INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 2, p. 1-20, 2022

REVIEW PAPER OPEN ACCESS

Antimicrobial and synergistic activity of bioactive molecules against drug-resistant bacteria: A review

Muhammad Afzal[1], Maryam Khan[1], Fiaz Ahmed[1,2,3], Hamadia[1], Arif Malik[1], Saba Shamim[*1]

[1]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road Campus, Lahore, Pakistan*

[2]*Shaikh Zayed Hospital, Lahore, Pakistan*

[3]*Shalamar Hospital, Lahore, Pakistan*

Key words: Phytochemicals, Antibacterial activity, Synergistic activity, Antimicrobial mode of action

http://dx.doi.org/10.12692/ijb/20.2.1-20 Article published on February 7, 2022

Abstract

This review outlines the antimicrobial and synergistic activities of bioactive molecules of plant origin against multidrug-resistant (MDR) bacteria. The pervasiveness of MDR microorganisms has become the most serious problem of public health concern. Overuse/misuse of antibiotics is a major reason for the development and propagation of MDR strains of several groups of bacterial strains. To overcome these circumstances, the scientists paved their way to perform researches on plants to find out possible antimicrobial compounds. The plant kingdom constitutes a vast reservoir of phytochemicals having medicinal values including antibacterial, antifungal and anticancer activities. Research investigations indicate that various plants contained many bioactive agents like as alkaloids, peptides, phenolic compounds, tannins, flavonoids and essential oils etc. These bioactive molecules have prospective therapeutic indications against resistance strains of human pathogens. The possible mechanism of plant extracts against antibiotic resistance bacteria is targeting distinct sites, not used by antibiotics. Contagious diseases are very common in third world countries. There is an urgent need to find out the novel molecular targets to circumvent resistant mechanism. Previous ethno-botanical records and recent research publications advocate that higher plants are napping giants of the pharmacological industry.

* **Corresponding Author:** Saba Shamim ✉ sabashamimgenetics@gmail.com

Introduction

Antimicrobial resistance has become the most serious problem of public health concern. Inappropriate practice of antibiotics has become a foremost aspect for the development and propagation of multidrug-resistant (MDR) bacteria of several groups. The phenomena of antibiotic resistance depends upon the precise nature of the bacteria-antibiotic relationship, the practice of antimicrobial drugs, host physiognomies and environmental aspects. Consequently, a decreased in therapeutic efficacy of antibiotics leading to treatment failure, recurrent infections and an increase in morbidity and mortality. This situation intended to search for novel bioactive molecules from medicinal plants (Cordell, 2000, Abiramasundari *et al*., 2011, Borges *et al*., 2015). Primitive people used plant sources as food, medicine and shelter for their survival. With passage of time as dependency on plants increased, man learn to utilize plants for various other purposes. Higher plant from wild origin have always been source of intention for their potential to demonstrate important properties for human comfort (Ali and Blunden, 2003). Phytoconstituents from higher plants possess anti-infective potential against various pathogens and can be applied as an alternative therapeutic agent in the management of infectious diseases not responding to antibiotics therapy. The interest in green medicines aroused from a believe that plant-sourced medicines are effective, safer and having minute adverse effects comparatively to the synthetic pharmaceutical drugs (Srinivasan *et al.,* 2001; Satish *et al.,* 2008; Abubakar, 2010).

According to western pharmacopoeia, ethno-medicines offered about 7000 pharmaceutically active compound like camptothecin, quinine, artemisinin etc. These different compounds have finding their way in the medicine industry including pharmaceuticals, cosmetics and neutraceuticals. These biologically active compounds also utilized in various food suppliments (Tshibangu *et al*., 2002). In spite of that plant-based medicines still underestimated particularly in the field of clinical microbiology (Karou *et al.,* 2005).

However bioactive molecules possessing the antimicrobial activity have always been attracting the scientists working in the field of clinical microbiology and this interest is growing with passage of time especially to combated phenomena of antimicrobial drug resistance (Clark and Hufford, 1993). Various studies argued the presence of significant compounds in plants which have potential therapeutic efficacy against bacteria, viruses and fungi. Some important knowing antimicrobial compounds are tannins, peptides, alkaloids, phenols, flavonoids and, essential oils. These substances may serve as an important antimicrobial agent against resistance human pathogens (Arora and Keur, 1999; Okigbo and Omodamiro, 2006). Previous ethno-botanical records and the recent research publications advocated that higher plants are napping the giants of the pharmacological industry (Hostettmann and Hamburger, 1991) (Figure 1).

Antimicrobial drug resistance

Antibiotics have ability to kill or inhibit the growth of bacteria by targeting different sites of microorganisms. Antibiotics are those substances produced by living organisms or derived synthetically by chemical means (Harrison and Svec, 1998; Kennedy *et al.,* 1998). Various drugs are isolated from microorganisms including bacteria and fungi. Some important antibiotics produced by bacteria are streptomycin, bacitracin, tetracyclines, chloramphenicol and polymyxin etc. whereas penicillin and cephalosporins are produced by fungi (McKeon *et al.,* 1995). Modern drugs including antibiotics helped humanity to overcome various fatal conditions of health concerned but on other hand a high ratio of antibiotics prescription paved the way of multidrug resistance in bacteria. Different factors like mutation in bacterial chromosomes, transfer of resistance genes by transposons and plasmid etc. contribute in the development of bacterial resistance in varied microbial species (Hart and Kariuki, 1998; Kimpe *et al.,* 2003, Salipante *et al.,* 2003; Shahid *et al*., 2003). A list of major risk factors for the development of anti-microbial resistance is given in Table 1.

Pharmaceutical industries have introduced new generations of antibiotics to combat resistance in microorganisms but these productions pose adverse impact with the development of multi-drug resistance bugs due to their genetic ability to acquire resistance against antibiotics and transmit resistance genes to microbial community (Cohen, 1992).

Hospital-acquired infections present more resistance to heal due to the frequent use of antibiotics in the patients and thus hospitals are initial place to spread resistant species. In this respect it seems that drug development programs delivered inadequate therapeutic cover in 10-20 years (Levy, 1998; Boucher *et al.*, 2009; Page and Heim, 2009). In some cases, even effective antibiotic therapy failed to overcome the infections. Some noticeable resistance pathogens i.e. *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* exhibit resistance to β-lactamases and *Staphylococcus aureus* may be found as methicillin-resistant and vancomycin-resistant species. Resistant strains cause treatment failure with lingering or relapsing infections which arises dilemmas in society about antibacterial therapy. Multidrug resistant pathogens render antibiotic therapy ineffective, hazardous and money consuming (Wormer and Bergman, 2002; Kepil, 2005; Alekshun and Levy, 2007; Ojha *et al.*, 2008).

Insufficient antibiotic therapy give rise to microbial resistance because causative pathogen was not susceptible to low doses of antibiotics rather they become resistant and result in treatment failure. This condition may associate with septicemia which may increases mortality rates especially in critically ill patients due to resistant pathogens including *S. aureus, E. coli, P. aeruginosa*, *K. pneumoniae*, *Enterobacter* spp. enterococci and coagulase-negative staphylococci (Ibrahim *et al.,* 2000; Kang *et al.,* 2005). Antibiotics largely used in veterinary field as prophylactics measures, growth promoters and therapeutic agents. This practice add antibiotic selection pressure which resulted in generation of multi-drug resistant pathogens. New generations of antibiotics have been introduced but seems ineffective against super bugs. Animal derived resistant strains posing great threat to animals as well as human community, and its call of time to design a methodology to control go up resistant pathogens (Endtz *et al.*, 1991; Wang *et al.,* 2011).

Despite of infection control programs, more logical medical practice and introduction of immunization, the up surging of resistant microorganisms causing more life-threatening infections and proposing great challenge to medical professions and scientists. In this respect most common Gram-positive resistant pathogens belong to staphylococci, enterococci and streptococci. The proposed mechanisms of antibiotic resistance in Gram-positive bacteria are inactivation or destruction of antibiotic, changes in active efflux or the target site (Russell *et al.*, 1998; Schiever *et al.,* 2005). On the other hand, resistant strains of Gram-negative bacteria also becoming more alarming and risky to the public health, and studies indicated that Gram-negative bacteria becoming resistant in faster way than Gram-positive bacteria (Tan, 2008; Cornaglia, 2009). The production of β-lactamases by Gram-negative bacilli is a significant mechanism of resistance against β-lactam antibiotics. Gram-negative bacteria acquired extended-spectrum β-lactamases (ESBLs) by horizontal gene transfer and produced resistance against oxyimino-cephalosporins. TEM and SHV are mutant derivatives of established ESBLs and widely produced by *Enterobacteriaceae* and some genes like CTX-M, mobilized from environmental bacteria (Albrich *et al.,* 2004). The β-lactams are chromosomally encoded by AmpC cephalosporinases. These are species-specific enzymes and commonly found in *Pseudomonaceae* and *Enterobacteriaceae*. Transmissible plasmids also mobilized these enzymes (AmpC) to the bacteria such as *K. pneumoniae, E. coli* and *Proteus mirabilis* which are lacking or have poor ability to express chromosomal *blaAmpC* gene (Jacoby, 2009). The development of metallo-β-lactamases has compromised the clinical efficacy of carbapenems against bacterial infections, and an increase in the production of AmpC may also induced resistance to

antibiotics of this class (Mena *et al.*, 2006; Walsh, 2008). Antibiotics resistance have been observed in various *in vitro* studies and clinical observations but no herb/spice resistance mentioned in literature. Garlic has been cultivated and used for centuries in human societies. In 1857, Louis Pasteur was the first who mentioned its antibacterial properties to the world (Pasteur, 1857). It seems logical to utilize plants-derived bioactive compounds to inhibit bacterial enzymes being an effective strategy to overcome bacterial resistance (Kim *et al.,* 1995).

