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Review Article

PHYTO-ANTIQUORUMONES: AN HERBAL APPROACH FOR BLOCKING BACTERIAL TRAFFICKING AND PATHOGENESIS

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ABSTRACT

Over centuries, plants are the richest resource of curative drugs as cited in folklore, traditional and modern medicinal systems and are been used as nutraceuticals, functional food supplements and in pharmaceuticals. Phytochemicals have exhibited beneficial effects against human acute and chronic ailments caused due to microbial pathogens. In recent years, phytochemicals and their derivatives have been extensively used as potent antimicrobials in humans and livestock due to their chemical stability, high bioavailability, low-molecular mass, safe consumption without any side-effect as seen in many antibiotic regimes. These phytocompounds have also been highlighted to function as Quorum Sensing Inhibitors (QSI) or antiquorumones in blocking bacterial pathogenesis preventing their regulatory mechanism and expression of specific set of virulence genes or cascades. However, the role of phytochemicals as QSI has been poorly identified but many of which remain unexplored. Therefore, this review summarizes most of the current scientific contributions focused on the use of plant phytochemicals as antiquorumones, highlighting the significance of plant derived molecules as bacterial inhibitors with larger emphasis on the mechanistic action of biofilm formation and quorum signaling networks mainly N-acylhomoserine lactones (AHLs), autoinducer-2 (AI-2) communications and their attributes in modulating the host immune response. A critical understanding of this complex trio-interaction between humans, microbes and phytochemicals has to be well explored, to exploit the usefulness of these metabolites ultimately paving newer paths for herbal drug discovery and their potential targets leading towards a better quality of life and human welfare.

Keywords: Herbal Drugs, Bacterial Biofilms, Antiquorumones, Autoinducer Signaling, Oligopeptides, N-Acyl Homoserine Lactones (AHLs), Food pathogens.

INTRODUCTION

Time immemorial, civilizations have explored and exploited plants for their wellbeing as food, shelter, medicines, etc. The latter has been extensively used as remedies from nature to improve health or to cure illnesses. Documentation of this can be found as far back in time as approximately 6000 y as mentioned in Charaka Samitha. Plants produce an array of metabolites that are found in their various parts which can be used for therapeutic cure. These metabolites possess various benefits and one among them is the protection against invisible pathogenic and spoilage microbes [1].

Natural plant antimicrobials have been recognized for centuries, to prevent or inhibit microbial growth and to extend shelf life of foods [2]. Most of the plants from Vedic literature are still in use both in traditional medicine, as formulations (Chuurna: solid; Leahaa: semi-solid; Kashaaya: liquid); also the active ingredients from these plants are used as purified single compounds in modern or complementary medicine [3]. The ethanobotonical study of indigenous food products and local medicinal knowledge may have practical implications for developing sustainable drug alternatives and novel drug targets. Nowadays, plant derived chemicals and genetic characters are been increasingly explored for human ethnopharmacological benefits for a better understanding of their application, effects and modes of action [4]. On the other hand, pharmacotoxic studies often explain the effectiveness of herbal drug, dose, duration, bioavailability and bioclearance. Plant-derived natural products have long been and will continue to be extremely important as sources of medicinal agents and models to design, synthesis, semisynthesis and retrosynthesis of novel substances for treating human diseases predominantly caused by microbes and their invasion through foods [5].

With a motto "make food as medicine" the current review therefore, attempts to serve as a basic primer on applicability of phytochemicals in blocking signaling pathways with special reference to foodborne bacterial pathogenes. There is no intent to present a comprehensive discussion of all of the important or attractive features of plants and their derived products. This review is designed for investigators with a primarily focus on phytochemists and microbiologists who wish to gain a perspective on how herbal drugs can either complement or even supplant drug discovery research underway in their laboratories as antiquorumones. This review should be particularly appealing to those in the pharmaceutical or herbal industry faced with the current difficulties of bacterial target identification and drug validation. The review has been divided into three major components for the better understanding of the readers. The first section of the review will provide an introduction to basic of phytochemicals and their classification with a brief mention on pharmacological significance. The second section illustrates the various forms of bacterial infections and their strategic approaches and transitions to cause food borne infections in humans. The third section highlights the mechanisms and mode of action to understand the effectiveness of phytochemicals to be used in blocking the signaling pathways ultimately leading towards the development of probable anti-biofilm herbal drug targets.

Phytochemicals

All plants produce chemical compounds as part of their normal metabolic activities for their survival, replication and defense. The latter, are predominantly secondary metabolites and possess pharmacological or toxicological effects in humans and animals [6]. These are commonly found in plant foods such as fruits, vegetables

and grains, although not classified as essential nutrients in the traditional sense (as is the case for protein, fat, vitamins and minerals), there is increasing evidence that, as dietary constituents, at least some of them may have a role in promoting health. It is these phytochemicals that can have therapeutic actions in humans and which can be refined to produce drugs [7]. Secondary metabolites are broadly classified on the basis of chemical structure (rings, contain a sugar), composition (with or without nitrogen), their solubility in various solvents, or the pathway by which they are been synthesized. A simple classification includes three main groups: the terpenes (made from mevalonic acid, composed almost entirely of carbon and hydrogen), phenolics (made from simple sugars, containing benzene rings, hydrogen and oxygen) and nitrogen containing compounds (extremely diverse, may also contain sulfur) as illustrated in table 1.

