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REVIEW ARTICLE

Antimicrobial betalains

Vindya Nilakshi Wijesinghe | **Wee Sim Choo**

School of Science, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

Correspondence
Wee Sim Choo, School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia.
Email: choo.wee.sim@monash.edu

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Abstract

Betalains are nitrogen-containing plant pigments that can be red-violet (betacyanins) or yellow-orange (betaxanthins), currently employed as natural colourants in the food and cosmetic sectors. Betalains exhibit antimicrobial activity against a broad spectrum of microbes including multidrug-resistant bacteria, as well as single-species and dual-species biofilm-producing bacteria, which is highly significant given the current antimicrobial resistance issue reported by The World Health Organization. Research demonstrating antiviral activity against dengue virus, in silico studies including SARS-CoV-2, and anti-fungal effects of betalains highlight the diversity of their antimicrobial properties. Though limited in vivo studies have been conducted, antimalarial and anti-infective activities of betacyanin have been observed in living infection models. Cellular mechanisms of antimicrobial activity of betalains are yet unknown; however existing research has laid the framework for a potentially novel antimicrobial agent. This review covers an overview of betalains as antimicrobial agents and discussions to fully exploit their potential as therapeutic agents to treat infectious diseases.

KEYWORDS

antibacterial, antibiofilm, anti-fungal, antiviral, betacyanin, ESKAPE pathogens

INTRODUCTION

Antimicrobial resistance (AMR) stands as one of the most fatal threats to humans, with a staggering 1.27 million deaths in just 2019 directly ascribed to drug-resistant infections (Murray et al., 2022). With the ongoing evolution of microbes, rapid emergence of new resistance mechanisms and the spread of drug-resistant pathogens, the race to discover new antimicrobial agents continues to grow. The development of novel drugs requires new chemical entities (NCE) with the requisite druggability and medicinal chemistry features to be identified; whether they be chemically produced or extracted from plants. Before the post-genomic age was attained and high-throughput screening was established, at least 80% of pharmaceuticals were (or were based on) natural compounds derived from plants (Katiyar et al., 2012). Compared to synthetic molecules, natural products are better tolerated (with fewer adverse reactions), more cost-effective and are 'naturally' intended to fulfil certain biological roles such as interacting/competing with other organisms and regulating endogenous defence mechanisms (Atanasov et al., 2021; Veeresham, 2012). Plants produce a variety of bioactive compounds that have a broad range of health benefits. Alkaloids (such as strychnine, quinine and morphine), carbohydrates, steroids and phenolics (such as flavonoids, lignins and tannins) have been the subject of numerous studies due to their potential medicinal/therapeutic

properties (Altemimi et al., 2017; Heinrich et al., 2021; Kurek, 2019).

Among these bioactive plant compounds are betalains—a naturally occurring class of pigments found in around 17 different plant families (Belhadj Slimen et al., 2017). Betalains are currently being used as natural colourants in the food industry as well as in cosmetics and pharmaceuticals (Choo, 2018; Esatbeyoglu et al., 2015), but they also have tremendous potential to be developed as functional food ingredients (Gengatharan et al., 2021) and therapeutic agents. Thus far, betalains have been found to be safe for consumption (up to a maximum concentration of 100 μM) (Sadowska-Bartosz & Bartosz, 2021), and non-toxic to proliferating cell cultures even at relatively high concentrations (Yong et al., 2018). This could be useful during drug development as it indicates that betalains would be safe and well-tolerated if used as a drug in fairly high doses.

Betalains have grown in popularity over the years due to their antioxidant and radical-scavenging properties, and continuous efforts are being made to explore their other health benefits. Recent studies have demonstrated various biological properties of betalains such as anti-inflammatory, anticancer, anti-lipidaemic and antimicrobial activity—the lattermost is the subject of this review. This review discusses the inhibitory effects of betalains on bacteria, viruses, fungi and a malaria-causing protozoan using in vitro, in vivo and in silico approaches.

Chemical properties of betalains: An overview

Betalains are water-soluble, nitrogen-containing pigments found in the vacuoles of plant cells (Sadowska-Bartosz & Bartosz, 2021), further divided into two structural groups: betacyanin (red-violet) and betaxanthin (yellow-orange) (Miguel, 2018). Betalamic acid, the core structure of all betalains, undergoes two types of condensation reactions: with amines (and/or its derivatives), and *cyclo*-DOPA (or sometimes its glucosyl derivatives) giving rise to betaxanthin and betacyanin respectively (Guerrero-Rubio et al., 2019; Miguel, 2018). Betanidin is the backbone of all betacyanin structures whereby glycosylation and acylation of the resulting 5-O- or 6-O-glucosides results in various betacyanin structures (Belhadj Slimen et al., 2017).

