# Natural products-current and future promising source of novel drugs: A review on their antimicrobial mechanism of actions

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The emergence of antibiotic-resistant pathogenic bacteria has given rise to the needs of antimicrobial compounds that derived from natural products which less likely to cause pathogen resistance. The most profound and traditional example of the antimicrobial from the nature is antimicrobial peptides (AMPs) that serve as natural innate barriers limiting microbial infection or act as an integral component in response to inflammation or microbial infection. Cytoplasmic membrane, appeared as the first targeting action of these peptides pertaining to the amphipathicity and cationic-charged of the compounds in nature, which allow them to attach, translocate and eventually inserted into the inner region of the microbial cells. In fact, many studies have proven that some of the AMPs can even adopt more than one mechanism in inhibiting the microbial cells. This chapter aims to review and explain in depth the antimicrobial mechanism of actions of natural proteinaceous and non-proteinaceous compounds from various plant and animal sources. Preparation/extraction, treatment and purification of the extracted antimicrobial compounds will also be discussed.

**Keywords** natural products; antimicrobial peptide; mechanism of action; plant extract; essential oils; purification

## 1. Introduction

### 1.1. The occurrence of antibiotic resistance

Penicillin is one of the most remarkable β-lactam antibiotic (including penicillin, methicillin, dicloxacillin, nafcillin and oxacillin), that has been a chronicle success in treating the life-threatening bacterial infections by substantially decreasing the fatality rate that caused by the nuisance in 1940 [1]. This discovery ushered in the modern medicinal therapy for bacterial infections and the world wide introduction of antibiotics. Antibiotics have saved millions of lives and help in reducing patient's suffering. Unfortunately, four years after the commercial introduction of penicillin in the market, penicillin-resistant *Staphylococcus aureus* has evolved [2, 3]. The resistant strain destroys penicillin by secreting enzyme β-lactamase [4]. In the late 1950s, penicillin was effective against only 15% of infections caused by *S. aureus*. By early 1970s, the existing therapies seemed to be inadequate as antibiotic resistance was emerging and resulting in the urgently need for new drugs with minimum side-effect–bacterial resistance. The spread of antibiotic resistance was continued with the growing clones of methicillin-resistant *S. aureus* (MRSA) [2, 4]. Thus, efforts were focused by researchers, academicians and pharmaceutical companies on developing a new or improved antibacterial agent with different mode of action from β-lactams. Shortly thereafter, glycopeptide antibiotics (vancomycin and its sister antibiotic teicoplanin), glycosylated cyclic or polycyclic non-ribosomal peptides that inhibit bacterial peptidoglycan synthesis were introduced. The effectiveness of the newly invented antibiotics in fighting bacterial resistance crisis for 15 years, guaranteed the glycopeptide antibiotics being considered as the last resort of drugs in combating Gram-positive bacterial infections including the multi-drug resistant superbug, MRSA [3–5]. Nonetheless, the long fear occurrence of the incidence of antibiotic resistance has emerged as major clinical challenge when the first incidence of vancomycin-resistant *S. aureus* was detected and reported in 1997 [6]. In fact, the appearance of the vancomycin resistance was not surprising, owing to the globally widespread overuse and misuse of the antibiotic [4]. The subsequent effort in overcoming the continuing glycopeptide resistance incidence has brew out the second generation of glycopeptide antibiotics, such as oritavancin, telavancin and dalbavancin. These glycopeptides have improved antibacterial efficacies as compared to vancomycin [7]. Although β-lactams and glycopeptides are still the first and last line of defence, there is a critical need for novel effective therapies against bacterial infections and the potential occurrence of antibiotic resistance.

### 1.2. Natural product-based antibiotics

In the 1980s, the discovery of antimicrobial peptides (AMPs) in insects and animals, has gained the attentions from the researchers and pharmaceutical companies towards these newly, promising alternative sources of today's antibiotic [8]. This finding indicates the importance of nature not only being the rich source for developing drugs against serious bacterial infection diseases. Human being, though, is the most evolved species in this planet; they still need the help from nature in fighting multi-drugs resistant bacteria since nature, is the scaffold that contains the key to bacterial vulnerability. Generally, AMPs exert broad spectrum of inhibition against pathogenic bacterial, fungi, yeast, parasites, protozoa, and even viruses [9, 10]. Their extended inhibitory activities probably due to the contrast mode of action with most antibiotics. Typically they are targeting specific protein in bacterial cell. Suggested killing mechanisms of AMPs

 

obtained from the natural products may involve the fundamental structure of the pathogenic bacteria, the cytoplasmic membrane, in which makes the bacterial resistance less likely to occur as compared to the action of traditional antibiotics [9]. Besides AMPs, antimicrobial substances could also be found in the plant extracts, phytochemicals and plant secondary metabolites such as essential oils (components like aldehydes, alcohols, phenols, ketones and esters), alkaloids, terpenoids, saponins and flavonoids [11, 12]. The antimicrobial actions of the compounds were reported to be associated with destabilization of bacterial cytoplasmic membrane, interfering energy metabolism, permitting loss of intracellular macromolecules and causing deprivation of substances required for microbial growth [13].

Thus, in this review, we aim to illustrate and explain in depth the antimicrobial mechanism of actions of natural proteinaceous and non-proteinaceous compounds from various plant and animal sources. Preparation/extraction of the antimicrobial compounds, their treatment and purification will also be discussed. Understanding the mode of action of such compounds would assist in overcoming the intimidating challenge of microbial antibiotic resistance.

## 2. Antimicrobial peptides (AMPs) from animals and insects

### 2.1. AMPs from animals and insects

An alternative source of pharmaceuticals for treating antibiotic resistant bacterial infection is the natural compound known as “antibiotic peptide” from plants, insects and animals, that exhibit potent antimicrobial activity and most of them have been established since the last three decades ago [14, 15]. This group of molecules are in fact termed as “antimicrobial peptides”, which are expressed, induced and secreted as the first line of defence in the innate immunological functions and mechanisms of eukaryotic organisms [16]. Prokaryote antimicrobial peptides (AMPs) are normally referred to as bacteriocins, colicins, or lantibiotics that are produced by the respective microorganism itself [16]. Animal, either in vertebrates or invertebrates is the most important source of natural AMPs [17]. Table 1 shows examples of AMPs isolated from animals and insects.

