

## Review Article

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# A review: Use of plant extracts and their phytochemical constituents to control antibiotic resistance in *S. aureus*

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### Abstract

Although the invention of antibiotics has reduced the morbidity and mortality due to infectious diseases but the inappropriate use of antibiotics both for humans and animals has led to the emergence of multidrug resistant strains of bacteria and fungi. Among the antibiotic resistant pathogens, *S. aureus* is the pathogen of greatest concern due to its intrinsic virulence, its ability to cause a large number of infections and to survive under various environmental conditions. Modification of antibiotic ( $\beta$ -lactamases), use of alternative target site (PBP2a), efflux of antibiotics (NorA) and biofilm formation are some of the mechanisms utilized by *S. aureus* to develop resistance against antibiotics. This has led to the increased interest in traditional medicine that has long been used to cure various infectious diseases at different regions of the world. Plant extracts serve as an important source to combat microbial resistance because they consist of complex mixture of phytochemical constituents against which the development of resistance is very slow. In addition, phytochemical constituents present in the plant extracts also act as immunomodulatory and anti-inflammatory agents. The current study aims at stimulating the search of plant extracts and their phytochemical constituents to control microbial resistance. In future, plant extracts as well as their purified constituents could be used as potent therapeutic agents for the treatment of infections caused by *S. aureus* without obvious side effects.

**Keywords:**  $\beta$ -lactamases; Biofilm formation; Efflux pump; Multidrug resistance; Phytochemical constituents; Plant extracts

### Introduction

Discovery of penicillin in 1929 not only revolutionized the field of antibacterial therapy but also led to the discovery of large number of effective antibiotics from bacteria and fungi. However, in 1940s, penicillin-resistant strains of microbes

began to appear due to its widespread use [1, 2]. Similarly, bacteria have developed resistance to other commonly used antibiotics [3]. Necessity is the mother of invention; therefore, a man is in continuous search for the discovery of new remedies to control the infectious diseases and

emerging resistance of microbes. Plants especially herbs have been used to cure diseases since ancient times. About 80% of the world population living in developing countries relies on herbs as well as other medicinal plants for therapeutic purposes and their uses are increasing day by day due to the side effects of allopathic medicine [4]. For the promotion and development of herbal medicine, World Health Organization has made a strategic plan which includes four areas: a) Recognition of herbal medicines as valuable resources and promotion of their use. b) Development of comprehensive program for identification, cultivation and recognition of herbal medicines as valuable products. c) Introduction of measures for the assessment of medicinal plants to ensure the quality control of plant based products in order to include the herbal medicines in the national standard or pharmacopoeia. d) Development of programs by international cooperation and coordination for the conservation of plants that serve as valuable source of traditional or modern drugs in order to prevent their extinction [5].

*S. aureus* as an alarm to human health

In early 1970s, optimistic view of physicians that virtually all bacterial infections can be controlled by using a wide array of antimicrobial agents, was shaken due to the emergence of antibiotic resistant strains of bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*). Several factors contributed to the evolution of these antibiotic-resistant pathogens and one of these factors is the widespread use of antibiotics to control both human and animal infections [6, 7]. Among the antibiotic resistant strains, *S. aureus* is the pathogen of greatest concern due to its intrinsic virulence, its ability to cause a large number of infections and to survive under different environmental conditions [8]. Besides the availability of effective antimicrobial agents, the mortality rate for *S. aureus* bacteremia remains approximately 20-40% [9]. Now, *S. aureus*

is the leading cause of a variety of community and hospital acquired infections ranging from mild skin infections to severe infections including osteomyelitis, pneumonia, endocarditis and sepsis [10].

### **Molecular mechanisms of antibiotic resistance**

#### **Resistance to penicillins**

Development of antibiotic resistance has changed the strategy to treat *S. aureus* associated infections. In the late 1960s, more than 80% of both community and hospital acquired *S. aureus* strains were found to be resistant to penicillins. Rapid emergence of penicillin resistance was due to the horizontal transfer of blaZ gene that encodes  $\beta$ -lactamase enzyme. This enzyme hydrolyzes the  $\beta$ -lactam ring of penicillins and thereby rendering them ineffective [11].