Betacyanins are divided into four structural types: betanin, amaranthine, gomphrenin and bougainvillein (Polturak & Aharoni, 2018) depending on the attachment of glucosyl groups to the oxygen atoms in the *o*-position on the *cyclo*-DOPA moiety (Belhadj Slimen et al., 2017). Betaxanthins, on the other hand, are divided into two structural groups: amino-acid-derived conjugate group and amine-derived conjugate group as the amino acid/amine side chains replace *cyclo*-DOPA units in betaxanthin molecules (Chung et al., 2015; Miguel, 2018). The differences in chemical structures between betacyanin and betaxanthin results in distinct absorption maxima for each, hence the variation in colour. Betacyanins exhibit two absorption maxima: one in the UV range between 270 and 280 nm as a result of the *cyclo*-DOPA unit, and the other in the visible range between 535 and 540 nm (depending on the solvent) (Azeredo, 2009). The absorption maximum of betaxanthins varies from 460 to 480 nm (Belhadj Slimen et al., 2017), and structures conjugated to amine groups have lower absorption maximum compared to those with respective amino acid side chains (Azeredo, 2009). Various forms of betalain pigment are still being discovered thanks to advances in technology and analytical methods (Skalicky et al., 2020). Approximately 80 distinct betalain structures have thus far been identified from 17 different plant sources (Sadowska-Bartosz & Bartosz, 2021).

Sources and functions of betalains

Betalains primarily occur in Caryophyllales order plants, followed by certain fungi of higher order such as *Amanita muscaria* (fly agaric) (Rahimi et al., 2019), and now *Gluconacetobacter diazotrophicus*—the first betalain-producing bacterium (Contreras-Llano et al., 2019). All plant families in the Caryophyllales order contain betalains, except for those that produce anthocyanins as both these pigments do not coexist in any living organism (Rahimi et al., 2019). Betalains are mainly found in edible portions of plants though they can also exist in the flowers, stems, leaves, and roots (Li et al., 2019). The most common source of betalains belongs to the Amaranthaceae and Cactaceae families which include *Beta vulgaris* L. (red beetroot), *Opuntia* spp. (prickly pear), *Hylocereus* spp. (red dragon fruit or red pitahaya), *Amaranthus* spp., *Beta vulgaris* L. var. cicla (Swiss chard) and *Ullucus tuberosus* (ulluco tubers) (Moreno-Ley et al., 2021).

The main function of betalains in plants is to attract pollinators and frugivores for seed distribution and fertilization. Evidence suggests that red-pigmented leaves are less susceptible to light or radiation than green leaves. This, together with the fact that betalain synthesis increases in the presence of light or UV radiation, suggests that they may have a photoprotective role (Polturak & Aharoni, 2018). Plants are said to benefit from betalains when they are exposed to saline conditions. Betacyanin accumulation has been observed in betacyanin-containing plant species such as *Amaranthus hypochondriacus*, *Alternanthera philoxeroides Mesembryanthemum crystallinum and Portulaca oleracea* when exposed to salt stress,

which has been linked to the induction of betalain biosynthetic gene expression (Zhou et al., 2021).

ANTIMICROBIAL ACTIVITY OF BETALAINS

Bacteria, fungi, viruses and other parasites are becoming more resistant to existing antimicrobial drugs. The hunt for novel antimicrobial agents is growing in importance as drug-resistant disease-causing pathogens are becoming more prevalent. This review covers research that investigated the inhibitory effects of betalains on a variety of microorganisms, including the highly virulent and multi-drug resistant ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.). In vitro tests using betalains from various sources revealed antimicrobial activity against bacteria (including Gram-positive (Table 1), Gram-negative (Table 2) and biofilm-forming species), fungi and dengue virus. Different techniques such as broth microdilution, agar well diffusion and virucidal assay were adopted based on the subject micro-organism in accordance with internationally accepted procedures. The ability of betacyanin to exhibit antiviral activities against SARS-CoV-2 was found and analysed using computational approaches. To date, only a few in vivo studies have been undertaken to explore the antimicrobial activity of betalains, although anti-infective and antimalarial activity has been proven using living infection models.