**Table 1** A comprehensive list of antimicrobial peptides (AMPs) from animals and insects

| Source | Peptide | Antimicrobial activity | References |
|---|---|---|---|
| Amphibian | Buforins | Bacteria, fungi | [14, 18] |
| | Magainin | Bacteria, protozoa | [14] |
| | Bombinins | Bacteria, fungi | [14] |
| | Temporins | Bacteria, fungi | [14] |
| | Dermaseptin | Bacteria, protozoa | [14] |
| | Brevinin | Bacteria | [14] |
| Bovine | Bac5 | Bacteria | [15] |
| | Bac7 | Bacteria | [15] |
| | BMAP-28 | Bacteria | [16] |
| | Indolicidin | Bacteria, virus | [14, 19] |
| | TAP | Bacteria | [20] |
| | BNBD-1 | Bacteria | [20] |
| Sheep | SMAP 28 | Bacteria | [15] |
| | SMAP 29 | Bacteria, fungi | [15] |
| | SMAP 34 | Bacteria, fungi | [15] |
| | OaBac5α,β | Broad-spectrum | [15] |
| | OaBac6 | Broad-spectrum | [15] |
| | OaBac7.5 | Broad-spectrum | [15] |
| | OaBac11 | Broad-spectrum | [15] |
| Porcine | PMAP 23 | Broad-spectrum | [15] |
| | PMAP 36 | Broad-spectrum | [15] |
| | PMAP 37 | Broad-spectrum | [15] |
| | PR-39 | Bacteria | [15] |
| | Cecropins | Broad-spectrum | [15, 21] |
| | Prophenin | bacteria | [15] |
| | Protegrins | Bacteria, fungi, virus | [14, 15] |
| | Tripticin | Fungi | [15, 16] |
| Horse | eCATH-1 | Bacteria | [15] |
| | eCATH-2 | Bacteria | [15] |
| | eCATH-3 | Bacteria | [15] |

| | | | |
|---|---|---|---|
| Avian | Gal 1/CHP1 | Bacteria, fungi | [15, 20] |
| | Gal 1α/CHP2 | Bacteria, fungi | [15, 20] |
| | Gal 2 | Bacteria, fungi | [15, 20] |
| | Gal 3 | Bacteria, fungi | [15, 20] |
| | THP 1 | Bacteria, fungi | [20] |
| | THP 2 | Bacteria, fungi | [20] |
| | THP 3 | Bacteria, fungi | [20] |
| | GPV-1 | Bacteria | [15] |
| | OSP-1 | Bacteria | [20] |
| | BNDBD-1 | Bacteria | [20] |
| | NP-1 | Bacteria | [20] |
| | Spheniscins | Bacteria, fungi | [20] |
| Marine | Tachyplesins | Bacteria, fungi, virus | [14, 22] |
| | Hepcidin | Bacteria | [23] |
| | Pelteobagrin | Broad-spectrum | [24] |
| | Piscidin 2 | Fungi | [24] |
| | Parasin I | Bacteria | [23] |
| | Penaeidins | Bacteria, fungi | [14, 22, 25] |
| | Crustins | Bacteria, fungi | [22] |
| | Astacidin 2 | Bacteria | [26] |
| | Arasin 1 | Bacteria | [26] |
| | Mytilin | Bacteria, fungi, virus | [22, 27] |
| | Protamine | Bacteria, fungi | [23, 28] |
| | CAP-1 | Bacteria, protozoa | [29] |
| | Piclavines | Fungi | [30] |
| | Didemnins | Bacteria, virus | [30] |
| | Halocyamine | Bacteria, virus | [30] |
| | Lissoclinotoxin | Bacteria, fungi | [30] |
| Insects | Apidaecins | Bacteria | [31] |
| | Drosocin | Bacteria | [31] |
| | Pyrrhocoricin | Bacteria | [31] |
| | Hymenoptaecin | Bacteria | [31] |
| | Coleoptericin | Bacteria | [31] |
| | Drosomycin | Bacteria | [31] |
| | Melittin | Bacteria, virus | [16, 19, 31] |
| | Bombolitins | Bacteria | [31] |
| | Diptericin | Bacteria | [16] |
| | Cecropins | Broad-spectrum | [16] |
| | Bicarinalin | Broad-spectrum | [32] |
| | Ponericins | Bacteria | [32] |
| | Gambicin | Bacteria, fungi, protozoa | [14] |
| | Attacin | Bacteria | [16] |
| | Holotricin | Bacteria | [16] |
| | Acaloleptin | Bacteria | [16] |

Insects and other animals AMPs are normally present and expressed in specialized cells, such as polymorphonuclear leukocytes, macrophages and mucosal epithelial cells [15]. Most of them are positively charged, amphipathic, small in size (10-50 amino acids), possessing at least 50% hydrophobic amino acids, and have the ability to kill a broad-range of microorganisms including Gram-positive and Gram-negative bacteria, fungi, protozoa and even viruses [28, 31, 33]. Based on the conformational structure and amino acids composition, AMPs can be classified into 5 groups [31]. The first group of the AMPs comprises of those anionic peptides. They can be isolated from bronchoalveolar lavage fluid in neonatal calves, mammalian epithelia and aspartic-acid-rich molecules isolated from sheep [34]. The second AMPs group consists of cationic peptides with linear or α-helical structure, for instance, cecropins from porcine small intestines, melittin from bee venom, buforins, magainin, bombinins, dermaseptin and brevinin from the amphibian, SMAP29 from sheep bone marrow and bombilitins from bees (Table 1) [14, 15]. Cecropins are isolated from the porcine small intestine and are found in dipterans and lepidopterans. These polypeptides show broad inhibitory activity against bacteria, fungi, protozoa, viruses, nematode worms as well as tumour cells [21]. Buforins, such as buforin I and buforin II, show much stronger antimicrobial activities against a broad spectrum of microorganisms as compared to other amphibian extracted peptides, like magainin. The striking feature that possessed by buforins is that, the peptide