Mechanism of antimicrobial drug resistance

Microorganisms have ability to developed antibiotic resistance as innate tendency or acquired by transferred resistant genes horizontally or vertically. Selective pressure of antibiotics lead to natural selection and ultimately evolution of resistance strains appeared in field. The antibiotics with broad spectrum activity resulted in more powerful resistant pathogens and an increase in lingering infections. There are four possible mechanism of bacterial resistance (Figure 2);

(1) Target modification which will lead to decreased affinity of antibiotics for target site or development of new target site which will prevent the attachment of drug.

(2) Enzyme production which will modify or inactivate antibiotic.

(3) Changes in antibiotic permeability by reduction of porin diameter or loss of porin in Gram-negative bacteria.

(4) Antibiotics efflux to outside the cells utilizing ATP pumps (Sefton, 2002; Pantosti and Moro, 2005; Vanderkooi *et al.,* 2005; Moselio, 2009).

Antibiotic resistance developed in the presence of two necessary elements:

(1) First element is the presence of an antibiotic, have capability to inhibit the vast number of microorganism existed in a colony.

(2) Second, at least one bacterium in a heterogeneous bacterial colony, which carries the gene capable of expressing the antibiotic resistance (Levy and Marshall, 2004).

Bacterial species can develop resistance by genetic means in two ways;

(1) Vertical evolution, by mutation of existing genes.

(2) Horizontal gene transfer, in which new resistant genes acquired from other bacterial strains or species.

This genetically mutated material carrying resistance ability to antibiotics, which can be transferred to other species by using different mechanisms including conjugation, transformation, and transduction (Martinez and Baquero, 2000; Jacoby and Munoz-Price, 2005; Palmer *et al.,* 2010).

In the environment, extra-chromosomal genes like naked DNA, transposons or plasmids served as mobile genetic elements and responsible for transfer of drug resistance genes among different taxonomical and ecological groups of bacteria. Resistance plasmids are extra chromosomal DNA molecules occurring in small, circular, covalently closed and double-stranded form which can transfer resistant determinants to bacterial populations mainly in Gram-negative bacteria. It is not essential for the bacterial growth and have ability to replicate independently. Transposons and integrons are also DNA mobile elements and presented on the plasmid. These DNA mobile elements cause rapid distribution of resistant genes among different groups of bacterial species. Methods like standardized plasmid typing, helped us to improve our understanding about host range and worldwide distribution of these mobile elements (Levy and Miller, 1989; Levy, 2002; McDermott *et al.,* 2002; Carattoli *et al.,* 2006; Carattoli, 2009; Jeters *et al.,* 2009; Wang *et al.,* 2011). It is need of time to adopt better strategies to combat an increase in the emergence of multi-drug resistant bacterial strains with development of novel antibiotics or modification of old ones (Tollefson and Miller, 2000).

Biofilm formation

Bacterial biofilms are colonization of unicellular organisms which come get together, attach to a solid

surface and enclosed by scaffold of exopolysaccharide matrix. Single or multiple bacterial species may involve in biofilm formation (Whittaker *et al.,* 1996). The bacterial biofilms can be developed on different surfaces, such as medical devices, living tissues, soil environments, natural aquatic or potable water pipes (Flemming and Wingender, 2001). Bacterial attraction and adhesion to the surface may involve different mechanisms including surface type and charge, Brownian motion, gravity and chemo-attraction (Jenkinson and Lappin-Scott, 2001). The process of biofilm formation is a general approach in the bacterial species to survive in nature, and to protect them from environmental stresses (O'Toole and Kolter, 1998; Flemming and Wingender 2001; Singh *et al.*, 2006; Ahimou *et al.,* 2007). Bacterial attachment with surface provide great opportunity to make strong bond with surface and association between bacterial communities. This proximity between bacteria confer an easy transfer of genes from one bacterium to another and these colonized bacteria metabolically more active than bacteria in plankton environment (Marshall, 1994). Biofilm interlinks produced by exopolysaccharide matrix, or glycocalyx, worked as barrier and prevents the penetration of antibiotics due to embedded bacterial cells and thus presented much more resistance in biofilm producing bacteria to antimicrobial drugs (Stewart, 1996; Mohamed *et al.,* 2007). The opportunity of biofilm formation allows bacteria to develop resistance to chemical biocides, antibiotics, host immune responses, amoebae, and bacteriophage in industrial and natural environments (Costerton *et al.,* 1999).

Quorum-sensing

Quorum sensing (QS) is a behavior coordination between one bacterium to another. This mechanism involves cell-to-cell communication among bacteria under changing situations. The phenomena of quorum sensing is ubiquitous and regulate a wide range of activities in many well-known bacterial classes. Quorum sensing contributes to produce phenotypic changes in bacteria by the variation of gene expression which helps bacteria to adjust in environmental circumstances during their growth (Camara *et al.,* 2002; Turovskiy *et al.,* 2007). Autoinducers are signaling molecules, produced by bacteria and play the most important role in mechanism of QS. These autoinducers diffuse out via the cell membrane and accumulate in the environment. QS is responsible to regulate various important function in bacteria which mainly involves in development of genetic capability, regulation of virulence factors, transfer of plasmid by conjugation, biofilm formation, synthesis of antimicrobial peptide, sporulation and symbiosis (Smith *et al.,* 2004).

There are two types of quorum sensing;

(1) Species-specific QS: There are two basic groups of signal molecules involved in species-specific QS, known as fatty acid derivatives and peptide derivatives signal molecules. In Gram-negative bacteria, species-specific QS is mediated by a fatty acid derivative signaling molecules know as acyl homoserine lactones (AHLs). These molecules have ability to coordinate in group-based behavior with varied species. Species-specific QS can be observed in Gram-positive bacteria, which is commonly aided through small peptide derivative signaling molecules.

(2) Interspecies QS: This type of communication associated with autoinducer-2 (AI-2), which are diester of furanosyl borate.

The mechanism of QS plays an important role in the development and regulation of pathogenicity and virulence factors in Gram-negative bacteria related to human and plant infections. In this regard some known genera including *Agrobacterium*, *Brucella*, *Bukholderia*, *Erwinia*, *Enterobacter*, *P. aeruginosa*, *Ralstonia*, *Serratia*, *Vibrio*, and *Yersinia* are can be evident (March and BentleyYa, 2004; Smith *et al.,* 2004; Williams, 2007). Since few decades, *P. aeruginosa* is to be consider an important microbe to understand the QS-related behavior and mechanisms in microorganisms. It is an opportunistic organism and responsible for infections in patients with cancer, cystic fibrosis and AIDS (Govan and Deretic, 1996; Smith *et al.,* 2002). A wide range of extracellular enzymes take part in *P. aeruginosa* virulence. These

enzymes are proficient to produce extensive tissue damage in humans. The well-known variety of these exofactors include protease, elastase, exotoxin A, phospholipase and rhamnolipid biosurfactants (Delden and Iglewski, 1985; van Delden and Iglewski, 1998). Biofilm formation and QS linked closely and this collaboration plays a central role in the development of pathogenicity of various bacteria in infections (Parsek and Greenberg 2005; Sakuragi and Kolter, 2007).

Table 1. Risk factors for the development of antibiotic resistance.

- Irrational, excessive and overuse of antibiotics
- Prophylactic use of antibiotics in hospitalized patients to prevent infections
- Frequent therapeutic prescription in recurrent infections
- Prophylactic and therapeutic use in poultry field
- Long term use of antibiotics in seriously ill patients, immunocompromised patients, having congenital diseases like cystic fibrosis and low sanitation and hygiene
- Ineffective measures for prevention and control of infection such as proper hand washing, logical use of antibiotics, isolation of patient having resistant infections
- Utilization of prosthetic devices amenable to super-infections with multi-drug resistant bacteria

(Adapted from Alfonso, 2005).