Plants and their products like leaves, stems, bark, roots, flowers, seeds and extracts in combination have been used in herbal drugs over the millennia. Herbal products have reached extensive adequacy as beneficial agents like anti-microbial, anti-diabetic, anti-fertility, anti-ageing, anti-arthritic, sedative, anti-depressant, anti-anxiety, anti-spasmodic, analgesic, anti-inflammatory, anti-HIV, vasodilatory, hepato protective, treatment of cirrhosis, asthma, acne, impotence, menopause, migraine, gall stones, chronic fatigue, Alzheimer's, Parkinson disease and memory enhancing activities [8, 9]. These drugs have survived real world testing and thousands of years of human testing. Some drugs have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to counterbalance side effects. Plant-derived foods include rich sources of a number of nutrients that may have a beneficial effect on health, for example carbohydrates, vitamin C, folate, dietary fibre, ß-carotene and vitamin K. In economically developed countries, diets abundant in a variety of plant foods, such as brightly colored fruits (berries) and vegetables (broccoli), are associated with reduced risk of developing chronic disease, and with general health and wellbeing [7].

In today's lifestyle due to technological advancement and changing norms have resulted in shifting demand of foods [10]. There is an increased demand for convenient foods due to lack of time, physical and mental effort associated with food preparation and hence people have shifted to much ready to eat and storage food products [11]. We would like to emphasis the point that, no food is junk, it's our habits which makes the food junk and inappropriate use of food both in terms of quality and quantity leads to many lifestyle acquired diseases like obesity, diabetics, hypertension and binge disorder/night eating syndrome.

Food spoilage is a disagreeable change from the food's normal state. Such a change can be detected with the senses of smell, taste, touch, or vision. Various physical, chemical and biological factors play contributing roles in food spoilage. The contamination of food products with microorganisms presents a problem of global concern, since the growth and metabolism of microorganisms can cause serious foodborne infection and intoxications leading to rapid spoilage of the food products [12].

Fruits, vegetables and canned foods are normally susceptible to bacterial, fungal and viral infections. These infections invade the food during various stages of their development and result in the subsequent spoilage [13]. Food spoilage is more frequently caused by bacteria. The common bacteria which cause food spoilage are *Salmonella, Campylobacter, Listeria monocytogenes, Escherichia coli, Helicobacter pylori, Staphylococcus, Clostridium* and other *Bacillus species*. Raw, cooked and canned foods have been reported to be the rich media for contamination of food samples rendering them unsafe for consumption. Bacterial contamination of raw vegetables and fruits can occur in the field or during post-harvest treatment, while cooked food and canned foods can be contaminated during moderate heat treatment, inadequate refrigeration or poor hygiene during processing with the production of toxins. Toxin produced by foodborne bacteria has raised public concern because of direct and associated food poisoning. Of all the foodborne bacteria, *Bacillus cereus* is the most widely distributed and can cause severe, local and systemic human infections, posing a serious public health problem. The pathogenesis and virulence of *B. cereus* varies according to strain, some being regarded as lethal or highly toxic [14].

Bacteria are responsible for the rapid spoilage of high-protein foods. The range of food-spoiling bacteria is huge, including lactic acid bacteria, Gram-negative rods (Pseudomonas, Shewanella), Gram-positive spore-formers (Bacillus, Clostridium) and other Gram-positive bacteria (Brochothrix, Micrococcus). Many fruits and vegetables present nearly ideal conditions for the survival and growth of bacteria. The internal tissues are nutrient rich and many, especially vegetables, have a pH near neutrality [15]. However many fruits and vegetables possess an outer protective epidermis containing phenolics and their derivatives which typically is covered by a natural waxy cuticle layer containing the polymer cutin providing the first-line of defense against the invading pathogenes.

Life in the slime

Bacteria occur as individual, free-floating (planktonic) cells or clustered together in aggregates of cells (biofilms). Bacteria in biofilms are a major source of food contamination which predisposes to foodborne disease outbreak. Bacterial transfer to food from biofilm can lead to food spoilage or the transmission of diseases [16]. Bacterial polysaccharides are a major component of the extracellular polymeric substance (EPS) or matrix of biofilms and mediate most of the cell-to-cell and cell-to-surface interactions required for biofilm formation and stabilization [17]. Biofilms are groups of microorganisms in which cells stick to each other on a surface, it is a polymeric mixture generally composed of extracellular DNA, proteins and polysaccharides [18]. Biofilms may form on living or non-living surfaces, on solid or liquid surfaces as well as on soft tissue in living organisms, and are typically resistant to conventional methods of disinfection [19]. Biofilms are generally pathogenic in the body, causing more diseases [20]. Some human disease associated with bacteria biofilms are been tabulated in table 2.