 

shares complete sequence identity with the N-terminal region of histone H2A, which contributes to the protein's DNA binding effect of the peptide [18]. Further explanation will be given in subsequent section. In fact, cecropin-derivative, cecropin A, melittin (haemolytic peptide isolated from the venom of European honeybee, *Apis mellifera*), magainin I and magainin II were reported to exhibit antiviral activity against the arenavirus Junin virus (JV), and herpes simplex virus type 1 (HSV-1) and 2 (HSV-2). Among these peptides, melittin exerts the greatest inhibitory effect against the viruses at a concentration of 3 μM compared to the 10-fold greater concentrations required to inhibit the viruses by cecropin and magainin [19]. SMAP29, a typical cathelicidin (defense peptide) found in sheep bone marrow, is effective against Gram-positive and Gram-negative bacteria and *Aspergillus fumigatus*. Greater antimicrobial activity was shown by the derivative of SMAP29, called ovispirin, which active against multiple antibiotic-resistant *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* [15, 35].

The third group of AMPs are those cationic peptides that rich in specific amino acids. For instance, astacidin 2, a 14-amino acid long peptide is found rich in proline [26]; bactenecin (Bac5 and Bac7), OaBac5α,β, PR-39, apidaecins, drosocin, pyrrhocoricin, and arasin 1, are rich in proline and arginine [14, 15, 26, 31]; hymenoptaecin which isolated from honeybees is rich in glycine [31]; coleoptericin from beetles is abundant in glycine and proline [31]; while prophenin, a peptide isolated from porcine leucocytes, reflected by its name, is abundant in proline and phenylalanine [15]. In contrast with the other AMPs which commonly rich in proline and arginine, indolicidin isolated from the bovine neutrophils, is a tryptophan-rich peptide, and exceptionally displays an extended structural conformation with the β-turns as its structural motifs [35]. The amino acids distribution of the AMPs is proportionally related and contributed to the charge of the peptides which is vital in enabling the peptides association with the bacterial membrane [35]. The forth group of AMPs comprises of those with anionic and cationic peptides which contain cysteine residues and form disulphide bonds and stabilized by β-sheets conformation as in brevinins, isolated from frog [31]. Protegrins, the peptide found in porcine leukocytes, is also categorized in this group and stabilized by two disulphide bonds in its structural conformation [14, 16]. Tachyplesins, a 17-18 amino acid long peptide isolated from the haemocytes of the Japanese horseshoe crab, *Tachypleus tridentatus*, possesses a β-hairpin motif and stabilized by two disulphide bonds [16]. Tachyplesins show potent inhibitory effect against a wide range of bacteria, fungi, and viruses [14]. β-defensin from avian and penaeidins, which purified from shrimp *Litopenaus vannamei*, consists of six cysteine residues and forms three pairs of disulphide bonds [20, 25]. Further, more than three disulphide bonds are displayed in drosomycin (peptide isolated from fruit flies *Drosophila melanogaster*) and mytilin (found in mussels *Ruditapes decussates* haemocytes). These peptides consist of eight cysteine residues and form four pairs of disulphide bonds [16, 27]. Drosomycin was found to have potent antifungal effect, while mytilin exerts broader inhibitory effect against bacteria, fungi and virus [27]. Finally, the fifth group of the AMPs are the anionic and cationic peptide fragments of a larger protein. For instance, lactoferricin from lactoferrin, are derivatives of human lysozyme, bovine α-lactalbumin, and avian eggs ovalbumin [31]. Glycoprotein lactoferrin which secreted in the human milk, has been well-known with its antimicrobial activity, but when the protein is digested by the acidic proteases such as pepsin found in the stomach, the products such as lactoferricin B are found to have enhanced inhibitory activity against bacteria and fungi [16]. Lysozyme, which commonly present in human secretory fluids and neutrophils, was found to possess inherent antimicrobial activities, and also releases antimicrobial fragments by proteolytic digestion [8, 36]. Bovine α-lactalbumin, the major milk proteins, constitutes potent antimicrobial peptides after undergoing protease digestion [36]. Whereas ovalbumin, the dominant protein in avian egg white, was reported to produce a number of antimicrobial peptides after chymotrypsin digestion, which are effective in inhibiting Gram-negative bacteria [37].

### 2.2. Antimicrobial mechanism of actions

Microbial cytoplasmic membrane which served as the protective barrier to prevent the extracellular localization of adhesins, invasions, or other virulence factors, always appeared as the first targeting action of antimicrobial peptides due to the amphipathicity and cationic-charged of the compounds in nature [16]. The phospholipids head group of the bacterial membrane are negatively charged, thus this feature contributes to the preferential activity of the cationic peptides against bacteria. The cationic (positively charged) residues in antimicrobial peptides interacting with the negatively charged phosphate moieties of the lipid head group, and further interrupting the integrity of the membrane by causing pore-formations and increase in permeability, and then the peptides may enter the inner region of the bacteria through membrane translocation [10, 38, 39]. There are several pathways proposed to describe the process of phospholipid membrane permeation by antimicrobial peptides which included "barrel-stave pore", "carpet mechanism", "toroidal pore" and "disordered toroidal pore" [40, 41]. Antimicrobial peptides which follow "barrel-stave pore" pathway of bacterial phospholipid membrane permeation tend to insert themselves perpendicularly to the membrane bilayer, subsequently causing pore formation. While for the "carpet mechanism", peptides adhere in parallel to the surface of membrane bilayer and disintegrate the membrane in such a way of the detergent-like effect. Similar as "barrel-stave pore" action, the peptides which practise the mechanism pattern of "toroidal pore" insert perpendicularly to the membrane bilayer, but then the peptides tend to induce the formation of a local membrane curvature where the pore lumen is lined partly by peptides and partly by phospholipid head groups of the membrane. Whereas the "disordered toroidal pore" pathway is almost the same as described for "toroidal pore" pathway, but involving a less-rigid peptide conformation within the bacterial membrane bilayer [40]. In fact, there is increasing evidence to indicate

that some of the AMPs can even adopting more than one mechanism in inhibiting the microbial cells apart from causing bacterial membrane rupture. Indeed, such a multiple cellular targeting of peptide is useful in attacking bacteria simultaneously by multiple mechanisms, which may help to prevent drug-resistant staphylococcal infections or antibiotic resistant bacteria on the long run [31, 42]. Table 2 includes some of the bacterial killing properties of AMPs isolated from animals and insects sources.

