Chapter

Antioxidants: Natural Antibiotics

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Abstract

The aim of this current piece of writing is to draw the attention of readers and researchers toward the natural antioxidants that can take the place of synthetic antibiotics to avoid bacterial resistance and gastrotoxicity/nephrotoxicity. Antioxidants such as polyphenols, vitamins, and carotenoids are the organic compounds mainly extracted from natural sources and dominantly involved in boosting the defense system of organisms. The main public health-related issue over the globe is ever-growing bacterial resistance to synthetic antibiotics, which is being continuously reported during the last decade. Further, the pipeline of the development of new synthetic antibacterial agents to replace the resistant antibiotics in clinical set-up is gradually drying up. This scenario originated the concept to revive the interest toward natural antibacterial products due to their chemical diversity, which provide important therapeutic effect and make the microbes unable to copy them for creating resistance. Natural products, especially polyphenols had been seen in antioxidant, antibacterial, anticancer, anti-inflammation, and antiviral activities with encouraging results. In this chapter, we will focus over the role of natural antioxidants as antibacterial agents.

Keywords: antioxidants, antibacterial agents, infection therapy, natural antibiotics

1. Introduction

Microbes are known to human civilization due to their beneficial and lethal effects. When the symbiotic relation of microorganisms goes beyond the limit, they may cause pathogenic infections and diseases, causing damage to the body and sometimes leading to death: this is a major concerning issue especially in developing countries. The determination of exact site of infection in the body is very critical for curing the pathogenesis caused by bacteria at their early stage. Antimicrobial agents especially antibiotics, which are either obtained from natural sources or through total synthetic procedures, are practiced against pathogens. Antibiotic may be obtained naturally from living organism (e.g., fungi, actinomycete, and bacillus species), prepared synthetically and semisynthetically in the laboratory. Its mechanism of action is divided into two spectrums (narrow and broad-range spectra).

Broad-spectrum antibiotics act against both Gram-positive and Gram-negative bacteria [1]. A good antibiotic should have the following characteristics: long shelf life, nontoxic to human body, soluble in the body fluid, low cost, show long-lasting antibacterial effect, and low possibility of bacterial resistance to the agent. However, all these standard parameters for an ideal antibiotic are difficult to meet, while developing synthetic antibacterial agents that is the reason a big threat is being felt from pathogenic bacterial resistance which is the main public health-related issue, all over the globe [2]. This appeared during the last decade in a more prominent way which mainly originated either due to wrong identification of bacterial strain and prescription of antibiotic or due to imbalance use of antibacterial agents. The transmission of bacterial resistance among the individuals and across the geological border is one way of antimicrobial resistance [3]. Further, on the other way, to handle the bacterial resistance threat, the pipeline of the development of new synthetic antibacterial agents is gradually drying up. And it might be possible, on the bases of continuously increasing level of bacterial resistance; at some stage pathogenic bacteria halt antibiotic therapy—that stage will be not good in the history of human being [4].

Antioxidants such as polyphenols, vitamins, and carotenoids are the organic compounds mainly extracted from natural sources and dominantly involved in living defense system. Due to continuously increasing resistance to synthetic antibiotics, there is an urgent need to shift our focus toward natural antioxidant-based antibacterial products due to their vast chemical diversity which provide potent therapeutic effect and make the microbes unable to copy them for creating resistance. Out of many natural products and antioxidants which are showing great healthy impact on human beings, polyphenols have been reported as natural agents that fight as antioxidants, antibacterial, anticancer, anti-inflammation, and antiviral agents. We, in this chapter, tried to review the role of antioxidants as natural antibiotics. In the following section, we will discuss the inflammation and infectious process and how antioxidants play their role in fixing them. Then we will also discuss antibacterial mechanism of natural antioxidants as antibiotics in animal bodies.

2. Inflammation and infection

Pathogens like bacteria are responsible for the infection or inflammation in animals. These infections may be mild inflammation which is hardly noticeable or which appeared to human being in history as a big threat. According to one estimation, based on growing bacterial resistance, up to 2050, the death rate due to bacterial infection may increase to 390,000 in Europe and similarly all over the world as shown in **Figure 1**. Inflammation is a nonspecific immunological response by the organism's body to any trauma, neoplasm, autoimmune attack, or invading of the microbes. At the site of inflammation, several processes can be noticed such as blood supply increase and leakage of cellular fluid and small molecules, and protein penetration may take place. In the case of acute injury, body defense mechanism, i.e., leucocyte and plasma protein migration to the site of infection become activated. Neutrophils invade bacteria when seeking entrance in the body and prevent the body from further infection. The process of infection starts within a second or minute and prolongs to hours or days to heal. It causes the sequential symptoms, like inflammation, redness, warmth, and pain, which consequently affect the functions of the tissue or organ. Inflammation may be a nonspecific process, but infection consists of stepwise progress in inflammation which might be chronic if it could not be addressed timely.