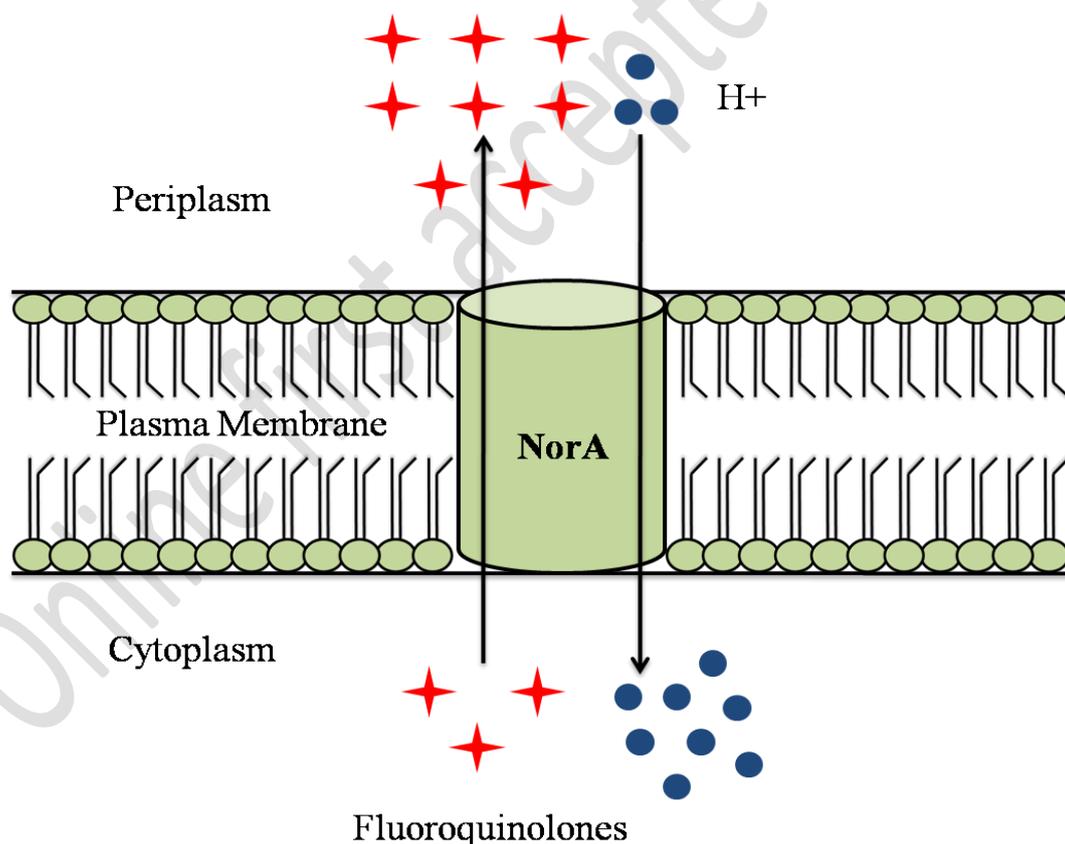
#### **Resistance to methicillin**

To overcome the problems of penicillins, semisynthetic penicillinase resistant penicillin called methicillin was introduced in 1961 but its introduction was followed by a large number of cases of methicillin resistant *S. aureus* [12]. Resistance to penicillin is due to the presence of mecA gene that encodes penicillin binding protein 2a (PBP2a or PBP2') and is located on chromosomal island designated as staphylococcal cassette chromosome mec (SCCmec). PBP2a is a membrane bound enzyme that catalyzes the cross-linking of peptidoglycan chains [13, 14]. PBP2a has low affinity with  $\beta$ -lactam antibiotics and thereby allowing the *S. aureus* to survive in the presence of high concentrations of  $\beta$ -lactam agents. mecA gene confers resistance not only to  $\beta$ -lactam agents but also to Cephalosporins [15]. Continued use of vancomycin to control MRSA infections in hospitals led to the emergence of vancomycin resistant MRSA strains. A case of an MRSA with reduced susceptibility to vancomycin was first reported in 1997 in Japan. Resistance to vancomycin is due to the mutations in the genes that are involved in the biosynthesis of cell envelope [16].

#### **Resistance to fluoroquinolones**

Another mechanism of resistance to antibiotics utilized by *S. aureus* is to reduce the intracellular concentration of antibiotics. Reduction in intracellular concentration of antibiotics is achieved either by decreasing the membrane permeability or by efflux of antibiotic out of the cell [17, 18]. The purpose of these efflux pumps is the elimination of intracellular metabolites that are toxic to cell. In addition, bacterial efflux pumps also play their role in the secretion of virulence determinants (hemolysins, proteases, coagulases and clumping factors) and maintenance of cell under stress conditions [19]. Some efflux pumps are specific, extruding only one antibiotic or a class of antibiotics whereas others can extrude several classes of antibiotics and are

designated as multidrug resistance (MDR) efflux pumps. Until now, more than ten MDR efflux pumps have been identified in *S. aureus*. NorA is one of the most studied MDR efflux pump of *S. aureus* that is encoded by the chromosomal gene *norA*. NorA efflux pump was first reported in fluoroquinolone-resistant (Figure 1) bacterial strain isolated from a patient at Japanese Hospital in 1986 [20]. NorA comprises 388 amino acids and it consists of 12 membrane spanning segments. It shares 44% identity with *Bacillus subtilis* MDR efflux pump Bmr while it shares 24% identity with *Escherichia coli* tetracycline resistant efflux pump TetA. NorA efflux pump plays a significant role in the efflux of fluoroquinolones and dyes such as ethidium bromide and rhodamine [21].