Mechanism of antimicrobial activity of phytoconstituents

In bacteria, biosynthesis of fatty acids is an essential step for the building of cell membrane and other lipid-containing components. Fatty acid synthesis (FAS) is carried out by a group of highly individualized enzymes known as type II fatty acid synthase (FAS II). In mammals, fatty acid synthesis is mediated by type I fatty acid synthase also known as acyl carrier protein (ACP) complex. Enoyl-acyl carrier protein reductase (ENR) plays a key role in the chain elongation process of the type II fatty acid synthesis. There is an overall difference in sequence and structural homolog between mammalian and bacterial fatty acid biosynthesis enzymes, thus ENR is a striking target for the development of narrow-spectrum antibiotics (Heath *et al.*, 2000; Payne *et al.,* 2001). Long-chain unsaturated fatty acids are well known for their antibacterial properties from a long time. Fatty acids are capable to inhibit the growth of undesirable microorganisms, thus functioning as a key antimicrobial additive in food. Despite of usual fatty acids, some fatty acids derivatives with potential antimicrobial activities are found in bacterial species, algae or plants, exist in the nature. These may also mediate chemical defenses against pathogenic microorganisms (Frees *et al.,* 1973; Dellar *et al.,* 1996; Pfefferle *et al.,* 1996). The possible mechanism of plant extracts against antibiotic resistant bacteria is to target some different sites, not used by antibiotics. However, the information about such activities is not fully clear (Hasegawa *et al.*, 1995; Lee *et al.*, 1998). Various *in vitro* studies demonstrate that different plant substances exhibit various antimicrobial mechanism by selecting different targets which includes cell membrane disruption, bind to adhesins, DNA gyrase disruption, enzymes inactivation etc. A list of phytoconstituents are given in Table 2 (Lanciotti *et al.,* 2004; Burt *et al.,* 2007; Arques, 2008; Proestos *et al.,* 2008).

Plants produced two types of basic compounds these are known as primary and secondary metabolites.

(1) Primary metabolites: These compounds contain carbohydrates, proteins, and lipids and have significant value for the growth and metabolism of plant.

(2) Secondary metabolites: Various secondary compounds and their derivatives are produced as a result of primary metabolism process known as secondary metabolites. These compounds serve as plant-based medicines including antimicrobial drugs

and provide defense to plants against microbes, pests, and herbivores (Ramawat, 2007; Vaghasiya *et al.*, 2011; Savoia, 2012) (Figure 3). To study the biological activities of plant constituents, raw materials were collected and analyzed for their bioactive compounds. A number of substances served as potential source for antimicrobial drugs production and depicted a significant therapeutic value against pathogenic organism including bacteria, fungi, and viruses.

The compounds exhibiting antimicrobial properties included peptides, alkaloids, phenols, flavonoids, tannins and essential oils (Arora and Keur, 1999; Okigbo and Omodamiro, 2006).

Table 2. Basic classes of antibacterial phyto-constituents.

Class	Subclass	Example(s)	Mechanism
Phenolic compounds	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Disruption of cell membrane
	Phenolic acids	Cinnamic acid	Binding to adhesins proteins, complex with the bacterial cell wall, enzymes inactivation
	Quinones	Hypericin	
	Flavonoids	Chrysin	Binding to adhesions proteins Complex with bacterial cell wall
	Flavones	Abyssinone	Enzymes inactivation Inhibit the HIV reverse transcriptase
	Flavonols	Totarol	?
	Tannins	Ellagitannin	Binding to adhesins proteins Enzyme inhibition Deprivation of substrate Complex with bacterial cell wall Disruption of bacterial cell membrane Metal ion complex formation
	Coumarins	Warfarin	Showing antiviral activity by interacting with eukaryotic DNA
Terpenoids, essential oils		Capsaicin	Bacterial cell membrane disruption
Alkaloids		Berberine Piperine	Inserted into the bacterial cell wall and/or DNA
Lectins and polypeptides		Mannose-specific agglutinin Fabatin	Block the viral fusion or adsorption Form disulfide bridges

(Adapted from Cowan, 1999).

Therapeutically active phyto-constituents may be utilized as anti-bacterial, fungicide, insecticide, anti-viral, anti-plasmodial, spasmolytic, laxative, febrifuge or antioxidant substance (Iqbal *et al.*, 2011). Quinine is a naturally occurring alkaloid and found in the bark of Cinchona tree. It is famous for the treatment of malaria and to relieve nocturnal leg cramps as well (Iwu *et al.,* 1999). Research studies claimed that phenolic compounds presented in different herbs and spices have ability to inhibit foodborne pathogens and showed antimicrobial properties against human pathogens (Nychas, 1995; Smid and Gorris, 1999; Prashanth *et al.,* 2001; Hara-Kudo *et al.,* 2004; Kim *et al.,* 2005). Various plant extracts contain highly hydroxylated phenolic compounds. Polyphenols are well known to have antimicrobial activities against massive strains of pathogenic microbes. They include hydroxycinnamate derivatives, hydroxycoumarins, flavanols, flavanones, flavonols, flavones, tannins, anthocyanins, aurones, hydroxystilbenes, etc. (Scalbert, 1991; Cowan, 1999). Hydroxylated phenols (catechol with two 2OH groups and pyrogallol with three 2OH groups) have toxic properties against microorganisms. It is supposed that site(s) for

hydroxyl groups on the hydroxylated phenols are responsible for their toxic action to microorganisms. It indicates that an increase in hydroxylation may result in an increase in its toxic properties (Geissman, 1963). Tannins and flavonoids, are important polyphenols having antibacterial activity. Tannins are abundantly found in plants and provide chemical defenses against pathogenic bacteria and herbivory. It is suggested that tannin-containing beverages, like red wines and green tea have therapeutic values to cure or prevent diseases (Serafini *et al.,* 1994; Machado *et al.,* 2002; Gedir *et al.,* 2005). Flavonoids destroy a wide range of microbes by different mechanism of actions possibly through the disruption of cytoplasmic membrane, to make complex with extracellular, soluble proteins, and bacterial cell walls, DNA gyrase inhibition and β-hydroxy acyl-acyl carrier protein dehydratase activities (Tsuchiya *et al.*, 1996; Cushnie and Lamb, 2005; Zhang *et al.,* 2008). Essential oils are profoundly rich with terpenes, which promote cell membrane disruption. Coumarin reduced cell respiration, and tannin inactivate enzymes thus effect the microorganisms in various ways (Cai *et al.,* 1988; Cowan, 1999; Ulanowska *et al.,* 2006).

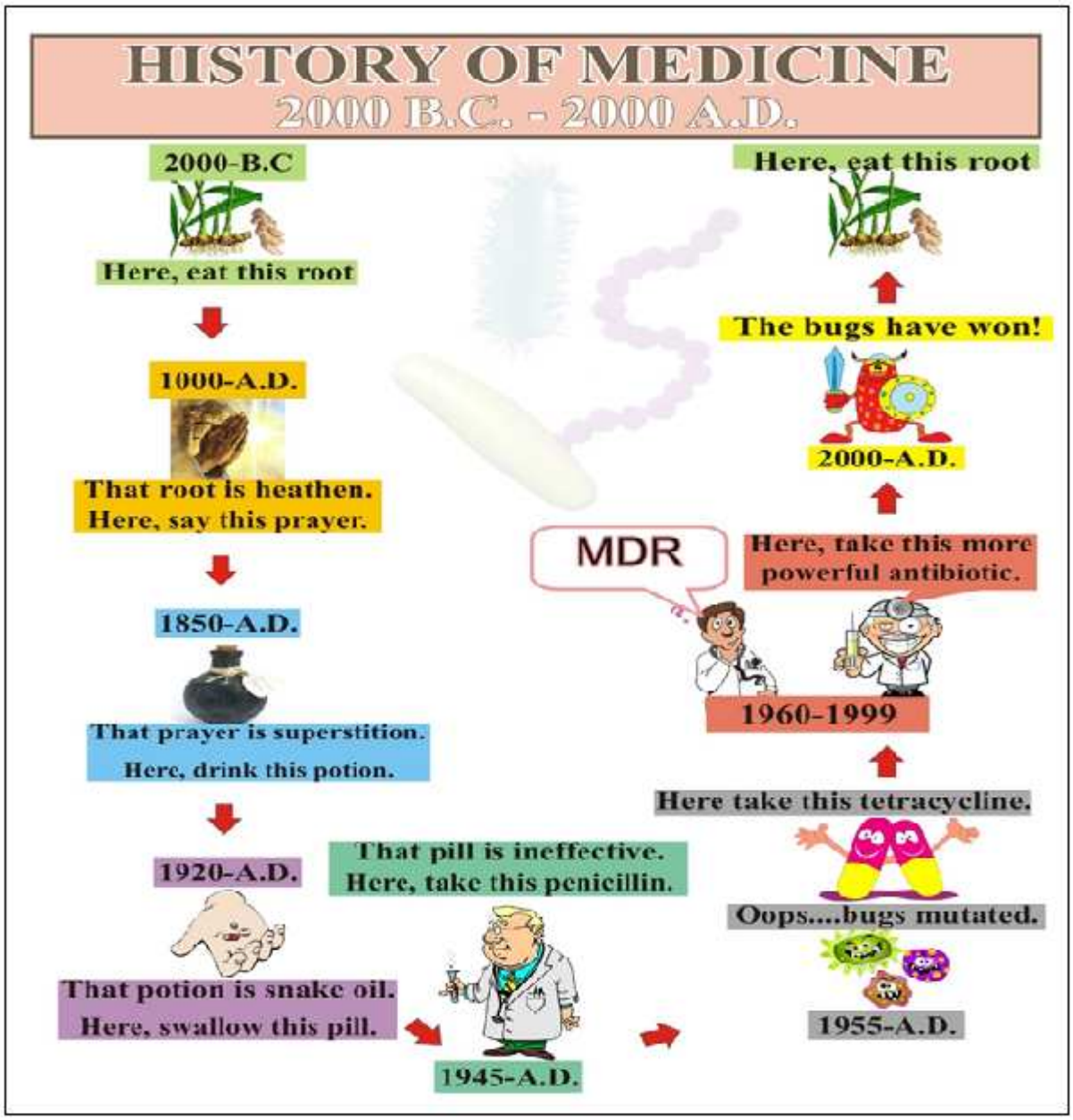

Fig. 1. Human history of using plants as a medicine.

