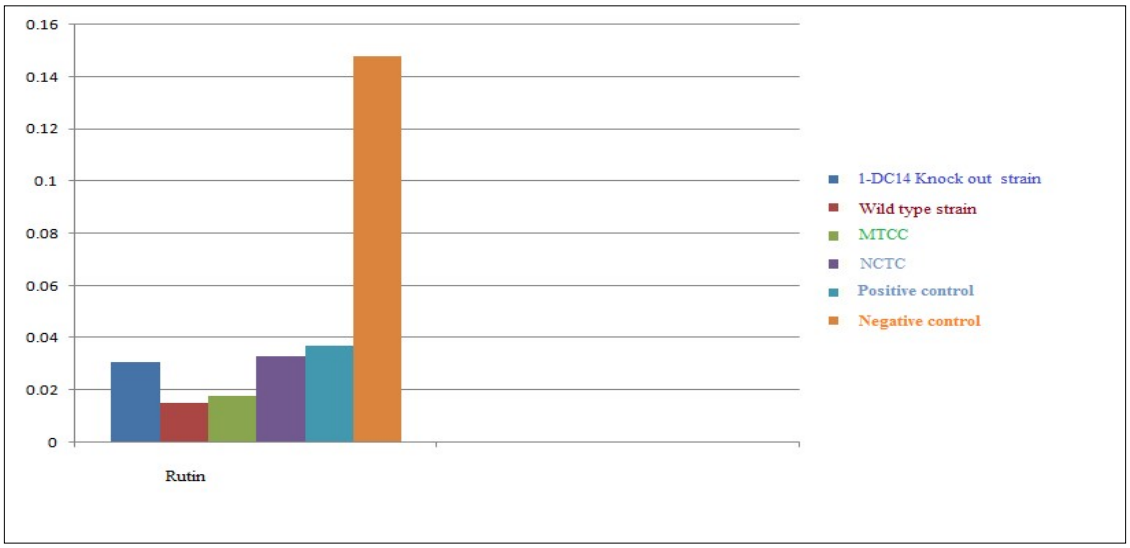

Figure 1: Absorbance shown by rutin at concentration of 1000 µg/ml against *E. coli* strains

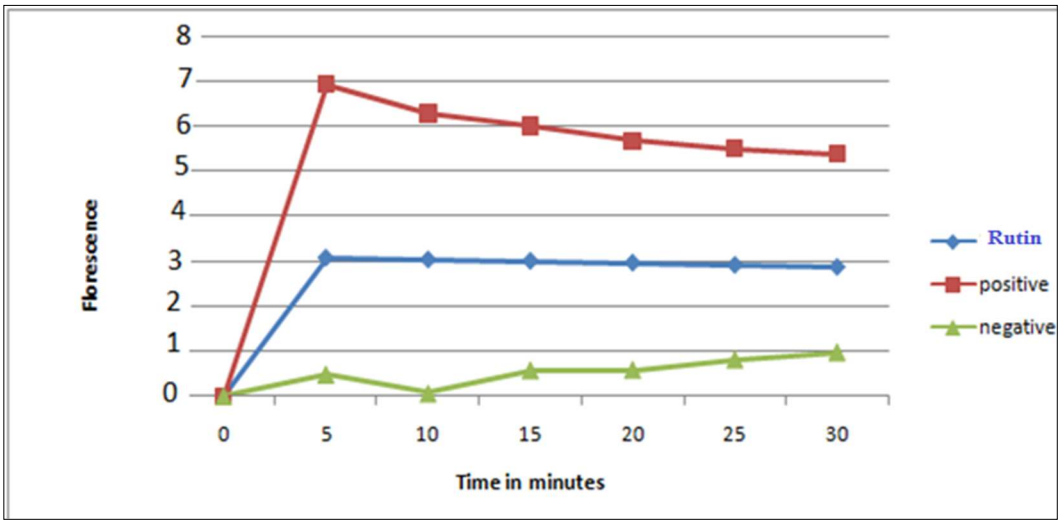

Figure 2: Effect of rutin on accumulation of Ethidium bromide at a concentration of 1000ug/ml against *E. coli* strains

Table 1: Minimum inhibitory concentration of active compound alone and in combination with antibiotics

S. No.	Name of strain	MIC of rutin (1000 µg/ml		MIC of ciprofloxacin		MIC of rutin (1000µg/ml)		MIC of Tetracycline		MIC of rutin (1000µg/ml)		MIC of Erythromycin	
		Without ciprofloxacin	With ciprofloxacin	Without plant extract	With plant extract	Without tetracycline	With tetracycline	Without rutin	With rutin	Without erythromycin	With Erythromycin	Without rutin	With rutin
1	1-DC14	0.06	0.03	1	0.5	1	0.003	2	0.125	1	0.5	2	0.5
2	K12-AG100	64	32	64	16	4	2	2	0.5	2	1	4	1
3	MTCC1302	0.003	0.003	0.5	0.5	1	1	0.125	0.125	0.003	0.003	0.06	0.06
4	NCTC	0.5	0.125	1	1	1	0.5	0.003	0.003	1	1	0.5	0.5

DISCUSSION:

In the berberine uptake assay and ethidium bromide accumulation assay rutin behaved as an efflux pump inhibitors as compare to Phe- Arg-β-naphthylamine only in case of ciprofloxacin and tetracycline two fold reduction in MIC was observed. Rutin inhibits the pump at a concentration of 1000µg/ml. Since rutin considerably enhances accumulation of ethidium bromide in ethidium bromide accumulation assay. From the previous reports we suggest that ability of efflux pump inhibitors to restore the susceptibility to different antibiotics is highly substrate specific.

