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REVIEW ARTICLE

A Review on Antibacterial Phytochemical Constitutions Present in *Aerva lanata* and their Mode of Action Against Bacterial Biofilm

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ABSTRACT

Antibacterial phytochemicals have unexplored chemical structures with high therapeutic potential, additionally; phytochemicals have several advantages, including green status, different mechanisms of action from antibiotics which could help to overcome the chemotherapeutic agent resistance problem and also ability to inhibit the growth of planktonic cell and biofilm. These phytochemicals are unmatched structural diversity, and it also has no target specific. In this study, an overview of the main classes of antibacterial phytochemicals present in *Aerva lanata* and their mode of action against bacterial biofilm is presented. A revision about the bacterial biofilm characteristics, biofilm formation, mechanism involved against antimicrobial agents, phytochemicals properties, and their targets to eradicate biofilm, anti-biofilm properties of various phytochemicals found in *A. lanata* is also done. The phytochemicals such as polyphenolics interfere with the adhesion potential, quorum sensing (QS) controlled, swarming motility and biofilm formation of *Escherichia coli,* and *Pseudomonas aeruginosa*. Catechin and tannic acid also present in *A. lanata* were able to promote a significant reduction in biofilm formation by *P. aeruginosa,* and it able to block biofilm formation by *E. coli* and *Pseudomonas putida.* Antibacterial phytochemicals isolated from the different plant part of *A. lanata* inhibited and reduced cell-surface adhesion, methicillin-resistant bacterial biofilm formation, inhibit bacterial motility, QS, and controls biofilms of *E. coli, P. aeruginosa,* and *Staphylo*coccus *aureus*. Phenolic acids increased the susceptibility of dual species biofilms. Peptides react against bacterial biofilm by the process of cell membrane permeabilization, intracellular targets, inhibiting nucleic acids and protein synthesis, and cell wall adhesion of Gram-negative and Gram-positive bacteria.

Keywords: *Aerva lanata,* anti-biofilm activity, antibacterial phytochemical.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds.[1] Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern.[2] The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens.[3]

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases.[4] Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.[5] The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants, for their potential antimicrobial activity.[6,7]

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Secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.[8] Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections.[9]

Advantage of using medicinal plants is that they do not causes any side effects when compared with synthetic drugs, because medicinal plants have high content of antioxidant compounds. This gives protective effects against diseases without reducing their therapeutic efficacy. Nowadays, herbal drugs have become world important objects, with both medicinal and economic implications.

BACTERIAL BIOFILM DEFINITION AND CHARACTERISTICS

Biofilms are not easily defined as they vary greatly in structure and composition from one environmental niche to another. Microbial biofilms are extremely complex microbial ecosystems consisting of microorganisms attached to a surface and embedded in an organic polymer matrix of microbial origin. As well as microbial components, non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, may also be found in the biofilm matrix. Biofilms, particularly in water systems, can be highly complex, while others such as those on medical devices, may be simpler, and composed of single, coccoid, or rod-shaped organisms.[10] Given these differences, it does not seem plausible to suggest that a true "biofilm model" can be defined that is applicable to every ecological, industrial and medical situation. Therefore, the definition of a biofilm has to be kept general and thus may be redefined as "microbial cells immobilized in a matrix of extracellular polymers acting as an independent functioning ecosystem, homeostatically regulated."[11]

Surface polysaccharides also are used by bacteria as a means of adherence. In many natural settings, bacteria from multilayer communities called biofilms. Biofilms usually contain more than one species of bacteria. The first layer of the biofilm builds to a pili or some other attachment mechanism, then succeeding layers adheren to the first layer, using a polysaccharide slime to cement them together. Bacterial biofilm causes a variety of problems, the national institute of health estimated that over 80% of microbial infections that occur in the human body involve biofilms. The most common diseases associated with biofilm formation are such as urinary tract infection (UTI) (*Escherichia coli* and *Klebsiella pneumonia*), burn wound (*Acinetobacter baumannii* and *Staphylococcus aureus*), dental plaque (*Streptococcus mutans*), respiratory infection (*Bordetella pertussis*), otitis media (*Haemophilus influenza*), and cystic fibrosis lung infection (*Pseudomonas aeruginosa*) are almost impossible to eliminate with antibiotics, necessitating the surgical removal of the implants. Bacteria in a biofilm are probably less susceptible to antibiotics than free-swimming (planktonic) bacteria because the antibiotic does not diffuse readily through the polysaccharide layer.[12]

