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Review

Acylated and non-acylated anthocyanins as antibacterial and antibiofilm agents

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Abstract
Natural products have served as an essential source of medicinal compounds in drug discovery, with their high abundance in nature and structural complexity being beneficial for various biological activities. Anthocyanins are a natural food colourant that belongs to the flavonoid group of compounds responsible for the colour of various fruits, vegetables, and flowers. There has been a growing interest in these compounds, especially for their health benefits. Antimicrobial resistance is on the rise, making the prognosis for bacterial infection treatment rather difficult. The discovery of alternative agents and treatment approaches is needed. Many in vitro and some in vivo studies demonstrated the potential effects of anthocyanins or their fraction from various natural sources to prevent and treat bacterial infections and biofilm formation. This review reports the recent literature and focuses on the potential role of anthocyanins and their acylation or functional groups for antibacterial and antibiofilm activities and their use as potential antibiotic substitutes or adjuvants. Their possible mechanism of action and prospects of their uses are also discussed.

Keywords Acylated anthocyanins · Antibiotics · Antimicrobial resistance · Biofilm · Flavonoids · Quorum sensing

1 Introduction

Antibiotics have been used to treat bacterial diseases since the early twentieth century [1]. Most infectious diseases were brought under control through the discovery of many classes of antibiotics. However, the increase in the usage of antibiotics with unnecessary and inappropriate prescriptions in clinical practice soon led to the emergence of antibiotic resistance within a few years, limiting the effectiveness of current drugs and significantly causing treatment failure of infections [2, 3]. Apart from that, the use of antibiotics to prevent infectious diseases and promote animal growth in food and animal industries have also contributed to antibiotic resistance [2]. More than 70% of pathogenic bacteria were resistant to at least one of the currently used antibiotics [4, 5]. The World Health Organisation (WHO) published a list of pathogens in 2017 requiring urgent development of new antimicrobials known as ESKAPE, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. Most antibiotics have a limited life due to pathogen variants with intrinsic or acquired resistance mechanisms. Microbial cells have developed intrinsic resistance towards antibiotics through various mechanisms such as inactivation of drugs via hydrolysis (e.g., via β-lactamase) or modification (e.g., aminoglycoside resistance); alteration of drug targets within cells making them unrecognisable to the drug (e.g., by mutating DNA gyrase in fluoroquinolone

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resistance) or bypassing the drug target; the use of permeation barriers, preventing access of drugs to the target (e.g., the Gram-negative outer membrane); and active efflux of drugs out of the cell via membrane-bound efflux transporters [6, 7]. Apart from intrinsic resistance, microbial communities are able to develop tolerance toward environmental stress through the formation of biofilms which enhances their resistance to antibiotics [8]. Resistance of biofilms to antimicrobial agents is contributed by several mechanisms and differs from those contributed by planktonic bacteria. Some of these resistance mechanisms can contribute together, resulting in the increase of resistance of biofilms [9]. The rapid emergence of antibiotic-resistant bacteria has caused an increase in financial burden as second or third-line drugs are more expensive, and this phenomenon may also soon lead to the shortage of available antibiotics [10, 11].

In regard to this challenge, there is a need to develop alternative approaches in addition to searching for new antimicrobial compounds [2]. Various strategies are being developed and have been reported to inhibit or disrupt biofilms, such as quorum sensing inhibitors, bacteriophages, enzymes, surfactants, nanoparticles, antimicrobial photodynamic therapy, ethnopharmacology, and diguanylate cyclase inhibitors [12].

Lately, considerable attention has been given to the use of isolated natural extracts for their therapeutic and prophylactic action in the treatment and prevention of several diseases and as a promising source of antimicrobial agents due to their eminent levels of biocompatibility and low levels of toxicity [13]. Many studies have reported on the various health benefits and bioactivities of anthocyanins, and there has been an increase in new findings on their therapeutic potential. Previous reviews have generally focused on the various bioactivities of different sources of anthocyanins including those which have not been fractionated or purified to enhance the anthocyanin content [14–16]. This review focuses on the potential and prospects of anthocyanins or their fractions from various sources that have been purified for their antibacterial and antibiofilm properties. Based on the major anthocyanin in the anthocyanin fractions, the acylation type of anthocyanins or functional groups (solely on carbohydrate moieties) was further discussed for its antibacterial and antibiofilm potential. This review also looks at the possibilities of using anthocyanins as potential substitutes or adjuvants for antibiotics to treat bacterial infections, of those involving biofilms and their mechanisms.

2 Biofilm formation and treatment challenges

Biofilm is a community of bacterial cells (single or multiple bacterial species) attached to either a living or non-living surface enclosed in a complex exopolymeric substance (EPS). Biofilm has a slime-like appearance, and it is known to occur in natural, industrial and hospital settings. It is composed mainly of polysaccharides, proteins, nucleic acids, lipids, extracellular DNA and water [17, 18]. Biofilms cause many health problems such as endocarditis, chronic wounds, otitis media, urinary tract infections and periodontitis [19]. Biofilms serve as a physiological barrier for bacterial cells, which involves the transition of the planktonic cells encountering environmental stress signals to attach to a surface to facilitate the formation of biofilm. Upon maturation of biofilm, the sessile bacterial cells are dispersed from the biofilm in order to spread and colonise new surfaces [20].

Bacterial cells enclosed in biofilms have a greater ability to withstand unfavourable environmental conditions (e.g. antibiotics, heat shock, changes in pH, oxygen and nutrient limitation), enabling their survival [20, 21]. Bacterial biofilms form tolerance over time to enable their survival through several mechanisms, such as the ability of biofilms to reduce the diffusion of antibiotics, formation of metabolic activity gradient in which bacterial cells in the inner layer of biofilms have a slower growth rate due to limitations to oxygen and nutrient, ability of persister bacteria to withstand antibiotic treatment to re-establish infection as well as conventional antibiotic resistance in biofilms facilitates bacterial biofilms to resist antibiotics, disinfectants, and innate and adaptive immune defence system of the host [17, 22, 23]. Among all microbial and chronic infections, 65% and 80%, respectively, are associated with biofilm formation, according to the National Institutes of Health [18].

Clinically biofilm-related infections require aggressive treatment with a combination of antibiotics which is challenging as infections may recur or persist with adequate treatment. It is due to its inability to eradicate biofilms [23, 24] and if it is device-related, it requires the removal and replacement of the device, requiring surgery, increases in cost, risks and complications [23]. Regarding the rise of failures in combating infectious diseases, there has been a realisation of the importance of developing antibiofilm drugs. Microbial cells in biofilms can produce compounds that enable their shift from biofilm to a planktonic mode of life. It is essential for the dispersal of biofilms that allow bacterial populations to colonise new habitats [25]. For instance, the bacterium *Bacillus subtilis* produces D-amino acids which help in the dispersal of biofilm formation. This particular property has been exploited in developing antibiofilm drugs via the identification and characterisation of such chemical cues [26]. Apart from that, cell signalling, such as quorum sensing, is essential in

forming and maintaining biofilms. Identifying drugs or molecules with structural similarity to quorum-sensing signals is another potential approach to prevent biofilm formation [27].

