IJBPAS, August, 2016, 5(8): 1965-1976

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SYNERGISM BETWEEN NATURAL PRODUCTS AND FLUOROQUINOLONES AGAINST *Staphylococcus aureus* STRAINS

URMILA[1*], JANDAIK S[1], MEHTA J[1] AND MOHAN M[2]

[1]Department of Microbiology, Shoolini University of Biotechnology and Management Sciences, Solan, Himachal Pradesh, India

[2]Uttarakhand Council for Biotechnology, Haldi, U.S. Nagar-263146, Uttarakhand, India

***Corresponding Author: E Mail: urmila.sharma406@gmail.com**

Received 25th April 2016; Revised 20th May 2016; Accepted 20th July 2016; Available online 1st August

ABSTRACT

The impact of *Staphylococcus aureus* infections on human health has led to intensive investigation of this organism over recent years. Antibiotic resistance in *Staphylococcus aureus* is of major concern because bacteria have evolved numerous defenses against antimicrobial agents. Efflux pumps extrude structurally diverse compounds including antibiotics and render them therapeutically ineffective. Multidrug efflux pumps (predominantly NorA) are important cause of antibiotic resistance in *Staphylococcus aureus.* It is therefore imperative that new efflux pump inhibitors are characterized. The aim of the current study was to evaluate efflux pump inhibitory activity of the plant extracts to facilitate the reintroduction of therapeutically ineffective antibiotics back into clinical use.

Keywords: Antibiotic resistance, *Staphylococcus aureus*, antibiotic efflux, efflux pump inhibitors

INTRODUCTION

Staphylococcus aureus are major concern pathogen and causes serious therapeutic difficulties particularly caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Development of the fluoroquinolones class of antimicrobial agents advanced the possibility of an effective option for therapy of these serious infections. Unfortunately, shorter after introduction of these agents into clinical use, fluoroquinolones for treatment of these infections is impaired by emergence of fluoroquinolone resistance [1,2]. Efflux mediated resistance overshadowed all

known mechanisms of drug resistance. Microbial resistance to several classes of antibiotics is contributed by bacterial multidrug efflux pumps. Multidrug efflux transporter (eg. NorA) play a major role in resistance to fluoroquinolones by actively extruding them from the cells. For gram positive bacteria, pumps belonging to Major Facilitator Superfamily play a vital role in the efflux of fluoroquinolones [3]. NorA MDR pump (member of MFS), of *Staphylococcus aureus* is chromosomally encoded and can efflux fluoroquinolones, quaternary ammonium compounds, Rhodamine, Ethidium bromide, and acridines [4]. The mechanism involved in quinolone resistance in *Staphylococcus aureus* is overexpression of *norA* gene. *norA* gene encodes multidrug efflux protein which is capable of transporting fluoroquinolones outside the bacteria [5-7]. The problem of antibiotic efflux can be overcome by addressing four strategies: (i) inhibiting drug binding to the cytoplasmic membrane pumps; (ii) inhibiting interaction of different components of multi-component pump; (iii) targeting the regulatory network that controls the expression of efflux pumps; (iv) targeting energy sources of the pump [8]. Inhibition of efflux pump can potentially improve the efficacy of fluoroquinolones and decrease the emergence of resistant mutants. Plants play a central role in healthcare settings as they are potential sources of antimicrobials and natural sources of phytoconstituents. This raises the prospects of obtaining novel chemotherapeutic compounds especially efflux pump inhibitors. Many chemical compounds with NorA efflux pump inhibitory activities have been investigated, but due to their pharmacokinetic properties and toxic effects none of the efflux pump inhibitors have entered clinical trial yet [9]. The plants of Western Himalaya region selected in present study have a wide range of biological and pharmacological properties.

So keeping in mind the pharmacological properties of selected medicinal plants and to overcome the problem of drug resistance in *Staphylococcus aureus* due to efflux of fluoroquinolones (Ciprofloxacin and Norfloxacin) by NorA efflux pump, the present study was undertaken.

METHODS

Plants

Leaves, roots, flowers, fruits and rhizomes of 12 medicinal plants (*Allium humile, Chenopodium album, Heracleum lanatum, Solena amplexicaulis, Picrorrhiza kurrooa, Rheum australe, Rubus ellipticus, Rumex hastatus, Rumex nepalensis, Trillium govanianum, Verbascum thapsus, Viola canesiens*) were collected from Bhanara, Kullu Himachal Pradesh, India of north western Himalaya. The plants specimens have been duly identified and deposited in

the herbarium of Botanical Survey of India (BSI), northern circle, Dehradun, Uttarakhand, India.