HISTORICAL PERSPECTIVES OF BIOFILM IN NATURE

In the 17th century, a dry-goods merchant named Antonie van Leeuwenhoek first observed "animalcules" swarming on living and dead matter. Leeuwenhoek's curiosity and inventiveness were remarkable; he discovered these "animalcules" in the tartar on his own teeth and even after meticulous cleansing, the remaining opaque deposits isolated between his teeth were still "as thick as if it were batter." These deposits contained a mat of various forms of "animalcules" that we now know where the bacteria of dental plaque. It is reasonable to suggest that this early study of dental plaque was the first documented evidence of the existence of microbial biofilms. Today, we, generally, define such biofilms as microbial communities adhered to a substratum and encased within an extracellular polymeric substance (EPS) produced by the microbial cells themselves. After van Leuwenhoek's early work, it was not until 1940 that the so-called "bottle effect" in marine microorganisms was first observed.[13] This showed that the growth of bacteria was substantially increased when they were attached to a surface. Further advancements in our knowledge of biofilms were made by Zobell in 1943 when he noted that bacteria on surfaces were greater in number compared with the surrounding seawater. From his studies, Zobell also postulated that the adhesion of bacteria consisted of a two-

stage process of reversible and then irreversible adhesion.

Despite the above studies being the first documented ones on biofilms, the extensive physical and chemical analysis of bacterial biofilms did not begin until the late 1960s and early 1970s, when a few investigators recognized the prevalence of bacterial biofilms[14-16] used scanning and transmission electron microscopy to examine biofilms on trickling filters in a wastewater treatment plant.[14] From this work, it was shown that biofilms were composed of a variety of different microorganisms and revealed that the matrix material or EPS was primarily composed of polysaccharides. The investigation of biofilms around this time was greatly aided by the use of electron microscopy, which provided information, not only on biofilm structure but also on the presence of EPS. In 1973, Characklis who investigated microbial slimes in industrial water revealed that biofilms were both tenacious and highly resistant to the antimicrobial effects of chlorine.[15] The true analysis of biofilms was not recognized until 1978. Many bacteria spent the majority of their existence within surface-attached, sessile communities. Work on dental plaque and sessile communities in mountain streams enabled[16] to hypothesis the mechanisms by which microorganism's adhered to living and non-living materials and derived benefit from this ecologic niche.

Since the 1970s, the study of biofilms in industrial, ecological, and medical settings has followed similar paths. Initial biofilm studies, generally, concentrated on composition, especially of the polymer matrix or "glycocalyx" that was thought to conserve and concentrate the digestive enzymes released by the bacteria, thus increasing the metabolic efficiency of the cells. Research[17] indicated that this glycocalyx also acted as anionic exchange matrix, trapping nutrients that were then transported into cells by highly efficient permeases. In 1981,[18] glycocalyx was characterized as a hydrated polyanionic polysaccharide matrix produced by polymerases affixed to the lipopolysaccharide component of the bacterial cell wall. In aqueous environments, biofilm production of glycocalyx is prevalent with organic and inorganic nutrients being concentrated at the solid/liquid interface. In addition, the glycocalyx provides a physical/chemical barrier that offers partial protection against antibacterial agents.

Since biofilms form under diverse conditions, and may be composed of single or multiple species, the structures of various biofilms will necessarily have distinct features. Nevertheless, biophysical, structural, and chemical studies have led to a useful basic concept of a "biofilm model."[19] In this model, microorganisms form microcolonies surrounded by copious amounts of exopolysaccharide. Between the microcolonies are water-filled channels, and it has been proposed that these promote the influx of nutrients and the efflux of waste products.

STAGES IN THE FORMATION OF BIOFILMS

The process of biofilm formation is complex, but generally recognized as consisting of five stages:[20]

1. Development of a surface conditioning film
2. Movement of microorganisms into close proximity with the surface
3. Adhesion (reversible and irreversible adhesion of microbes to the conditioned surface)
4. Growth and division of the organisms with the colonization of the surface, microcolony formation, and biofilm formation; phenotype and genotype changes
5. Biofilm cell detachment/dispersal.