**Table 2** Antimicrobial mechanism of action(s) of some AMPs isolated from animals and insects

| Peptide | Antimicrobial mechanism of action(s) | References |
|---|---|---|
| SMAP 29 | Interferes outer membrane protein 1 | [8] |
| Cecropin | Transmembrane pore-forming (carpet model) | [15, 31] |
| Protegrins | Toroidal transmembrane pore-forming | [31] |
| Magainin | Toroidal transmembrane pore-forming | [31] |
| Bombilitins | Transmembrane pore-forming | [21] |
| Diptericin | Increases membrane permeability | [16] |
| Attacin | Inhibits synthesis of outer membrane proteins | [16] |
| Melittin | Haemolytic, transmembrane pore-forming (carpet and toroidal model) | [16, 31] |
| Apidaecins | Binding to bacterial heat shock protein, inhibit bacterial enzymatic activity | [16, 18] |
| Drosocin | Binding to bacterial heat shock protein, inhibit bacterial enzymatic activity | [18] |
| Pyrrhocoricin | Binding to bacterial heat shock protein, inhibits bacterial enzymatic activity | [18] |
| Tachyplesins | Nucleic acid binding | [8, 43] |
| Buforins | DNA binding | [8, 31] |
| Dermaseptin | Transmembrane pore-forming (carpet model), ion channel formation, inhibits synthesis of nuclei acid | [31, 38] |
| Bac7 | Inhibit macromolecular synthesis, membranolytic effect | [44] |
| Indolicidin | Induces septum formation, inhibits the synthesis of DNA and protein | [31, 43] |
| PR-39 | Induces septum formation, inhibit DNA and RNA synthesis | [43] |
| Protamine | Transmembrane pore-forming, inhibits bacterial enzymatic activity | [28] |

The amino acids distribution of AMPs contributes to the charge of AMPs which is essential in assisting the peptide to interact with the bacterial membrane and subsequently traverse the inner region to cause cell death [35]. For instance, cationic peptides, such as apidaecins, drosocin, pyrrhocoricin and PR-39 which rich in glycine and proline, are found to substantially interact with the negatively charged membrane, like lipopolysaccharide (LPS) and teichoic acid. Thus, allowing the peptides to penetrate deeper into the inner region and involve in intracellular destruction activities [35]. For apidaecin, its antimicrobial mode of action was demonstrated to bind to the bacterial heat shock protein, DnaK, preventing chaperone-assisted host protein folding and inhibiting the strongly related ATPase activity of DnaK [18]. Similar intracellular target also exerted in drosocin and pyrrhocoricin [18]. Peptide rich in a particular amino acid, for example, indolicidin which rich in tryptophan, was found highly membrane-active, and tends to inhibit bacterial cell division. The peptide induces septum formation in Enterobacteriaceae strains. With the hydrophobic character contributed by tryptophan, enables the peptide to penetrate easily into the intracellular region of the cell, and shows inhibition effect on protein and DNA synthesis of bacterial cells [38, 43]. Another remarkable example of peptide which appears to cause septum formation in bacteria as its mode of action is the PR-39. The peptide is rich in proline and arginine, alters bacterial cytoplasmic membrane septum formation and interferes with the nuclei acid and protein synthesis of the cells [43]. Buforin has attracted the attention of researchers due to their unique antimicrobial mode of action. This peptide easily binds to nuclei acids of bacteria without permeabilizing the membrane. This characteristic is owing to the histone H2A region that found in the sequence of the peptide [45]. The antimicrobial activity of AMPs sometimes is concentration dependent. The peptide Bac7, demonstrated two different mode of actions depending on its concentration that applied. With the concentration nearly minimum inhibitory concentration (MIC), the peptide tends to interrupt the macromolecular synthesis and the transport and energy metabolism of the bacteria. Whereas with the concentration ten-fold higher than the MIC, the peptide is likely to cause membranolytic effect on the bacteria [44]. Most AMPs act selectively against different microorganism and with nonspecific mechanism of actions [28]. Gram-negative bacteria are more resistant to AMPs compared to Gram-positive bacteria. Gram-negative bacteria reduce their susceptibility to antimicrobial peptides by obstructing peptide attachment to the outer membrane, reducing net negative

 

surface charges by altering the Lipid A moiety of the lipopolysaccharide (LPS) which is absent in Gram-positive bacteria [31, 43].

## 3. Antimicrobial peptides (AMPs) from plants and their mechanism of actions

When plants continuously exposed to environmental stresses such as temperature, humidity, and especially the intruding pathogens, the plants' innate immune system will develop numerous defence mechanism to produce the compounds such as phenols, alkaloid, secondary metabolites and AMPs in response to the stresses [46]. In this section, we attempt to discuss in-depth regarding the plant AMPs. Plant AMPs are commonly found in the roots, leaves and seeds. Those AMPs are defensins, thionins, knottin-like peptides, maize basic protein, lipid transfer proteins, hevein-type peptides, snakins, cyclotides and 2S albumins [46, 47]. Recently, AMPs are also found to be present in flower, such as defensin γ-thionins, basic lipid transfer protein and snakins. The possible mechanisms of action of the AMPs isolated from flowers involve lipid bilayer permeabilization, inducing ion-channel formation, and thus causing osmotic-imbalance and subsequently leading to cell death [46]. For instance, floral defensin which has antifungal activity, was reported to have electrostatic interaction with the fungal hyphal membrane, and further induces the uptake of $Ca^{2+}$ and efflux of $K^{+}$, which would greatly interfere the growth of the fungi [48].