Bacterial biofilms account for more than 80% of all microbial infections in humans [21]. Biofilm formation protects and enables single-cell organisms to assume a multicellular lifestyle, in which "group behavior" facilitates survival in adverse environments. Transition from planktonic growth to biofilm occurs in response to environmental changes and involves multiple regulatory networks, which translate signals to concerted gene expression changes [22].

Formation of biofilms from placktonic population can be generalized in five basic steps as depicted in fig. 1: 1). Interaction among free-floating forms. 2) Deposition of the conditioning film which alters the surface properties of the substratum and allows microbes to adhere to the surface. 3) Microbial (planktonic) attachment to the conditioning film. 4) Growth and bacterial colonization, where production of polysaccharides that anchor the bacteria to the surface allow colonies to grow and 5) biofilm formation, where a fully developed biofilm will contain an EPS matrix and vertical structures separated by interstitial spaces and will detach to form new or secondary colonies.

Antimicrobials are used to control the growth of food borne pathogens; the screening of antimicrobial assays reports the inhibition of cell growth on planktonic bacterial cells [23]. The bacteria embedded in a biofilm exist in a low metabolic state or at a stationary growth phase, and they are much less susceptible to the host immune system and to antimicrobial agents. Bacteria in a biofilm are 10-1000 times more resistant to the effects of antimicrobial agents because the EPS matrix delays or prevents antimicrobials from reaching target microorganisms within the biofilm by diffusion limitation and/or chemical interaction with the extracellular proteins and polysaccharides [17, 24]. EPS is their general protective effect on biofilm microorganisms against adverse conditions.

The biofilm life-cycle consists of several overlapping steps. First planktonic (i.e. motile) bacteria encounter a solid surface and undergo a phenotypic change to 'biofilm'-forming bacteria. This phenotypic switch is controlled by a quorum-sensing system. Bacteria continuously produce signaling molecules that diffuse freely across the cell walls [25]. When a bacterium transiently attaches near a solid surface, the rate of diffusion out of the

bacterium is reduced and the signaling molecule builds up inside the cell. Once a critical threshold is reached, the bacterium activates multiple gene-networks that tend to induce EPS [18].

Regulation signals

The propensity to form a bacterial biofilm is guided by numerous environmental signals mainly (i) Nutritional and metabolic cues, (ii) Inorganic molecules, (iii) Osmolarity, (iv) Host-derived signals, (v) Antimicrobials and (vi) Quorum signals, some of which have been identified and many of which remain unstudied. Among these various factors the latter is predominant and crucial dependent factor for the successful development of biofilms and have been more extensively studied and are common to diverse bacteria [17, 18, 26].

Quorum signals (QS): cell to cell cross talks

Bacteria behave as single cellular organisms at low cell densities; however, they may shift their behaviour to 'multicellular' type by sensing that their population density has reached a threshold level. At this stage, they communicate through small signaling molecules, which enable them to express genes for different phenotypes, especially those responsible for their virulent behavior [27, 28]. This phenomenon is termed as bacterial Quorum sensing (QS). QS operates through a wide range of signals such as: (1) N-acyl homoserine lactones (AHLs), (2) Oligopeptides (5-10 amino acid cyclic thiolactone), (3) Furanosyl borate (Autoinducer-2, AI-2), (4) Hydroxyl-palmitic acid methylester and (5) Methyl dodecanoic acid.

General mechanism of QS in bacterial community

Different bacterial species may produce different types of quorum-sensing signals but they appear to adopt only two general mechanisms for detecting and responding to these signals. Most of the bacteria seem to use one or other of the above quorum-sensing systems in modulating the target gene expression [29]. The two most widely studied QS signals are: (i) AHLs produced by more than 70% species of Gram-negative bacteria, which diffuse across the cell membrane and bind to regulatory proteins within the cell and (ii) peptide based QS system in Gram-positive bacteria, which operate through membrane bound receptor histidine kinases [30] and a few use hybrid communication mechanisms. All the three mechanisms are well illustrated in fig. 2.

AHL based QS

In AHL dependent bacterial QS systems, a single synthase-regulator complex is responsible for the expression of specific genes. In this case, the signal molecules are synthesized constitutively at a low concentration by the synthase gene (such as luxI) and are distributed in and around the cells [31]. At high cell density, it binds to its receptor and activates the transcriptional regulator (LuxR). The AI-LuxR binds to the DNA (promoter region of the gene) and triggers the expression of genes regulated by QS system (fig. 2).

Peptide based QS

Gram-positive bacteria regulate the QS induced gene expression through oligopeptides which are secreted into the environment. These small (approximately 10 amino acid) extracellular peptides (Auto inducer peptide, AIP) operate through a 2-component signaling system. Among the gram-positive bacteria, *Bacillus subtilis* has at least 4 groups to communicate. The variation exists in the 4 groups with respect to ComX (an extracellular pheromone) and sensor domain position of ComP (histidine kinase). Similarly, *the Staphylococcus aureus* Agr system is also responsible for their segregation in to 4 distinct groups [27, 28, 31]. The uniqueness of the system is maintained independently in each of them (fig. 2).