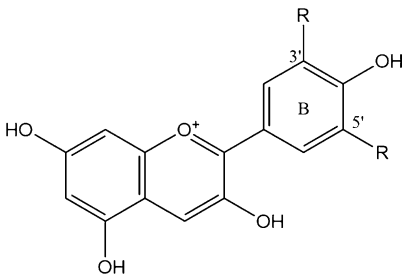
In order to treat bacterial infections effectively, it is important that the drugs which are used are able to prevent biofilm formation and dispersal as well as affect the growth of individual cells. This is important as to prevent the re-establishment of biofilms by existing planktonic cells. Therefore, a combination therapy applying antibiofilm drugs in conjunction with traditional antibiotics that target cell growth would probably be a better alternative to control biofilm-related infectious diseases. In such combination therapy, the antibiofilm drugs will reduce its resistance in combating biofilm formation and facilitate the targeting of pathogens at the cellular level by traditional antibiotics [1].

3 Anthocyanins

Many current antibiotics are derived from natural products that provide diverse and complex structures with densely packed functional groups [28, 29]. Plants are rich in a variety of secondary metabolites, such as flavonoids, tannins, alkaloids and terpenoids, which have been shown to have antimicrobial properties in vitro [30, 31]. Furthermore, polyphenols in fruits and vegetables, such as flavonoids and tannins, have shown promising antimicrobial activity [32, 33].

Anthocyanins are classified under the flavonoid group of polyphenol compounds, which gives rise to the red, blue and purple colours of plants [34] in which more than 700 types of anthocyanin derivatives of aglycones known as anthocyanidins are known to occur in nature. The six major types of anthocyanidins commonly present in nature are malvidin, pelargonidin, cyanidin, delphinidin, peonidin and petunidin (Table 1). These anthocyanins are usually either acylated or non-acylated and are commonly attached to one or more sugar moieties, making them glycosides [35]. The most common sugars attached to the anthocyanins are glucose, galactose, rhamnose, arabinose and xylose, while common acylating agents are cinnamic, ferulic and sinapic acids. Anthocyanins have high solubility in water and have higher stability at low pH. Studies have shown acylated anthocyanins to have higher stability against factors such as high temperature, changes in pH, UV radiation, light exposure, processing and storage [36]. Current knowledge on the sources and stabilities of acylated and non-acylated anthocyanins has been previously covered in a review [37]. Anthocyanins are being used as natural colourants in the food industry as an alternative food colourant to synthetic colourants due

Table 1 Chemical structures of anthocyanidins



Name	Substitution	
	$R_{3'}$	$R_{5'}$
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3
Pelargonidin	H	H

to safety concerns of their use in food products. The use of anthocyanins as food colourants is gaining interest also due to their multiple health benefits.

Various studies have shown anthocyanins to have a wide range of biological activities such as antimicrobial, antioxidant, cardiovascular protection and anticancer [38–41]. Apart from that, studies have also shown anthocyanins and their metabolites to exert a positive modulation on gut bacterial growth [42], anti-inflammatory properties [43], inhibition of platelet aggregation [44], control diabetes [45], chemoprotective [46], and radiation-protective agents [47]. Several methods, such as disc diffusion, agar dilution, broth microdilution and macrodilution, are suitable and commonly used for in vitro antimicrobial susceptibility testing, following the guidelines that are internationally accepted procedures like Clinical and Laboratory Standards Institute (CLSI). The following sections discuss the antibacterial and antibiofilm potential of anthocyanins from various sources and examine their possible mode of action in exerting the observed activity.

3.1 Antibacterial activity of anthocyanins

In search of alternatives for treating bacterial infections, anthocyanins have been researched for their potential in numerous studies owing to their various health benefits. Studies have shown bilberry and blueberry extracts to inhibit the growth of bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeuruginosa* while blackcurrant extract was shown to inhibit the growth of *S. aureus* and *Enterococcus faecium* strains and having only mild effects on *E. coli*. These studies showed that microbial strains have different susceptibilities to various berry extracts [48, 49]. Anthocyanins have been shown to exert their antibacterial activity through various mechanisms such as inducing damage to bacterial cell damage, destroying the structural integrity of the wall, membrane, and intracellular matrix, inhibiting extracellular microbial enzymes, antibiofilm activity as well as affecting microbial metabolism and growth [48, 50, 51].

The antibacterial properties displayed by anthocyanins from various sources are shown in Table 2 and Fig. 1. The anthocyanin-rich extracts of four different varieties of blueberries (Snowchaser, Star, Stella Blue and Cristina Blue) were found to have potent antibacterial activity against Gram-positive and Gram-negative bacterial strains from patients with urinary tract infections (UTIs) and standard strains from American Type Culture Collection (ATCC). The minimum inhibitory concentration (MIC) values ranged from 0.4 to 9.52 mg/mL while the minimum bactericidal concentration (MBC) value ranged from 1.03 to 9.52 mg/mL. The anthocyanin compounds were identified in the anthocyanin-rich extracts and were composed mainly of cyanidin, delphinidin, peonidin, petunidin and malvidin derivatives. The highest effect was against *Pseudomonas aeruginosa* (MIC value = 0.4–0.85 mg/mL) with a potent effect against other uropathogenic strains such as *Klebsiella pneumoniae*, *Providencia stuartii* and *Micrococcus* spp. strains isolated from UTIs [52]. The anthocyanin extract of strawberries was found to have activity against *Staphylococcus aureus* associated with bovine mastitis, a common disease affecting dairy cattle worldwide, contributing to great economic losses in the dairy industry. The anthocyanin extract was found to have at least 40% or higher growth inhibition at 100 µg/disc in disc diffusion assay [53]. The anthocyanin extract of dark purple-fleshed potato (major pigments = malvidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside and petunidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside) showed potent antibacterial activity against the standard and clinically isolated bacterial strains. Gram-positive bacteria were most prone to the action of the extract with MIC values of 15.6–31.3 µg/mL compared to the Gram-negative bacterial strains (MIC value 31.3–250 µg/mL) [54]. A similar pattern was also obtained in a disc diffusion assay of the anthocyanin fraction of *Thymus kotschyanus* against Gram-positive and Gram-negative bacterial strains [55]. However, the anthocyanin extract of cranberry displayed an equipotent effect against both Gram-positive and Gram-negative bacterial strains apart from *Listeria monocytogenes*, which had the least susceptibility to the extract [56].

Several studies have reported cranberries as a natural remedy to treat UTIs by preventing bacterial attachment to surfaces, impairing bacterial motility, and interfering with quorum sensing [57, 58]. The anthocyanin fraction of cranberry was reported for potential antibacterial activity against *E. coli* with MIC and MBC value of 14.8 mg/L [50]. It was found to cause disintegration of the outer member of *E. coli* with cytoplasm leakage. Another study reported the proanthocyanidins of cranberry (at a concentration that does not affect bacterial growth) prevented the resistance to various antibiotics in Gram-negative bacterial strains (*P. aeruginosa*, *E. coli*, *Proteus. mirabilis*) [58]. The combination treatment of proanthocyanins with antibiotics was found to have potentiated the effect of the antibiotics requiring up to less than 98% of antibiotics than treatment without the proanthocyanidins. It was also found to have synergistic activity by reducing the MIC value up to 64-fold against *Proteus. mirabilis*. The ability of the proanthocyanidins at concentrations that did not affect the bacterial growth against tested bacterial strains is unlikely to lead to resistance. Interestingly, the co-administration of proanthocyanidins with tetracycline prevented the evolution of resistance in *E. coli* and *P. aeruginosa*.