EPS AND THE GLYCOCALYX

EPS and glycocalyx are terms used to describe the polysaccharide produced by bacterial cells. EPS refers to one of the major components of biofilms, and glycocalyx refers to the polysaccharide matrix surrounding individual cells. EPS has an important role in biofilm structure and function and has a complex physical and chemical nature. Its functions are mostly protective in nature and this is one of the benefits for bacteria in the sessile state. Because the glycocalyx is the outermost component of bacterial cells, this layer mediates virtually all bacterial associations with surfaces and other cells: It dictates location, juxtaposition, and the eventual success in the ecosystem.[16] The physical properties of the biofilm are largely determined by the EPS, while the physiological properties

are determined by the bacterial cells. There are majorly eight processes in the development of biofilms.[21] These can be condensed to three main processes: The attachment of cells to a surface (colonization), growth of the attached cells into a mature biofilm, and the detachment of single cells (erosion) or large pieces (sloughing).

BIOFILM STRUCTURE

Biofilm structure is the spatial arrangement of bacteria, cell clusters, EPS, and particulates. Since the structure can influence transport resistance, it is a significant determinant in the activity of the biofilm. Various conceptual and mathematical models have been proposed to describe the structure and function of biofilms.[21-23] Mathematical models describing transport, conversion, cell growth, and biofilm development are based on conceptual models. Biofilms and mats are matrices of cells and extracellular polymers (EPS). The EPS is produced by the cells and consists of polysaccharides, polyuronic acids, proteins, nucleic acids, and lipids.[24-26]

MECHANISMS OF ANTIMICROBIAL RESISTANCE

The bacteria enclosed within the biofilm are extremely resistant to antibiotic treatments. Such resistance can be explained by different hypotheses, not necessarily limited to the following ones. First, the EPS secreted by biofilm bacteria, acts as a physical/chemical barrier, thus preventing penetration by antibodies or many antibiotics.[19] Second, embedded biofilm bacteria are generally not actively engaged in cell division and are smaller in size and less permeable to antibiotics. Third, the antibiotic degrading enzyme β-lactamase produced by several bacterial species can inhibit the activity of β-lactam ring structured antibiotics such as penicillins and cephamycins. β-lactamase may also be immobilized and accumulate in the EPS matrix so that the incoming antibiotic molecules can be inactivated effectively.[27] Fourth, up to 40% of the cell-wall protein composition of bacteria in biofilms is altered from that of its planktonic brethren. The membranes of biofilm bacteria might be better equipped to pump out antibiotics before they can cause damage, or even antibiotics targets may disappear. Fifth, the antimicrobial agent is deactivated with the help of antimicrobial oxidants such as hypochlorite and H_2O_2. It is, however, known that the biofilm matrix does not form a completely impenetrable barrier to antimicrobial agents.[28]

There are six different characteristic mechanisms of bacterial resistance to antimicrobials.[29] They are (1) modification of the target site; (2) acquisition of alternative metabolic pathways to those inhibited by the drug; (3) alteration of permeability of the bacterial cell wall/membrane that restrict antibacterial agent access to target sites; (4) enzymatic modification or degradation of the antimicrobial agent; (5) over-expression of the drug target; and (6) active efflux pumps that extrude the antibiotic from the cell.

TARGETS INVOLVED TO ERADICATE BIOFILM

Biofilm formation is regulated by combination of several mechanisms that are intrinsically related, such as adhesion, EPS synthesis, bacterial motility, and cell-to-cell interaction or QS.[30,31] Therefore, these cellular processes can be possible targets for the discovery of new drugs. This led to an increased interest in the search of natural products that have been proven to be able to restrict the capability of bacteria to adhere, communicate, and form complex biofilms.[32]

Phytochemicals may represent a natural antimicrobial strategy with considerable impact not only against free-living bacteria (planktonic) but also on bacterial biofilm formation.[33] Nevertheless, studies on biofilm prevention and control with phytochemicals are scarce. Diverse researchers already identified new strategies for biofilm control.[30,33,34] The use of phytochemicals in biofilm prevention and control is a relevant strategy. There are evidences that phytochemicals can interfere with diverse biofilm formation processes[35] such as inhibition of adhesion, degradation of EPS, interference with EPS production, motility inhibition adhesion, and QS inhibition. Other possible targets also possible, e.g., inhibition of cell viability of biofilms and reduction of metabolic activity of biofilm cells, Inhibition of biofilm formation, interference with virulence factors production, inhibition of nucleic acid synthesis, etc., are summarized in Table 1.