Synergistic interaction of herbs

Synergism is the state in which two or more substances/ herbs interact or combined in such a way that their collective effect is greater than the effects of individual substance. In contrast, antagonism occurs when one substance in combination of two or more substances reduce or block the effects of others (Burt, 2004). The increasing risk of fatal infections due to resistant microbes, diverting the attention of medical scientist to the plants as a source of biologically active compounds. Plants and herbs are rich in various substances having ability to kill or inhibit pathogens. To find out the novel bio-compound, will help to reduce resistance and increase susceptibility of bacteria for antibacterial drugs. In the view of this situation various studies have been evaluated to observe the synergistic effects of plant isolated compounds with commonly used antibiotics. The positive outcomes of these interactions will help to develop new approaches to overcome the antibiotic resistance (Nostro *et al.,* 2006, Horiuchi *et al.,* 2007; Stefanovic *et al.,* 2009 a-b).

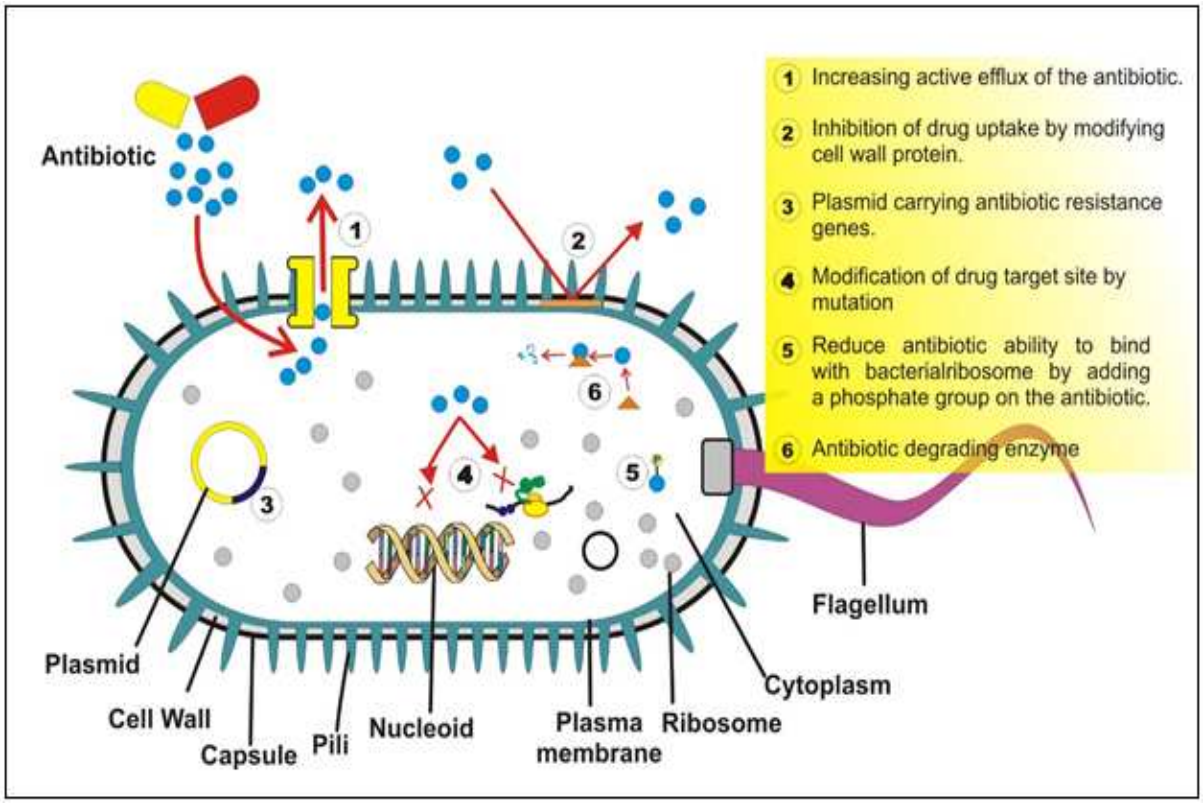

Fig. 2. Potential targets for antimicrobial drugs in a bacterial cell.

The synergistic approach to combining well-studied antimicrobials from plant source and synthetically produced antibiotics, will result in more powerful and effective therapeutics against multidrug resistant bacteria causing serious health problems. *In vitro* investigations indicated that even in a plant, a single substance is not responsible as a potential antimicrobial rather a combination of secondary metabolites interact in a synergistic way to produce a noticeable effect as an antimicrobial agent. A morphological difference between the cell wall of Gram-positive and -negative bacteria make them less or more sensitive to plant extracts. The cell membrane of Gram-negative bacteria becomes impermeable to organic solutes due to the presence of structural lipopolysaccharide in outer phospholipidic membrane and porins in cell membrane act as barrier for aqueous solutes. On the other hand, the Gram-positive bacteria is more sensitive to antimicrobials due to the lack of above features in cell membrane. In this regard, various bioactive compounds may produce synergy by combining with antibiotics, as these substances may not have ability to kill the bacteria rather these harbor ability to make the

pathogen sensitive for previously ineffective antibiotics (Hooton *et al.,* 1984; Arias *et al.,* 2004; Betoni *et al.*, 2006; Olgica and Ljiljana, 2011). The synergistic interaction may produce more powerful effects and expand the spectrum of therapeutic action which will help to prevent the evolution of resistant species and resolve the stubborn bacterial infections which are not responding to standard treatment with antibiotics. In addition, this synergy may reduce the possible toxicity and avoid unwanted side effects due to the overuse of antibiotics (Kubo *et al.,* 1996; Kamatou *et al.,* 2006).

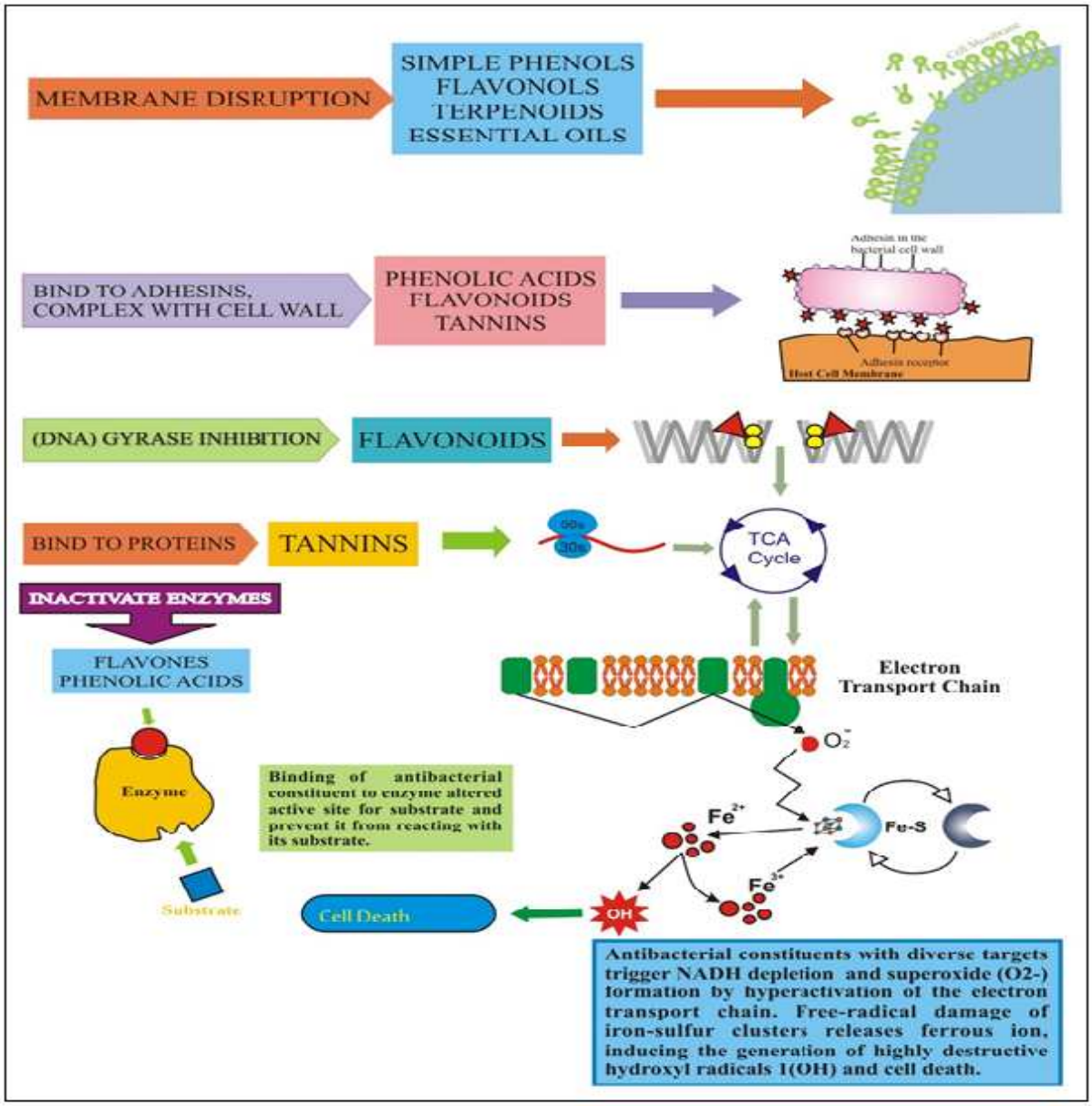

Fig. 3. Mechanism of action of antibacterial bioactive molecules in a bacterial cell.