Table 2 Antibacterial activity of anthocyanins from various sources towards Gram-positive and Gram-negative bacterial strains

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibacterial activity	Possible mechanism of action	References
Nonacylated anthocyanins	Dark roselle petals (*Hibiscus sabdariffa*)	Anthocyanin fraction	Disc diffusion assay Gram-positive *S. aureus* (1.62 cm), *B. subtilis* (3.93 cm) Gram-negative *E. coli* (4.83 cm), *Salmonella* sp. (4.98 cm)	–	[77]
	Eggplant peels (*Solanum melongena*)	Anthocyanin fraction	Disc diffusion assay Gram-positive *S. aureus* (3.55 cm), *B. subtilis* (2.13 mm) Gram-negative *E. coli* (3.03 cm), *Salmonella* sp. (3.05 cm)	–	[77]
	Cranberry (*Vaccinium macrocarpon*)	Anthocyanin fraction	Gram-negative *E. coli* MIC and MBC value (14.8 mg/L)	Disintegration of bacterial outer membrane	[50]
	Cranberry (*Vaccinium macrocarpon*)	Anthocyanin extract	Broth microdilution assay Gram-positive (MIC value 62.12–124.3 µg phenol/well) *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecium* resistant to vancomycin Gram-negative (MIC value 31.07–62.12 µg phenol/well) *E. coli*, *P. aeruginosa*, *Salmonella typhimurium*	–	[56]
	Sweet cherry (*Prunus avium*)	Cyanidin-3-glucoside	Disc diffusion assay Gram-negative = *E. coli* (16 mm inhibition zone)	–	[78]
	Commercial anthocyanin	Cyanidin 3-*O*-glucoside	Gram-negative *Helicobacter pylori*	At 100 µM (unaffected bacterial numbers) inhibited secretion of VacA and CagA toxins through suppression of secA transcription	[65]
	Blackberries (*Rubus eubatus* cv. "Hull")	Anthocyanin-enriched fraction	Gram-negative *Fusobacterium nucleatum*	At 27.8 µg/mL, 70% reduction of metabolic activity at 24 h	[79]
	Black soybean (*Glycine max*)	Anthocyanin extract [Cyanidin-3-glucoside (72%), delphinidin-3-glucoside (20%) and petunidin-3-glucoside (6%)]	Gram-negative *Helicobacter pylori*	At 50 µg/mL inhibited H. pylori-induced ROS, MAPKs, NF-kβ, COX-2, iNOS, and IL-8 cytokines in human gastric epithelial cells	[66]

Table 2 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibacterial activity	Possible mechanism of action	References
	Lowbush wild blueberries (*Vaccinium angustifolium*)	Anthocyanin fraction	Gram-negative *E. coli* > 5-log CFU/mL reduction at 0.65 g/L GAE	Increased membrane permeability damage	[80]
	Sour cherry (*Prunus cerasus*)	Anthocyanin-rich extract	Disc diffusion assay Gram-positive *S. aureus* (15.0 – 27.0 mm) Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* (14.0–32.0 mm)	–	[81]
	Blackcurrant (*Ribes nigrum*)	Anthocyanin-rich extract	Disc diffusion assay Gram-positive *S. aureus* (10.3 – 11.3 mm) Gram-negative *Escherichia coli* (9.3 – 10.7 mm)	–	[81]
	Lingonberry (*Vaccinium vitis-idaea*)	Anthocyanin and proanthocyanidin fraction	Broth microdilution assay Gram-positive *S. mutans* (MIC 125 µg/mL) Gram-negative *Fusobacterium nucleatum* (MIC 250 µg/mL)	–	[82]
	Thymus kotschyanus	Anthocyanin fraction	Disc diffusion assay Gram-positive *S. aureus* (11.5 mm); *B. subtilis* (10.5 mm) Gram-negative *E. coli* (13.0 mm); *P. aeruginosa* (12.5 mm)	–	[55]
	Black plum (*Syzygium cumini*)	Anthocyanin-enriched extract	Gram-negative *Klebsiella pneumonia*	At 1 mg/mL, 80% inhibition of violacein production (anti-QS activity), synergistic antibacterial activity with conventional antibiotics	[63]
	Commercial anthocyanin	Malvidin	Gram-negative *Klebsiella pneumonia*	At 20 µg/mL, highest ligand binding to LasR receptor protein in molecular docking analysis, 80% inhibition of violacein production (anti-QS activity)	[63]

Table 2 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibacterial activity	Possible mechanism of action	References
	Chilean berry "murta" (*Ugni molinae*)	Anthocyanin fraction (Cyanidin-3-glucoside, pelargonidin-3-arabinose and delphinidin-3-glucoside	Gram-negative *E. coli*, *S. typhi* - Showed inhibitory activity against tested strains in disc diffusion assay, comparable to standard antibiotic agents	–	[83]
	Blueberry wine pomace	Anthocyanin extract	Broth microdilution assay Gram-positive *S. aureus* (MIC 5 mg/mL) Gram-negative *Escherichia coli* (MIC 10 mg/mL), *Salmonella* (MIC 20 mg/mL)	–	[84]
	Commercial anthocyanin	Cyanidin	Gram-negative *Klebsiella pneumonia*	High ligand binding to LasR receptor protein in molecular docking analysis, inhibited violacein production (anti-QS activity) with 74% inhibition at 80 µg/mL, synergistic antibacterial activity with conventional antibiotics	[64]
	Blueberries (*Vaccinium corymbosum*)	Anthocyanin-rich extract	Broth microdilution assay Gram-positive *S. aureus* (MIC 500 µg/mL)	–	[85]
	Red wine by-product	Anthocyanin extract [malvidin-3-(6-*O*-pcoumaroyl-glucoside and malvidin-3-*O*-glucoside, malvidin-3-(6″-acetyl-glucoside)]	Disc diffusion assay Gram-positive *S. aureus*, *B. cereus*, *B. subtilis* (4.08 – 5.08 mm) Gram-negative *E. coli* (5.01 mm)	–	[86]
	Black chokeberry (*Aronia melanocarpa*)	Anthocyanin fraction (Cyanidin 3-galactoside, cyanidin 3-xyloside, cyanidin 3-glucoside, cyanidin 3-arabinoside)	Broth microdilution assay Gram-positive (MIC 2.5–10 mg/mL) *S. aureus*, *E. faecium*, *E. faecalis* Gram-negative (MIC 2.5–10 mg/mL) *E. cloacae*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *Morganella morganii*, *Acinetobacter baumanii*	–	[61]
	Commercial anthocyanidins	Pelargonidin, cyanidin, and delphinidin	Broth microdilution assay Gram-negative *P. aeruginosa* (MIC 0.45 – 1.0 mg/mL; MBC 0.9 – 1.35 mg/mL)	–	[87]