Processing of Plant Materials

Plant materials were washed with distilled water and roots were cut into small bits to facilitate drying. Then plant materials were allowed to air dry and were ground into fine powder. The powdered root samples were stored in a clean glassware container until needed for analysis.

Extraction of plant materials

Plant material was extracted with 70% methanol, ethyl acetate and distilled water. 10g of plant material was soaked in 100ml of solvents for four days with shaking at orbital rotary shaker. Extract was clarified by filtration through Whatman filter paper No. 1 and was concentrated using water bath at 40°C for 12 hrs [10].

Similarly aqueous and ethyl acetate extracts were prepared.

Bacterial strains tested

Staphylococcus aureus 8325-4 (wild type), K1758 (nor A Knockout), K2378 (nor A over expressing) were obtained from Northeastern University, Boston.

Antibiotics used

Fluoroquinolones in powder form were purchased from different sources; Norfloxacin (SRL); Ciprofloxacin (Himedia).

Well Diffusion method

Well diffusion assay was performed according to the NCCLS, 1993. Mueller Hinton agar plates were seeded with a 24 h culture of the bacterial strains and inoculum was adjusted to an O.D of 0.8. Wells (6 mm diameter) were punched in the agar and plant extracts and antibiotics were added in a concentration of 100mg/ml and 5µg respectively. Plates were incubated at 37^0C for 24 h. For strain where antibiotic did not show any inhibition zone alone, the combination of plant extract with antibiotic was calculated on the basis of GIIs (Growth Inhibitory Indices) values and the combination was considered as synergistic, additive and antagonistic when GIIs >1, 1 and <1 respectively, where as antibiotic showing inhibition alone and as well as in combination, synergistic, additive and antagonistic activities of plant extracts with antibiotics were defined with GIIs >0.5, 0.5 and <0.5 respectively [11].

$$\text{GIIs} = \frac{\text{Zone of inhibition in combination (antibiotic + plant extract)}}{\text{Total of zone of inhibition alone}}$$

The method was performed in triplicates. After screening for synergistic activity, plants showing maximum synergistic activity were taken for further study.

Screening of plant extract for efflux pump inhibitory activity

Berberine Uptake Assay

Berberine uptake assay was performed for the isolation of efflux pump inhibitors [12].

Serial 2-fold dilutions of Berberine and a plant extract were mixed in each well of a 96-well microtiter plate. Each row (and column) contained a fixed amount of one agent and increasing amounts of the second agent. The concentrations of Berberine (row) ranged from 30 to 0.5µg/mL (89-1.5 µM), while plant compound (column) concentrations ranged from 15 to 0.015µg/mL. Inoculums were added to each well at a final concentration of 5×10^6 CFU/mL, and plates were incubated at 37 °C for 24 h. Growth was assayed by absorption at 600 nm with a microtiter plate reader. An OD less than 0.04 were considered to reveal no bacterial growth. Three replicates were performed.

Ethidium Bromide Efflux Inhibition Assay

Accumulation of Ethidium bromide was assessed by Ethidium bromide efflux inhibition assay [16]. Sterile microtitre plates were used for this assay. 175 µl of bacterial inoculum was added to each well of 96 well microtitre plate. 10 µl of test compound was added to columns 1-10. 30 µl of 100 µM EtBr was added in each well. 10 µl of positive control (CCCP) was added to column 11. 10 µl of negative control (DMSO) was added to column 12. Plates were immediately placed in Fluoroscan Ascent Fl and fluorescence of the accumulated ethidium bromide for 30 mins at excitation = 530nm and emission = 600nm was determined. Three replicates were performed.

RESULTS

In the present investigation, aqueous, Ethyl acetate and Methanolic extracts of plants were evaluated for efflux pump inhibitory activity against *Staphylococcus aureus* strains. Synergistic activity of plant extracts with fluoroquinolones (Ciprofloxacin and Norfloxacin) was evaluated by well diffusion method. Efflux pump inhibitory activity of effective plants was assessed by Berberine uptake assay and Ethidium bromide efflux inhibition assay.

Results demonstrated that the most of test plant extracts contains potential synergistic activity with fluoroquinolones against *Staphylococcus aureus* strains while aqueous extract of all plants and methanolic and ethyl acetate extract of *Allium humile, Rumex hastatus, Rumex nepalensis* and *Trillium govanianum* did not show any activity.