Another AMPs isolated from potato, called as "potide-G", was demonstrated to exert antibacterial and antifungal effect. This may be resulted from the presence of small proteins such as the proteinase inhibitors which responsible in mediating defence mechanism against invading pathogens [49]. Potamin-1 (PT-1), a thermostable, trypsin-chymotrypsin protease inhibitor which isolated from the potato tubers (*Solanum tuberosum* L cv. Gogu), was found to possess potent antifungal activity as well. This peptide strongly inhibited human pathogenic fungal and plant pathogenic bacterial strains such as *Candida albicans*, *Rhizoctonia solani*, and *Clavibacter michiganense subsp. michiganinse*. Disulphide bridge(s) which found in the polypeptide chains of potamin-1 were found essential in contributing to the antimicrobial efficacy of the peptide [50]. This probably indicates the hydrophobicity characteristic of potamin-1 which contributed from the disulphide bonds enables the peptide to interact with the bacterial membrane as its antimicrobial mode of action [50]. Cereals, dry beans, oilseeds (including certain legumes), nuts, tree nuts, fruit and vegetable seeds, and other miscellaneous edible seeds are also valuable source of AMPs. Defensin and lipid transfer proteins (LTPs) in cowpeas could inhibit the early growth of several fungi and cause many hyphal morphological alterations [51]. There are four novel antimicrobial proteins were isolated from chickpeas namely cicerin, arietin, cicerarian, and cyclophilin-like protein. Cicerin shows potent antifungal activity against *Mycosphaerella arachidicola*, *Fusarium oxysporum*, and *Botrytis cinerea*. Meanwhile, cicerarian was reported to exhibit heat-stable antifungal activity against *Botrytis cinerea*, *Mycosphaerella arachidicola*, and *Physalospora piricola* [52]. Another antifungal peptide (*Pe*-AFP1) was discovered in passion fruit (*Passiflora edulis*) seeds. In vitro assays show that the peptide was able to inhibit the growth of filamentous fungi *Trichoderma harzianum*, *Fusarium oxysporum*, and *Aspergillus fumigatus* [53].

The specific low molecular weight proteins, such as hirudine and linusitin which found in flaxseed protein were reported to show antifungal activities as well [54]. Apart from that, cationic protein, glycinin which isolated from soy protein isolate was found to have inhibition effect against *Listeria monocytogenes*, *Bacillus subtilis* and *Salmonella enteritidis*. Hydrophobicity of the protein allows it to interact with bacterial cell membrane as proven with the signs of irregular wrinkled outer surface, fragmentation, adhesion and aggregation of damaged cells or cellular debris through observation by scanning electron microscopy [55]. Subsequently, sulphur-rich protein (γ-thionin like) in soybean, namely SE60, shows antimicrobial effect against *Pseudomonas syringae*, a plant pathogenic Gram-negative bacterium. This thionin-like antimicrobial protein was proposed to cause pore formation on the bacterial cell membrane. Previous studies showed that thionins' antimicrobial mechanism could relate to its toxicological effect which resulted from a detergent-like interaction with the biological membranes. The membrane disruption would eventually end up with cell leakage and leading to cell death [56]. AMPs derived from cereal, for instance, peptide from foxtail millet (*Setaria italic*) meal, after being fermented by *Lactobacillus paracasei* Fn032 showed fairly inhibition activity against *Escherichia coli* ATCC 8099. The net positively charged and hydrophobicity characteristics of the peptide are reported to involve in the exertion of its antimicrobial activity [57]. AMPs were also found in green coconut (*Cocos nucifera* L.) water. The peptides, named *Cn*-AMPs were extremely efficient in inhibiting the growth of Gram-positive and Gram-negative bacteria such as *B. subtilis*, *Staphylococcus aureus*, *E. coli* and *P. aeruginosa*. These peptides are rich in arginine and aromatic amino acid residues which indicating a possible membrane interaction by hydrophobic attraction [58]. Protein hydrolysates from palm kernel cake were found to effectively inhibit the growth of *B. cereus*. The hydrolysates were demonstrated to disrupt the bacterial membrane integrity, causing efflux of $K^{+}$, depleting ATP molecules and inhibit the synthesis of RNA [59]. Encouraging finding indicates that an antiviral peptide was successfully isolated from the seeds of *Sorghum bicolor* L. The peptide is relatively small in size, with only 2000 Da; showed strong inhibition in the replication of herpes simplex virus type 1 (HSV-1). The virucidal activity of the peptide may be caused by the disintegration of the HSV particles and the virus envelope; or degradation, masking of some of the essential envelope proteins [19]. In fact, understanding the mechanism or mode of actions of every single antimicrobial compound that has been developed is of important in order to prevent bacterial resistance in the future and to ensure safety usage of the antimicrobial agent in a long run.