Table 2 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibacterial activity	Possible mechanism of action	References
	Strawberry (*Fragaria x ananassa* Duch.)	Anthocyanin extract	Disc diffusion assay Gram-positive *Staphylococcus aureus* associated to bovine mastitis: ≥ 40% growth inhibition at 100 µg/disc	–	[53]
	Chinese wild blueberries (*Vaccinium uliginosum*)	Anthocyanin-rich extract	Broth microdilution assay Gram-positive *S. aureus* (MIC 0.21 mg/mL); *Listeria monocytogenes* (MIC 0.27 mg/mL) Gram-negative *Salmonella enteritidis* (MIC 0.27 mg/mL); *Vibrio parahaemolyticus* (MIC 0.03 mg/mL)	Cell membrane damage, reduction in energy-transducing system and TCA cycle	[60]
	Cranberry (*Vaccinium macrocarpon*)	Commercial cranberry proanthocyanidins	Gram negative *E. coli*, *P. mirabilis*, *P. aeruginosa*	At 50 µg/mL potentiated antibacterial effect of antibiotics and increased synergistic activity, prevented the evolution of resistance towards antibiotics, enhanced survival of infected fruit flies and the larvae of the greater wax moth, repressed selective membrane permeability multidrug efflux pumps in selected bacterial strains	[58]
	Pomegranate peel (*Punica granatum*)	Anthocyanin extract	Broth microdilution assay Gram-positive *S. aureus* (MIC 125 ppm), *B. cereus* (MIC 250 ppm) Gram-negative *E. coli* (MIC 125 ppm), *S. typhi* (MIC 250 ppm)	–	[88]
	Bluberry varieties: Cristina Blue, Snowchaser, Stella Blue, and Star (*Vaccinum corymbosum*)	Anthocyanin-rich extract	Broth microdilution assay Gram-positive (MIC 0.76 – 4.54 mg/mL) *Listeria monocytogenes*, *Micrococcus* spp. Gram-negative (MIC 0.4 – 9.52 mg/mL) *Escherichia coli*, *Salmonella enteritidis*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* ssp.	–	[52]

Table 2 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibacterial activity	Possible mechanism of action	References
	Brightwell blueberries (*Vaccinium virgatum*)	Anthocyanin-rich extract	Disc diffusion assay Gram-negative *E. coli* (16.04 mm), *Salmonella enterica* (10.95 mm)	–	[89]
	Cranberry (*Vaccinium macrocarpon*)	Commercial cranberry anthocyanins	Agar dilution method Gram-positive *S. aureus* (MIC 5 mg/mL)	Cell membrane depolarization, reduced intracellular ATP, reduced bacterial protein content, destruction of cell morphology	[59]
	Black chokeberry (*Aronia melanocarpa*)	Anthocyanin fraction (Cyanidin-3-xyloside, cyanidin-3-galactoside, cyanidin-3-arabinoside, and cyanidin-3-glucoside)	Broth microdilution assay Gram-negative *E. coli* (MIC 0.625 mg/mL, MBC 1.25 mg/mL)	Destroy integrity of cell wall and membrane, cellular protein leakage, disruption of DNA replication, transcription, and expression	[62]
	Commercial anthocyanin	Cyanidin 3-*O*-glucoside	Broth microdilution assay Gram-positive *S. aureus* (MIC 2.0 mg/mL)	Cell wall and membrane damage, leakage of DNA, AKP, LDH, and protein, DNA Topo IV activity inhibition with reduction of DNA biosynthesis	[67]
	Blueberry (*Vaccinium myrtillus)*	Anthocyanin-rich extract	Broth microdilution assay Gram-positive *S. aureus (*MIC 2.5 – 15.0 mg/mL), *E. faecalis* (MIC 10.0 mg/mL) Gram-negative *E. coli* (MIC 25.0 mg/mL)	–	[90]
	Black chokeberry (*Aronia melanocarpa)*	Anthocyanin-rich extract	Broth microdilution assay Gram-positive *S. aureus* (MIC 10.0 – 15.0 mg/mL), *E. faecalis* (MIC 10.0 mg/mL)	–	[90]
	Blueberry (*Vaccinium corymbosum)*	Anthocyanin-rich extract	Broth microdilution assay Gram-positive *S. aureus* (MIC 12.5 mg/mL) Gram-negative *E. coli* (MIC 12.5 mg/mL)	–	[90]
	Onion peel (*Allium ascalonicum*)	Anthocyanin fraction	Disc diffusion assay Gram-positive *S. aureus* (18.0 mm), *B. subtilis* (14.0–16.0 mm)	–	[91]
	Squirrel's claws plant seed coat (*Caesalpinia crista*)	Anthocyanin fraction	Disc diffusion assay Gram-positive *S. aureus* (20.0 mm)	–	[92]

Table 2 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibacterial activity	Possible mechanism of action	References
	Wild cabbage (*Brassica oleracea*)	Anthocyanin extract	Disc diffusion assay Gram negative *E. coli* (18.21 mm)	–	[93]
Monoacylated anthocyanins	Dark purple-fleshed potato (*Solanum tuberosum*)	Anthocyanin extract (Petunidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside and malvidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside)	Broth microdilution assay Gram-positive (MIC value 15.6–31.3 µg/mL) *S. aureus*, *E. faecalis* Gram-negative (MIC value 31.3–250 µg/mL) *P. vulgaris*, *P. mirabilis*, *S. typhi*, *E. cloacae*, *E. aerogenes*, *P. aeruginosa*, *K. pneumonia*	–	[54]
Polyacylated anthocyanins	Blue pea flowers (*Clitoria ternatea*)	Anthocyanin extract	Disc diffusion assay Gram-positive *S. aureus* (7.0 mm), *B. subtilis* (10.0 mm) Gram-negative *E. coli* (8.0 mm)	–	[94]

AKP, Alkaline phosphatase; ATP, Adenosine triphosphate; CFU, Colony-forming units; COX-2, Cyclooxygenase-2; GAE, Gallic acid equivalent; LDH, Lactate dehydrogenase; IL-8, Interleukin-8; iNOS, Inducible nitric oxide synthases; MBC, Minimum bactericidal concentration; MAPKs, Mitogen-activated protein kinases; MIC, Minimum inhibitory concentration; NF-kβ, Nuclear factor-kappa β; QS, Quorum sensing; ROS, Reactive oxygen species; TCA, Tricarboxylic acid