Table 1: Phytochemical targets involved to eradicate biofilm

S.no	Targets	Reference[s]
1	Microbial membrane associated with –OH group	[36,37]
2	Inhibition of adhesion	[38,39]
3	Motility inhibition (swimming and swarming), inhibition of EPS production	[40-42]
4	Antiquorum sensing or QSI	[43-49]
5	Inhibition of nucleic acid synthesis	[50]
6	Inhibition of enzyme involved in the radical generation, Destabilize, and permeabilize the cytoplasmic membrane	[50], [51-54]
7	Increase the membrane fluidity and permeability, disturb the membrane-embedded proteins, inhibit respiration and alter ion transport processes in both Gram-positive and Gram-negative bacteria	[55-57], [57-60]
8	Bind to sulfhydryl groups of external proteins of cell membrane	[61-63]
9	Disrupt the cell membrane; inhibit the nucleic acids and protein synthesis	[64-67]
10	Inhibits antibiotic efflux pumps	[68]
11	Intercalate with DNA, RNA polymerase, DNA gyrase, and topoisomerase IV	[69,70]
12	Attenuate the level of penicillin-binding protein	[71]
13	Inhibition of cell viability of biofilms	[72]
14	Interference with QS and inhibition of biofilm formation. Decrease of EPS production, reduction of metabolic activity of biofilm cells	[73-79]
15	Interference with virulence factors production	[80,81]
16	Reduction of viable bacterial cells counts of multispecies biofilms	[82]
17	Degradation of cell wall, disruption of cytoplasmatic membrane, damage of membrane proteins, leakage of intracellular contents, coagulation of cytoplasm, and depletion of proton	[36,37,51,83,84]
18	Interference with motility, adhesion, biofilm formation, and QSI	[85-87]
19	Inhibition of enzymes β-lactamases	[88]

QSI: QS inhibition, EPS: Extracellular polymeric substance

IMPORTANCE AND PROPERTIES OF ANTIBACTERIAL PHYTOCHEMICALS

Phytochemicals have demonstrated distinctive properties.[33] Unlike synthetic molecules, phytochemical products display an unmatched structural diversity with complex and novel multilayer mechanisms of action. The synthetic chemotherapeutic agent are react against microbial growth in a specific manner (single target) such as some molecules breakdown the bacterial cell wall, some molecules alternation in membrane function etc, so these all are target specific. All the chemotherapeutic agent, targets and their functions are controlled by specific genes present in the pathogenic organism. Some currently used antibiotics act also through multiple modes of action (multiple molecular targets and/or targets encoded by multiple genes.[89] Therefore, compounds that inhibit bacterial growth by different mechanisms than the presently used by conventional antibiotics can provide an interesting approach to control drug-resistant infections. Moreover, contrarily to the previously considered strategy"one drug, one target, one disease," it is now extensively recognized that the use of a single molecule able to operate simultaneously in various targets is more advantageous for the treatment of complex infectious diseases.[90] In fact, there are no evidences on the emergence of resistance to phytochemicals.

The antibacterial mechanism of action of phytochemicals is not completely understood.[33] Hence, more studies are needed to know their exact antimicrobial targets. Degradation of the cell wall, disruption of cytoplasmatic membrane, damage of membrane proteins, leakage of intracellular contents, coagulation of cytoplasm, and depletion of proton have been currently reported as the mechanisms responsible for cell death, caused by some of these compounds[36,37,51,83,84] Useful phytochemicals with antimicrobial activity can be divided into several classes that include: Phenolics and polyphenolics, terpenoids, and other essential oils constituents, alkaloids, lectins, peptides, and polyacetylenes among much other.[91,92]

Phytoconstituents employed by plants to protect them against pathogenic insects, bacteria, fungi, or protozoa have found applications in human medicine.[93] Some phytochemicals such as phenolic acids act essentially by helping in the reduction of particular adherence of organisms to the cells lining the bladder, and the teeth, which ultimately lowers the incidence of UTI and the

usual dental caries. Plants can also exert either bacteriostatic or bactericidal activity of microbes.