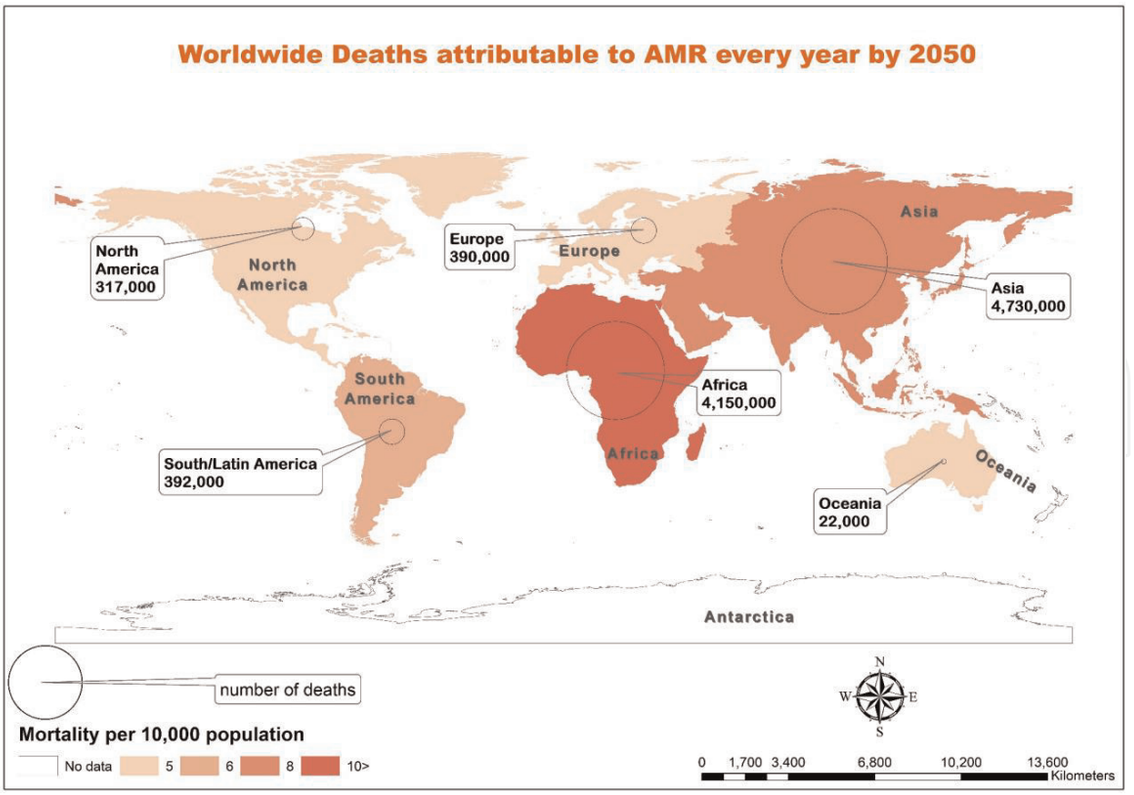


Figure 1.
An estimation of deaths caused by bacterial infection worldwide [5].

3. Types of infection

Infections can be categorized as viral, bacterial, fungal, protozoan, prion, and parasitic. Some **viral** infections are influenza, rotavirus, chicken pox, HIV, and herpes. Pneumonia, tetanus, cellulites, chlamydia, gonorrhea, etc. are the common **bacterial** infections. **Fungal** infections are ringworm, candidiasis, and athlete's foot. Malaria and African sleeping sickness are common **protozoan** infections transmitted through plasmodium and tsetse fly, respectively. **Prion** is the poisonous entities or protein infection particles which cause fatal neurodegenerative disease. Amebiasis, dysentery, and coccidiosis infections are caused by **parasites.** Antibiotics are the major agents to cope and fix these infections. There are mainly two types of antibacterial agents, i.e., synthetic antibiotics and natural antibiotics. In the following section, we will discuss antibiotics, natural antibiotics, and their mode of action.

4. Antibiotics

In the ancient times, it is believed that antibiotics were the chemicals released by microorganisms, causing prompt deleterious effect on humans. However, later this notion was reversed, i.e., these compounds were used against microbes instead of isolating from them. Antibiotics are generally of two types, **bactericidal** which kill the bacterial cell and **bacteriostatic** which inhibit the bacterial growth and may kill the bacteria. The first antibiotic was discovered by Alexander Fleming in 1928 from *Penicillium notatum*, a soil-inhabiting fungus, and the clinical trials on humans are conducted in 1940. There are five generations of different classes of antibiotics, up till now, which have been discovered and are in clinical practice.

5. Natural antioxidants as antibiotics

The increased resistance of pathogenic microorganism against antibiotic becomes a major issue around the globe from the last decade. To overcome this serious problem, it is necessary to discover a new world of antimicrobials, which are not only beneficial in bacterial infection but show long-lasting effect by boosting the immunity of the body. However, we do not skip the usage of previously practiced antibiotics as some of them show a very effective result in bacterial infection, but there is a need of advanced or may say strong antibacterials whose chemical makeup bacteria cannot be copied.

Plant synthesizes a variety of secondary metabolites (phytochemicals) which are involved in plant defense mechanism, and it is recognized that major classes of these molecules have beneficial effects on health including antioxidants and antimicrobial. The attractive antioxidant as well as antibacterial activity of phytochemicals seeks attention as it may replace the synthetic antioxidants, which cause deleterious effect on human health such as cancer. The plant kingdom is rich in various phytochemicals like phenolic acid, flavonoids, gingerol, curcumin, etc.