## 4. Non-proteinaceous antimicrobial compounds and their mechanism of action

### 4.1. Non-proteinaceous antimicrobial compounds from animals and insects

Humans from the ancient times tend to utilize wild animals or insects in the preparation of their traditional medicines for therapeutic purposes as they believed those wild animals and insects comprise of essential ingredients for the preparation of drugs. For instance, lava lizard (*Tropidurus hispidus*) was used to treat injuries caused by boil and sore throat, tonsillitis and pharyngitis. *Ameiva ameiva*, another species of lizard was used in the preparation of traditional medicines for treating inflammation, dermatitis, venereal diseases, and snake bites [60]. In fact, these traditional medicines have been proven for their antimicrobial efficacy and pharmacological usage. Methanolic extract of the skin of lava lizard (*T. hispidus*) and *Ameiva ameiva* showed synergistic effect in inhibiting *S. aureus* growth when combined with gentamicin and against *E. coli* and *S. aureus* when combined with amikacin and gentamycin, respectively. However, no significant inhibition of bacterial growth was observed at clinically relevant concentrations when the extract was used alone. The presence of alkaloids was accounted for the antimicrobial action of the extracts [60]. Subsequently, secondary metabolites from marine gastropod egg capsule, egg masses, for instance, kabiramide C, aplysianin E, thisaplysianin E and tyrian purple demonstrated potent antifungal and antibacterial efficacy. Kabiramide C, the lipophilic extract of egg masses from nudibranch showed considerable antifungal activity. While aplysianin E, a component found in the eggs of sea hare (*Aplysia kurodai*) exerts bacteriostatic activity against Gram-positive and Gram-negative bacteria. The compound was found to suppress the synthesis of RNA and DNA of the bacteria. On the other hand, thisaplysianin E, which also a component found in sea hare, showed inhibitory effect against *E. coli* and *S. aureus* with minimum inhibitory concentration (MIC) as low as 0.4 pg/ml and 0.13 pg/ml respectively. Tyrian purple, natural dye extracted from gastropod mollusks, exhibited inhibition effect on marine pathogens, as well as *Candida albicans* [61]. Also a substance derived from marine, squalamine, an antimicrobial steroid from shark, showed broad spectrum of inhibition against bacteria, fungi and protozoa [62, 63].

Fatty acids have been reported for their antimicrobial efficacy several decades ago especially those unsaturated fatty acids and medium chain fatty acids. Free lipid content of extract from the spawn of molluscs which dominantly comprises of palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1) was proven to possess inhibitory effect against marine pathogens such as *Lactococcus garvieae*, *Vibrio harveyi*, *V. anguillarium* and *V. alginocolyticus* [64]. Next, crocodile (*Crocodylus niloticus*) oil which traditionally used as treatment for ailments such as skin rashes, skin ulcers and cancer, and can promoting wound healing, was justified by scientists to exert antifungal and antibacterial effect against *Candida albicans*, *S. aureus* and *Klebsiella pneumoniae*. Oleic, palmitic and linoleic acid (18:2) were identified as the major components in the crocodile oil [65]. Medium chain fatty acid, lauric acid and its liposomal derivatives were evaluated and proven to give strong bactericidal activity against *Propionibacterium acnes*. Lauric acid and its liposomal derivatives were found to interact with *P. acnes* through fusion, but not aggregation or adsorption. The compounds fused with the bacterial membrane and released active ingredients into the membrane [66]. The effectiveness of lauric acid in inhibiting the bacterial growth especially Gram-positive bacteria was again, demonstrated in Enterococcae. Short chain fatty acid, caprylic acid (8:0), was a potent antimicrobial against coliforms (Gram-negative) [67].

The hygroscopic secretion produced by the secretory setae of terrestrial larvae of the biting midge *Forcipomyia nigra* which found abundant in oleic, palmitic, palmitoleic (16:1) and linoleic acid, is effective in inhibiting Gram positive such as *Bacillus cereus*, *B. subtilis*, *Enterococcus faecalis* and Gram-negative bacteria such as *Citrobacter freundii* and *Pseudomonas aeruginosa*. The secretion also demonstrated certain fungistatic activity against entomopathogenic fungi [68]. Apart from that, cuticular and internal extracts obtained from *Calliphora vomitoria* larvae, pupae, adult males and females which rich in saturated and unsaturated fatty acids revealed strong inhibitory effect against Gram-positive bacteria but not Gram-negative bacteria. This is likely due to the outer membrane that present in Gram-negative bacteria which act as a protective barrier to the bacteria [69].

### 4.2. Non-proteinaceous antimicrobial compounds from plants

There is an arsenal of antimicrobial compounds being manifested in plant besides AMPs. Plant extracts, phytochemicals and secondary metabolites such as essential oil, alkaloids, naphtha-, terpenoids, saponins and flavonoids could in fact serve as antimicrobial agents [70]. First of all, crude coconut fat hydrolysate which mainly consists of 70% lauric acid was found to confer enhanced antimicrobial activity by solid state cultivation using *Yarrowia lipolytica*. The fat hydrolysate demonstrated high antimicrobial potential against Gram-positive (*B. cereus* and *Listeria monocytogenes*) and Gram-negative (*E. coli* and *Salmonella enteritidis*) bacteria [71]. Ethyl acetate extract of *Thymelaea hirsuta* which found to contain phenolic compounds such as p-hydroxybenzoic, p-coumaric, ferulic and caffeic acids, exhibited potent antibacterial and antifungal activity. The extract tends to interfere with the cellular metabolism such as the $H^+$-ATPase pump [72]. Volatile components from *Aurinia sinuata* (L.) which mainly were glucosinolate degradation products originating from glucoberteroin, glucobrassicanapin and glucoalyssin showed broad spectrum of inhibition against Gram-positive, Gram-negative bacteria and fungi [73]. Next, liquorize (root and rhizome of *Glycyrrhiza* spp.) which has been used for centuries as the medicine for the relief of rheumatic and healing of ulcers,

 

comprises of glycyrrhizin (triterpene glycoside), flavonoids, isoflavonoids, chalcones and coumarins, is effective in inhibiting the growth of Gram-positive bacteria, yeast and fungi. The compounds tend to interfere with the energy metabolism and macromolecular biosynthesis of the bacteria [74]. Besides, the extract from cranberry, which was found to contain anthocyanins and phenolic compounds, is having potent antimicrobial activity against Gram-positive and Gram-negative bacteria such as *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa*, and *S*. Typhimurium. Anthocyanins and phenolic compounds are demonstrated to destabilize the bacterial cytoplasm membrane, permeabilize the membrane, inhibit the extracellular microbial enzymes, and interfere with the energy metabolism. In fact, the antimicrobial effect of polyphenols is concentration-dependent [13]. Phenols tend to affect the enzyme activity which associated with energy production at low concentration. While at greater concentrations, phenols could actually cause the denaturation of the protein. Besides, phenols or polyphenols could alter the permeability of the bacterial membrane and subsequently allow the loss of macromolecules from the intracellular region [75].