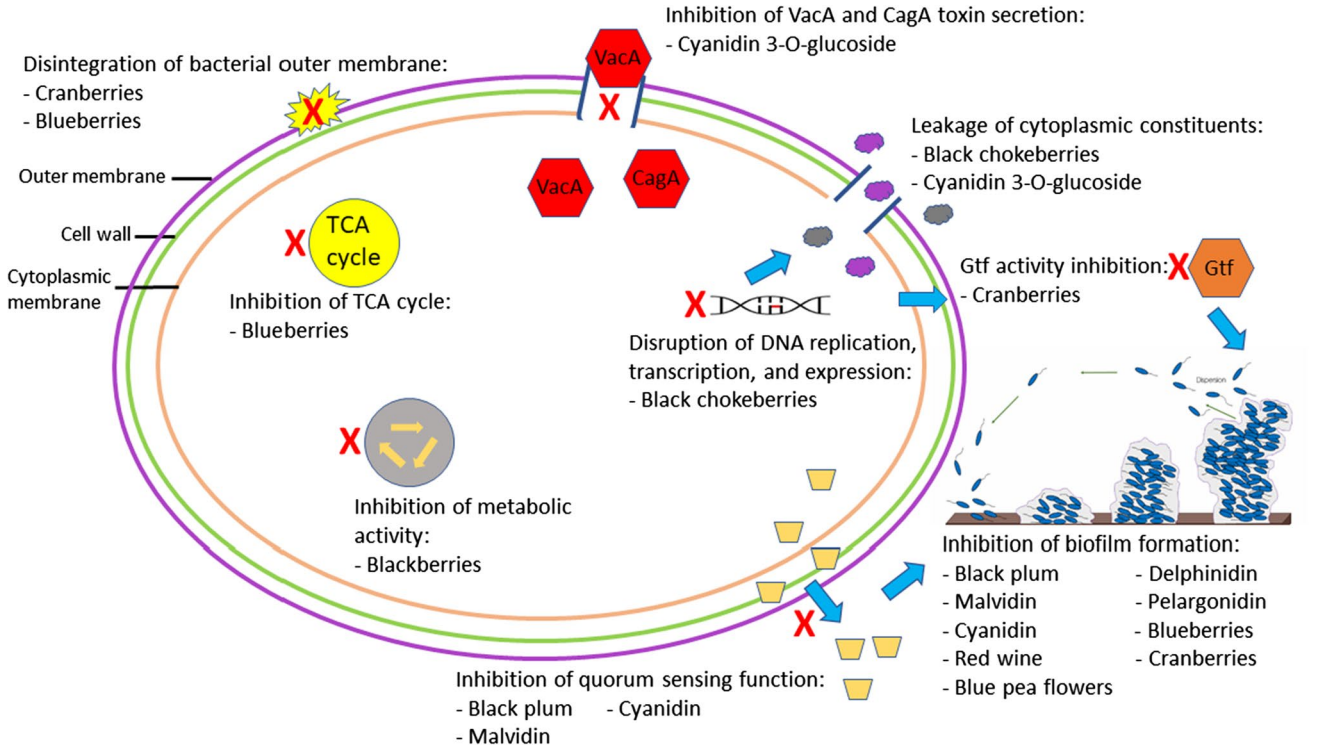


Fig. 1 Antibacterial and antibiofilm mechanism of action of various sources of anthocyanin fraction/enriched extract and those available commercially. *Abbreviations*: CagA, cytotoxin-associated gene A; DNA, deoxyribonucleic acid; Gtf, glucosyltransferase; TCA, tricarboxylic acid; VacA, vacuolating cytotoxin A

The continuous treatment of these strains with tetracycline alone for 21 days was found to have 128-fold and 32-fold increase in MIC values, respectively.

In vivo studies revealed that the combination treatment of the proanthocyanidins of cranberries with sulfamethoxazole increased the survival of *Drosophila melanogaster* fruit flies and *Galleria mellonella* larvae of the greater wax moth infected with *P. aeruginosa* compared to treatment with antibiotics alone. The proanthocyanidins had antibiotic potentiating effects in vitro and in vivo on various antibiotics and had the ability to repress selective membrane permeability and multidrug efflux pumps of tested bacterial strains [58]. In another study, the anthocyanins of cranberry showed potential activity against various *S. aureus* strains with a MIC of 5 mg/mL and their growth was completely inhibited at 3 h [59]. The anthocyanins reduced the level of intracellular (adenosine triphosphate) ATP in *S. aureus* strains and displayed depolarisation of membrane potential which led to membrane damage. Apart from that, the anthocyanins induced the leakage of cellular proteins and destruction of cell morphology where bacterial cells were observed to have irregular wrinkles, rough surface, separation of cytoplasmic membrane from cell wall and cytoplasmic leakage [59].

The anthocyanin-rich extract of Chinese wild blueberries was found to have potent antibacterial activity against various foodborne pathogens (*L. monocytogenes*, *S. aureus*, *S. enteritidis* and *Vibrio parahaemolyticus*). The Gram-negative bacteria *V. parahaemolyticus* was the most susceptible to the treatment, with an MIC value of 0.03 mg/mL. All strains displayed distorted membrane morphology, aggregation and leakage of cellular contents upon treatment with the anthocyanin extract. It also increased the nucleic acid leakage from bacterial cytoplasm, leakage of proteins through membrane damage as well as affecting the membrane-associated energy-transducing system. Alkaline phosphatase (AKP), adenosine triphosphatase (ATPase), and superoxide dismutase (SOD) activity were also decreased. The effects observed, together with the decrease of the tricarboxylic acid (TCA) cycle led to the reduction of energy transfer of pathogens leading to the inhibition of their growth and reproduction [60].

A study by Dorneanu and group investigated the antibacterial potential of the anthocyanin fraction of black chokeberry against standard bacterial strains and clinical isolates and was found to have equipotency against the Gram-positive and Gram-negative strains tested (MIC = 2.5–10 mg/mL) [61]. The anthocyanin fraction also displayed moderate synergism with its respective clinical antibiotics against *E. coli* and *P. aeruginosa* in disc diffusion assay. However, another study by Deng and group reported the anthocyanins of black chokeberry to have strong antibacterial activity against

E. coli (MIC = 0.625 mg/mL) [62]. The anthocyanins were able to destroy the integrity of the cell wall and cell membrane of *E. coli* followed by leakage of cellular proteins, disruption of protein synthesis and degradation of bacterial proteins. The anthocyanins were also found to bind to DNA, potentially causing the inhibition of replication, transcription, and expression of DNA in cells which ultimately leads to cell death [62]. The black chokeberry anthocyanin extract in the study was about 16 times more potent against *E. coli* than that reported by Dorneanu and group based on their MIC values [62]. This could probably be contributed by the anthocyanin extraction and purification process adapted in the study. A semi-preparative high performance liquid chromatography (HPLC) was used to isolate, identify, and collect the respective anthocyanins (cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, and cyanidin-3-xyloside) based on the peaks observed by Deng and group. However, the collection of ethanol fraction (containing anthocyanins) by Dorneanu and group was not further purified, which may cause it to have a lower content of anthocyanins as well as the presence of other compounds.