Phenolic acids and flavonoids are a very important class of antioxidants as it directly affects bacterial growth and causes hindrance in their pathogenic activity. The mechanism of action of antioxidants as antibacterial is still not fully understood, but some researches reveal that the attributable antibacterial activity involves three basic mechanisms: outer membrane permeability, cytoplasm leakage, and inhibition of nucleic acid formation. The interaction of polyphenols with nonspecific forces like hydrogen bonding and hydrophobic effect lipophilic forces as well as by covalent bond formation was related to microbial adhesion and enzyme and cell envelope transport protein. The antibacterial activity of polyphenols may also due to the capacity of these compounds to chelate iron, vital for the survival of almost all bacteria. Polyphenols rupture the wall, increase the permeability of cytoplasm membrane, and release lipopolysaccharides (LPS) [6].

The cell wall composition of Gram-positive and Gram-negative differs significantly as Gram-positive bacteria have thick layer of peptidoglycan along with lipoteichoic acid but lack of outer membrane. Gram-negative bacterial outer membrane consists of phospholipid, protein, and LPS and a thin layer of peptidoglycan. Both Gram-negative and Gram-positive bacterial cell walls play a very important role in osmotic protection of cell. Any damage to cell wall will decrease the tolerance of cell against osmotic pressure and ionic strength. Many researchers have demonstrated that the interaction of polyphenols with bacterial cell wall is different for Gram-negative and Gram-positive bacteria. Different interaction cites for antibacterial agents in variety of bacterial strains are shown in (**Tables 1–4**).

6. Mechanisms of action

The activity of antioxidants against bacterial inflammations is being progressively recognized. They also work synergistically with current antibacterial agents against the resistant strains of bacteria. The diversity in the structure of natural products makes it impossible for bacteria to copy its functional moieties, unlike the synthetic agents. The structure of antioxidants holds the key role in determining the antibacterial activity. Different groups of researchers investigating the relationship between flavonoid structure and their antibacterial activity generalized that active compounds share common structural features. Moreover, the unique structural

Antibacterial Action mechanism	Interaction cites	Example	References
Interaction between the bacterial cell wall	Outer membrane	Trans-anethole cause membrane integrity on *S. albus, B. subtilis, S. typhimurium, S. dysenteriae, E. coli*	[4]
		Thyme essential oils have severe effect on the listerial cell wall of L. monocytogenes leading to cell wall rupture.	[6]
		Flower essential oil of Bidens pilosa rich in monoterpene hydrocarbons and oxygenated monoterpenes which is strongly affect the membranes of gram negative bacteria	[7]
		Flavonoids (epigallocatechin, catechin, myricetin, quercetin) and aromatic phenolic compound (thymol, carvacrol, eugenol, cinnamic aldehyde)	[8]
		Obtained from dietary spice and medicinal herbs show antibacterial potential for S. aureus, E. coli, L. monocytogenes.	
	Membrane protein	Quercetin, 3-O-caffeoylquinic acid and anthocyanidins are contained within the berry leaves and branches that alter the mechanism to repair DNA thus showing antibacterial potential.	[9]
		Phenolic compound obtained from Eucalyptus globules show more activity against gram negative bacteria due to their outer lipopolysaccharide membrane.	[10]
		Quercetin obtained from yellow onion (allium cepa) has inhibitory effect on antibiotic resistant bacteria H. pylori	[11]
Interaction with cell membrane	Bilayer	Phenolic compound extracted from herbs alter the permeability of microbial cell, disrupt energy metabolism and proton motive force causing cell death.	[12]
		Essential oil of S. cumini shows activity especially for S. typhimurium.	
		Extract of Tunisian ruta chalepensis organs(leave, flower, stem) were rich in vanillic acid and coumarin which show remarkable antibacterial properties against P. aeruginosa .it is revealed that the activity was strain and origin dependent .	[13]

Table 1.
Natural antioxidants and their role in inhibiting bacterial growth in living system showing interaction between the bacterial cell wall and with cell membrane.

Antibacterial Action mechanism	Interaction cites	Example	References
Metal ion deficiency due to chelating ability	Ionic iron	Tannins from hydnora africanathunb are used for different strains of gram negative and positive bacteria.	[14]
		Spice and herbs extract rich in phenolic, flavonoids, proanthocyanidins and hydrolysable tannins show antimicrobial activity against three bacterial strains(L. monocytogenes, S. aureus, S. enterica)	
Microbial enzyme inhibition and substrate deprivation	Microbial enzymes	Quercetin cause hindrance in the production of exopolysaccharide production in K. pneumoniae, P. aeruginosa ,and Y. enterocolitica	[3]
		Ferulic acid completely inhibit colony spreading of S. aureus	
		Tannic acid along with other antioxidants inhibit B. subtilis cyclic di-AMP synthase	
Protein regulation	Repression / stimulation of the bacterial genes	Treatment of P. aeruginosa with cranberry proanthocyanidins down regulated 2 proteins implicated in ATP synthesis, a cytochrome C(PA2482 and hypothetical protein PA2481 and protein involved in DNA and RNA synthesis and acid cycle proteins(subunits of acetyl-CoA carboxylase and fumarase)	
		Treatment of P. aeruginosa with cranberry proanthocyanidins up- regulated 12 proteins related to cation transporters (such as PchD, PvdN, PhuS), 5 protein involved I amino acid synthesis (such as PA0335,PA2044, HutG) protein involved in response to stress (such as OsmC, SodM) and a hypothetical protein involved in flavonoid metabolism.	
Change in cell morphology and inhibition of nucleic acid synthesis	Formation of filamentous cells, DNA & RNA	Genistein, an isoflavone show antibacterial activity against V. harveyi and B. subtilis	[15]
Inhibition of respiratory chains of bacterial membranes	Respiratory chains	Retrochalcones from Glycyrrhiza Inflata inhibits the growth of M. luteus, S. aureus and P. aeruginosa.	[16]
Inhibition of DNA and RNA synthesis	DNA and RNA	(-)-Epigallocatechin, a flavonoid inhibits DNA and RNA synthesis in P. vulgaris and S. aureus.	[17]