The Well diffusion test results depicted in table1 revealed that for 2378 strain, antibiotic did not show any inhibition zone alone. Methanolic and Ethyl acetate extract of *Rubus ellipticus* was antagonistic (GII = 0.72), while all other Methanolic and Ethyl acetate extracts of plants showed synergism (GIIs >1). Methanolic extract of *Solena amplexicaulis* and *Rheum australe* showed maximum (GIIs = 1.67) and minimum (GIIs = 1.06) synergistic activities

respectively. For 8325-4 and 1758 strain, antibiotic was showing inhibition alone and as well as in combination. Ethyl acetate extract of *Rheum australe* and Methanolic extract of *Heracleum lanatum* showed maximum synergism (GII = 0.67) in 1758 strain (table1).

GII values for Norfloxacin in combination with plant extracts ranged from 0.40 to 1.55 (Table 2). Maximum synergism was shown by methanolic extract of *Viola canesiens* (GII = 1.55) in case of K2378 strain, while ethyl acetate extract of *Rheum australe* showed maximum synergism in 8325-4 strain. In K1758 strain GII values ranged from 0.42 to 0.71.

Six plants (*Rheum australe* (EE); *Heracleum lanatum* (ME); *Solena amplexicaulis* (ME); *Verbascum thapsus* (EE); *Viola canescens* (ME); *Picrorhiza kurrooa* (EE)) showing maximum synergistic activity were selected for efflux pump inhibitory activity. Berberine is an efflux pump substrate and hence acts as a marker to find out efflux pump inhibitors. A fixed concentration of berberine [89µM (30 µg/ml)] and serial two fold dilution of plant extracts (ranged from15-0.015 µg/ml) were used in this study. An O.D ≤0.04 revealed no bacterial growth. The absorbance at different MICs are presented in the Figure 1. Out of six plant extracts, *Heracleum lanatum* showed maximum inhibition of growth when compared with Berberine alone as negative control and CCCP as positive control in all the three test strains (Figure 1).

Ethidium bromide is also a substrate of efflux pumps and is effluxed by strains having efflux pumps. In presence of efflux pump inhibitors Ethidium bromide gets accumulated and shows fluorescence (Figure 2).

Methanolic extract of *Heracleum lanatum* was having maximum potential of Ethidium bromide accumulation and maximum efflux pump inhibition. Ethyl acetate extract of *Picrorhiza kurrooa* was showing least efflux pump inhibition (Figure 3, 4).

Table1: Synergistic effect between plant extracts (Methanolic and Ethyl acetate) with Ciprofloxacin (Average ± S D) by well diffusion method.

Plants (Part used)	Type of extract	Zone of inhibition in mm											
		2378 (NorA Overexpressing)				8325-4 (Wild Type)				1758 (NorA Knockout)			
		P.E	P.E+Cx	Cx	GIIs	P.E	P.E+Cx	Cx	GIIs	P.E	P.E+Cx	Cx	GIIs
Chenopodium album (Leaves)	ME	13±0.4	16±0.4	-	1.23	14±0.4	18±0.3	13±0.5	0.67	14±0.5	18±0.6	18±0.5	0.56
	EE	13±0.3	15±0.2	-	1.15	13±0.5	15±0.3	13±0.5	0.58	13±0.5	19±0.6	18±0.4	0.61
Heracleum lanatum (Root)	ME	13±0.8	18±0.4	-	1.38	13±0.7	18±0.6	12±1	0.72	14±0.3	22±0.4	19±0.8	0.67
	EE	10±0.8	13±0.4	-	1.30	11±0.8	17±0.7	15±0.8	0.65	13±0.5	19±0.7	18±0.6	0.61
Solena amplexicaulis (Leaves)	ME	12±0.7	20±1.1	-	1.67	12±0.7	18±0.4	14±0.7	0.69	15±0.8	21±0.7	18±0.4	0.64
	EE	12±0.4	16±0.7	-	1.33	14±0.7	15±0.6	13±0.9	0.56	14±0.7	19±0.3	17±0.4	0.61
Picrorrhiza kurrooa (Root)	ME	16±0.4	18±0.6	-	1.13	14±0.8	19±0.4	15±0.7	0.66	12±0.8	19±0.7	19±0.8	0.61
	EE	14±0.6	19±0.7	-	1.36	14±0.7	19±1.3	13±0.8	0.70	17±0.8	23±1	18±0.9	0.66
Rheum australe (Root)	ME	16±0.3	17±0.6	-	1.06	18±0.6	18±0.7	14±0.2	0.56	16±0.3	19±0.7	18±0.9	0.56
	EE	16±0.6	23±0.2	-	1.44	18±0.8	24±0.4	14±0.8	0.75	16±0.6	22±0.9	17±0.5	0.67
Rubus ellipticus (Fruit)	ME	12±0.3	9±0.4	-	0.75	12±0.3	10±0.3	12±0.4	0.42	13±0.4	13±0.2	18±0.4	0.42
	EE	11±0.4	8±0.4	-	0.72	12±0.4	10±0.1	13±0.2	0.40	14±0.3	14±0.3	18±0.4	0.43
Verbascum Thapsus (Leaves)	ME	15±0.1	17±0.2	-	1.13	18±0.3	20±0.4	13±0.3	0.65	18±0.2	20±0.4	18±0.3	0.56
	EE	-	12±0.4	-	-	11±0.2	13±0.3	12±0.4	0.57	15±0.2	17±0.3	17±0.2	0.53
Viola canesiens (Leaves)	ME	13±0.3	19±0.5	-	1.46	12±0.8	17±1	13±0.6	0.68	13±0.6	19±0.8	17±0.3	0.63
	EE	-	18±0.5	-	-	11±0.2	16±0.1	14±1	0.64	13±0.4	18±0.6	16±0.7	0.62