AERVA LANATA PLANT DESCRIPTION

A. lanata belongs to the family Amaranthaceae. This family consists of about 169 genera and 2300 species. *A. lanata* (L) Juss Ex. Schult commonly called Polphala of Amaranthaceae is a perennial shrub which is seen commonly in different waste parts of India, Eritrea and another part of the world. Most of the plants of this family are herbs, erect/with climbing branches. Leaves are opposite or alternate, ex-stipulate. Flowers are usually hermaphrodite, small usually in terminal simple or paniculate spikes, cymes or cluster; bracts hyaline never leafy, bracteoles. Fruits are membranous utricle, irregularly rupturing capsule, rarely berry. Seeds inverted or erect, orbicular or kidney shaped.[94] *A. lanata* grows wild on the mountain slopes, fields, and bare patches of ground up to an altitude, 900 m in the hills, and a native of Asia, Africa, and Australia.[94-97]

PHYTOCHEMICAL CONSTITUTIONS IN *A. LANATA*

Phytochemical studies have been carried out by several workers with the report of different kinds of bioactive compounds such as alkaloids, flavonoids, oxalate, phytic acid, phytin phosphorus, phenolic compounds, phytosterols, carbohydrates, proteins, amino acids, quinines, anthraquinones, catechins, coumarins, phenols, quinones, saponins, steroids, glycosides, tannins, and xanthoproteins.[98,99] The phytochemical constituents present in the plant include alkaloids (ervine, methylervine, ervoside, aervine, methylaervine, aervoside, ervolanine, and aervolanine) and flavonoids (kaempferol, quercetin, isorhamnetin, persinol, and persinosides A and B).[100] The whole plant of *A. lanata* contains β-sitosterol, α-amyrin, betulin, hentriacontane, sitosteryl palmitate, D-glucoside, glycosides, kaempferol-3-galactoside and kaempferol-3- rhamnoglucoside, starch, and free: Sugars (fructose, galactose, rhamnose, and sucrose).[95,101]

The antinutrient levels also revealed the presence of tannic acid, saponin, alkaloids, flavonoids, and oxalate. The phytic acid and phytin phosphorus were also in low amount.[102-105] *A. lanata* also contains miscellaneous phytoconstituents such as methyl grevillate, lupeol, lupeol acetate benzoic acid, β-sitosteryl acetate, and tannic acid.[102] Different studies revealed the presence of 30 different types of steroids, 21 different types of saponins, 27 varieties of terpenoids, and 24 types of tannins in the methanolic extract of root, stem, leaves, and seeds of *A. lanata*[106-108] using high-performance thin-layer chromatography. Fourier-transform infrared (FT-IR) spectral analysis[109] of plant parts such as flowers, leaves, stem, and roots of *A. lanata* showed the presence of characteristic functional groups of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, alcohols, carboxylic acids, esters, aliphatic amines, and alkyl halides compounds derivatives which were responsible for medicinal properties of this plants.

The presence of different kinds of bioactive compounds particularly alkaloids such as canthin-6-one and beta-carboline, aervine (10-hydroxycanthin-6-one), methylaervine (10-methoxycanthin-6-one), aervoside (10-β-Dglucopyranosyloxycanthin-6-one), and aervolanine (6-methyoxy-β-carbolin-1-yl) propionic acid from leaves of *A. lanata*.[110-116] The alkaloids, phenolic compounds, phytosterols, carbohydrates, proteins, amino acids, flavonoids, and quinones were identified in different solvents extracts.[117]

A new type diterpene was identified[118] in methanol extract of dried seeds of the *A. lanata*. Qualitative phytochemical analysis[119] of the methanol and ethanol extracts prepared from *A. lanata* whole plant revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, sugar, glycosides, tannins, and xanthoproteins. The FT-IR spectrum confirmed the presence of alkyl group, methyl group, alcohol group, ethers, esters, carboxylic acid, and anhydrides. Phytochemical analysis carried out for leaf extracts of the plant showed the presence of several secondary metabolites. The methanol extract of leaf showed the highest phenolic and flavonoid content.[120] The isolation of above-mentioned bioactive compounds from the different research and review report concluded the whole plant of *A. lanata* would be useful to find out the novel drugs.