The anthocyanin-enriched extract of black plum was reported to have potential anti-quorum sensing (QS) activity against *Klebsiella pneumoniae* induced acyl-homoserine lactone (AHL) mediated violacein production with about 70% inhibition at 500 µg/mL [63]. Molecular docking studies showed the anthocyanin malvidin to have the highest docking score with LasR receptor protein out of 43 active components of the extract. The anthocyanin-enriched extract also exhibited synergistic activity with ofloxacine, tetracycline and chloramphenicol antibiotics on the growth inhibition of *K. pneumonia*. The most potent effect was observed with ofloxacine, having increased the sensitivity of *K. pneumonia* up to 55.6% when treated with the extract at 1 mg/mL. The anthocyanin malvidin (20 µg/mL) showed a remarkable inhibitory effect (85.4%) against QS regulated violacein production at against *K. pneumonia* [63]. Later on the same group investigated the potential of the anthocyanin cyanidin, which showed a high docking score with LasR receptor protein in molecular docking studies. Cyanidin (80 µg/mL) inhibited violacein production (anti-QS activity) by 74% and exhibited synergistic activity with erythromycin, tetracycline and kanamycin antibiotics on the growth inhibition of *K. pneumonia*. The most potent effect was observed with kanamycin having increased the sensitivity of *K. pneumonia* to 86.5% when treated together with cyanidin at 150 µg/mL [64].

A study determined the antibacterial activity of several commercially available anthocyanins [65]. Cyanidin 3-*O*-glucoside (100 µM) was found to have potential activity against *Helicobacter pylori* which the infection is known to lead to gastritis, peptic ulcers and gastric cancer. It inhibited the secretion of CagA and VacA toxins via suppression of secA transcription of *H. pylori*. Later on, the same group investigated the potential of the anthocyanins from black soybean, which was mainly composed of cyanidin-3-glucoside to inhibit gastric epithelial cell inflammation due to *H. pylori* infection [66]. Cyanidin-3-glucoside (50 µg/mL) was found to decrease *H. pylori* induced reactive oxygen species (ROS), inhibit mitogen-activated protein kinases (MAPKs) phosphorylation, nuclear factor-kappa β (NF-kβ) translocation and Ikβα degradation. It also inhibited *H. pylori*-induced inducible nitric oxide synthases (iNOS) and cyclooxygenase-2 (COX-2) mRNA expression and interleukin-8 (IL-8) cytokine production [66]. Cyanidin 3-*O*-glucoside was also reported by another study to have potential antibacterial activity against *S. aureus* with an MIC value of 2 mg/mL [67]. The treated bacterial cells exhibited rough and tattered surface, disintegration in the cell membrane and cytoplasm leakage leading to irreversible rupture of cell wall and cytoplasmic membrane. The increase in the leakage of alkaline phosphatase (AKP) as detected in the supernatant indicated damage to the cell wall while damage to cell membrane was observed with the increase in leakage of lactate dehydrogenase (LDH), protein and DNA. It was identified as a DNA intercalator that showed strong inhibitory activity on topoisomerase (Topo IV) affecting the synthesis of bacterial DNA and protein [67].

Monoacylated anthocyanins from dark purple-fleshed potato (*Solanum tuberosum*) demonstrated the lowest MIC against two gram-positive bacterial strains, *S. aureus*, *E. faecalis* based on broth microdilution assay (Table 2). Monoacylated anthocyanins in dark purple-fleshed potatoes consist of more hydroxyl groups compared to anthocyanin sources, with nonacylated anthocyanins having higher MIC values. In a study by Sun et al. [68], petunidin-derived diacylated anthocyanins isolated from purple sweet potato [*Ipomoea batatas* (L.) Lam] had lower MIC against *Staphylococcus aureus* and *Salmonella typhimurium* compared to the MIC values of nonacylated anthocyanins, indicating that the acylation increases the antibacterial properties of anthocyanins. Interestingly, Zhang et al. [69] reported that acylated anthocyanins from purple sweet potato [*Ipomoea batatas* (L.) Lam] enhanced the growth of *Lactobacillus* spp. and *Bifidobacterium*, but inhibited the abundance of *Clostridium histolyticum* and *Prevotella*. This indicates that the acylated anthocyanins impart their antibacterial effect only against harmful bacteria. Considering nonacylated anthocyanins, anthocyanin fractions from dark roselle petals (*Hibiscus sabdariffa*) and eggplant peels (*Solanum melongena*) demonstrated higher disc diffusion diameters against *B. subtilis* than onion peel (*Allium ascalonicum*) anthocyanins (Table 2). Main anthocyanin from both dark roselle petals (delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside) and eggplant peels (delphinidin-3-*O*-rutinoside) consists a higher number of hydroxyl groups compared to onion peel anthocyanins

(cyanidin-3-*O*-glucoside). The higher antimicrobial activity was related to the presence of hydroxyl groups (phenolic and alcohol compounds), whereas hydrocarbons resulted in less activity of compounds in essential oils [70]. However, anthocyanins from blue pea flower (*Clitoria ternatea*) which contains polyacylated anthocyanins with a large number of hydroxyl groups did not show higher disc diffusion diameters compared to nonacylated anthocyanins (Table 2). This could be due to the steric hindrance imparted by the acyl groups in the polyacylated anthocyanins. Acylation decreases the polarity of the anthocyanin molecule and causes steric hindrance [71].

3.2 Antibiofilm activity of anthocyanins

The search for alternative agents or approaches apart from antibiotics is urgent to tackle the rise of multidrug-resistant bacterial infections. Therefore, various strategies are being explored to target the virulence factors of pathogens to impair their ability to cause infections such as those associated with biofilms. The antibiofilm properties displayed by anthocyanins from various sources are shown in Table 3 and Fig. 1. The anthocyanin-enriched extract of black plum was found to have potential antibiofilm activity against *K. pneumonia* [63]. The extract (1 mg/mL) inhibited biofilm production by 79.9% and exopolysaccharide formation by 64.3%. It was also found to have antibacterial (growth inhibition) activity (Table 2). Among the underlying mechanisms for the effects observed was its ability to inhibit the quorum sensing signalling pathway, which is essential for bacterial growth, virulence factors, and biofilm formation [63]. A similar mechanism was also found by the same group the following year using a commercial anthocyanin cyanidin (150 µg/mL) for its antibiofilm potential in *K. pneumonia* [64].

Grape marc anthocyanin extract was determined for its potential in preventing dental caries in vivo in white rats (*Rata albicans*) infected with *S. mutans* and were fed with a cariogenic diet. The treatment group (topical application of 5% anthocyanin extract for 60 days) had reduced number of cavities (1.62 fold) as well as reduction in surface roughness (2.13 fold) compared to the control untreated group [72]. Another study reported the anthocyanin extract of soybean and walnuts to have antibiofilm activity against various *Pseudomonas* and *Klebsiella* bacterial species. It was found to have inhibited biofilm formation by reducing bacterial cell hydrophobicity having the capacity to inhibit attachment to surfaces [73].