GIIs = Growth inhibitory indices, ME = Methanolic extract, EE = ethyl acetate extract, P.E = Plant extract, Cx =Ciprofloxacin, - = No inhibition zone.

Table 2: Synergistic effect between plants extracts (Methanolic and Ethyl acetate) with Norfloxacin (Average ± S D) by well diffusion method.

Plants (Part used)	Type of extract	Zone of inhibition in mm											
		2378 (NorA Overexpressing)				8325-4 (Wild Type)				1758 (NorA Knockout)			
		P.E	P.E+Nx	Nx	GII	P.E	P.E+Nx	Nx	GII	P.E	P.E+Nx	Nx	GII
Chenopodium album (Leaves)	ME	15±0.2	16±0.3	-	1.07	14±0.2	16±0.3	14±0.2	0.57	15±0.3	18±0.2	17±0.3	0.56
	EE	13±0.3	16±0.4	-	1.23	13±0.3	14±0.5	13±0.3	0.54	16±0.3	19±0.4	18±0.3	0.56
Heracleum lanatum (Root)	ME	11±0.4	16±0.2	-	1.45	11±0.3	17±0.2	14±0.2	0.68	11±0.6	20±0.3	17±0.4	0.71
	EE		15±0.4	-		9±0.3	15±0.7	14±0.7	0.65	12±0.3	18±0.7	18±0.6	0.60
Solena amplexicaulis (Leaves)	ME	16±0.4	21±0.4	-	1.31	17±0.5	22±0.6	13±0.5	0.73	15±0.4	21±0.3	18±0.3	0.64
	EE	13±0.4	16±0.5	-	1.23	14±0.5	15±0.5	13±0.4	0.56	14±0.3	19±0.5	18±0.5	0.59
Picrorrhiza kurooa (Root)	ME	11±0.4	14±0.2	-	1.27	11±0.4	15±0.5	13±0.5	0.63	14±0.5	19±0.1	18±0.4	0.59
	EE	13±0.3	17±0.3	-	1.31	11±0.6	17±0.4	13±0.4	0.71	15±0.5	20±0.3	17±0.6	0.63
Rheum australe (Root)	ME	20±0.5	23±0.5	-	1.15	21±0.2	22±0.4	14±0.4	0.63	20±0.5	20±0.3	17±0.6	0.54
	EE	18±0.4	24±0.3	-	1.33	19±0.6	25±0.4	13±0.4	0.78	18±0.5	22±0.1	18±0.4	0.61
Rubus ellipticus (Fruit)	ME	12±0.3	9±0.4	-	0.75	12±0.3	10±0.3	12±0.4	0.42	13±0.4	13±0.2	18±0.4	0.42
	EE	11±0.4	8±0.4	-	0.72	12±0.4	10±0.1	13±0.2	0.40	14±0.3	14±0.3	18±0.4	0.43
Verbascum Thapsus (Leaves)	ME	12±0.4	14±0.5	-	1.17	12±0.3	14±0.2	13±0.4	0.56	14±0.5	17±0.5	17±0.4	0.55
	EE	-	19±0.4	-	-	16±0.5	18±0.3	12±0.4	0.64	17±0.4	19±0.4	17±0.4	0.56
Viola canesiens (Leaves)	ME	11±0.5	17±0.3	-	1.55	12±0.3	17±0.7	12±1	0.71	14±0.4	19±0.5	17±0.6	0.61
	EE	-	15±0.4	-	-	12±0.2	14±0.3	11±0.5	0.61	10±0.5	17±0.3	18±0.6	0.61