**Figure 1. Efflux of fluoroquinolones by NorA. NorA utilizes the energy derived from proton motive force for pumping fluoroquinolones out of the cell, thereby providing resistance to *S. aureus* against fluoroquinolone antibiotics**

#### **Biofilm formation**

In addition to  $\beta$ -lactamases, PBP2a and

efflux pumps, another weapon utilized by *S. aureus* to develop resistance to antibiotics

is the formation of biofilms. Biofilm formation is used as a defense mechanism by *S. aureus* as it provides protection against host defense system and antibiotic therapy. Moreover, biofilm associated infections are more chronic and mostly occur in hospitals [22]. Biofilm formation in *S. aureus* involves three steps: initial attachment of bacteria to biotic or abiotic surface, proliferation which is preceded by the production of ECM (extracellular matrix) that consists of extracellular polysaccharides, teichoic acid and DNA released by lysed cells, and final step of biofilm production is the detachment. Detachment of biofilm is under the control of quorum sensing system Agr but the quorum sensing control of biofilm detachment in *S. aureus* is not yet defined. However, the enzymes that can degrade the biofilm polymers have been identified in *S. aureus* [23-25]. Due to biofilm formation, *S. aureus* including MRSA are found to be resistant to Clindamycin which was once effectively used to control *S. aureus* associated infections. Now, Linezolid and Vancomycin can be used to control the serious infections of *S. aureus* [26]. At present, there is a need for the search and development of new antimicrobial agents other than antibiotics to overcome the microbial resistance.

#### **Use of botanicals to combat bacterial antibiotic resistance**

In order to combat emerging microbial drug resistance, botanicals including herbs, shrubs and traditional medicine could be used as alternative to antibiotics. A variety of compounds is produced by the plants that are not required for their primary metabolism but significantly increases the adaptive capability of the plant to survive under unfavorable biotic and abiotic environments. The secondary metabolites of the plants (alkaloids, phenols, flavonoids, quinones, tannins, terpenes and lectins) are well known to provide defense against herbivores and microbes [27, 28]. Methanolic leaf extract of *Yucca aloifolia* was found to be rich source of alkaloids,

flavonoids, saponins, tannins and steroids [29]. Besides this, hydro-alcoholic extract of the flowers of *Calliandra haematocephala* also serves as a rich source of phytochemical constituents (alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates and steroids) and exhibited strong antibacterial activity against *S. aureus* at the concentration of 100µg/ml by agar well diffusion assay [30].

#### **Alkaloids**

Among the secondary metabolites of plants, alkaloids are the group of heterocyclic nitrogen containing compounds that act as pain relievers and can also be used as narcotics. Alkaloids such as harmaline and berberine also intercalate with DNA and thereby resulting in impairment of cell division [31].

#### **Phenols**

Phenols are aromatic secondary metabolites of plants that are further divided into flavonoids, quinones, tannins and coumarins. Flavonoids are the phenolic compounds of plants that are present in edible parts of plants (vegetables, fruits and nuts). Antimicrobial activity of flavonoids resides in their ability to increase the permeability and disruption of plasma membrane by interacting with membrane proteins present on bacterial cell wall. In addition, flavonoids also have anti-inflammatory and antitumor activities [31]. Quinones are the aromatic compounds with two ketone substitutions. Antimicrobial activity of quinones is due to their binding to cell surface adhesin proteins, cell wall polypeptides and enzymes bound to membranes [32]. Tannins are the phenolic compounds that are found in all parts of a plant and are known to have antibacterial and antifungal activities. Their antimicrobial activity is due to their ability to inactivate transport proteins as well as cell surface adhesins [33]. Coumarins such as chalcones and scopoletin which have been recently isolated from *Fatoua pilosa*, consist of benzene ring fused to alpha pyrone ring and have antitubercular activity [34].

## Terpenes

Terpenes form the largest group of natural compounds and their identification is done on the basis of isoprene units they possess. Terpenoids which are the derivatives of terpenes exhibit antibacterial effects but their mechanism of antibacterial activity is not clearly defined. It is estimated that terpenoids work by disrupting the bacterial membranes [35].

## Lectins

Lectins are larger than polypeptides and contain mannose containing molecules. Their antimicrobial activity is due to their ability to compete with bacterial cell surface adhesion molecules for binding to host polysaccharide receptors [36, 37].