Hybrid languages: the quorum sensing systems

The free-living marine luminous bacterium *V. harveyi* possesses two auto inducer-response systems that function in parallel to control the density-dependent expression of the luciferase structural operon *luxCDABE*. This complex quorum sensing circuit has features found in both Gram-negative and Gram-positive bacteria. The two *V. harveyi* autoinducers, AI-1 and AI-2, are recognized by cognate sensor kinase proteins named LuxN and LuxQ, respectively. Additionally, a periplasmic-binding protein called LuxP is hypothesised to interact with LuxQ to recognize AI-2 [31]. Sensory information from both systems is transduced by phosphorylation and dephosphorylation to a shared signal integrator protein called LuxU, which subsequently conveys the signal to the response regulator protein LuxO (fig. 2).

Brief overview on state switching, based on secondary messengers

Bacteria have two major sensory systems for the recognition of the signals: two-component systems (TCSs) and the c-di-GMP-mediated signal transduction network. Briefly we here discuss these regulatory networks that have been more extensively studied.

Two-component systems (TCSs)

Prokaryotes process environmental information through phosphoryl group transfer. This is done by TCSs, at their simplest form, are composed of a sensor histidine kinase, which directly or indirectly senses a signal, and a response regulator, which receives the information from the histidine kinase and brings about the relevant response. The signal is relayed from the histidine kinase to the response regulator as a phosphoryl group transfer. Many of them are composed of multiple components and hybrid kinase-response regulators [26]. TCSs are involved in regulating biofilm formation in a number of bacteria. The best-characterized example of a TCS driving the motile-sessile switch is the intricate LadS/RetS/Gac/Rsm signal transduction system of *P. aeruginosa*, where the GacS/GacA TCS represses the expression of the CsrA homolog RsmA [32].

c-di-GMP

In c-di-GMP signaling, the sensor protein domain reacts to the stimulus by activating an output domain located in the same protein that triggers the synthesis [diguanylate cyclase (DGC), GGDEF domain-containing proteins] or degradation [phosphodiesterase (PDE), EAL and HD-GYP domain-containing proteins] of c-di-GMP. Then, the resulting c-di-GMP interacts with specific effectors that finally relay the signals to cellular processes. Based on *in vitro* studies carried out with different bacterial species, it is widely accepted that a high concentration of c-di-GMP enhances biofilm development and represses virulence factor expression, whereas low c-di-GMP levels promote bacterial motility and a planktonic lifestyle. When bacteria start producing a biofilm on the surface of a host tissue, the switch from a planktonic to a biofilm lifestyle will also depend on the accumulation of c-di-GMP through the activation of specific GGDEF domain-containing proteins or the inhibition of EAL/HD-GYP domain proteins [32]. Because very often bacteria contain several GGDEF and EAL/HD-GYP domains linked to signal input domains [including PAS, REC, globin, blue light sensing (BLUF), hemerythrin, GAF, CHASE and MASE domains], it is conceivable that one or more of these proteins sense specific signals on the surface of host tissues, either upon surface contact or as a prerequisite for attachment, to promote increased c-di-GMP accumulation.

Strategies to inhibit bacterial biofilms and their signaling network–Preventive mode

Removal of cells from the biofilm colony is an essential stage of the biofilm life cycle. Strategies to plan against bacterial biofilm must be achieved by prevention of biofilm formation rather than dispersal of the formed biofilm. Strategies for prevention of biofilm formation include both "Chemical" and "Mechanical" methods [21, 33].

Chemical methods

Antimicrobial coatings

Antibiotics, biocides and ion coatings are commonly used chemical methods of biofilm prevention. These methods prevent biofilm formation by interfering with the attachment and expansion of immature biofilms and are effective only for a short time period (about 1 w), after which leaching of the antimicrobial agent reduces the effectiveness of the coating. Use of silver coatings for antimicrobial purposes has been benificial. The antimicrobial property of silver is known as an oligodynamic effect, a process in which metal ions interfere with the growth and function of bacteria.

Several *in vitro* studies have confirmed the effectiveness of silver at preventing infection, both in coating form and as nanoparticles dispersed in a polymer matrix [21, 33]. However, application of silver in the *in vivo* system is associated with warnings due to the toxic effect of silver on human tissue.

Polymer modifications

Antimicrobial agents can be immobilized on device surfaces using long, flexible polymeric chains. These chains are anchored to the device surface by covalent bonds, producing non-leaching, contact-killing surfaces capable of inactivating ≥ 99% of *S. epidermidis, E. coli,* and *P. aeruginosa* bacteria. Dispersion forces between the polymer chains and the bacterial cells prevent bacteria from binding to the surface and initiating biofilm growth. The concept is similar to that of steric stabilization of colloids [21, 33].