GIIs = Growth inhibitory indices, ME = Methanolic extract, EE = ethyl acetate extract, P.E = Plant extract, Nx = Norfloxacin, - = No inhibition zone

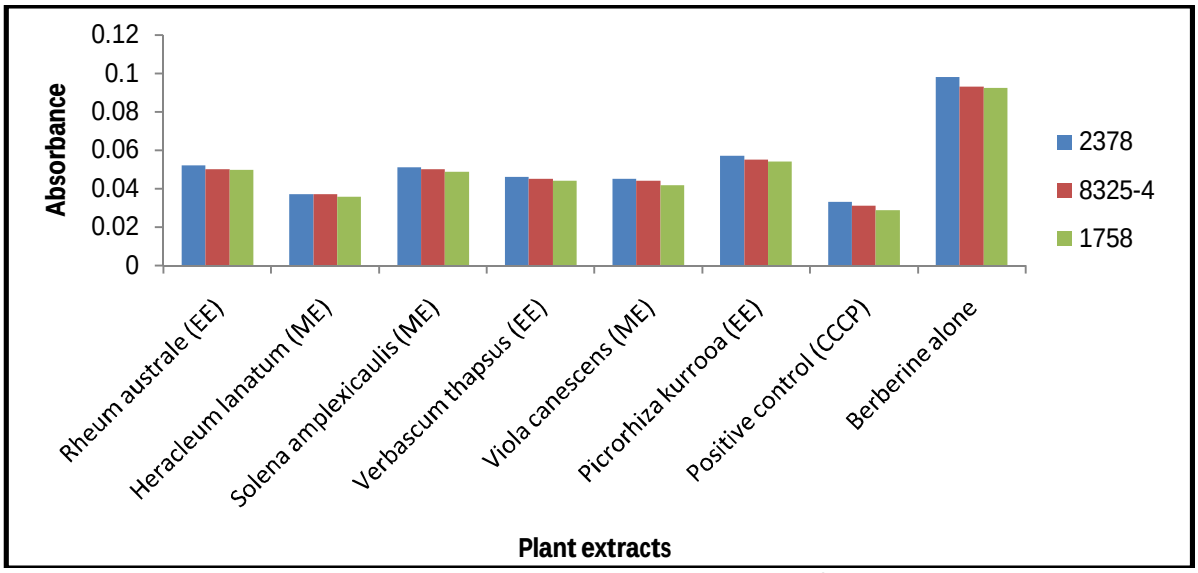

Fig. 1: Absorbance shown by plant extracts at 89µM of Berberine

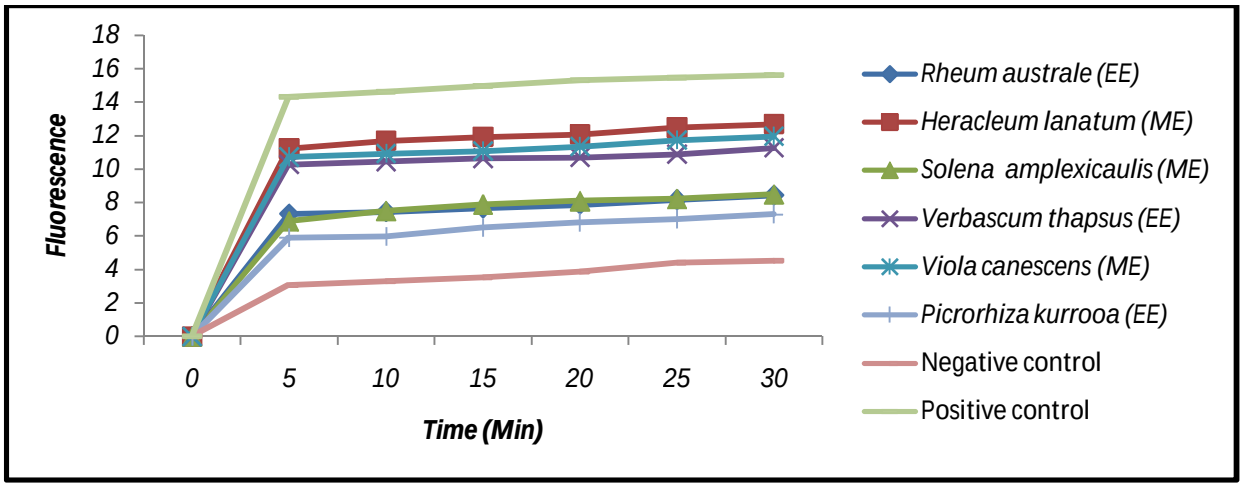

Fig. 2: Effect of plant extract on accumulation of Ethidium bromide by 2378 over expressing strain