Cumin seeds exhibit synergistic activity along with antibiotics and antibiotic/cumin interaction also assessed in dose-response preclinical studies. This combination proves very effective therapeutic tool against resistant strain of bacteria which is difficult to eradicate from antibiotic (Sivam, 2001). A vast number of commonly used antibiotics include ampicillin, chloramphenicol, co-trimoxazole, doxycycline, erythromycin, gentamicin, lincomycin, lincomycin, nalidixic acid, nalidixic acid, spectinomycin, Streptomycin, and tobramycin are experimented with diethyl ether extract of *N. sativa*. These interactions demonstrate synergistic and additive effects as antimicrobial agents. These studies

also revealed that *N. sativa* extracts increased sensitivity and reduced drug-resistance of Gram-positive and Gram-negative bacteria for antibiotics (Hanafy and Hatem, 1991; Morsi, 2000). A composition of extracts from *Azadirachta indica*, *Cucumis sativus* and *Citrullus colocynthis* is prepared to evaluate their synergistic efficacy in tooth and gums problems. This formulation was utilized as mouth wash to rinse the mouth. This combination prove effective to prevent inflammatory processes and infections like gingivitis and periodontal diseases and also works as anti-plaque agent (Behl *et al.,* 2004). It is anticipated that such synergistic combinations may inhibit different drug resistance mechanism in bacteria i.e. drug efflux pump (Zhao *et al.*, 2001; Lewis and Ausubel, 2006). Numerous *in vitro* studies point out significant synergistic effects between different crude plant extracts and antibiotics against resistance strain of *S. aureus*. This interaction also reduced the minimum inhibitory concentrations (MICs) of antibiotics (Yam *et al.,* 1998; Aqil *et al.,* 2005; Braga *et al.,* 2005; Betoni *et al.,* 2006; Esimone *et al.,* 2006; Adwan *et al.*, 2008).

Diverse variety of antimicrobial bio-molecules and synthetically produced antibiotics may affect promptly in a synergistic way by using low dose from each side and thus therapeutic out comes are more result oriented. It seems that pharmacologically active both drugs may clear the infection from body by different mechanism in a harmonious way. Thus, the synergistic relations help to prevent, delayed or reduced depressive resistances in the pathogens (Williamson, 2001; Lupetti *et al.,* 2002).

Conclusion

From the ancient time, plants are well recognized for their therapeutic value in different ailments. Various *in vitro* screenings of different plants and herbal extracts established a prominent space in pharmaceutical as a potential antibacterial chemotherapeutic agent in infectious diseases (Elvin-Lewis, 1980; Tona *et al.*, 1998). Antibacterial screenings proposed that Gram positive bacteria are likely more sensitive to herbal extracts than Gram negative species. This statement indicates the morphological differences in cell membrane structure among both bacteria (Zaika, 1988; Arias *et al.,* 2004; Ceylan and Fung, 2004; Lopez *et al.,* 2005; Betoni *et al.*, 2006).

In developing countries, contagious diseases are very common due to poor sanitation and hygienic situations. A frequent use of antibiotic therapy is a potential cause for drug resistant mutants. Drug resistant strains have invaded developed countries as well, so its call of time to find novel bio-molecules from natural sources to circumvent this condition. *In vitro* applications of drugs combination to study synergism against resistant bacteria showed the favorable results. The synergy of antimicrobial from natural and synthetic source will boost the therapeutic efficacy of antibacterial drug in clinical application to overwhelmed fatal infections from microbial origin. This review demonstrates the utilization of bioactive compound from plants and herbal origin in alone or combination with modern drugs to established more effective products to tackle rapidly spreading resistant pathogens (Hooton *et al.,* 1984; Williamson, 2001; Patra, 2012).

References

Abiramasundari P, Priya V, Jeyanthi GP, Gayathri DS. 2011. Evaluation of the antibacterial activity of *Cocculus hirsutus*. Hygeia Journal for Drugs and Medicines **3(2),** 26-31.

Abubakar EMM. 2010. Antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria. African Journal of Plant Science **4(6),** 202-209.
http://dx.doi.org/10.5897/AJPS

Adwan GM, Abu-Shanab BA, Adwan KM. 2008. *In vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections. Pakistan Journal of Medical Sciences **24(4),** 541-544.

Ahimou F, Semmens MJ, Haugstad G, Novak

PJ. 2007. Effect of protein, polysaccharide, and oxygen concentration profiles on biofilm cohesiveness. Applied and Environmental Microbiology **73(9),** 2905-2910. http://dx.doi.org/10.1128/AEM.02420-06

Albrich WC, Monnet DL, Harbarth S. 2004. Antibiotic selection pressure and resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Emerging Infectious Diseases **10(3),** 514-517. http://dx.doi.org/10.3201/eid1003.030252

Alekshun MN, Levy SB. 2007. Molecular mechanisms of antibacterial multidrug resistance. Cell **128(6),** 1037-1050. http://dx.doi.org/10.1016/j.cell.2007.03.004

Alfonso JA. 2005. Resistance to antibiotics: Are we in the post-antibiotic era? Archives of Medical Research **36(6),** 697-705. http://dx.doi.org/10.1016/j.arcmed.2005.06.009

Aqil F, Khan MSA, Owais M, Ahmad I. 2005. Effect of certain bioactive plant extracts on clinical isolates of β-lactamase producing methicillin resistant *Staphylococcus aureus*. Journal of Basic Microbiology **45(2),** 106-114. http://dx.doi.org/10.1002/jobm.200410355

Arias ME, Gomez JD, Cudmani NM, Vattuone MA, Isla MI. 2004. Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill. Ex Hook et Arn. Life Sciences **75(2),** 191-202. http://dx.doi.org/10.1016/j.lfs.2003.12.007

Arora DS, Kaur J. 1999. Antimicrobial activity of spices. International Journal of Antimicrobial Agents **12(3),** 257-262.

Arqués JL, Rodríguez E, Nuñez M, Medina M. 2008. Inactivation of Gram-negative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. European Food Research and Technology **227,** 77-82. http://dx.doi.org/10.1007/s00217-007-0695-8

Betoni JEC, Mantovani RP, Barbosa LN, Di Stasi LC, Junior AF. 2006. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Memórias do Instituto Oswaldo Cruz **101(4),** 387-390. http://dx.doi.org/10.1590/s0074-02762006000400007

Borges A, Saavedra MJ, Simoes M. 2015. Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. Current Medicinal Chemistry **22(21),** 2590-2614. http://dx.doi.org/10.2174/092986732266615053021 0522

Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical Infectious Diseases **48(1),** 1-12. http://dx.doi.org/10.1086/595011

Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone-Souza E and Nascimento AMA. 2005. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. Canadian Journal of Microbiology **51(7),** 541-547. http://dx.doi.org/10.1139/w05-022

Burt S. 2004. Essential oils: Their antibacterial properties and potential applications in foods-A review. International Journal of Food Microbiology **94(3),** 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Burt SA, van der Zee R, Koets AP, de Graaff AM, van Knapen F, Gaastra W, Haagsman HP, Veldhuizen EJA. 2007. Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157:H7. Applied and Environmental Microbiology **73(14),** 4484-4490. https://dx.doi.org/10.1128/AEM.00340-07

Cai Y, Gaffney SH, Lilley TH, Haslam E. 1988. Carbohydrate - Polyphenol complexation. In: Chemistry and significance of condensed tannins (Eds. Hemingway RW, Karchesy JJ.). Plenum Press, New York. p 307-322.