Subsequently, ultrasound-assisted extraction of the leaves of *Cyclocarya paliurus* showed moderate inhibition effect against both Gram-positive and Gram-negative bacteria. The extract was found to contain polysaccharide component [76]. Another polysaccharide compound which was extracted from red seaweed (*Gracilaria corticata*) exhibited potent antimicrobial activity as well against Gram-positive and Gram-negative bacteria and fungi [77]. The antimicrobial mechanism of action of polysaccharide or sugar probably could refer to the action of sugar residue in antimicrobial glycoprotein. The sugar residue tends to increase the affinity for ligands of the antimicrobial agent and induce dimerization which would result in the enhancement of antimicrobial efficacy [78]. On the other hand, dioscin (steroidal saponin) which extracted from the root bark of wild yam *Dioscorea nipponica*, was proven to possess antifungal activity. The compound disrupts the membrane and leads to morphological changes of the fungi. The compound was then invading into the fungal membrane and subsequently resulting in cell death [79].

Next, the plant volatiles or plant essential oils (EOs) are plant secondary metabolites, aromatic oily liquid obtained from plant materials such as flowers, buds, seeds, leaves, barks, roots and etc. Steam distillation is the common method used to produce EOs in bulk. Besides being used as perfumes, flavourings and natural preservatives in food, EOs was demonstrated to possess antibacterial activity and it was first reported in the year 1881 [80]. The biological efficacy of EOs probably is due to the presence of the phenolic and terpene components in their compounds. Table 3 shows some examples of essential oil components, their plant source and their targeted bacterial or fungi species. In fact, the composition of EOs from a certain species of plant can be affected by the harvesting seasons and geographical factor.

**Table 3** Minimum inhibitory concentrations (MIC) of essential oils against bacteria and fungi.

| Plant source | EOs component | Bacteria/fungi species | MIC | References |
|---|---|---|---|---|
| Angelica root, Juniper berry, nutmeg | α-Pinene | *S*. Typhimurium | 8 – 16[a] | [80] |
| | | *Salmonella enteritidis* | 8[a] | [80] |
| Oregano, savory | Carvacrol | *S*. Typhimurium | 0.225 – 0.25[a] | [80] |
| | | *L. monocytogenes* | 0.375 – 5[a] | [81] |
| | | *E. coli* | 0.5 – 1.2[a] | [81] |
| | | *S. aureus* | 0.5 – 1.2[a] | [81] |
| Thyme | Thymol | *S*. Typhimurium | 0.056[a] | [80] |
| | | *E. coli* | 0.5 – 1.2[a] | [81] |
| | | *S. aureus* | 0.5 – 1.2[a] | [81] |
| Clove (bud) | Eugenol | *S*. Typhimurium | 0.0125% | [80] |
| | | *E. coli* | 0.4 – 2.5[a] | [81] |
| | | *S. aureus* | 0.4 – 2.5[a] | [81] |
| | | *L. monocytogenes* | 0.3[a] | [81] |
| Coriander, cilantro, rosewood, lavandin | Linalool | *Candida* strains | 0.05 – 0.4% | [82] |
| Lemongrass | Citral | *S*. Typhimurium | 0.5[a] | [80] |
| | | *E. coli* | 0.6[a] | [81] |
| | | *S. aureus* | 0.6[a] | [81] |
| | | *L. monocytogenes* | 0.5[a] | [81] |
| Cinnamon | Cinnamaldehyde | *Salmonella* sp. | 500[b] | [80] |
| Turmeric (*Curcuma aeruginosa*, *Curcuma mangga*) | Turmeric oil | *B. cereus* | 11.1[a] | [83] |
| | | *S. aureus* | 1.2[a] | [83] |
| | | *C. albicans* | 3.7[a] | [83] |
| | | *C. neoformans* | 0.1[a] | [83] |
| Caraway seed, dill | Carvone | *S*. Typhimurium | 10 mM | [80] |

| | | | | |
|---|---|---|---|---|
| Sage, bergamot | β-Pinene | *E. coli* | 3.5 – 5[a] | [81] |
| | | *S.* Typhimurium | 10 – 20[a] | [81] |
| | | *S. aureus* | 0.75 – 10[a] | [81] |
| | | *L. monocytogenes* | 0.2[a] | [81] |
| Zinger | Camphene, β-Bisabolene, Ar-Curcumene | *S.* Typhimurium | >2[a] | [80] |

[a] Values in µl/ml
[b] Values in µg/ml

As shown in Table 3, essential oil components such as carvacrol, thymol and eugenol are effective in the bacterial growth inhibition. These EO components are showing great inhibition against mold as well such as *Aspergillus flavus*. The combined effect of the phenol components and EOs in the particular plant source appeared as the important natural antimicrobial agents. Hydrophobic characteristic of EOs eases the compound to interact with the hydrophobic region of cell membrane. The compound may further react and inactivates the vital enzymes and interferes with the normal function of the gene materials in the cell [75]. Besides, citral from lemongrass was reported to exhibit potent antifungal effect as well [84]. Previous study showed that citral might be feasible to be involved in the treatment of *Helicobacter pylori* infections as it exhibited strong anti-*H. pylori* effect [84]. Exciting finding also shows that citral component from *Melissa officinalis* L. is able to inhibit the replication of Herpes Simplex Virus type-2 (HSV-2) [85]. Other EOs component, cinnamaldehyde was demonstrated to have bactericidal effect against *E. coli* O157:H7 as the compound inhibited the membrane-bound ATPase activity in *E. coli*. Previous studies Yossa et al [86] proved that cinnamaldehyde had excellent antitermite, antibacterial, antimite, antimildew, antimosquito and antipathogenic activities. The mechanism of action of coriander oil (linolool as a major EOs component) which is effective in inhibiting fungus, was reported to associate with interfering membrane integrity and membrane potential of the fungal cells and consequently causing leakage of intracellular components [82]. Linolool, which also found in lavandin oil, exert good inhibition effect against Gram-positive and Gram-negative bacteria such *S. aureus*, *B. cereus* and *E. coli*. The compound altered bacterial membrane fluidity and tends to increase the permeability of the membrane [87]. Another EOs component, β-pinene, not only showing growth inhibition activity against bacteria, but they are also active against yeasts and molds as well due to the presence of compounds bridge bicyclic terpenes [88]. Generally, the antimicrobial mechanism of actions of essential oils are associated with the disturbance of cytoplasmic membrane, disrupting proton motive force, electron flow, active transport and eventually interfere with the biosynthesis of the intracellular components due to their naturally lipophilic and hydrophobic characteristics [81].