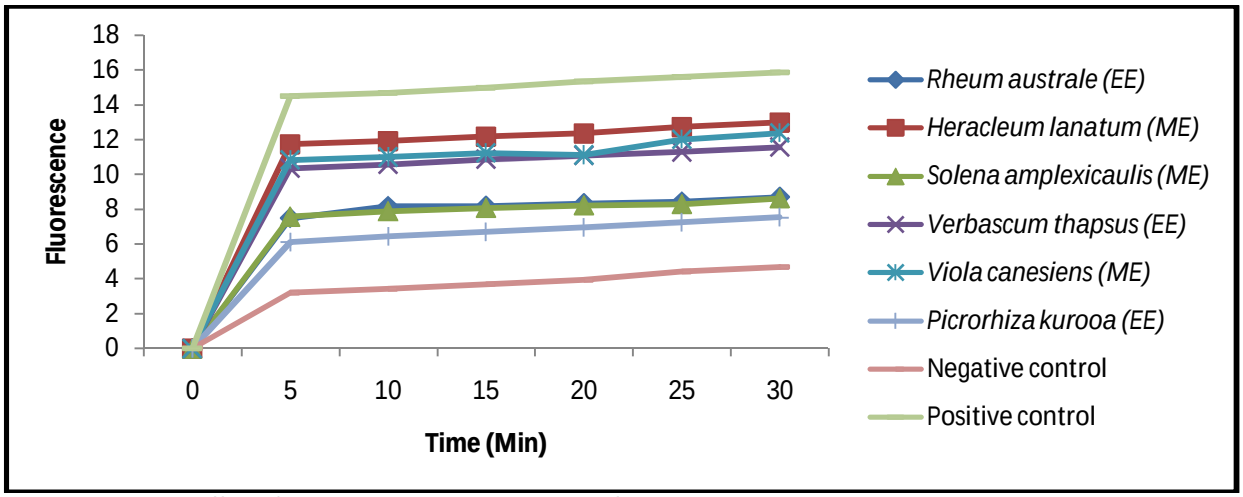

Fig. 3: Effect of plant extract on accumulation of Ethidium bromide by 8325-4 wild type strain

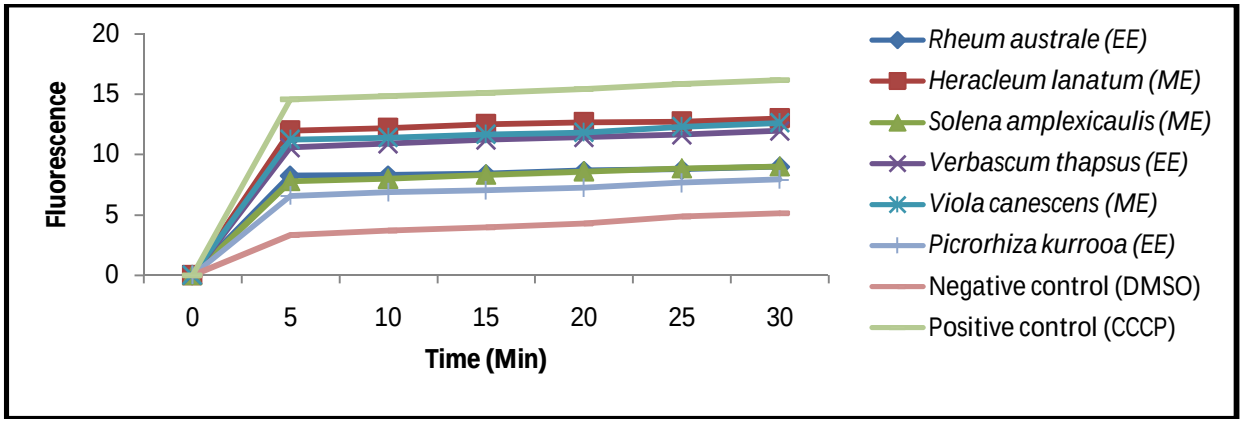

Fig. 4: Effect of plant extract on accumulation of Ethidium bromide by 1758 knockout strain