Camara M, Williams P, Hardman A. 2002. Controlling infection by tuning in and turning down the volume of bacterial small-talk. The Lancet Infectious Diseases **2(11),** 667-676. http://dx.doi.org/10.1016/s1473-3099(02)00447-4

Carattoli A. 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrobial Agents and Chemotherapy **53(6),** 2227-2238. http://dx.doi.org/10.1128/AAC.01707-08

Carattoli A, Miriagou V, Bertini A, Loli A, Colinon C, Villa L, Whichard JM, Rossolini GM. 2006. Replicon typing of plasmids encoding resistance to newer beta-lactams. Emerging Infectious Diseases **12(7),** 1145-1148. http://dx.doi.org/10.3201/eid1207.051555

Ceylan E, Fung DYC. 2004. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology **12(1),** 1-55. http://dx.doi.org/10.1111/j.17454581.2004.tb00046.x

Clark AM, Hufford CD. 1993. Discovery and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency syndrome. Human Medicinal Agents from plants, ACS Symposium series, Washington, DC **534,** 228-241. http://dx.doi.org/10.1021/bk-1993-0534.ch016

Cohen ML. 1992. Epidemiology of drug resistance: Implications for a post antimicrobial era. Science **257(5073),** 1050-1055. http://dx.doi.org/10.1126/science.257.5073.1050

Cordell GA. 2000. Biodiversity and drug discovery-a symbiotic relationship. Phytochemistry **55(6),** 463-480. http://dx.doi.org/10.1016/s0031-9422(00)00230-2

Cornaglia G. 2009. Fighting infections due to multidrug-resistant Gram-positive pathogens. Clinical Microbiology and Infection **15(3),** 209-211. http://dx.doi.org/10.1111/j.1469-0691.2009.02737.x

Costerton JW, Stewart PS, Greenberg EP. 1999. Bacterial biofilms: A common cause of persistent infections. Science **284(5418),** 1318-1322. http://dx.doi.org/10.1126/science.284.5418.1318

Cowan MM. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews **12(4),** 564-582. http://dx.doi.org/10.1128/CMR.12.4.564

Cushnie TPT, Lamb AJ. 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents **26(5)**, 343-356. http://dx.doi.org/10.1016/j.ijantimicag.2005.09.002

Delden CV, Iglewski BH. 1998. Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerging Infectious Diseases **4(4),** 551-560.

Dellar JE, Cole MD, Waterman PG. 1996. Unusual antimicrobial compounds from *Aeollanthus buchnerianus*. Experientia **52,** 175-179.

Elvin-Lewis M. 1980. Plants used for teeth cleaning throughout the world. The Journal of Preventive Dentistry **6,** 71-73.

Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP. 1991. Quinolone resistance in *Campylobacter* isolated from man and poultry following introduction of fluroquinolones in veterinary medicine. Journal of Antimicrobial Chemotherapy **27(2),** 199-208.

Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM. 2006. In vitro evaluation of the interaction between tea extracts and penicillin against *Staphylococcus aureus*. African Journal of

Biotechnology **5(11),** 1082-1086.
http://dx.doi.org/10.4314/ajb.v5i11.42972

Flemming HC, Wingender J. 2001. Relevance of microbial extracellular polymeric substances (EPSs)-Part I: Structural and ecological aspects. Water Science and Technology **43(6),** 1-8.

Freese E, Shew CW, Galliers E. 1973. Function of lipophilic acids as antimicrobial food additives. Nature **241(5388),** 321-325.
http://dx.doi.org/10.1038/241321a0

Gedir JV, Sporns P, Hudson RJ. 2005. Extraction of condensed tannins from cervid feed and feces and quantification using a radial diffusion assay. Journal of Chemical Ecology **31,** 2761-2773.
http://dx.doi.org/10.1007/s10886-005-8392-1

Geissman TA. 1963. Flavonoid compounds, tannins, lignins and related compounds. (Eds. Florkin M, Stotz EH). Comprehensive Biochemistry. Elsevier, New York **9(1963),** 213-250.
http://dx.doi.org/10.1016/B978-1-4831-9718-0.50018-7

Govan JRW, Deretic V. 1996. Microbial pathogenesis in cystic fibrosis: Mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Microbiology Reviews **60(3)**, 539-574.

Hanafy MS, Hatem ME. 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (Black cumin). Journal of Ethnopharmacology **34(2-3),** 275-278.
http://dx.doi.org/10.1016/0378-8741(91)90047-h

Hara-Kudo Y, Kobayashi A, Sugita-Konishi Y, Kondo K. 2004. Antibacterial activity of plants used in cooking for aroma and taste. Journal of Food Protection **67(12),** 2820-2824.
http://dx.doi.org/10.4315/0362-028x-67.12.2820

Harrison JW, Svec TA. 1998. The beginning of the end of the antibiotic era? Part I. The problem: Abuse of the "miracle drugs". Quintessence International **29(3),** 151-162.

Hart CA, Kariuki S. 1998. Antimicrobial resistance in developing countries. BMJ **317(7159),** 647-650.
http://dx.doi.org/10.1136/bmj.317.7159.647

Hasegawa H, Matsumya S, Yamasak K. 1995. Reversal of efflux mediated tetracycline resistance in *Staphylococcus aureus* clinical isolates by *Ginseng* prosaponenins. Phytotherapy Research **9(4),** 260-263.
http://dx.doi.org/10.1002/ptr.2650090406

Heath RJ, Su N, Murphy CK, Rock CO. 2000. The enoyl-[acyl-carrier-protein] reductases FabI and FabL from *Bacillus subtilis*. The Journal of Biological Chemistry **275(51),** 40128-40133.
http://dx.doi.org/10.1074/jbc.M005611200

Hooton TM, Blair AD, Turck M, Counts GW. 1984. Synergism at clinically attainable concentrations of aminoglycoside and beta-lactam antibiotics. Antimicrobial Agents and Chemotherapy **26(4),** 535-538.
http://dx.doi.org/10.1128/aac.26.4.535

Horiuchi K, Shiota S, Kuroda T, Hatano T, Yoshida T, Tsuchiya T. 2007. Potentiation of antimicrobial activity of aminoglycosides by carnosol from *Salvia officinalis*. Biological & Pharmaceutical Bulletin **30(2),** 287-290.
http://dx.doi.org/10.1248/bpb.30.287

Hostettmann K, Hamburger M. 1991. Bioactivity in plants: The link between phytochemistry and medicine. Phytochemistry **30(12),** 3864-3874.
http://dx.doi.org/10.1016/0031-9422(91)83425-K

Hussain I, Khattak MMR, Ullah R, Muhammad Z, Khan N, Khan FA, Ullah Z, Haider S. 2011. Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyber-Pakhtunkhwa Pakistan. African Journal of Pharmacy and Pharmacology **5(6),** 746-750.

http://dx.doi.org/10.5897/AJPP11.175

Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. 2000. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest **118(1),** 146-155.
http://dx.doi.org/10.1378/chest.118.1.146

Iwu MM, Duncan AR, Okunji CO. 1999. New antimicrobials of plant origin. Perspectives on New Crops and New Uses. ASHS Press. Alexandria, VA. 457-462.

Jacoby GA, Munoz-Price LS. 2005. The new β-lactamases. The New England Journal of Medicine **352,** 380-391.
http://dx.doi.org/10.1056/NEJMra041359

Jacoby GA. 2009. AmpC β-lactamases. Clinical Microbiology Reviews **22,** 161-182.

Jenkinson HF, Lappin-Scott HM. 2001. Biofilms adhere to stay. Trends in Microbiology **9(1),** 9-10.
http://dx.doi.org/10.1016/s0966-842x(00)01891-6

Jeters RT, Wang GR, Moon K, Shoemaker NB, Salyers AA. 2009. Tetracycline-associated transcriptional regulation of transfer genes of the Bacteroides conjugative transposon CTnDOT. Journal of Bacteriology **191,** 6374-6382.
http://dx.doi.org/10.1128/JB.00739-09

Kamatou GPP, van Zyl RL, van Vuuren SF, Viljoen AM, Figueiredo AC, Barroso JG, Pedro LG, Tilney PM. 2006. Chemical composition, leaf trichome types and biological activities of the essential oils of four related *Salvia* species indigenous to Southern Africa. Journal of Essential Oil Research **18,** 72-79.

Kang HY, Jeong YS, Oh JY, Tae SH, Choi CH, Moon DC, Lee WK, Lee YC, Seol SY, Cho DT, Lee JC. 2005. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. The Journal of Antimicrobial Chemotherapy **55(5),** 639-644.
http://dx.doi.org/10.1093/jac/dki076

Karou D, Dicko MH, Simpore J, AS Traore. 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. African Journal of Biotechnology **4(8),** 823-828.
http://dx.doi.org/10.4314/AJB.V4I8.15190

Kennedy DG, McCracken RJ, Cannavan A, Hewitt SA. 1998. Use of liquid chromatography mass spectrometry in the analysis of residues of antibiotics in meat and milk. Journal of Chromatography A **812(1-2),** 77-98.
http://dx.doi.org/10.1016/s0021-9673(98)00048-x

Kepil A. 2005. The challenge of antibiotic resistance: Need to contemplate. The Indian Journal of Medical Research **121(2),** 83-91.

Kim H, Park SW, Park JM, Moon KH, Lee CK. 1995. Screening and isolation of antibiotic resistant inhibitors from herb materials - Resistant inhibition of 21 Korean Plants. Natural Product Sciences **1(1),** 50-54.