Table 2.
Natural antioxidants and their role in inhibiting bacterial growth in living system showing metal ion deficiency due to chelating ability, microbial enzyme inhibition and substrate deprivation and other inhibition mechanism.

features may be essential for flavonoids to gain adjacency or uptake into the bacterial cell. Like, polyhydroxylated flavonoids show more pronounced antibacterial activity than mono-hydroxylated or non-hydroxylated flavonoids. Structural

Class	Compound	Bacteria	MIC	MBC	Mode of action
Phenolic acids	Ferulic acid	E. coli	1500	5000	Cytoplasmic membrane integrity
		P. aeruginosa	500	500	
		S. aureus	1750	5250	
		L. monocytogenes	2000	5500	
	Gallic acid	E. coli	100	2500	
		P. aeruginosa	100	500	
		S. aureus	1100	5000	
		L. monocytogenes	1250	5300	
		S. mutans	>1.2 mg/ml		
	Tannic acid	S. mutans	>0.4 mg/ml		
	Salicylic acid	S. mutans	>3.8 mg/ml		
Flavones	Flavone	Staphylococcus	50		
	7,8-	Staphylococcus	100		
	6,7-	Staphylococcus	50	_	
		S. aureus	100		
	3(OH)-flavone	S. aureus AM-176	257		
	5,7(OH)$_2$-flavone	S. aureus AM-176	103		
Flavonols	Datiscetin	P. vulgaris	100		DNA and RNA synthesis
		Staphylococcus	50		
	Morin	P. vulgaris	100		
	Quercetagetin	P. vulgaris	100		
	Robinetin	S. aureus	100		
		P. vulgaris	100		
	Myricetin	S. aureus	100		
		P. vulgaris	50		
		Staphylococcus	100		
	Galangin	Staphylococcus	6.3		
	kaempferol	Staphylococcus	50		
	fisetin	Staphylococcus	100		
	Quercetin	S. mutant	>1.5 mg/ml		
Flavanono	(+)-Dihydrorobinetin	S. aureus	200		DNA and RNA synthesis
		P. vulgaris	200		
Catechins	(-)-Epigallocatechin	S. aureus	100		DNA and RNA synthesis
		P. vulgaris	50		
		Staphylococcus	50		
vitamins	Ascorbic acid	S. mutans	>2.0		

Table 3.
Purified antioxidants extracted from plant sources and their antibacterial potential: phenolic acid, flavones, flavanonols, catechins, and vitamins.

similarity among flavonoids is too dominant that there are three probable hypotheses regarding their mechanism of action:

a. Flavonoids of same structure take same mechanism.

b. All flavonoids follow multiple mechanisms of actions.

c. All flavonoids have same sole mechanism of action.

According to a recent development, the study of mechanism of actions is not that reliable as it was assumed earlier. Like epigallocatechin gallate only induce clumping of FabG enzyme and have no such effect on other enzymes. Another such development is that flavonoids cause aggregation of bacterial cells. Clumping of bacterial cells on treatment with flavonoids causes reduction in surface area of

Class	Compound	Bacteria	MIC
Chalcones	Chalcone	AM-51 (MRSA	38.5
		AM-72 (MRSA	15.5
		AM-172 (MRSA	32.0
		AM-176 (MRSA	36.2
	2'(OH)-chalcone	AM-51 (MRSA	38.6
		AM-72 (MRSA	13.6
		AM-172 (MRSA	33.0
		AM-176 (MRSA	37.1
	2',4'$(OH)_2$-chalcone	AM-51 (MRSA	38.4
		AM-72 (MRSA	12.7
	chalcone	AM-172 (MRSA	31.0
		AM-176	34.2
	2',4'$(OH)_2$-chalcone	AM-51 (MRSA	38.7
		AM-72 (MRSA	16.3
		AM-172 (MRSA	31.6
		AM-176 (MRSA	36.6
Flavanone	Sophoraflavanone	MRSA strains	3.13-6.25
	Exiguaflavanone D	MRSA strains	3.13-6.25
	Kenusanone D	MRSA strains	3.13-12.5
	Exiguaflavanonne	MRSA strains	6.25
	Sophoraflavanone	MRSA strains	3.13-12.5
	Sophoraflavanone	MRSA strains	6.25-12.5
	Kenusanone A	MRSA strains	6.25-12.5
	Naringenin	MRSA strains	200-400
	Exiguaflavanone C	MRSA strains	12.5
	Exiguaflavannone	MRSA strains	12.5
	Leachianone G	MRSA strains	12.5