Antibacterial activity

Gram-positive bacteria

Beetroot pomace extract (BPE), which contains betanin and vulgaxanthin, demonstrated antibacterial activity against five Gram-positive bacteria: *Staphylococcus sciuri*, *S. aureus*, *Staphylococcus saprophyticus*, *Staphylococcus equorum* and *Bacillus cereus*. (Vulic et al., 2013). The bacteria were grown on Müeller-Hinton agar slants and incubated for 24 h at 37°C after being treated with 100 mg ml^{-1}of BPE in distilled water. The inhibition zones produced by BPE were used to assess its antibacterial activity. Inhibitory effects were not observed against *Bacillus* sp., *Enterococcus faecalis* and *Listeria monocytogenes*. The smallest inhibitory zone was observed in *S. sciuri* (12.33 ± 0.58 mm), while the remaining four produced inhibition zones of the same size (20 mm). All inhibitory zones observed after BPE treatment were smaller than that of the control (cefotaxime/clavulanic acid) for each of the bacteria. Čanadanović-Brunet et al. (2011) investigated the antibacterial activity of beetroot pomace ethanol extract against *S. aureus* and *B. cereus* at concentrations of 10, 20 and 50 mg ml^{-1}. Their study showed susceptibility of *S. aureus* and *B. cereus* to the extract with minimum inhibitory concentration (MIC) values of 0.75 and 0.5 mg ml^{-1}, control (chloramphenicol) MIC values of 5.0 and 10.0 μg ml^{-1} and inhibition zones of 8 ± 1.0 and 10.3 ± 0.58 mm respectively. Velićanski et al. (2011) found that *S. aureus* and *B. cereus* were the most susceptible Gram-positive bacteria to all tested volumes of BPE (15, 50 and 100 μl) and inhibition zones were observed in a dose-dependent manner post-treatment. However, inhibitory zones produced by the control (cefotaxime/clavulanic acid) were larger than those produced by the bacteria post-treatment with BPE. Similar to Vulic et al. (2013), no inhibitory effects were observed toward any of the tested BPE volumes against *Bacillus* sp., *E. faecalis*, *L. monocytogenes* and *Staphylococcus cohnii* spp. *cohnii*, while a minimum of 100 μl was required to produce inhibitory effects against *Staphylococcus epidermidis*. The lack of inhibitory effects could be due to the presence of other components in the BPE with the increased volumes which may have triggered an antagonistic effect (Velićanski et al., 2011; Vulic et al., 2013). *S. aureus* is an ESKAPE pathogen among the high priority group of Gram-positive bacteria for which new antimicrobial agents are urgently needed due to the emergence of methicillin-resistant strains (Tacconelli et al., 2018). *S. aureus* infections are common because they can cause a variety of infections in both hospital and community settings, including endocarditis, meningitis, gastroenteritis, urinary tract infections (UTIs), pneumonia, skin and soft tissue infections (Taylor & Unakal, 2021). *B. cereus*, on the other hand, is a toxin-producing pathogen linked to diarrheal and emetic foodborne diseases (Qu et al., 2021). While it has not been designated as a priority in the antimicrobial resistance crisis, it has gained attention due to its resistance to penicillin and other β-lactam antibiotics, as well as its ability to acquire resistance to commonly used antibiotics such as ciprofloxacin, cloxacillin, erythromycin, streptomycin and tetracycline (Fiedler et al., 2019).

Betalains from red beetroot ethanol extract inhibited *E. faecalis* growth in a dose-dependent manner (Vijaya & Thangaraj, 2019). Although the composition of betalain had not been determined, it has been reported that red beetroot primarily contains betanin, isobetanin and vulgaxanthin (Sawicki et al., 2016). Inhibitory zones of 8.3 ± 0.47, 10.3 ± 0.47, 12.6 ± 0.47 and 14.6 ± 0.47 mm were produced after treatment with 1, 2, 4 and 8 mg of extract respectively (Vijaya & Thangaraj, 2019). The purpose of this investigation was to see if the betalain-containing extract had any effect on isolates that generate extended

TABLE 1 In vitro antibacterial activity of betalains from different sources against Gram-positive bacteria