Mechanical methods

Hydrophobicity and Surface phenomenon

Hydrophobicity plays an important role in determining the ability of bacteria to form biofilms. Some species are not able to attach to a surface and are sometimes able to establish themselves directly to earlier colonists due to their limited motility. Non motile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria. Modification of the surface charge of polymers has also proven to be an effective means of biofilm prevention. Based on the principles of electrostatics, charged particles will repel other particles of like charge. The hydrophobicity and the charge of polymeric chains can be controlled by using several backbone compounds and antimicrobial agents. Positively-charged polycationic chains enable the molecule to stretch out and generate bactericidal activity. Surface roughness can also affect biofilm adhesion. Rough, high-energy surfaces are more conducive to biofilm formation and maturation, while smooth surfaces are less susceptible to biofilm adhesion. The roughness of a surface can affect the hydrophobicity or hydrophilicity of the contacting substance, which in turn affects its ability to adhere [21, 33]. It is, thus, desirable to maintain a smooth surface on any products that may come in contact with bacteria.

Strategies for dispersal of formed bacterial biofilm–destructive mode

Most recent advances in strategies are designed to prevent biofilm formation by killing the bacteria or targeting different biofilm developmental stages [21]. Some strategies and mechanisms for biofilm inhibition are discussed below.

Bacterial antibiofilm polysaccharides

Lectins are proteins that specifically recognize and bind sugars without modifying native molecules. In bacteria, the primary function of lectins is to facilitate attachment or adherence of bacteria to host cells. Several plant, microbial and milk polysaccharides have been shown to block various lectins from human pathogenic bacteria by competitive inhibition. Polysaccharides mediate cell-to-surface and cell-to-cell interactions that are critical for biofilm formation and stabilization. Antibiofilm properties of polysaccharides are believed to lie on their ability to: a) alter the physical characteristics of bacterial cells or abiotic surfaces.

b) act as signaling molecules that impact the gene expression patterns of susceptible bacteria. or c) competitively inhibit multivalent carbohydrate–protein interactions, thereby interfering with adhesion. Many studies are reported about the ability of some bacterial polysaccharides to inhibit biofilm formation by several bacteria, including *E. coli strains, L. monocytogenes, P. aeruginosa, Klebsiella pneumoniae, Staphylococcus* and *Enterococcus* [17-19, 21].

Table 1: Broad classification of phytochemicals and their derivatives with potent pharmacological uses

Class of phytochemicals	Compounds derived	Sources	Pharmacological Activity/Function
Terpenes/Terpenoids			
Monoterpenes	Menthol, Linalool	Mint and relatives, many plants	modulate neural reflux, ion transport, anesthetic
Sesquiterpenes	Parthenolid	Parthenium and relatives (*Asteraceae)*	Contact dermatitis
Diterpenes	Gossypol	Cotton	Block phosphorylation
Triterpenes/cardiac glycosides	Digitogenin	*Digitalis purpurea*, *Digitalis lanata*, Ehrhart	Stimulate heart muscle, alter ion transport
Tetraterpenoids	Carotene	Carrot and berries	Antioxidant, orange coloring
Terpene polymers	Rubber	Hevea trees, dandelion	Antiulcer agent and prevents constipation
Steriods/Sterols	Diosgenin, Stigmasterol, Spinasterol	*Dioscorea spp.*(Mexican yams), Soyabean, Spinach	Oral contraceptives, other steroid drugs and animal hormones
Phenolics			
Phenolic acids	Caffeic acid, Chlorogenic acid	All plants	Cause oxidative damage, browning in fruits and wine
Coumarins	Umbelliferone	Banana pseudo stem, carrots, parsnip	Cross-link DNA, block cell division
Lignans	Podophyllin Urushiol	Mayapple poison ivy	Cathartic, vomiting, allergic dermatitis
Flavonoids	Anthocyanin, Flavonols, Flavanones, Anthocyanidins	Almost all plants	Inhibit inflammatory enzymes, anti-and pro-oxidants, estrogenic effect
Tannins	Gallotannin	Oak, hemlock trees and legumes	Bind to proteins, enzymes, block digestion, antioxidants
Nitrogen containing			
Alkaloids	Atropine, Nicotine, Cocaine, Theobromine	*Atropa belladonna*, *Datura stramonium*, *Hyoscyamus niger*, *Mandragora* officinarum, Tobacco and coca plant	Anticholinergics (parasympatholytics), Interfere with neurotransmission, block enzyme action
Nitrogen and Sulphur containing			
Glucosinolates	Sinigrin	Cabbage and other leafy vegetables	Anti-inflammatory benefits and anticancer

Anti-biofilm enzymes

Enzymes that degrade biofilm extracellular matrix may play a role in biofilm dispersal and may be useful as anti-biofilm agents. N-acetyl-D-glucosamine-1-phosphate acetyl transferase is an essential peptidoglycan and lipopolysaccharide precursor in Gram-positive and Gram-negative pathogens, respectively, is among the enzymes targeted for matrix disruption. Treatment with such enzymes prevents *Staphylococcus* and *Enterococcus* biofilm formation and dispersal. For example, Dispersin-B is a glycoside hydrolase that cleaves β 1–6 N-acetylglucosamine polymers in the bacterial peptidoglycan layer has been shown to be effective against *S. aureus* and *S. epidermidis* biofilms and bacteria [19, 21].