Table 4.
Purified antioxidants extracted from plant sources and their antibacterial potential: chalcone and flavanone.

bacterial population which reduce the oxygen consumption by bacteria (interruption respiratory chains). Reduction in surface area of cells decrease the nutritional uptake like uridine and thymidine (specify nucleic acid inhibition). Moreover, the prospect of baffling and the cause and effect of mechanisms of actions exist. For example, the interruption of membrane integrity by an antibacterial agent will impart negative effects on proton-motive force that directly influence the synthesis of ATP and solute transport into the bacterial cell. The deterioration of bacterial capability to produce energy and to attain nutrients results in declining capability of bacterial cell to make DNA and peptidoglycan. So, one mechanism of action may be misunderstood as multiple.

Correspondingly, if any enzyme of bacteria-like DNA gyrase is obstructed by an antibacterial agent, then this swift automated cell death and lysis. Likewise, the

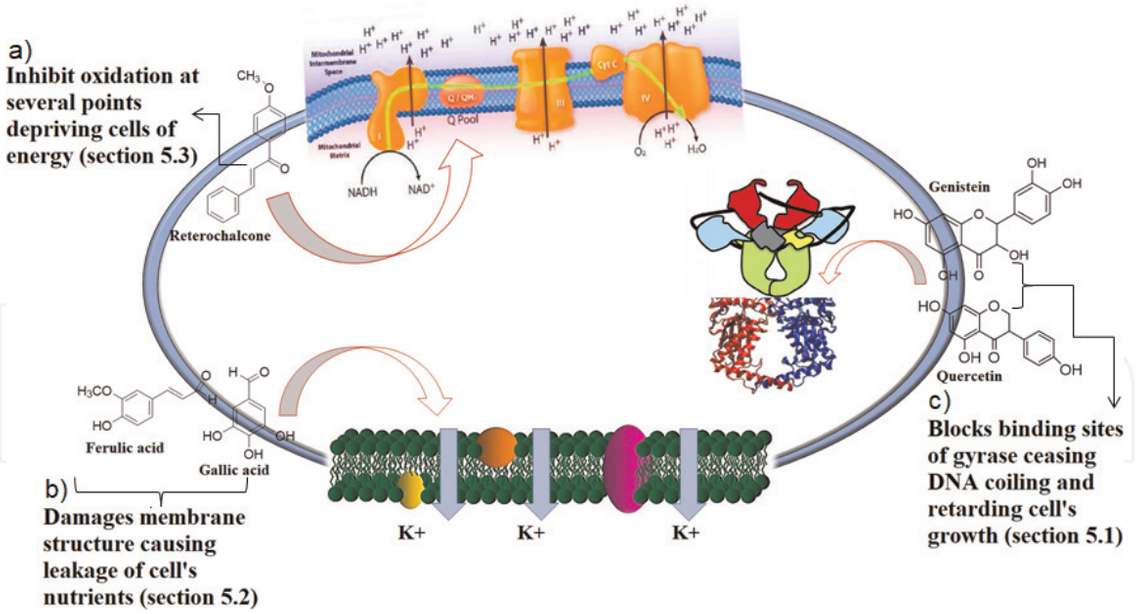


Figure 2.
The schematic layout demonstrating the natural antioxidant role as antibacterial: (a) representing inhibition of energy metabolism; (b) representing disruption of membranes; and (c) representing interruption in nucleic acid synthesis.

antibacterial agent that impedes the synthesis of nucleic acid may be misinterpreted as the agent that alters the cytoplasmic membrane functions [7].

The following mechanisms of actions are attributed to the antibacterial action of flavonoids as reported by different groups of researchers:

- Impairment of membrane functions
    a. Alteration of cytoplasmic membrane fluidity
    b. Inhibition of cell wall formation
    c. Inhibition of cell membrane formation
- Interruption of synthesis of nucleic acid
- Inhibition of respiratory metabolism

The mechanisms of action of antioxidants as an antibacterial are shown in **Figure 2**.

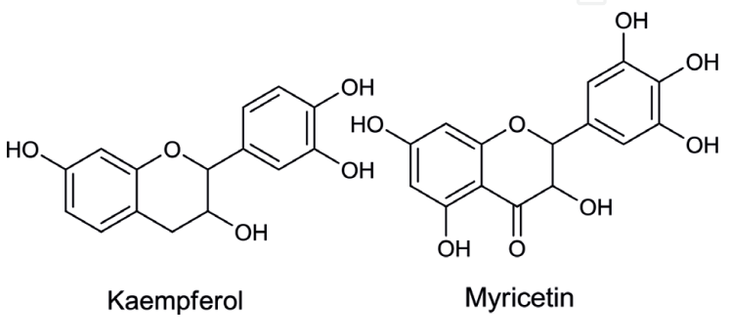
6.1 Inhibition of nucleic acid synthesis