DISCUSSION

Transport mechanisms including efflux processes are primary tools in prokaryotic and eukaryotic cells in acquisition of essential nutrients, establishment of proper charge and pH gradient across the cytoplasmic membrane and extrusion of potentially toxic compounds [13,14]. This protective function empowers bacterial cells to survive in hostile environments, including in the presence of antibiotics during the treatment of infections. The up-regulation of efflux systems can significantly lower the intracellular concentration of many antibiotics causing an impact on the clinical efficacy of the antibiotics [15]. Medicinal plants are important elements of indigenous medical systems in India where different parts of various medicinal plants are used to cure specific ailment and interest in medicinal plants has revived as a consequence of current problems associated with the use of antibiotics [16,17]. Bacterial resistance can be restored by synergistic action of antibiotics and bioactive plant extracts which is a novel concept and could be beneficial [18].

In the present study, Methanolic, Ethyl acetate and aqueous extracts of 12 plants were screened for synergistic activity with two fluoroquinolones (Ciprofloxacin and Norfloxacin) against S*taphylococcus aureus* strains (2378 NorA Over expressing strain, 8325-4 wild type strain, 1758 NorA Knockout strain). Among the three extracts, Methanolic extract was found most effective followed by Ethyl acetate extract, whereas aqueous extract did not show any activity. Many plants exhibited synergistic activity but the results obtained were variable in all the three test strains and the drug.

In this study Ethyl acetate extract of *Verbascum thapsus* and *Viola canescens* have been found to be synergistic enhancer though it does not have any antimicrobial properties alone, but when taken concurrently with standard fluoroquinolones it enhance the effect of that drug. Except *Rubus ellipticus* which

showed antagonism, all other plant extracts showed synergism with the fluoroquinolones (Ciprofloxcin and Norfloxacin).

Various other authors have also reported the antimicrobial and synergistic activity of most of the plant (*Allium sativum, Baccharis trimera, Cymbopogon citrates, Zingiber officinale, Laurus nobilis, Majorana syriaca, Mentha piperita, Mikania glomerata, Ocimum basilicum, Psidium guajava, Rosa damascene, Rosamarinus officinalis, Rubia cordifolia, Salvia fruticosa and Syzygium aromaticum)* extracts against *Staphylococcus aureus* strains [11, 19-23].

In the present study, six plants were selected on the basis of synergistic activity for detection of efflux pump inhibitory activity by Berberine potentiation assay and Ethidium bromide assay. All the plant extracts showed efflux pump inhibitory activity, but maximum activity was shown by Methanolic extract of *Heracleum lanatum*. Our results are in conformity with Rana *et al.,* 2014 [24], who reported that The above study concluded that the problem of antimicrobial resistance due to efflux is growing and the outlook for the use of antimicrobial drugs against *Staphylococcus aureus* in the future is still uncertain. The synergistic effect of antibiotic with efflux pump inhibitors isolated from plant sources against plants *Artemisia absinthium, Berberis plant, Geranium caespitosum, Rosmarinus officinalis, Dalea versicolor, Lycopus europaeus, Thymus vulgaris, Jatropha elliptica, Piper nigrum, Piper longum, Salix alba, Momordica balsamina, Lupinus argenteus, Artemisia annua, Ipomoea murucoides, Herissantia tiubae, Mezoneuron benthamianum, Securinega virosa* exhibit NorA efflux pump inhibitory activity.

Thus the present findings suggest that the efflux pump inhibitory activity of plant extracts with fluoroquinolones observed in this study was attributable to such compounds, showing synergistic activity, present in crude extracts of plants. The plants used in the present study might be potential source of non-antibiotic drugs that might potentially improve the performance of fluoroquinolones against infections by inhibiting the efflux. The bioactive compounds having efflux inhibitory activity of *Heracleum lanatum* (ME) can be used in future as adjuvants with antibiotics.

CONCLUSION

Staphylococcus aureus leads to new choices for the treatment of Staphylococcal diseases. This study has shown that methanolic extract of *Heracleum lanatum* exhibits potentials of synergy in combination with selected antibiotics against *Staphylococcus aureus* strains, often presenting with problems of drug

resistance (mainly due to efflux pumps). Moreover, this study established a good base for developing future NorA efflux pump inhibitors from *Heracleum lanatum* (ME) as adjuvant of antibiotics.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Kim Lewis, Northeastern University, Boston, USA for providing strains *Staphylococcus aureus* 8325-4 (wild type) and K1758 (NorA Knockout), K2378 (NorA over expressing).

REFERENCES

[1] Harnett N, Brown S, Krishnan C, Emergence of quinolone resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in Ontario, Canada, Antimicrob Agents Chemother, 1991; 35: 1911-3.