Kim HJ, T Yokozawa, YH Kim, C Tohda, TP Rao and LR Juneja. 2005. Influence of amla (*Emblica officinalis* Gaertn.) on hypocholesterolemia and lipid peroxidation in cholesterol-fed rats. Journal of Nutritional Science and Vitaminology **51(6),** 413-418.
http://dx.doi.org/10.3177/jnsv.51.413

Kimpe A, Decostere A, Martel A, Devriese L, Haesebrouck F. 2003. Phenotypic and genetic characterization of resistance against macrolides and lincosamides in *Streptococcus gallolyticus* strains isolated from Pigeons and humans. Microbial Drug Resistance **9(Suppl 1),** S35-S38.
http://dx.doi.org/10.1089/107662903322541874

Kubo A, Lunde CS, Kubo I. 1996. Indole and (E)-2-hexenal, phytochemical potentiators of polymyxins against *Pseudomonas aeruginosa* and *Eschericia coli*. Antimicrobial Agents and Chemotherapy **40(6),** 1438-1441.
http://dx.doi.org/10.1128/AAC.40.6.1438

Lanciotti R, Gianotti A, Patrignani F, Belletti N, Guerzoni ME, Gardini F. 2004. Use of natural aroma compounds to improve shelf life and safety of minimally processed fruits. Trends in Food Science & Technology **15(3-4),** 201-208.
http://dx.doi./10.1016/j.tifs.2003.10.004

Lee CK, H Kin, KH Moon, KH Shun. 1998. Screening and isolation of antibiotic resistance inhibitors from herb materials-resistance inhibition of volatile components of Korean aromatic herbs. Archives of Pharmaceutical Research **21,** 62-66.
http://dx.doi.org/10.1007/BF03216754

Levy SB. 1998. The challenge of antibiotic resistance. Scientific American **278(3),** 46-53.

Levy SB, Miller RV. 1989. Gene transfer in the environment. In: Environmental Biotechnology. McGraw Hill, New York.
http://dx.doi.org/10.1126/science.247.4940.350

Levy SB, Marshall B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine **10,** S122-S129.
http://dx.doi.org/10.1038/nm1145

Lewis K, Ausubel FM. 2006. Prospects for plant-derived antibacterials. Nature Biotechnology **24,** 1504-1507.
http://dx.doi.org/10.1038/nbt1206-1504

López P, Sánchez C, Batlle R, Nerín C. 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. Journal of Agricultural and Food Chemistry **53(17),** 6939-6946.
http://dx.doi.org/10.1021/jf050709v

Lupetti A, Danesi R, Campa M, Tacca MD, Kelly S. 2002. Molecular basis of resistance to azole antifungals. Trends in Molecular Medicine **8(2),** 76-81.
http://dx.doi.org/10.1016/s1471-4912(02)02280-3

Machado TB, Leal ICR, Amaral ACF, dos Santos KRN, da Silva MG, Kuster RM. 2002. Antimicrobial ellagitannin of *Punica granatum* fruits. Journal of the Brazilian Chemical Society **13(05),** 606-610.
http://dx.doi.org/10.1590/S0103505320020005000 10

March JC, Bentley WE. 2004. Quorum sensing and bacterial cross-talk in biotechnology. Current Opinion in Biotechnology **15(5),** 495-502.
http://dx.doi.org/10.1016/j.copbio.2004.08.013

Marshall KC. 1994. Microbial adhesion in biotechnological process. Current Opinion in Biotechnology **5(3),** 296-301.
http://dx.doi.org/10.1016/0958-1669(94)90032-9

Martinez JL, Baquero F. 2000. Mutation frequencies and antibiotic resistance. Antimicrobial Agents and Chemotherapy **44(7),** 1771-1777.
http://dx.doi.org/10.1128/AAC.44.7.1771-1777.2000

Mc Dermott R, Ziylan U, Spehner D, Bausinger H, Lipsker D, Mommaas M, Cazenave J-P, Raposo G, Goud B, de la Salle H, Salamero J, Hanau D. 2002. Birbeck granules are subdomains of endosomal recycling compartment in human epidermal langerhans cells, which form where Langerin accumulates. Molecular Biology of the Cell **13(1),** 317-335.
http://dx.doi.org/10.1091/mbc.0106-0300

McDevitt JT. 1996. Berberine: a candidate for the treatment of diarrhea in AIDS patients, abstr. 175. In: Program and Abstracts of the 36th Interscience Conference on XXI Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

McKeon DM, Calabrese JP, Bissonnette GK. 1995. Antibiotic resistant Gram-negative bacteria in rural groundwater supplies. Water Research **29(8),** 1902-1908.
http://dx.doi.org/10.1016/0043-1354(95)00013-B

Mena A, Plasencia V, García L, Hidalgo O, Ayestarán JI, Alberti S, Borrell N, Pérez JL, Oliver A. 2006. Characterization of a large outbreak by CTX-M-1-producing *Klebsiella pneumoniae* and mechanisms leading to *in vivo* carbapenem resistance development. Journal of Clinical Microbiology **44(8),** 2831-2837.
http://dx.doi.org/10.1128/JCM.00418-06

Mohamed JA, Huang DB, Jiang Z-D, DuPont HL, Nataro JP, Belkind-Gerson J, Okhuysen PC. 2007. Association of putative Enteroaggregative *Escherichia coli* virulence genes and biofilm production in isolates from travelers to developing countries. Journal of Clinical Microbiology **45(1),** 121-126.
http://dx.doi.org/10.1128/JCM.01128-06

Moselio S. 2009. Desk Encyclopedia of Microbiology, 2nd edition, Elsevier Inc. San Diego, USA.

Nostro A, Cellini L, Bartolomeo SD, Cannatelli MA, Campli ED, Procopio F, Grande R, Marzio L, Alonzo V. 2006. Effects of combining extracts (from propolis or *Zingiber officinale*) with clarithromycin on *Helicobacter pylori*. Phytotherapy Research **20,** 187-190.
http://dx.doi.org/10.1002/ptr.1830

Nychas GJE. 1995. Natural antimicrobials from plants. In: New Methods of Food Preservation. (Ed. Gould GW). Blackie Academic, London. p 58-89.

O'Toole GA, Kolter R. 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. Molecular Microbiology **28(3),** 449-461.
http://dx.doi.org/10.1046/j.1365-2958.1998.00797.x.

Ojha AK, Baughn AD, Sambandan D, Hsu T, Trivelli X, Guerardel Y, Alahari A, Kremer L, Jacobs WR Jr, Hatfull GF. 2008. Growth of Mycobacterium tuberculosis biofilms containing free mycolic acids and harbouring drug-tolerant bacteria. Molecular Microbiology **69(1),** 164-174.
http://dx.doi.org/10.1111/j.1365-2958.2008.06274.x

Okigbo RN, Omodamiro OD. 2006. Antimicrobial effects of leaf extracts of pigeon pea (*Cajanus cajan* (L.) Millsp.) on some human pathogens. Journal of Herbs, Spices and Medicinal Plants **12(1-2),** 117-127.
http://dx.doi.org/10.1300/J044v12n01_11

Olgica S, Ljiljana C. 2011. Inhibitory effect of *Cytisus nigricans* L. and *Cytisus capitatus* Scop. on growth of bacteria. African Journal of Microbiology Research **5(27),** 4725-4730.
http://dx.doi.org/10.5897/AJMR10.650

Page MGP, Heim J. 2009. Prospects for the next anti-*Pseudomonas* drug. Current Opinion in Pharmacology **9(5),** 558-565.
http://dx.doi.org/10.1016/j.coph.2009.08.006

Palmer KL, Kos VN, Gilmore MS. 2010. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. Current Opinion in Microbiology **13(5),** 632-639.
http://dx.doi.org/10.1016/j.mib.2010.08.004

Pantosti A, Moro ML. 2005. Antibiotic use: the crystal ball for predicting antibiotic resistance. Clinical Infectious Disease **40(9),** 1298-1300.
http://dx.doi.org/10.1086/429248

Parsek MR, Greenberg EP. 2005. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends in Microbiology **13(1),** 27-33.
http://dx.doi.org/10.1016/j.tim/2004.11.007

Pasteur L. 1857. Mémoire sur la fermentation

appelée lactique. Comptes rendus des séances de l' Academie des Sciences **45,** 913-916.

Patra AK. 2012. Dietary phytochemicals and microbes. Springer, Dordrecht Heidelberg, New York, London.
http://dx.doi.org/10.1007/978-94-007-3926-0

Payne DJ, PV Warren, DJ Holmes, Y Ji and JT Lonsdale. 2001. Bacterial fatty-acid biosynthesis: a genomic-driven target for antibacterial drug discovery. Drug Discovery Today **6(10),** 537-544. http://dx.doi.org/10.1016/s1359-6446(01)01774-3

Pfefferle C, Kempter C, Metzger J, Fiedler H-P. 1996. E-4-oxonon-2-enoic acid, an antibiotically active fatty acid produced by *Streptomyces olivaceus* Tue 4018. The Journal of Antibiotics **49(8),** 826-828.
http://dx.doi.org/10.7164/antibiotics.49.826

Prashanth D, Asha MK, Amit A. 2001. Antibacterial activity of *Punica granatum*. Fitoterapia **72(2),** 171-173.
http://dx.doi.org/10.1016/S0367-326X(00)00270-7

Proestos C, Boziaris IS, Kapsokefalou M, Komaitis M. 2008. Natural antioxidant constituents from selected aromatic plants and their antimicrobial activity against selected pathogenic microorganisms. Food Technology and Biotechnology **46(2),** 151-156.