Among the classes of antioxidants, flavonoids significantly show inhibitory activity against nucleic acid synthesis. Interaction of flavonoids with DNA- or with ATP-binding site of gyrase finally leads to the inhibition of nucleic acid synthesis as shown in **Figure 2**. Metabolism of DNA in bacteria comprises transcription, recombination, DNA replication, and transport of genetic information. A vital enzyme to control vigorous changes of nucleic acid is DNA gyrase. Gyrases, characteristic and crucial bacterial enzymes that change the topology of DNA, are the amiable aim to hit for the antibacterial agents. DNA gyrase, in a reaction that depends on ATP,

enhances the supercoiling of DNA of bacteria, and its inactivation leads to bacterial death. Estimation of DNA supercoiling is the important parameter in assessment of flavonoids activity to inhibit DNA gyrase. It has two subunits, gyrase A that takes part in DNA breakage-resealing and gyrase B that is involved in the hydrolysis of ATP, the driving force for the DNA supercoiling. The topoisomerase (DNA gyrase) inhibitors form a cleavable complex of agent-topoisomerase-DNA or interfere with the gyrase binding to DNA (see **Table 2**).

6.1.1 Kaempferol and PMFs

Kaempferol show the strongest antibacterial activity (MIC_{50} = 25 μg/ml) against *E. coli* DNA gyrase. It inhibits the activity of gyrase enzyme that holds the key role in DNA supercoiling and bacterial growth.

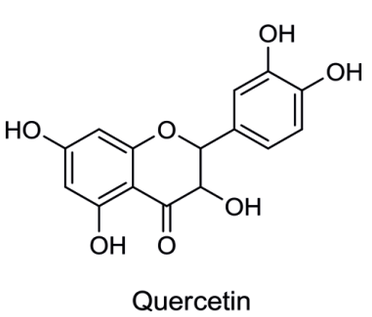


Structure of Polymethoxylated flavones

Polymethoxylated flavones (shown in structure) usually found in citrus peel possess broad spectrum antimicrobial activity. It shows antibacterial activity against *E. coli* and *S. aureus* with IC_{50} values ranging from 1.45 to 1.89 mg/ml [8] (see **Table 3** for MIC values).

6.1.2 Quercetin

Quercetin is one of the ubiquitous flavonoids, impedes the DNA supercoiling, and causes DNA to cleave. Quercetin encourages DNA scission by forming gyrase-DNA-quercetin cleavable complex. Cleavage of DNA was promoted at quercetin concentration above 80 μM in the presence of gyrase, and at 640 μM the maximum cleavage was obtained [9]. MIC values of quercetin are listed in **Table 3**. Quercetin obtained from yellow onion skin has inhibitory effect on antibiotic-resistant bacteria *H. pylori*. Sulfur and quercetin have synergistic growth inhibitory effect with beta lactam, a very functional antibiotic [10].



Structure of quercetin

Mode of action of quercetin inhibition includes two mechanisms:

First inhibition pathway involves rivalry at binding site of ATP at gyrase B that prevents DNA supercoiling. The second mechanism involves binding to DNA that

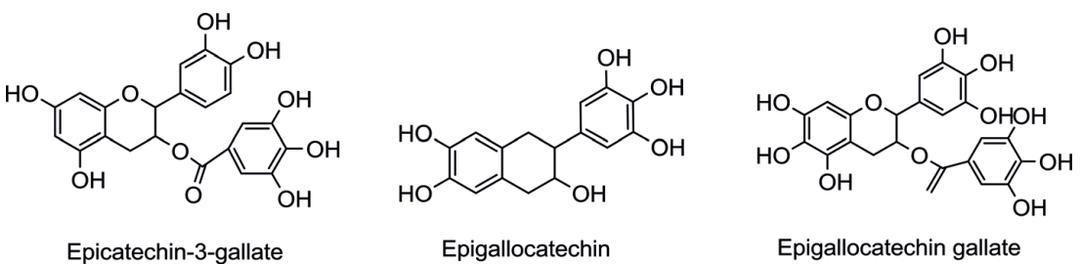
stabilizes DNA topoisomerase II complex causing DNA to cleave [11]. MIC values for different bacterial strains are listed in **Table 3**.

6.1.3 Glycosylated flavones

Glycosylated flavones (isolated from cottonseed flour) promote topoisomerase IV-dependent cleavage of DNA in *E. coli*. Rutin is the most potent glycosylated isoflavone in exciting topoisomerase IV-dependent cleavage of DNA (CC_{50} = 1 μg/ml). It blocks the catalytic activity of type II topoisomerase in addition to alleviate the cleavable complex. At CC_{50} = 64 μg/ml, rutin inhibited the decantation action of topoisomerase IV.

6.1.4 Catechins

Catechins like epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (structure is shown below) inhibit ATPase action because the ATP-binding site of B subunit of gyrase shares the structural similarity with these catechins. So, owing to this similarity with ATP-binding site, the catechins occupy these sites and as a result inhibits of ATPase activity. Catechins inhibit the ATPase activity in the following order, EGC < ECG < EGCG, while EC had no affect at all (for MIC values see **Table 4**) [12]. ATP hydrolysis provides vigorous force for DNA supercoiling. The inhibition of ATPase activity by catechins prevents ATP hydrolysis. In this way the DNA supercoiling is affected and so the bacterial growth.