## 5. Preparation of antimicrobial compounds

Solid-liquid extraction is the common method used for the separation of antimicrobial compounds in plants or animals. This section will emphasize on the extraction and purification of AMPs. Many types of solvent are adopted in extracting the antimicrobial compounds, depends on the solubility of the solute (sources that containing antimicrobial compounds) in the solvent [22]. Table 4 shows the extraction and purification strategy of some AMPs. In fact, the extraction and purification steps adopted are highly dependent on the amount of the active ingredients available. Normally extraction is always followed by the steps of centrifugation or ultrafiltration in order to remove unwanted impurities and larger proteins as AMPs are relatively small in size. Subsequently, for the preparative purification method, size exclusion chromatography (SEC, or also called as gel filtration chromatography), solid phase extraction (SPE) and ion exchange chromatography (IEC) are commonly used. Reversed-phase high performance liquid chromatography (RP-HPLC) is the final purification method that most researchers would practise. The partially purified peptides would be fractionated based on the differences in hydrophobicity using RP-HPLC [22]. As shown in Table 4, the solvent used to extract antimicrobial peptide from mussel haemocytes, horseshoe crab and shrimp is an acidic solution. Before purification step is carried out, neutralization of the extracted peptide solution should be undertaken. Hence, this will lead to the formation of salt in the solution. High salt content would probably contribute to the false positive result during the antimicrobial screening analysis. Moreover, high salt content may cause difficulties during analytical separation steps using HPLC and characterization analysis using mass spectrometry. Thus, ultrafiltration or SPE is suggested for effective desalting purpose [22]. Dialysis is also an alternative way to remove salt and used as the initial purification step as shown in the extraction of AMPs from shrimp (Table 4). Besides, antimicrobial compounds could also be prepared from fermented food such as the foxtail millet meal [57]. Water-soluble antimicrobial peptide solution was successfully prepared from the fermented foxtail millet meal. The extracts were subjected to centrifugation and the resulting supernatant was collected and proceeds with freeze-drying before antimicrobial screening analysis is carried out.

 

**Table 4** Extraction and purification strategies of animals and plants AMPs.

| AMPs (source) | Extraction and Purification Strategy* | References |
|---|---|---|
| Mytilins (mussel haemocytes) | 1) Extraction: acetic acid<br>2) SPE (C18)<br>3) RP-HPLC<br>4) SEC<br>5) RP-HPLC | [22] |
| Tachyplesins (horseshoe crab haemocytes) | 1) Extraction: 30% acetic acid or 20 mM HCl<br>2) SEC<br>3) IEC (cationic) or RP-HPLC or SEC | [22] |
| Arasin, crustins (sea hare body wall, spider crab haemocytes) | 1) Extraction: 60% acetonitrile + 0.1% trifluroacetic acid (TFA)<br>2) Ultrafiltration or SPE (C18)<br>3) RP-HPLC | [22] |
| Penaeidins (shrimp) | 1) Extraction: 5% or 2 M acetic acid<br>2) SPE (C18) or SEC<br>3) RP-HPLC | [22] |
| Bactenecin-like (shore crab haemocytes) | 1) SPE (C18)<br>2) SEC<br>3) RP-HPLC | [22] |
| Peptide from *Sorghum bicolor* | 1) Extraction: 50% ethanol + 3.3% TFA<br>2) SEC<br>3) IEC<br>4) RP-HPLC | [19] |
| *Cn*-AMPs (green coconut water) | 1) Dialysis<br>2) RP-HPLC | [58] |
| Potamin-1 (potato tubers) | 1) Extraction: distilled water<br>2) Dialysis<br>3) RP-HPLC | [50] |
| Peptide from foxtail millet meal | 1) Solid-state fermentation<br>2) Extraction: water<br>3) RP-HPLC | [57] |

* SPE=solid phase extraction, SEC=size exclusion chromatography, RP-HPLC=reversed-phase high performance liquid chromatography, IEC= ion exchange chromatography

## 6. Conclusions

"Natural scaffolds contain the key to bacterial vulnerability". This statement is exactly reflecting the important role which played by natural products, either from animal or plant sources in combating with the rapid emergence of incidents of antibiotic resistance and the occurrence of infectious diseases. After looking insight into how antimicrobial peptides (AMPs) and non-proteinaceous antimicrobial compounds targeting the bacterial membrane by increasing the permeability of the membranes, disrupting the structure of the membrane and then translocate into the inner region of the cell and causing further destruction to the key intracellular genetic materials, and eventually leading to the cell death, a continuous effort in finding a better antimicrobial or antibiotic is vital and should not be decelerated as there is still a possibility for the occurrence of AMPs resistance. In fact, besides putting emphasis on the biological effects of the natural products, factors such as the in-vivo stability of such compounds, their production cost and the side effects on human health that may be generated should also be considered. Anti-quorum sensing activity is an advanced research area that focused on controlling the pathogenicity of the bacteria by inhibiting the intracellular communication to occur between the cells. In fact, this anti-quorum sensing compound could be found in some medicinal plants and the seaweeds from southern Florida. For instance, the red alga, *Delisea pulchra* was reported to contain anti-quorum sensing compounds [89]. The advantage of interrupting the quorum sensing or cell to cell communication among bacteria is that, the virulence factors of the bacteria would be greatly reduced or affected. Thus, future work should pay more attention in this anti-quorum sensing activity as an effort to overcome the alarming increase of the incidents of antibiotic resistance.

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