Ramawat KG, Merillon JM. 2007. Biotechnology: Secondary metabolites; plants and microbes (Ed. Enfield NH). 2nd Edition. Science Publishers p 21-57.

Sakuragi Y, Kolter R. 2007. Quorum-sensing regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. Journal of Bacteriology **189(14),** 5383-5386.
http://dx.doi.org/10.1128/JB.00137-07

Salipante SJ, Barlow M, Hall BG. 2003. Genehunter, a transposon tool for identification and isolation of cryptic antibiotic resistance genes. Antimicrobial Agents and Chemotherapy **47,** 3840-3845.
http://dx.doi.org/10.1128/AAC/47.12.38403845.2003

Satish S, Raghvendra Mp, Anandarao RK. 2008. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. Advances in Biological Research **2(3-4),** 44-48.

Savoia D. 2012. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiology **7(8),** 979-990.
http://dx.doi.org/10.2217/fmb.12.68

Scalbert A. 1991. Antimicrobial properties of tannins. Phytochemistry **30(12),** 3875-3883.
http://dx.doi.org/10.1016/0031-9422(91)83426-L

Schiever CA, Fernandez C, Rodvold KA, Danziger LH. 2005. Daptomycin: a novel cyclic lipopeptide antimicrobial. American Journal Health-System Pharmacy **62(11),** 1145-1158.
http://dx.doi.org/10.1093/ajhp/62.11.1145

Sefton AM. 2002. Mechanisms of antimicrobial resistance: their clinical relevance in the new millennium. Drugs **62(4),** 557-566.
http://dx.doi.org/10.2165/00003495-200262040-00001

Serafini M, A Ghiselli, Ferro-Luzzi A. 1994. Red wine, tea, and anti-oxidants. Lancet **344(8922),** 626.

Shahid M, Malik A, Sheeba. 2003. Multidrug resistant *Pseudomonas aeruginosa* strains harboring R-plasmids and AmpC β-lactamases isolated from hospitalised burn patients in a tertiary care hospital of North India. FEMS Microbiology Letters **228(2),** 181-186.
http://dx.doi.org/10.1016/S0378-1097(03)00756-0

Singh R, Paul D, Jain RK. 2006. Biofilms:

implications in bioremediation. Trends Microbiology **14(9),** 389-397.
http://dx.doi.org/10.1016/j.tim.2006.07.001

Sivam GP. 2001. Protection against *Helicobacter pylori* and other bacterial infections by cumin seed extracts. The Journal of Nutrition **131(3),** 1106S-1108S.
http://dx.doi.org/10.1093/jn/131.3.1106S

Smid EJ, Gorris LGM. 1999. Natural antimicrobials for food preservation. In: Handbook of Food Preservation (Ed. M Shafiur Rahman). 2nd Edition. CRC Press. Taylor and Francis Group. New York. p 237-258.

Smith RS, Harris SG, Phipps R, Iglewski B. 2002. The *Pseudomonas aeruginosa* quorum-sensing molecule N-(3-oxododecanoyl) homoserine lactone contributes to virulence and induces inflammation *in vivo*. Journal of Bacteriology **184(4),** 1132-1139.
http://dx.doi.org/10.1128/jb.184.4.1132-1139.2002

Smith JL, Fratamico PM, Novak JS. 2004. Quorum sensing: a primer for food microbiologists. Journal of Food Protection **67(5),** 1053-1070.
http://dx.doi.org/10.4315/0362-028x-67.5.1053

Srinivasan D, Nathan S, Suresh T, Perumalsamy O. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology **74(3),** 217-220.
http://dx.doi.org/10.1016/S0378-8741(00)00345-7

Stefanovic O, Comic L, D Stanojevic D, Sukdolak SS. 2009. Antibacterial activity of *Aegopodium podagraria* L. extracts and interaction between extracts and antibiotics. Turkish Journal of Biology **33,** 145-150.

Stefanovic O, Stanojevic D, Čomić L. 2009. Inhibitory effects of *Torilis anthriscus* on growth of microorganisms. Central European Journal of Biology **4(4),** 493-498.
http://dx.doi.org/10.2478/s11535-009-0045-x

Stewart PS. 1996. Theoretical aspects of antibiotic diffusion into microbial biofilms. Antimicrobial Agents and Chemotherapy **40(11),** 2517-2522.

Tan TT. 2008. "Future" threat of Gram-negative resistance in Singapore. Annals of the Academy of Medicine, Singapore **37(10),** 884-890.

Tollefson L, Miller MA. 2000. Antibiotic use in food animals: controlling the human health impact. Journal of AOAC International **83(2),** 245-256.

Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of Ethnopharmacology **61(1),** 57-65.
http://dx.doi.org/10.1016/S0378-8741(98)00015-4

Tshibangu JN, Chifundera K, Kaminsky R, Wright AD, König GM. 2002. Screening of African medicinal plants for antimicrobial and enzyme inhibitory activity. Journal of Ethnopharmacology **80(1),** 25-35.
http://dx.doi.org/10.1016/S0378-8741(01)00409-3

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of Ethnopharmacology **50(1),** 27-34.
http://dx.doi.org/10.1016/0378-8741(96)85514-0

Turovskiy Y, Kashtanov D, Paskhover B, Chikindas ML. 2007. Quorum sensing: fact, fiction, and everything in between. Advances in Applied Microbiology **62,** 191-234.
http://dx.doi.org/10.1016/S0065-2164(07)62007-3

Ulanowska K, Tkaczyk A, Konopa G, Węgrzyn G. 2006. Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA

and protein synthesis in some bacterial strains. Archives of Microbiology **184(5),** 271-278. http://dx.doi.org/10.1007/s00203-005-0063-7

Vaghasiya Y, Dave R, Chanda S. 2011. Phytochemical analysis of some medicinal plants from Western region of India. Research Journal of Medicial Plant **5(5),** 567-576. http://dx.doi.org/10.3923/rjmp.2011.567.576

Vanderkooi OG, Low DE, Green K, Powis JE, McGeer A. 2005. Predicting antimicrobial resistance in invasive pneumococcal infections. Clinical Infectious Diseases **40(9),** 1288-1297.

Walsh TR. 2008. Clinically significant carbapenemases: an update. Current Opinion in Infectious Diseases **21(4),** 367-371. http://dx.doi.org/10.1097/QCO.0b013e328303670b

Wang CG, Zhang T, Zhao XH. 2011. Plasmid fingerprinting profiles of chicken-derived pathogenic strains of *Escherichia coli*. Advanced Materials Research **178,** 355-360. http:/dx.doi.org/10.4028/www.scientific.net/AMR.178.355

Whittaker CJ, Klier CM, Kolenbrander PE. 1996. Mechanisms of adhesion by oral bacteria. Annual Review of Microbiology **50,** 513-552. http://dx.doi.org/10.1146/annurev.micro.50.1.513

Williams P. 2007. Quorum sensing, communication and cross-kingdom signaling in the bacterial world. Microbiology **153(12),** 3923-3938. http://dx.doi.org/10.1099/mic.0.2007/012856-0

Williamson EM. 2001. Synergy and other interactions in phytomedicines. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology **8(5),** 401-409. http://dx.doi.org/10.1078/0944-7113-00060

Wormer GP, Bergman MM. 2003. The antibiotic paradox: How the misuse of antibiotics destroys their curative powers (Ed. Stuart B. Levy). 2nd Edition. Cambridge, Massachusetts, Perseus Publishing. Clinical Infectious Diseases **36(2),** 238. http://dx.doi.org/10.1086/344957

Yam TS, Hamilton-Miller JM, Shah S. 1998. The effect of a component of tea (*Camellia sinensis*) on methicillin resistance, PBP2' synthesis, and beta-lactamase production in *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy **42(2),** 211-216. http://dx.doi.org/10.1093/jac/42.2.211

Yildirim A, Mavi A, Kara AA. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. Journal of Agricultural and Food Chemistry **49(8),** 4083-4089. http://dx.doi.org/10.1021/jf0103572

Zaika LL. 1988. Spices and herbs: their antimicrobial activity and its determination. Journal of Food Safety **9(2),** 97-118. http://dx.doi.org/10.1111/j.1745-4565.`988.tb00511.x

Zhang L, Kong Y, Wu D, Zhang H, Wu J, Chen J, Ding J, Hu L, Jiang H, Shen X. 2008. Three flavonoids targeting the β-hydroxyacyl-acyl carrier protein dehydratase from *Helicobacter pylori*: Crystal structure characterization with enzymatic inhibition assay. Protein Science **17(11),** 1971-1978. http://dx.doi.org/10.1110/ps.036186.108

Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T. 2001. Mechanism of synergy between epigallochatechin gallate and β-lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy **45(6),** 1737-1742. http://dx.doi.org/10.1128/AAC.45.6.1737-1742.2001