Table 2: Various bacterial biofilm infections associated with human pathogenesis

Biofilm-forming Bacteria	Human disease
Listeria monocytogenes	Listeriosis, meningitis and multiple abortions in female
Helicobacter pylori	Peptic ulcers, gut infections and colorectal cancer
Pseudomonas aeruginosa	Cystic fibrosis pneumonia
Burkholderia cepacia	
Pseudomonas pseudomallei	Meloidosis
Group A *streptococci*	Necrotizing fasciitis
Staphylococci and other Gram-positive cocci	Musculoskeletal infections
Haemophilus influenzae (Non-typable strains)	Otitis media
E. coli and other enteric bacteria	Biliary tract infection
E. coli and other Gram-negative rods	Urinary catheter cystitis
E. coli and other Gram-negative bacteria	Bacterial prostatitis
Gram negative anaerobic oral bacteria	Periodontitis
Streptococcus spp. and other acidogenic Gram-positive cocci	Dental caries

Table 3: Phyto-antiquorumone and their targeted bacterial biofilms

By-products	Major component	Target organisms
Leucas aspera leaves and flower	Phenolic and flavonoids (Leucasin)	*L. monocytogenes, P. aeruginosa, S. aureus* and *H. pylori*
Pomegranate fruit peels	Phenolic, tannins and flavonoids	*L. monocytogenes, S. aureus, E. coli, Y. enterocolitica* and *P. fluorescens*
Apple peels	Polyphenolic compounds	*S. aureus* and *P. fluorescens*
Almond skin extracts	Polyphenols	*S. aureus* and *L. monocytogenes*
Coconut husk	Phytochemical including phenolics and tannins	*L. monocytogenes, B. sublitis, S. aureus* and *V. cholera*
Green tea waste	Tannins	*S. aureus, E. coli, L. monocytogenes, B. coagulans* and *S. flexneri*
Acorn, chestnut, and persimmon hull	Tannins	*S. aureus, E. coli* and *L. monocytogenes*
Tomato seeds	Fatty acids, carotenoids, saponins, phenolic compounds	*S. aureus, S. epidermidis, M. luteus, E. faecalis* and *B. cereus*
Quince fruit peel	chloogenic acid, catechin, quercetin and kaempferol	*S. aureus, P. aeruginosa* and *E. coli*
Potato peels	Chlorogenic, caffeic, gallic and protocatechuic acids	Bacteriostatic effect on *E. coli* and *S. typhimurim*
Walnut green husk	Antioxidants such as phenolic compounds	*S. aureus, B. cereus* and *B. subtilis*
Mango seed kernel extract	Phenolic compounds, oleic acid, tocopherols, squaene and different sterol fractions	Coliforms and *E. coli*
Peels,seeds, pulp of mexican lime	Phytochemicals: flavonones,polymethoxylated flavones,tannins	*E. coli* 0157:H7, *S. typhimurium* and *S. sonnei*
Grape pomace	Phenolic acids, flavonoids, stilbenes	*S. aureus*, Salmonella and Enterococci
Olive pomace	Phenolic compounds oleocanthal, deoxyloganic acid lauryl ester	*E. coli* 0157:H7, *S. enterica*, *L. monocytogenes* and *S. aureus*
Beet root pomace extract	Phenolics, flavonoids, betacyanins, betaxanthins	*S. aureus, B. cereus, E. coli* and *P. aeruginosa*
Buckwheat hull extracts	Phenolics, flavonoids, tocopherols, rutin, quercetin derivatives	Gram-positive (*B. cereus, S. aureus, E. faecalis*) and Gram-negtive bacteria (*S. choleraesuis, E. coli* and *P. mirabilis*)
Legume hulls (Vigna radiate)	Polyphecolic compounds, flavonoids	*B. cereus* and *S. aureus*
Grape fruit seed extracts	catechins, epicatechin, epocatechin, procyanidins	Pseudomonas spp.
Oriental mustard	Phenolic compounds (sinapic acid and several sinapoyl conjugates)	*B. subtilis, E. coli, L. monocytogenes, P. fluorescens* and *S. aureus*
Coffee pulp	flavan-3-ols, hydroxycinnamic acids, flavonols anthocyanidins	Coliform and *E. coli*