ANTIBACTERIAL PHYTOCHEMICALS PRESENT IN *A. LANATA* AND THEIR MODE OF ACTION AGAINST BACTERIAL GROWTH

Natural products from various plants have a potential control microbial growth in diverse situations and the specific case of disease treatment, and numerous studies have aimed to describe the chemical composition of different plant antimicrobials and the mechanisms involved in microbial growth inhibition.

Phytochemicals are non-proteinaceous secondary metabolites or non-nutritive bioactive compounds as protective agents against external pathogenic stress.[121] Some of the phytochemicals interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents[122]. Phytochemicals induce membrane permeabilization by forming pores or disrupting membrane integrity and induced lysis of cell wall. In general, phytochemicals have diverse antimicrobial mechanisms including damaging cell wall and cytoplasmic membrane as shown in Table 1.

Antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study. It is, therefore, important that scientific protocols be clearly identified and adequately followed and reported. The different phytochemical constitutions present in *A. lanata* and their mode of antibacterial actions will be presented below.

Alkaloids

Numerous plant families are known to produce alkaloids and have been reported that several of them possess high antimicrobial activity and could, therefore, be a good alternative for actual drugs.[37] Number of reports revealed extracts from different parts of *A. lanata* containing alkaloids[98-105,110-117,119] showed antimicrobial activity against different multidrug-resistant bacteria, namely, *E. coli, P. aeruginosa, P. mirabilis, S. aureus, B. subtilis, Raoultella planticola, Enterobacter aerogenes, Agrobacterium tumefaciens*, and *K. pneumonia.*[123] Their mechanism of action can be attributed to their ability to increase membrane permeability and to intercalate with DNA. RNA polymerase, DNA gyrase, and topoisomerase IV are also possible targets.[69,70,124]

Anthraquinones

These are derivatives of phenolic and glycosidic compound phytochemical present in *A. lanata*.[119] They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols.[124,125]

Catechin

Catechins are one of the subclasses/components of flavonoids present in different plants including *A. lanata*[119] have been identified as potent antimicrobial agents and were suggested as a therapeutic possibility.[126] It forms complexes with the bacterial cell wall of intestinal microorganisms.[127]

Flavonoids

Flavonoids are one of the biggest classes of secondary metabolites found in various types of edible plants including *A. lanata*.[98-100,102-105,117,119,120] They have been identified as potent antimicrobial agents and were suggested as a therapeutic possibility.[126] Their activity is arguably due to the ability to form a complex with extracellular proteins, which then binds to the bacterial cell wall, increasing their permeability of the inner membrane of *E. coli* and also dissipation of membrane potential.[128] Moreover, their targets are the membranes with −OH groups.[36,37] Interference with metabolism and inhibition of nucleic acid synthesis was also reported as possible mechanisms of action.[50] Quinones also found in *A. lanata,*[124] it is a flavonoids derivative, the mechanism of bioactivity also similar as

flavonoids. Some of the flavonoid subclasses (quercetin and robinetin) found in various types of edible plants. This flavonoid can inhibit DNA gyrase, inactivate specific bacterial enzymes, inhibit antibiotic efflux in methicillin-resistant *S. aureus* (MRSA),[68] and inhibit the synthesis of nucleic acids of both Gram-negative and Gram-positive bacteria.[50] There are no evidences of these flavonoid subclasses found in *A. lanata*. More work is needed to be done to establish this assumption.

Phenols

Phenolic compounds constitute one of the most diverse groups of phytochemicals, being widely distributed in plants and protecting them from microbial infections. They have antioxidant properties but are also potent anti-infective.[37,129] Phytochemical studies have been carried out by several workers with the report various solvent extract of *A. lanata* contain phenol.[109,117,119,120,124] The antimicrobial activity of plant phenols has been extensively studied against human pathogens, to characterize and develop new healthy food ingredients, medical compounds, and pharmaceuticals.[37,129] The phenolic derivatives differ in the patterns of the hydroxylations and methoxylations of their aromatic rings. The common hydroxycinnamic acids are ferulic acid and vanillic acid found in *A. lanata*. Their antimicrobial activity can be due to their ability to destabilize and permeabilize the cytoplasmatic membrane, inhibition of enzymes involved in the radical generation and also the inhibition of the synthesis of nucleic acids of bacteria.[50-54] Antibacterial activity was also obtained ferulic acids against lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus hammesii,* and *Listeria monocytogenes*), *E. coli, P. aeruginosa,* and *S. aureus* (including MRSA).[31,130-134] Moreover, it was observed synergistic effects between these compounds and the antibiotic streptomycin.[134]