Catechin based antioxidants

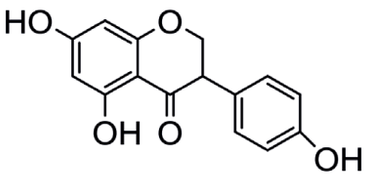
6.1.5 Soybean isoflavone

SI (soybean isoflavone) could alter the supercoiling of double-stranded DNA could be altered by affecting DNA topoisomerase. By increasing the concentration of soybean isoflavone, the supercoiling activity increases and the quantity of linear and open circular DNA decreases.

At 6.4 mg/ml concentration, SI significantly inhibited the activity of both topoisomerase I and II so stops the bacterial growth by affecting nucleic acid synthesis. Topoisomerase inhibitors form a drug-gyrase-DNA cleavable complex or disrupt the topoisomerase binding to DNA [13].

6.1.6 Genistein

Genistein is an isoflavone (shown in structure below) and characteristic of other flavonoids, apigenin, daidzein, and kaempferol for common use on the laboratory strains bacterial species like *B. subtilis*, *E. coli*, and *V. harveyi*. Addition of this flavonoid to bacterial cultures imposes drastic effects on the synthesis of DNA and RNA in almost 15 min [14].



Genistein

Three hours after addition, genistein caused the bacterial cells to become elongate that cause troubled cell division and chromosome replication. MIC values are listed in **Table 3**.

Protein synthesis was also significantly inhibited by genistein but was delayed a little, suggesting that repression of translation by genistein is secondary effect.

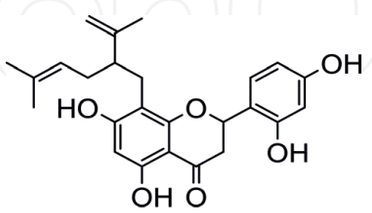
6.2 Disruption of membranes

The outer bacterial membranes safeguard the bacterial cells from harsh environment, causing them to survive in extreme conditions. The inner membrane or the so-called cytoplasmic membrane regulates the uptake of solutes and minerals into the cell as well as the transport of proteins and other macromolecules. The alteration in membranes causes many adverse effects on functions of bacterial cells that might be very important for bacterial integrity, like uptake of mineral ions and nutrients. To study the membrane effects of antioxidants, fluorescence polarization methods using model membranes (consisting of two component: DPPC and POPC) are used. Fluorescence polarization increasingly alters in correspondence with reduction in membrane fluidity. MIC values are given in **Table 3**.

The antioxidants like polyhydroxyl flavans and catechin of green tea hinder the development of certain bacteria and affect Gram-positive and Gram-negative bacteria by damaging the structure of membranes of the bacterial cells (see **Table 1**).

6.2.1 Sophoraflavanone G

Sophoraflavanone G, a phytochemical with intensive antibacterial activity, shows very low MICs (1.56—12.5 μg/ml) against Gram-positive bacteria than Gram-negative bacteria by altering membrane functions. Increased polarization in DPPC and POPC liposomes implies that sophoraflavanone G decreases membrane fluidity (for MIC value see **Table 4**) [15].



Sophoraflavanone G

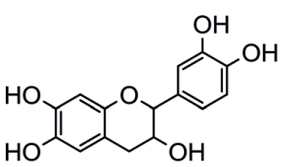
6.2.2 Catechins

The pathogenicity of Gram-negative bacteria is linked to the lipopolysaccharide layer (reduce the sensitivity against antibacterial agents). That is why, the antibacterial agents demonstrate more activity for Gram-positive bacteria. Catechins intermingle and target bacterial membrane protein, fatty acid synthase, beta lactamase, and such other bacterial enzymes. Antibacterial catechins were reported to alter membrane fluidity [16]. Tea catechins impart specific agitation in

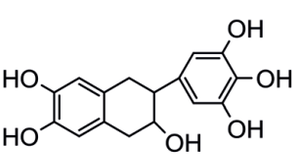
the well-organized phosphatidylcholine and phosphatidylethanolamine bilayers that makeup membranes of bacteria. Epigallocatechin gallate (EGCG), a polyphenol obtained from green tea, black tea, and cocoa shows intensive activity, perturbs membranes of bacteria, and causes leakage of membranes isolated from *E. coli*. The antibacterial effects result from the interaction of catechins which interacts with oxygen, genes, and cell membranes, and these interactions produce their antibacterial effects. MIC values are given in **Table 3**.

EGCG binds straight to the peptidoglycan of *S. aureus*, affects integrity of cell and thereby decreases the acceptance of the cell to high osmotic pressure and less ionic strength. EGCG induces changes in morphology of Gram-negative bacteria depending on the acquittance of H_2O_2 and causes oxidative stress in bacteria. Flavonoids (epigallocatechin, myricetin, quercetin; structure shown below), damage membrane protein, and coagulate cytoplasm alter constituents of fatty acids and phospholipids, weaken mechanism of energy formation and metabolism, impacts the production of RNA and DNA, and abolishes translocation of proteins [17].