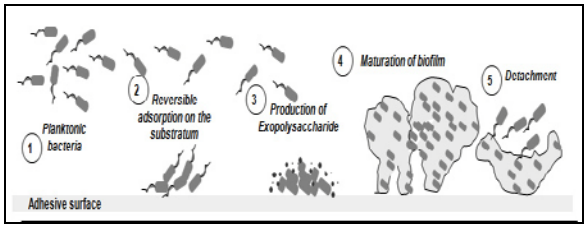

Fig. 1: Stage of bacterial biofilm development

Chelating agents

Metal cations, such as calcium, magnesium and iron have been implicated in maintaining matrix integrity. Consistent with this observation, chelating agents have been shown to destabilize biofilm architecture besides interfering with bacterial membrane stability. For example, sodium citrate inhibited biofilm formation by several *Staphylococci* species *in vitro* [21]. In addition, tetrasodium-EDTA eradicated biofilms in an *in vitro* biofilm model and on explanted hemodialysis catheters, whereas disodium-EDTA, in combination with tigecyclin or gentamicin, reduced biofilm formation by *Staphylococcus* species and *P. aeruginosa.*

Antimicrobial peptides

Antimicrobial peptides are produced by the innate immune response system and have been proposed as attractive candidates for the development of novel types of antibiotics. However, their activity spectrum and mechanism of action need to be more precisely defined before they can be considered as possible therapeutic strategies [21]. Lytic peptides are group of antimicrobial peptides assessed for their inhibitory effects on biofilm formation. Lytic peptides bind the lipopolysaccharide moieties of the bacterial cell membrane, disrupting membrane stability. Studies in *Staphylococcus aureus* have shown that a lytic peptide prevents *in vitro* biofilm formation and was also capable

of diffusing into the deep layer of preformed biofilm, killing 99.9% of biofilm bacteria. This peptide retained activity under highly acidic environments and in the presence of excess of metals, conditions that mimic the *S. aureus* biofilm environment.

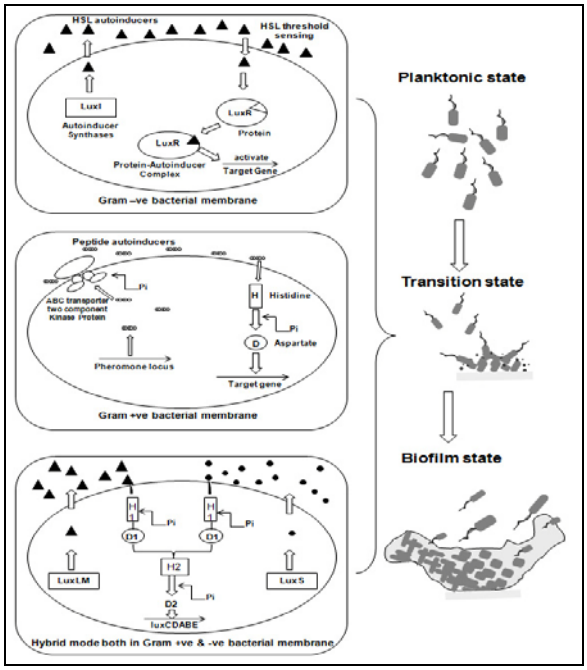

Fig. 2: Quorum sensing mechanisms in Gram–ve, Gram+ve and Hybrid bacterium cross talks from planktonic to mature biofilm state *via* transition state (fig. **adopted, modified and reconstructed from Bassler, *et al.*, 1999)**

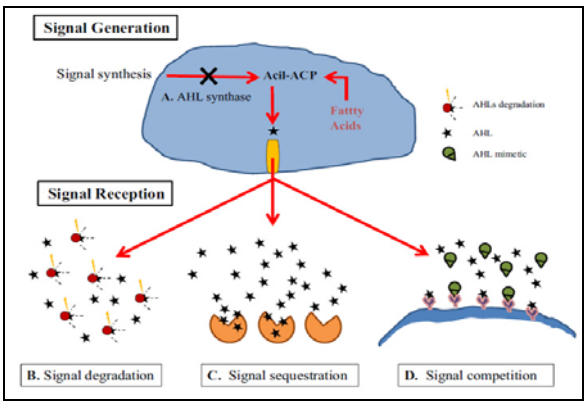

Fig. 3: Potential mechanisms of action of phyto-antiquorumones on bacterial biofilms (fig. adopted from Truchadoa, *et al.*, 2015)

Anti-adhesion agents

Attachment constitutes the first step in virtually all types of biofilm formation, thus numerous studies have focused on preventing bacterial adherence. Efforts have been made to inhibit assembly of different types of pili, through the use of pilicides, which are compounds rationally designed to interfere with export of the corresponding pilin subunits. Pilicides are shown to inhibit biofilm formation *in vitro* by 50%, at lower concentrations [21, 34].