REFERENCES:

[1] Ma D., Cook D.N., Alberti M., Pon N.G., Nikaido H and Hearst J.E. Molecular cloning and characterization of acrA and acrE genes of *Escherichia coli* J. Bacteriol 1993; 175: 6299-6313.

[2] Fralick J.A. Evidence that TolC is required for functioning of the Mar/ AcrAB efflux pump of *Escherichia coli*. J. Bacteriol 1996; 178: 5803-5805.

[3] Bohnert JA., Schuster S., Seeger MA., Fahnrich E., Pos KM., Kern WV. Site-directed mutagenesis reveals putative substrate binding residues in the *Escherichia coli* RND efflux pump AcrB. J. Bacteriol 2008: 190: 8225-9.

[4] Bohnert JA., Schuster S., Szymaniak-Vits M., Kern WV. Determination of real time efflux phenotypes in *Escherichia coli* AcrB binding pocket phenylalanine mutants using a 1,2 '-Dinaphthylamine Efflux assay. Plos one 2011; 6(6): e21196.

[5] Eicher T., Cha HJ., Seeger MA, *et al*. Transport of drugs by the multidrug transporter AcrB involves an access and a deep binding pocket that are separated by a switch- loop. Proc Natl Acad Sci USA 2012; 109: 5687-92.

[6] Husain F., Nikaido H. Substrate path in the AcrB multidrug efflux pump of

Escherichia col. Mol Microbiol; 2010; 78 (2): 320-30.

[7] Koronakis V., Sharff A., Koronakis E., Luisi B., Hughes C. Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. Nature 2000; 405: 914-

[8] Mikolosko J., Bobyk K, Zgurskaya HI, Ghosh P. Conformational flexibility in multidrug efflux system protein AcrA. Structure 2006; 14: 577-87.

[9] Murakami S., Nakashima R., Yamashita E., Matsumoto T., Yamagu- chi A. Crystal structure of a multidrug transporter reveal a functionally rotating mechanism. Nature 2006; 443:173-9.

[10] Nakashima R., Sakurai K., Yamasaki S., Nishino K., Yamaguchi A. Structures of the multidrug exporter AcrB reveal a proximal multisite drug binding pocket. Nature 2011; 480: 565- 9.

[11] Seeger MA., Schiefner A., Eicher T., Verrey F., Diederichs K., Pos KM. Structural asymmetry of AcrB trimer suggests a peristaltic pump mechanism. Science 2006; 313: 1295-8.

[12] Takatsuka Y., Nikaido H. Covalently linked trimer of the AcrB multidrug efflux pump provides support for the functional rotating mechanism. J. Bacteriol 2009; 191: 1729-37.

[13] Kern WV., Oethinger M., Jellen-Ritter AS, Levy SB. Non- target gene mutations in the development of fluoroquinolone resistance in *Escherichia coli*. Antimicrob Agents Chemother 2000; 44: 814-20.

[14] Bouzada Maria LM., Fabri Rodrigo L., Mauro Nogueira., Konno Tatiana UP., Duarte Gizele G., Elita Scio: Antibacterial, cytotoxic and phytochemical screening of some traditional medicinal plants in Brazil. Pharmaceutical Biology 2009, 47: 44-52.

[15] Alam Sher. Antimicrobial activity of natural products from medicinal plants. Gomal journal of Medical Sciences 2009, 7: 72-78.

[16] Osawa K, Matsumoto T, Maruyama T., Takiguchi T., Okuda K., Takazoe I. Studies on the antibacterial activity of plant extracts and their constituents against periodontopathic bacteria. Bulletin of Tokyo dental college 1990, 31: 17-21.

[17] Chakraborty A., Brantner AH. Antibacterial steroid alkaloids from the stem bark of Holarrhena pubescens. Journal of Ethnopharmacology 1999, 68: 339-344.

[18] Cowan M. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999, 12: 564-582.

[19] Vivanco JM, Savary BJ, Flores HE. Characterization of two novel type 1 ribosome- inactivating proteins from the storage roots of the Andean crop Mirabilllis expansa. Plant Physiology 1999, 119: 1447-1456.

[20] Bisignano G., Sanogo R., Marino A., Aquino R., Dangelo V., German MP., De Pasquale R., Pizza C. Antimicrobial activity of *Mitracarpus scaber* extract and isolated constituents. Letters in Applied Microbiology 2000, 30: 105- 108.

[21] Habibi Z., Eftekhar F., Samiee K., Rustaiyan A. Structure and antibacterial activity of new labdane diterpenoid from *Salvia leriaefolia*. Journal of Natural products 2000, 63: 270- 271.

[22] Setzer WN., Setzer MC., Bates RB., Jakes BR. Biologically active triterpenoid of *Syncarpia glomulifera* bark extract from Paluma, North Queensland, Australia. Planta Medica 2000, 66: 176-177.

[23] Al-Momani W, Abu- Basha E, Janakat S, Nicholas RAJ, Ayling RD. In vitro antimycoplasmal activity of six Jordanian medicinal plants against three Mycoplasma species. Tropical Animal Health and Production 2007, 39: 515-519.

[24] Renau TE, Leger R, Flamme EM, *et al*. Inhibitors of efflux pumps in *Pseudomonas aeruginosa* potentiate the activity of the fluoro- quinolone antibacterial levofloxacin. J Med Chem. 1999; 42: 4928-31.