INTRODUCTION

Throughout the history of human population, infections have been the major cause of concern. Infectious agents that were once thought to be controlled by use of antibiotics are now becoming resistant to these therapies (Levy, 2003). The use of new novel antibiotics to treat infectious diseases is frequently accompanied by the development of antibiotic resistance by the bacteria (Schito, 2006). Individuals may have infected with multidrug-resistant (MDR) organisms due to the failure of all available drugs (Levy, 2003). Nowadays, at least one mechanism of resistance is described for each class of commonly used antibiotics (Davies and Davies, 2010). Antibiotic resistance is developed by prescribed drug regime’s failure and human errors (Goldstein, 2007). The extensive use of antibiotic has promoted the emergence of antibiotic resistance among pathogens, including MDR strains. Apart four other mechanisms, activation of multidrug efflux pumps also play a key role in the emergence of MDR bacteria by the extrusion of multiple drugs, unrelated compounds. Efflux pumps are used as a first-line defense mechanism by the bacteria, which avoids the drug to reach lethal concentrations. Many MDR bacteria cause life-threatening infections, being a major concern both in the hospital and the community. The ability of pathogens to resist the action of various antibiotics has made their eradication extremely challenging. Therefore, it has become imperative to investigate new therapeutic agents with new modes of action (Zhang et al., 2006).

In Staphylococcus aureus, antibiotic resistance has become a severe problem because of its ability to acquire resistance against the currently used antibacterial agents (Stavri et al., 2007). This organism is facultative anaerobic, Gram positive cocci (1 to 1.3 µm), also known as golden staph. It appears as grape like clusters and has round, ß-hemolytic golden yellow colonies (Ryan and Ray, 2004). It is one of the major human pathogen that possesses many virulence factors such as coagulase, extracellular enzymes and toxins which contribute to its pathogenicity (Gemmell et al., 1997). It is the main causative agents of device-associated infection (DAI) and hospital-associated infection (HAI), including bacteremia, skin and soft tissue infections, pneumonia, blood stream infections, bone and joint infections and endocarditis. The emergence of MDR strains of S. aureus render antimicrobial agents ineffective and poses a significant problem for the clinical personnel, as it is often associated with prolonged hospitalization and increased morbidity and mortality (Paulsen et al., 1995 and Boucher et al., 2009). The emergence of MDR strains of S. aureus has demonstrated a unique ability to respond to each
new antibiotic with development of resistance to β-lactams (penicillin, methicillin), lincosamides, streptomycin, aminoglycosides, macrolides fluoroquinolones (FQs), chloramphenicol, sulfonamides, tetracycline, linezolid and daptomycin. Therefore, *S. aureus* has become an epidemic problem owing to limited therapeutic options (Schito, 2006). Drug resistance in *S. aureus* arises due to various mechanisms such as enzymatic inactivation, mutation of the target site and decreased penetration or increased efflux (Blair *et al*., 2015). Target site modification and antibiotic inactivation have accounted for resistance to a single class of compounds. MDR in *S. aureus* is attained by efflux of drugs through efflux pumps, which are chromosomally encoded (Stavri *et al*., 2007). Extrusion of diverse classes of structurally unrelated drugs through the efflux pumps is one of the favoured and broadly recognized mechanisms adopted by *S. aureus* strains (Winkler *et al*., 2015; Hashem *et al*., 2013). Efflux pumps serves as a primary mechanism to withstand antimicrobial stress and export an extensive range of structurally unrelated antibiotics from the cell by providing self defense to bacteria, resulting in reduced intracellular concentration and thus reduced susceptibility and promotes appearance of MDR phenotypes (Costa *et al*., 2015). The physiological role of efflux pumps in bacteria is to eliminate endogenous metabolites that get entry into the cell (Piddock, 2006b; Poole, 2008). Efflux pumps may selectively extrude one substrate or may transport various compounds with different structures (including antibiotics of multiple classes) conferring a MDR phenotypes. A single efflux pump can make bacteria resistant to a wide array of structurally and chemically diverse compounds (Piddock, 2006a). Efflux mediated resistance to single or multiple antimicrobial agents has not only raised serious concerns, but also has constricted the treatment therapies against bacterial infections (Nikaido, 1996). In the prokaryotic kingdom, MDR efflux pumps based on sequence comparison are classified in five major families of efflux transporter such as MFS (Major Facilitator Superfamily), RND (Resistance-Nodulation-Division), SMR (Small Multidrug Resistance), ABC (ATP binding cassette) and MATE (Multidrug and Toxic Efflux) (Lomovskaya *et al*., 2001). *S. aureus* belongs to MFS family which is an ancient, large, diverse, ubiquitous and main drug resistance conferring superfamily in Gram positive bacteria. Genome sequencing data suggests that there are numerous MDR efflux pumps present in this organism and more than ten chromosomally or plasmid encoded multidrug efflux pumps have been characterized. The efflux pumps characterized in *S. aureus* include NorA, NorB, NorC, MdeA, MepA, SepA and TetK (Jarmula
NorA is a chromosomally encoded predominant protein efflux pump in *S. aureus*, which uses proton motive force to energize the transportation of antimicrobial compounds across the cell membrane, via H\(^+\) drug antiport mechanism (Ubukata et al., 1989; Sabatini et al., 2012). The overexpression of NorA efflux pump due to its promiscuous substrate specificity is associated with resistance to hydrophobic fluoroquinolones (Norfloxacin and Ciprofloxacin), dyes (Ethidium bromide) and biocides (quaternary ammonium compounds) (Kaatz et al., 1993; Neyfakh, 1992). The chromosomal gene *norA* that codes for NorA pump is expressed in bacterial genome and increased expression of this gene is responsible for the resistance of these compounds by decreasing the intracellular concentration (Kaatz et al., 1993; Kaatz and Seo, 1995; Couto et al., 2008).