Steroids and glycosides

Plant steroids (or steroid glycosides) also referred to as "cardiac glycosides" are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs.[126] The phytochemical also reported in *A. lanata*[95,98,99,101,106-108,117,119,136-138] with antibacterial actions have been reported as anti-inflammatory or cytotoxic activity against different harmful bacteria.[135]

Tannins

Tannins are found in almost every plant part including *A. lanata*[107,109,139] nutritional and biological properties of tannins have been described.[140] In addition, antibacterial actions of tannins have been reported as bacteriostatic and bactericidal against different harmful bacteria, including *E. coli, Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., and *Streptococcus* spp.[141-143] Their mode of action is apparently related to their ability to inactivate microbial adhesins, enzymes, membrane proteins, and formation of complexes with cell wall. Furthermore, they can form complex with polysaccharide, which is suggested to be the main reason for their inhibitory effects on bacteria.[144,145]

Terpenoids

Terpenoids are the largest group of natural compounds reported in *A. lanata*.[119,120,124,136,137,146,147] These bioactive products have a lot of biological properties, including antioxidant and antimicrobial activities. Due to their recognized antimicrobial potential, terpenoids have been the subject of several studies along the years.[148] The mechanism of action of terpenoids is not fully understood, but it is speculated that involves membrane disruption by the lipophilic compounds and their activity depend largely of the structure of the compound, as recently demonstrated by some authors.[55-57] This antibacterial action can result in the increase of membrane fluidity/permeability, disruption of membrane-embedded proteins, and change of ion transport processes in both Gram-positive and Gram-negative bacteria.[55-57] The triterpenes include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal, or cytotoxic activity.[135]

Coumarin

Few researchers reported coumarin is phytochemical compound present in *A. lanata*.[98,99,119] It is a phenolic compound with fused benzene and pyrone groups[51,36,83] had broad-spectrum antibacterial activities against

S. aureus, E. coli, S. typhimurium, Salmonella enteritidis, A. hydrophila, Yersinia sp., *Shigella* sp., and *Vibrio parahaemolyticus*.[37]

Amino acids and peptides

Short-length peptides (between 15 and 30 amino acids) with microbicidal activity are commonly named as antimicrobial peptides.[65] These biologically active molecules are an important component of the innate immune system of wide variety of organisms.[149,150] They comprise several protein groups with different features, as regard to the total charge of the molecule and the content of disulfide bonds.[66] Peptides with antimicrobial properties are present in all organs of a variety of plant species including *A. lanata*[117] constitutively or in response to microbial infections.[151-153] Antimicrobial peptides are effective against a wide range of microorganisms, namely, Gram-negative and Gram-positive bacteria, including multidrug-resistant strains,[67,154] Their mechanism of action is believed be the damage or destabilization of the microbial cell membranes by formation of ion channels, transmembrane pores, or extensive membrane rupture.[64,65]

PHYTOCHEMICALS PRESENT IN *A. LANATA* AND THEIR MODE OF ACTION AGAINST BACTERIAL BIOFILM

Antibiotic resistance is a significant public health problem that is worsened when microorganisms are in biofilms.[155-157] The main weapons used to control harmful biofilms have been the antimicrobial products; nonetheless, there are no antimicrobials with ensured efficacy.[34] Natural products that have been proven to be able to restrict the capability of bacteria to adhere, communicate, and form complex biofilms.[32] There are evidences that phytochemicals can interfere with diverse biofilm formation processes.[30,33] Biofilm formation is regulated mainly by adhesion, EPS synthesis, bacterial motility, and QS.[30,84] Not all of the phytochemicals present in *A. lanata* involved to control the biofilm formation and growth. However, very few of the phytochemicals and their derivatives present in *A. lanata* have anti-biofilm properties. The phytochemicals present in *A. lanata* have an anti-biofilm properties are discussed below. The anti-biofilm phytochemicals are not target specific because the phytochemicals are multi target compounds and it interfere or inhibit the biofilm formation and growth in different mechanism compare to synthetic drug.