[2] Peterson LR, Quick JN, Jensen B, Homann S, Johnson S, *et al*., Emergence of ciprofloxacin resistance in nosocomial methicillin-resistant *Staphylococcus aureus* isolates, Arch Intern Med, 1990; 150: 2151-5.

[3] Neyfakh AA, Bidnenko VE, Chen LB, Efflux-mediated multi-drug resistance in Bacillus subtilis: similarities and dissimilarities with the mammalian system, Proc Natl Acad Sci, 1991; 88:4781- 5.

[4] Markham PN, Westhaus E, Klyachko K, Johnson ME, Neyfakh AA, Multiple Novel Inhibitors of the NorA Multidrug Transporter of *Staphylococcus aureus*, Antimicrob Agents Chemother, 1999; 43: 2404-8.

[5] Kaatz GW, Seo SM, Inducible NorA-mediated multidrug resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother, 1995; 39: 2650-5.

[6] Kaatz, GW, Seo SM, Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*, Antimicrob Agents Chemother, 1997; 41:2733-7.

[7] Yamada H, Kurose-Hamada S, Fukuda Y, Mitsuyama J, Takahata M, Minami S, Watanabe Y, Narita H, Quinolone susceptibility of *norA*-disrupted *Staphylococcus aureus*. Antimicrob. Agents Chemother, 1997; 41**:** 2308–9.

[8] Lawrence LE, Barrett JF, Inhibition of bacterial efflux: needs, opportunities, and strategies, Curr Opin Antiinfect Investig Drugs, 2000; 2: 145–53.

[9] Handzlik J, Matys A, Kiec-Kononowicz K, Recent Advances in Multi-Drug Resistance (MDR) Efflux Pump Inhibitors of Gram-Positive Bacteria *S. aureus*, Antibiotics 2013; 2: 28-45.

[10] Sultana B, Anwar F, Ashraf M. Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules 2009; 14: 2167-80.

[11] Yang ZC, Wang BC, Yang XS, Wang Q, Ran L, The synergistic activity of antibiotics combined with eight

traditional Chinese medicines against two different strains of *Staphylococcus aureus,* Colloids Surf 2005; 41: 79-81.

[12] Belofsky G, Carreno R, Lewis K, Ball A, Casadei G, Tegos GP, Metabolites of the "smoke tree", Dalea spinosa, potentiate antibiotic activity against multidrug-resistant *Staphylococcus aureus* J Nat Prod, 2006; 69: 261-4.

[13] Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, Lodish HF, Sequence and structure of a human glucose transporter, Science, 1985; 229: 941-5.

[14] Tisa LS, Rosen BP, Transport systems encoded by bacterial plasmids, J Bioenerg Biomembr, 1990; 22: 493-507.

[15] Mullin S, Mani N, Grossman TH, Inhibition of antibiotic efflux in bacteria by the novel multidrug resistance inhibitor biricodar (VX-710) and timcodar (VX-853), Antimicrob Agents Chemother, 2004; 48: 4171–6.

[16] Emori TG, Gaynes RP, An overview of nosocomial infections, including the role of the Microbiology laboratory, Clin Microbiol Rev, 1993; 6: 428-42.

[17] Kunin CM, Resistance to antimicrobial drugs- a world-wide calamity, Ann Intern Med, 1993; 118: 557-61.

[18] Adwan MG, Shanab ABA, Adwan MK, *In Vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections, Pak J Med, 2008; 24: 541-4.

[19] Betoni JEC, Mantovani RP, Barbosa LN, Stasi LCD, Fernandes AJ, Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases, Mem Inst Oswaldo Cruz, 2006; 101: 387-90.

[20] Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM, *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus,* Afr J Biotechnol, 2006; 5: 1082-6.

[21] Darwish RM, Aburjai T, Khalil SA, Mahafzah A, Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Staphylococcus aureus*, Ethnopharmacol, 2002; 79: 359-64.

[22] Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone SE, Nascimento AMA, Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus* aureus, Can J Microbiol, 2005; 51: 541-7.

[23] Yam TS, Miller JMH, Shah S, The effect of acomponent of tea (*Camellia sinensis*) on methicillin resistance, PBP2' synthesis, and beta-lactamase production in *Staphylococcus aureus,* J Antimicrob Chemother, 1998; 42: 211-6.

[24] Rana T, Singh S, Kaur N, Pathania K, Farooq U, A Review on Efflux Pump Inhibitors of Medically Important Bacteria from Plant Sources, Int J Pharm Sci Rev Res, 2014; 19: 101-11.