Inhibition of an efflux pump may potentially improve the clinical efficacy of an antibiotic and decrease the selection of resistant mutants (Lomovskaya et al., 1999). Novel and promising approach to deal with multidrug resistance of various antibiotics may be done by employing potent resistance modifying agents or efflux pump inhibitors (EPIs), which can restore and increase the susceptibility of resistant strains to antibacterial agents (Sabatini et al., 2012; Mahamoud et al., 2007; Poole and Lomovskaya, 2006). Such inhibitors can bind directly to the pump and block it in a competitive manner or a non-competitive manner with the substrates (Hasdemir et al., 2004). The combination of antibiotic with EPIs would be expected to re-establish susceptibility of the bacteria to antibiotics that are currently not in use due to resistance (Holler et al., 2012). Also, EPIs are expected to decrease the intrinsic and reverse acquired resistance and decrease the frequency of emergence of efflux proficient strains (Lomovskaya and Watkins, 2001). EPIs can also overcome the efflux of antibiotics and combat the drug efficacy by inhibiting drug binding to cytoplasmic membrane pumps and targeting energy sources and regulatory network that controls expression of efflux pump genes (Kumar and Schweizer, 2005).

Many efflux pumps possess significant structural homology, it is hoped that one inhibitor compound may be active against a range of efflux pumps from different bacterial species (Lomovskaya et al., 2001). Using the EPIs together with antibiotics can reduce the invasiveness of *S. aureus* besides its role in lowering the concentration of antibiotic (Hirakata et al., 2009).

Drug discovery from natural sources is an area pertinent to complementary and alternative medicines and provide basis for the isolation of unique and potentially effective bioactive compounds (Dhillion et al., 2002). Medicinal plant extracts play very important role in the
healthcare systems of large extents of the world’s population, particularly in the developing countries, where the herbal medicine has a longer and uninterrupted history of use (Koduru et al., 2007). Plants have been explored comprehensively as resistance modifying agent’s as potential sources of antimicrobials and play a significant role in the development of novel drugs (Bellini et al., 2008). These resistance modifying, modulating or reversal agents can specifically target resistance mechanisms or may act in synergistic manner because many diseases possess a multicausal agents and complex pathophysiology requiring treatment with different drug combinations (Gibbons et al., 2003). In combination with an antibiotic, resistance modifying agent will increase the cellular concentration of the antibiotic thus restoring its efficacy and will also reduce the emergence of antibiotic resistance (Markham et al., 1999). These drug-herbal combinations can also prevent the global increase of undesirable side effects of certain antibiotics and could facilitate the reintroduction of therapeutically ineffective antibiotics back into clinical use. Synergism between known antibiotics and bioactive plant compounds is a novel concept and it could be beneficial for control of S. aureus infections (Michael et al., 2007). Combination therapy might even synergistically increase the susceptibility of the bacteria. However at present no such EPI/antibiotic combination is in clinical use against S. aureus and current therapeutic problem is still uncertain (Holler et al., 2012). A number of novel compounds based on their NorA efflux pump inhibition properties have been investigated, but none of such compounds have entered in clinical trials in human or veterinary settings due to their pharmacokinetic properties and toxic effects. Thus, it is of great importance to isolate and identify bioactive compounds with pharmacokinetic properties and less toxic effects. The new active compounds present in medicinal plants may give new hope for their future therapeutic usage as an adjuvant of antibiotic (Koduru et al., 2007).

Keeping in view, the problem of drug resistance due to efflux of hydrophobic fluoroquinolones (Ciprofloxacin and Norfloxacin), mediated by NorA pump of S. aureus, the present study was planned to explore the bioactive molecules having efflux pump inhibitory activity against NorA pump of S. aureus strains from natural resources by screening medicinal plants found in Western Himalayas.
1.1 AIM AND OBJECTIVES

The aim of the present study was isolation and characterization of efflux pump inhibitors of \textit{S. aureus} from medicinal plants of Western Himalayas. To achieve the aim, following objectives were designed:

1. To evaluate synergistic effect of antibiotics with plant extracts against NorA proficient \textit{Staphylococcus aureus} strains.

2. Screening of crude plant extracts for efflux pump inhibitory activity of NorA strains of \textit{Staphylococcus aureus}.

3. Isolation and characterization of bioactive compound(s).

4. Evaluation of bioactive compound in lowering the MIC of chosen antimicrobials (antibiotics and Quaternary ammonium compounds) against NorA strains of \textit{Staphylococcus aureus}. 