Polyphenolics demonstrated the ability to interfere with the adhesion potential of *Streptococcus*.[158-160] Polyphenol, ellagic acid, and glycosylated derivatives inhibited biofilm formation of *S. aureus*.[161] Pure ellagic acid also displayed anti-biofilm properties against *S. aureus* and *E. coli*.[161,162] Polyphenol, catechin, and tannic acid were able to promote a significant reduction in biofilm formation by *P. aeruginosa*.[163] Polyphenol inhibited QS controlled, swarming motility and biofilm formation of *E. coli* K-12 and *P. aeruginosa* PAO1 in a concentration-dependent manner.[164] Bioactive fraction isolated from leaves of *A. lanata* contains alkaloids, tannins, saponins, steroids, glycosides, and flavonoids inhibited MRSA biofilm formation. Anti-biofilm activities of phytochemicals reduce cell-surface adhesion and attenuate the level of penicillin-binding protein. Ferulic acids present in *A. lanata*[50-54] to inhibit bacterial motility, adhesion and to prevent and control biofilms of *E. coli, P. aeruginosa, S. aureus,* and *L. monocytogenes*. Catechin and tannic acid also present in *A. lanata*[165] were able to block N-acyl homoserine lactones synthesis[73] and biofilm formation[74] of *E. coli* and *Pseudomonas putida*. Moreover, (-)-epigallocatechin gallate present in *A. lanata* inhibited biofilm formation of *Staphylococcus* spp. by reduction of EPS production.[75] Ferulic acid is a phenolic derivatives hydroxycinnamic acid phytochemical present in *A. lanata* were able to inhibit biofilm formation of *S. aureus*.[166] Ferulic inhibit swimming motility and QS[167] of *Bacillus cereus* and *Pseudomonas fluorescens*. In addition, the development of biofilms in the presence of these phenolic acids increased the susceptibility of dual-species biofilms (*B. cereus* and *P. fluorescens*) to a second exposure to the chemicals. Several researchers reported *A. lanata* contain phenolic acids it increased the susceptibility of dual-species biofilms.[168] The antimicrobial peptides present in *A. lanata* react against bacterial biofilm by the process of cell membrane permeabilization, antimicrobial peptides can also act on intracellular targets, inhibiting nucleic acids and protein synthesis, and enzymatic activity.[64,67] These peptides have the ability to inhibit biofilm growth by competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors has also been observed.[51] Due to their cationic and hydrophobic features,

antibacterial phytochemicals interact primarily with negatively charged components of the bacterial envelope, such as lipopolysaccharides of the outer membrane of Gram-negative bacteria or lipoteichoic acids present on the cell wall of both Gram-negative and Gram-positive bacteria.[64,65,151,169]

CONCLUSION

Most of the synthetic and microbial-derived chemotherapeutic (Antibiotics) agents are failed to control the growth of the microbial life involved in the biofilm. Bacterial members involved to form biofilm are multidrug-resistant microbes; it causes a variety of problems including human diseases such as a dental plaque and urinary tract infection. The bacterial biofilm has variety of special mechanism to neutralize, tolerate and escape the antimicrobials. The biochemical features are also completely differing from planktonic cell. Therefore, new compounds are required to eradicate the biofilm. Plant secondary metabolites (phytochemicals) play a key role in plants defense and have evolve to inhibit the growth of biofilm bacteria and planktonic cell; these are simply known as antibacterial phytochemicals. Compare to the antimicrobials antibacterial phytochemicals are structurally differ, and their mode of action and targets also completely differ from chemotherapeutic agents. The *A. lanata* is widely used the herb. The pharmacological studies conform and support the therapeutic utility of the plant in various disorders mainly in diseases of the urinary system. These plants contain numerous phytochemicals among that few have an anti-biofilm activity. However, more work should be needed to isolate, identified and purified the main classes/subclasses of anti-biofilm phytochemicals present in *A. lanata.*

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