Some studies have shown the proanthocyanidins for their potential antibiofilm activity. The proanthocyanidin fraction of red wine was reported by a study for potent antibiofilm activity against *Streptococcus mutans*. *S. mutans* is known to colonize tooth surfaces and form biofilm (dental plaque) that leads to the development of oral infectious diseases, such as caries, gingivitis and periodontal inflammation [74]. The proanthocyanidin fraction inhibited the adhesion (79.0–81.5%) of *S. mutans* to saliva coated hydroxyapatite (HA) beads in the presence of sucrose and the activity was found to be much higher than dealcoholized red wine. The proanthocyanidin fraction was also able to detach *S. mutans* attached to HA beads and had a very high biofilm inhibitory activity (89.0%) on the occlusal surface of natural human teeth, being a potential anticariogenic agent [74]. The proanthocyanidin fraction of cranberry was determined by another study for its antibiofilm activity against *S. mutans* [75]. It reduced the biomass (dry weight) and the total amount of extracellular insoluble polysaccharides of *S. mutans* biofilms (35–40%) on saliva-coated hydroxyapatite (sHA) discs as well reducing acidogenicity of the biofilms without affecting the viability of the bacterial cells. The glucosyltransferase (GtfB) enzyme activity either in solution or adsorbed on the sHA surface was also effectively inhibited. The incidence and severity of smooth-surface caries were significantly reduced (40–45%) in the in vivo dental caries model using Sprague–Dawley rats infected by mouth with an actively growing culture of *S. mutans* [75]. Another study also found the proanthocyanidins of cranberry (100 µg/mL) decreased the biofilm formation of *E. coli*, *Proteus. mirabilis*, and *P. aeruginosa* [58]. It also exerted a synergistic antibiofilm activity when combined with the antibiotic sulfamethoxazole which had minimal effect on biofilms when used alone. The A-type proanthocyanidins was found in a study to have potential biofilm inhibitory activity against *Porphyromonas gingivalis*, a key oral bacterium for periodontitis [76]. At 100 µg/mL, it exerted 60% inhibition of biofilm formation and inhibited adherence of *P. gingivalis* to epithelial cells (54.1%). The proanthocyanidins also decreased the secretion of IL-8 and chemokine ligand 5 (CCL5) of *P. gingivalis*-induced inflammatory response in oral epithelial cells and decreased the activity of NF-κB p65 pathway. Various other anthocyanin sources or its fraction, such blueberry, chokeberry, purple barley and blue pea flowers have been shown to have antibiofilm activity (Table 3).

Studies on the antibiofilm activity of acylated anthocyanins are limited. However, when comparing the available literature, the polyacylated anthocyanins from blue pea flowers demonstrated lower antibiofilm activity compared to nonacylated anthocyanins against gram-negative bacteria. For instance, the biofilm inhibition of anthocyanins from blue pea flowers at 5.0 mg/mL concentration was 64% [98] whereas nonacylated anthocyanins from blueberries (*Vaccinium corymbosum*) demonstrated biofilm inhibition up to 60% at a concentration of 250 µg/mL [85]. However, the mode of

Table 3 Antibiofilm activity of anthocyanins from various sources towards Gram-positive and Gram-negative bacterial strains

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibiofilm activity	Possible mechanism of action	References
Nonacylated anthocyanins	Cranberry (*Vaccinium macrocarpon*)	Proanthocyanidin fraction	Gram-positive *S. mutans*	At 500 µg/mL inhibited glucosyltransferase (Gtf) B and C activity (30–60%) and membrane associated F-ATPase (> 85%), reduced the acidogenicity, formation and accumulation of biofilms	[94]
	Cranberry (*Vaccinium macrocarpon*)	Proanthocyanidin fraction	Gram-positive *S. mutans*	At 1.5 mg/mL inhibited the formation of oral biofilms and glucosyltransferase (Gtf) activity, inhibited dental caries development in vivo	[75]
	Red wine	Proanthocyanidin fraction	Gram-positive *S. mutans*	Adhesion inhibitory activity (79.0–81.5%), detachment of *S. mutans* to saliva coated hydroxyapatite (HA) beads, biofilm inhibitory activity (89.0%)	[74]
	Cranberry (*Vaccinium macrocarpon*)	A-type cranberry proanthocyanidins	Gram-negative *Porphyromonas gingivalis*	Inhibition of biofilm formation (60%) at 100 µg/mL, reduced adherence of *P. gingivalis* to oral cells with a reduction in inflammatory responses	[76]
	Black plum (*Syzygium cumini*)	Anthocyanin-enriched extract	Gram-negative *Klebsiella pneumonia* − 1 mg/mL inhibited biofilm production (79.9%) and expolysaccharide formation (64.3%)	–	[63]
	Commercial anthocyanin	Cyanidin	Gram-negative *Klebsiella pneumonia* − 150 µg/mL inhibited biofilm production (72.4%) and exopolysaccharide formation (68.7%)	–	[64]
	Blueberries (*Vaccinium corymbosum*)	Anthocyanin-rich extract	Biofilm inhibition at 250 µg/mL (4% to < 60%) Gram-positive *S. aureus* Gram-negative *E. coli*, *P. aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*	Inhibited bacterial adhesion to polystyrene surface	[85]
	Grape marc (*Vitis vinifera*)	Anthocyanin extract	Gram-positive *S. mutans* Reduction of dental caries and enamel roughness in rat model infected with *S. mutans* treated with 5% anthocyanin extract	–	[72]

Table 3 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibiofilm activity	Possible mechanism of action	References
	Black chokeberry (*Aronia melanocarpa*)	Anthocyanin fraction (Cyanidin 3-glucoside, cyanidin 3-xyloside, cyanidin 3-arabinoside, and cyanidin 3-galactoside)	Minimum biofilm eradication concentration (MBEC) Gram-positive (MBEC 3.5–5 mg/mL) *S. aureus*, *E. faecalis*, *E. faecium* Gram-negative (MBEC 2.5–5 mg/mL) *E. coli*, *P. aeruginosa*, *Morganella morganii*	–	[61]
	Commercial anthocyanidins	Delphinidin, Pelargonidin, Cyanidin	Gram-negative *P. aeruginosa* Biofilm inhibition at 0.125 MIC (21–43%) against *P. aeruginosa*	Reduced bacterial twitching motility	[87]
	Cranberry (*Vaccinium macrocarpon*)	Commercial cranberry proanthocyanidins	Gram negative *E. coli*, *P. aeruginosa*, *P. mirabilis* Antibiofilm activity (100 µg/mL), potentiated antibiofilm effect of antibiotics and increased synergistic activity	–	[58]
	Soybean (*Glycine max*)	Anthocyanin extract	Minimum biofilm inhibitory concentration (MBIC): Gram-negative *P. aeruginosa*, *P. mimosa*, *P. maltophilia*, *K. pneumoniae*, *K. oxytoca* and *K. granulomatis* MBIC 0.1–0.48 mg/mL, cell surface hydrophobicity 35.2 – 42.9%	Reduction of bacterial cell surface hydrophobicity	[73]
	Walnut (*Juglan regia*)	Anthocyanin extract	Gram-negative *P. aeruginosa*, *P. mimosa*, *P. maltophilia*, *K. pneumoniae*, *K. oxytoca* and *K. granulomatis* MBIC: 0.18–1.1 mg/mL, cell surface hydrophobicity 8.1 – 46.3%	Reduction of bacterial cell surface hydrophobicity	[73]
	Purple highland barley (*Hordeum vulgare*)	Anthocyanin-rich extract	Biofilm inhibition (highest activity at sub-MIC concentration 4 mg/mL) Gram-negative *P. aeruginosa* (60.2%), *S. enterica* (47.3%)	Decreased biofilm thickness, reduced surface coverage of coverslips and bacteria density	[95]