Inhibition of quorum sensing signals

The process of QS can be disrupted by different mechanisms: (i) reducing the activity of AHL cognate receptor protein or AHL synthase, (ii) inhibiting the production of QS signal molecules, (iii) degradation of the AHL, and (iv) mimicking the signal molecules primarily by using synthetic compounds as analogues of signal molecules [27]. A complete holistic approach is been depicted in fig. 3. For example plant extracts have been found to act as QS inhibitors because of similarity in their chemical structure to those of QS signals (AHL) and also because of their ability to degrade signal receptors (LuxR/lasR), leading to inactivation of AHL signals. AHL-dependent QS can be disrupted by inactivation of the cognate AHL through lactonolysis at alkaline pHs or by enzymatic disruption. AHL-inactivating enzymes, either lactonases or acylases (amidases) are widely distributed in microbes and higher organisms. Lactonases are generally active against a broad spectrum of AHL signal molecules, probably because they target the conserved homoserine lactone ring [35]. Conversely, the AHL inactivating acylases which cleave the AHL amide bond exhibit a marked degree of substrate specificity. *P. aeruginosa* for example expresses three enzymes PvdQ, QuiP and HacB with acylase activity towards AHLs [36].

Phyto anti-quorumone and their potential bacterial targets

The presence of biofilms is a potential source of contamination that may lead to food spoilage and disease transmission. Bacteria included in biofilm structure are generally more resistant to antimicrobial agents than planktonic cells. Plants produce an array of secondary metabolites that can be found in the edible, medicinal and herbal plants and their derived products. These secondary metabolites possess various benefits including antimicrobial properties against pathogenic and spoilage microbes. In general plants have much greater inhibition effect against Gram-positive than Gram-negative bacteria. The antimicrobial efficacy of components in plants depends on the chemical structure of active components and their concentration. There are various chemical components present in plants with antimicrobial effect including saponin, flavonoids, thiosulfinates, glucosinolates, phenolics, and organic acids commonly found in herbs and spices, fruits and vegetables, seeds and leaves and roots. Fruits and vegetables generally contain high amounts phenolics and organic acids that are well known to possess antibiofilm activity [2, 37, 38]. A few examples are been tabulated in table 3 adopted from Gyawali and Ibrahim, 2014.

Merits of use of plant molecules in preventing bacterial food spoilage

Plant antimicrobials are phytochemicals which are important for the proper functioning of the plant. Phytochemicals in plants contribute to the sensory properties when added to food and possess antioxidant and antimicrobial properties, characteristics that are useful in extending the shelf-life of food [39, 40]. The antioxidant and other biological properties in phytochemicals have been attributed to beneficial health effects. Incorporating antimicrobial compounds in films rather than directly mixing it with the food allows for the functional effect at the food surface, where most of the microbial growth is localized [41]. Antimicrobial packaging would include systems such as adding a sachet into the package, dispersing bioactive agents in the packaging, coating bioactive agents on the surface of the packaging material, or using antimicrobial macromolecules with film forming properties as edible packaging material.

Demerits

Plant products are highly finicky. The antimicrobial effect of natural products could be affected by different factors including botanical source, time of harvesting, stage of development, and method of extraction. In food applications, these natural antimicrobial compounds could be also influenced by food components, processing and storage and thus could require higher concentrations than that used in laboratory media. The addition of natural antimicrobials to the food products may affect the sensory characteristics of the final product. Thus the challenge for practical application of natural antimicrobials is to develop an optimized combination of low doses of antimicrobial agents that could maintain product safety and extend the shelf life but minimize undesirable flavor and sensory changes associated with the addition of high concentrations of natural antimicrobials [41]. Thus, more research on possible low cost production of natural products is required in order to be used in food systems. In most cases natural

antimicrobials are extracted and purified to be tested or applied to food products. An extraction method with minimal processing such as direct extraction seems to be a promising method to avoid possible alteration or destruction of active ingredients. Natural antimicrobial appears to be the most promising solution for many food safety and food quality concerns. Thus, the future will anticipate more investigation of naturally antimicrobials to food products, especially in the areas of synergistic effectiveness and optimum concentrations.

Final remarks and conclusion

It has been estimated that about 65% of human bacterial infections involve biofilms. Biofilms have become the leading cause of infections related to indwelling, especially those caused by antibiotic-resistant bacterial strains by forming some specific virulent factors. Particular attention is oriented nowadays towards the need for antimicrobials from plants that are able to reduce or eliminate pathogenic bacteria, and to extend the shelf life of foods, reducing and increasing overall quality of food products. Biofilm is one of the major virulent factors of most of the pathogenic microorganism. Therefore, the developments of effective and safe medicine particularly plant extracts with antimicrobial properties have recently received growing interest from both academic and industrial sectors. The present review demonstrates the effective biofilm formation and the mechanism of plants as antiquoromones. Further study will be helpful to understand molecular mechanism of anti-biofilm effect by utilization of natural antimicrobial agents. The challenges of using plant antimicrobials of some plant extracts having flavours associated with them may pose a problem, therefore it is important to match the food and the plant extract flavour or understand the synergies to decide on the concentration used. Incorporation of plant antimicrobials in food can give rise to the growth and virulence of certain pathogens due to the changes in microbial ecology. In conclusion, it is critical to understand the effect of plant extracts on the behavior of these microbial populations in complex food systems and this would be a challenge in using them as a functional food ingredient. The successfulness new findings will ultimately pave newer paths for herbal drug discovery and their potential targets leading towards a better quality of life and hygiene.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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