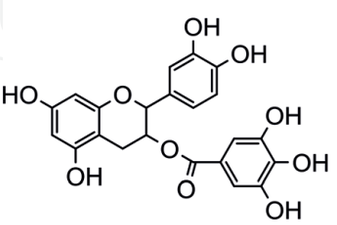
Epicatechin gallate (ECG) dramatically alters the physical properties of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) bilayers. They cause leakage from the membranes that is known to be pronounce by the presence of PE. So, at membrane level the antibacterial properties of catechins are due to their damage to PE. So, to estimate the phospholipid specificity, in the presence and absence of PE, egg yolk model was used. The results showed that only galloylated catechins affected PE and caused prominent leakage at 6.3 mol%. Protein translocation and other such processes are to some extent related to phospholipids; so, any effect on these can significantly alter the cell metabolism of bacteria [18]. Liposome membranes are damaged by EGCG and the leakage of intraliposomal CF occurs. This damage to membranes increases the permeability of catechins for catechins to penetrate in the cell. But how catechins damage the bilayer and penetrate the cell is still unanswered [19].



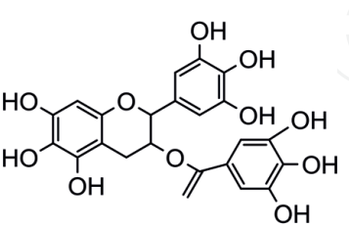
Epicatechin



Epigallocatechin



Epicatechin-3-gallate



Epigallocatechin gallate

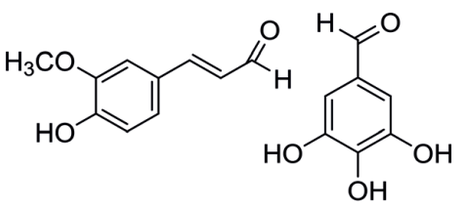
6.2.3 Ferulic and gallic acid

FA and GA cause severe and irreversible damage to the membranes causing constant leakage of the essential cell constituents. Different physiological terms are used to access the antimicrobial activities: MIC, MBC, and K^+ release in the cell. The MIC values of ferulic and gallic acids against some bacterial strains are listed in **Table 5**.

	MIC values μg/ml		
	E. coli	*P. aeruginosa*	*S. aureus*
Gallic acid	1500	500	1750
Ferulic acid	100	100	1100

Table 5.
MIC values of antioxidants against bacterial strains.

At 100 μg/ml ferulic acid and gallic acid cause 60% damage to the cytoplasmic membranes of *P. aeruginosa*. The uptake of propidium iodine (nucleic acid strain to which cell is impermeable) shows that FA and GA alter membrane integrity. In the outer membrane of Gram-positive and Gram-negative bacteria, porins (hydrophilic channels) are present that stops the hydrophobic substances from entering the cell. But some natural agents disintegrate the lipopolysaccharide layer and so damaging the permeability of the membrane causing nutrients to leak and effecting bacterial growth [20].



Ferulic acid Gallic acid

Moreover, bacterial cells have negative surface charge because of the ionic groups. The exposure to phenolic acids decreases this charge and the transport of solutes. Excess of phenolic acids cause hyper acidification that makes the cytoplasm acidic and denature the proteins present in the cytoplasm. So, the damage to membrane by acidification potentially explains the activity of phenolic acid.

Another factor that indicates the membrane damage is K^+ leakage. Because the cell's internal environment is rich in K^+, any damage to the cytoplasmic membranes causes its leakage that indicates the damage as shown in **Figure 2**.

6.3 Inhibition of energy metabolism

In bacterial cell the energy is required for the transport of solutes, uptake of metabolites, and biosynthesis of macromolecules. This energy comes from the respiratory chains like electron transport chain. Some antioxidants inhibit the respiratory chains at any step and thus depriving the cell of the energy necessary for growth (see **Table 1**).

6.3.1 Reterochalcones

Reterochalcones stops the oxygen consumption in the targeted cells and inhibits the NADH oxidation in the membranes of bacteria. The electron transport chain is inhibited in between the CoQ and cytochrome c sites as shown in **Figure 2**. The inhibition of respiratory chains stops the supply of energy to the cells thus retarding their growth [8, 21].

7. Conclusion

There is no doubt that synthetic antibiotics show quick therapeutic effect while treating bacterial infections but in parallel imposes the threat of serious gastrotoxicity, nephrotoxicity, and bacterial resistance. All these issues required special attention because we are gradually losing the game by treating bacterial infections with synthetic antibiotics—as we discussed in previous sections, natural antioxidants in its pure (isolated from raw extracts) had showed excellent potential against common infection causing bacteria, and no study has yet been reported of bacterial resistance to these compounds which firm our enthusiasm to study natural products with the aim to replace synthetic antibiotics. Finally, we can conclude that although antioxidants work slowly against bacterial growth, directly or indirectly, their action is steady and healthy—the continuous and careful evaluation in establishing antibacterial profile of isolated antioxidant can help in the utilization of natural products against bacterial infections with negligible toxicity and the fear of bacterial resistance.

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Conflict of interest

The authors declare "no conflict of interest."

Thanks declarations

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