Table 3 (continued)

Major anthocyanins	Anthocyanin source	Anthocyanin content/type	Antibiofilm activity	Possible mechanism of action	References
	Synthetic anthocyanins	Luteolinidin, deoxymalvidin, cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, and its synthetic-synthesized compounds	Highest antibiofilm activity Gram-positive *S. aureus* (carboxypyCy-3-glc, MethylpyCy-3-glc) Gram-negative *P. aeruginosa* (carboxypyCy-3-glc, deoxyMv, cy-3-glc, dimethyl-amino-cin-but-pyCy-3-glc and carboxypyMv-3-glc)		[96]
	Synthetic cyanidin- and malvidin-3-*O*-glucosides (obtained by the fractionation of blackberries and young red wine extract)	Pyranoanthocyanin extract (elderberry) & Pyranoanthocyanin extract (red wine)	Highest antibiofilm activity Gram-positive *S. aureus* (Pyranoanthocyanin extract of red wine) Gram-negative *P. aeruginosa* (Pyranoanthocyanin extract of elderberry and red wine)	–	[96]
	Reaction of cyanidin-3-*O*-glucoside and red wine anthocyanins with pyruvic acid	Carboxypyranocyanidin-3-*O*-glucoside (carboxypyranocy-3-glc), carboxypyranoanthocyanins extract (carboxypyrano-ant extract)	Gram-positive *S. aureus* (biofilm inhibition at 64 µg/mL) Gram-negative = *P. aeruginosa* (no biofilm inhibition)	Only carboxypyranocy-3-glc inhibited violacein production in *C. violaceum*, both compounds interfered with the expression of several QS-related genes in *P. aeruginosa* and *S. aureus* biofilms	[97]
	Brightwell blueberries (*Vaccinium virgatum*)	Anthocyanin-rich extract	Biofilm inhibition Gram-positive *Listeria monocytogenes* −2 mg/mL showed maximum reduction of biofilm biomass (84.6%)		[89]
	Commercial anthocyanin	Cyanidin 3-*O*-glucoside	Biofilm inhibition at ¼–4 MIC Gram-positive *S. aureus* −71.3% inhibition of biofilm formation at 4 MIC	Decreased cellular metabolism in biofilms	[67]
Polyacylated anthocyanins	Blue pea flowers (*Clitoria ternatea*)	Anthocyanin fraction	Gram negative: *P. aeruginosa* strains Antibiofilm activity (64%) at 5 mg/mL, MBIC values: 0.63 to 5.0 mg/mL	Reduction of bacterial attachment on polystyrene surface by 1.1 log CFU/cm^2 at 24 h	[98]

ATP, Adenosine triphosphate; CFU, Colony-forming units; MBEC, Minimum biofilm eradication concentration; MBIC, Minimum biofilm inhibitory concentration; MIC, Minimum inhibitory concentration

action of biofilm inhibition activity of acylated anthocyanins is not fully understood. Therefore, further in-depth studies looking into its mechanism of action would be beneficial in understanding the activity observed.

4 Conclusions and future recommendations

Various research studies are being done to obtain alternatives to antibiotics to treat bacterial infections with the rise of antimicrobial resistance. The anthocyanins or anthocyanin fraction/extract from various sources (cyanidin, malvidin and delphinidin derivatives), being mostly from fruits, have been studied for their potential antibacterial and antibiofilm activities. Many studies investigating anthocyanins against the Gram-positive or Gram-negative bacterial strains have shown much better activity against the Gram-positive strains. However, some studies have also shown it to be equally potent against Gram-positive and Gram-negative strains. The findings of these studies serve as a guide for the potential application of anthocyanins in treating bacterial infections in clinical settings. Further investigation and understanding of the causative pathogen/s of infected patients and the use of relevant anthocyanin source or type (being more selective to that particular bacterial strain) may be used, therefore being more specifically curated to treat the infection based on the causative pathogen. At the same time, infections involving a mix of Gram-positive and Gram-negative bacterial strains may be treated with anthocyanins which are effective against both strain types. Considering the potential of these anthocyanins or their fraction for their antibacterial or antibiofilm activity, further research on treating the co-culture of different bacterial colonies/strains would be beneficial as they may differ in their pathogenicity.

Some of the studies have explored the combination treatment of anthocyanins with antibiotics and which had a potentiating effect. This effect is highly beneficial as anthocyanins alone do not affect bacterial viability but are able to synergise the effect when combined with antibiotics to further reduce bacterial growth and viability. This combination approach would be beneficial in clinical settings as it may help prevent the unnecessary increase in the antibiotic concentration for treatment of infections which may eventually lead to antibiotic resistance. These anthocyanins at concentrations that do not affect bacterial growth have potential antibiofilm activity. Most of the current antibiotics are only effective against the planktonic growth mode of bacteria but not against biofilms, making it challenging for the antibiotics to penetrate into the biofilms to target their growth. Therefore, the combinatorial approach serves as an arsenal to target two different areas of bacterial cell communication and signalling pathway, thus providing a much more significant effect and leading to better treatment outcomes. Research studies looking further into the combinatorial approach of various other anthocyanins to treat different bacterial infections would provide useful information for its potential application for treatment in clinical settings. Several in vivo studies have been done so far, especially in the treatment and prevention of dental caries which was found to have a positive impact. Future in vivo studies looking at different infection models would also be needed to understand the potential of anthocyanins, especially in chronic wounds and biofilm infections involving medical implants or devices.

Most studies have used the anthocyanin fractions of various sources to study its antibacterial or antibiofilm properties. However, quite a number have not characterised the anthocyanin composition. It is also unknown if the cocktail of anthocyanins or some impurities in the fraction may have potentiated or reduced the observed effect. Further isolation of the anthocyanins (major compounds) would be needed to determine the compound most possibly being responsible for the activity, and it may even have much better activity. Obtaining the pure compound responsible for the effect would be important to standardise the potential application (e.g. concentration and dosage) of anthocyanins in clinical settings. Some studies have also shown the potential activity of individual commercial anthocyanins. Thus further studies exploring the chemical structure modification and synthesis of these anthocyanin compounds may yield compounds with improved activity.

With the growing interest and knowledge of the health benefits of anthocyanins, consumers are also looking forward to health or food products containing these active compounds. Incorporating anthocyanins in the food and beverages industries will represent an important value. Focusing on improving formulations and packaging together with consumption safety and compound stability studies is beneficial. Findings obtained from these studies further support the anthocyanins (natural compounds) as alternative sources of antibacterial or anti-infective agent, which has the potential for application in treating bacterial infections and may have the possibility of preventing the development of antimicrobial resistance. Further research into these compounds would be highly beneficial and recommended and their incorporation into health supplements and food products for general well-being.

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Declarations

Competing interests The authors declare that they have no competing interests.

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