## Other antimicrobial phytochemicals

Besides alkaloids, phenolic compounds and lectins, many other phytochemicals have also been reported to exert antibacterial activities. There are some reports on the antibacterial activities of polyamines (spermidine), glucosides, thiosulfinates and isothiocyanates. In Brazil, acetylene compounds and flavonoids derived from plant are traditionally used for the treatment of malaria and liver disorders. Cranberry and blueberry juices have been used for therapeutic purposes for the treatment of urinary tract infections. In 1990s, researchers found that monosaccharide fructose present in cranberry and blueberry juices competitively inhibit the adsorption of pathogenic *E. coli* to epithelial cells of urinary tract [38].

## Mechanism of action of botanicals

Bacteria used efflux pumps as a mechanism to develop resistance to antibiotics so interfering with the functions of these efflux pumps would be an interesting therapy to control infectious diseases. Various plant derived compounds such as reserpine, berberine, methoxylated flavones and isoflavones have putative inhibitory effects on efflux pumps [39]. The alkaloid reserpine from *Rauvolfia vomitoria*, terpenecarnosic acid from *Rosmarinus officinalis* and the diterpenetotarol from *Chamaecyparis*

*nootkatensis* inhibit the function of NorA of *S. aureus*. Another inhibitor of NorA is 5-methoxyhydnocarpin. It was reported that 5-methoxyhydnocarpin significantly increased the antimicrobial activity of berberine (an alkaloid) in the same plant [40]. Besides inhibiting the function of efflux pumps, various plant extracts affect the virulence factors of bacteria especially *P. aeruginosa* with altering the quorum sensing (QS) gene expression [41]. Extracts of *Berberis aristata* and *Camellia sinensis* showed antibacterial effects by targeting the hemolysins and hemagglutination on bacterial cell surface [42]. Sub-inhibitory concentrations of various plant extracts affect the virulence of both gram positive and gram negative bacteria [43, 44]. In addition, various plant extracts have immunomodulatory effects. Since the plant extracts consist of complex mixture of phytochemical constituents, emergence of microbial resistance to the complex mixture of compounds may be much slower than those of antibiotics [45].

## Prevention of skin-associated infections by plant based antiseptic soaps

In order to prevent, control and reduce microbial infections, hand washing is crucial. Health care workers are potent source of transmission of MDR pathogens from one person to another. Proper hand washing and drying terminate the chain of transmission of pathogens from hands to other body parts. Hence, hand sanitizers and antiseptic soaps are being utilized for hand washing purposes. Although, chemical antiseptic soaps have played a vital role in reducing the transmission of infectious diseases but they have some adverse effects including skin irritation and microbial resistance [46]. In order to overcome the problems associated with chemical antiseptic soaps, there is a need for the development of plant based antiseptic

soaps that will be less toxic and inexpensive [47]. Researchers are now in continuous search for the development and evaluation of plant based soaps that may be used as alternatives to harmful synthetic soap products. It is not surprising to find that synthetic foaming agent sodium lauryl sulfate (SLS) in soaps is replaced by saponins [48], synthetic antibacterial agents such as Triclosan are replaced with natural antimicrobial agents [49] and synthetic antioxidant agents such as BHT (butylated hydroxytoluene) are replaced with phenolic compounds [50].

### Conclusion

Discovery of antibiotics is marked as a significant scientific advancement in the field of medicine as they helped in controlling *S. aureus* associated infections. But an inappropriate use of antibiotics has led the evolution of *S. aureus* strains that are not only resistant to conventional antibiotics like penicillins but also resistant to other previously used antibiotics like fluoroquinolones. To overcome the emerging resistance of microbes, plant extracts and their phytochemical constituents are of great importance. Plant extracts are complex mixture of phytochemical constituents against which the development of microbial resistance is very slow. In addition, purified phytochemical constituents of plants have strong antimicrobial properties and are of great interest in scientific community. Not surprisingly, traditional medicinal plants and their purified phytochemical constituents are now increasingly employed to control microbial infections. Another application of plant extracts and their phytochemical constituents in their incorporation in antiseptic hand washing soaps as appropriate hand washing can terminate the chain of transmission of *S. aureus* from hands to other body sites of the same or different person.

### Authors' contributions

Conceived and designed the experiments: A Nazir, K Malik, H Qamar, MH Basit, A

Liaqat, M Shahid, MI Khan, A Fatima, A Irshad & H Sadia, Performed the experiments: A Nazir, K Malik, H Qamar, MH Basit, A Liaqat, M Shahid, MI Khan, A Fatima, A Irshad & H Sadia, Analyzed the data: A Nazir, K Malik, H Qamar, MH Basit, A Liaqat, M Shahid, MI Khan, A Fatima, A Irshad & H Sadia, Contributed materials/ analysis/ tools: A Nazir, K Malik, H Qamar, MH Basit, A Liaqat, M Shahid, MI Khan, A Fatima, A Irshad & H Sadia, Wrote the paper: A Nazir, K Malik, H Qamar, MH Basit, A Liaqat, M Shahid, MI Khan, A Fatima, A Irshad & H Sadia.

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