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion Zone of inhibition (mm)	Broth dilution MIC	Control MIC	Reference
Beetroot pomace extract (100 mg ml^{-1})	Betanin, vulgaxanthin	*Staphylococcus aureus* ATCC 11632	—	20.00 ± 1.00	—	—	Vulic et al. (2013)
			12.50 ± 0.55	16.00 ± 0.00, 20.00 ± 1.00	—	—	Velićanski et al. (2011)
		Bacillus cereus ATCC 10876	—	20.33 ± 0.58	—	—	Vulic et al. (2013)
			10.67 ± 1.03	17.00 ± 1.00, 20.33 ± 0.58	—	—	Velićanski et al. (2011)
		Bacillus sp.	—	nd		—	Vulic et al. (2013)
		Bacillus spp.	nd	nd	—	—	Velićanski et al. (2011)
		Enterococcus faecalis	—	nd	—	—	Vulic et al. (2013)
			nd	nd	—	—	Velićanski et al. (2011)
		Staphylococcus saprophyticus	—	20.00 ± 0.00	—	—	Vulic et al. (2013)
		Staphylococcus equorum	—	20.33 ± 0.58	—	—	Vulic et al. (2013)
		Staphylococcus sciuri	—	12.33 ± 0.58	—	—	Vulic et al. (2013)
		Staphylococcus cohnii spp. *cohnii*	nd	nd	—	—	Velićanski et al. (2011)
		Staphylococcus epidermidis	nd	13.33 ± 0.58		—	Velićanski et al. (2011)
		Listeria monocytogenes	—	nd	—	—	Vulic et al. (2013)
			nd	nd	—	—	Velićanski et al. (2011)
Beetroot pomace extract (10, 20, 50 mg ml^{-1})	Betanin, vulgaxanthin	*Staphylococcus aureus* ATCC 11632	8.00 ± 1.00	—	0.75 mg ml^{-1}	CAM: 5.00 μg ml^{-1}	Čanadanović-Brunet et al. (2011)
		Bacillus cereus ATCC 10876	10.30 ± 0.58	—	0.50 mg ml^{-1}	CAM: 10.00 μg ml^{-1}	Čanadanović-Brunet et al. (2011)
Red beetroot (1, 2, 4, 8 mg/100 g)	Not determined	*Enterococcus faecalis*	—	8.30 ± 0.47 to 14.60 ± 0.47	—	—	Vijaya and Thangaraj (2019)
Red pitahaya (pulp)	Betanin, isobetanin, phyllocactin, bougainvillein	*Bacillus cereus* ATCC 11778	—	—	7.80 ± 0.20 μg ml^{-1}	PEN: 7.50 ± 0.00 μg ml^{-1} TET: Resistant CTAX: Resistant	Tenore et al. (2012)
		Staphylococcus aureus ATCC 13709	—	—	7.80 ± 0.20 μg ml^{-1}	PEN: 0.03 ± 0.00 μg ml^{-1} TET: 2.00 ± 0.30 μg ml^{-1} CTAX: 2.00 ± 0.40 μg ml^{-1}	Tenore et al. (2012)
		Enterococcus faecalis ATCC 14428	—	—	7.80 ± 0.10 μg ml^{-1}	PEN: 8.00 ± 0.00 μg ml^{-1} TET: 2.00 ± 0.60 μg ml^{-1} CTAX: Resistant	Tenore et al. (2012)
		Listeria monocytogenes ATCC 15313	—	—	7.80 ± 0.10 μg ml^{-1}	PEN: Resistant TET: Resistant CTAX: 16.00 ± 0.00 μg ml^{-1}	Tenore et al. (2012)

TABLE 1 (Continued)

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion	Broth dilution MIC	Control MIC	Reference
	Betanin, isobetanin, phyllocactin, hylocerenin	*Staphylococcus aureus* ATCC 6538P	—	—	6.25 mg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
			—	—	6250 μg ml^{-1}	CAM: 6.000 μg ml^{-1}	Yong et al. (2018)
		Staphylococcus aureus ATCC 29213	—	—	3.13 mg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
					3130 μg ml^{-1}	CAM: 6.000 μg ml^{-1}	Yong et al. (2018)
		MRSA ATCC 700699	—	—	6.25 mg ml^{-1}	CAM: 0.013 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 13.000 μg ml^{-1}	Yong et al. (2018)
		MRSA ATCC 33591	—	—	3.13 mg ml^{-1}	CAM: 0.050 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 50.000 μg ml^{-1}	Yong et al. (2018)
		MRSA ATCC 43300	—	—	3.13 mg ml^{-1}	CAM: 0.013 mg ml^{-1}	Yong et al. (2017)
					3130 μg ml^{-1}	CAM: 13.000 μg ml^{-1}	Yong et al. (2018)
		Enterococcus faecalis ATCC 29212	—	—	3.13 mg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
					3130 μg ml^{-1}	CAM: 6.000 μg ml^{-1}	Yong et al. (2018)
		Enterococcus faecium ATCC 19434	—	—	3.13 mg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
					3130 μg ml^{-1}	CAM: 3.000 μg ml^{-1}	Yong et al. (2018)
		VRE ATCC 700802	—	—	6.25 mg ml^{-1}	CAM: 0.050 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 50.000 μg ml^{-1}	Yong et al. (2018)
		Bacillus cereus ATCC 14579	—	—	6.25 mg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 3.000 μg ml^{-1}	Yong et al. (2018)
		Bacillus subtilis ATCC 8188	—	—	6.25 mg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 6.000 μg ml^{-1}	Yong et al. (2018)
Red pitahaya (peel)	Betanin, isobetanin, phyllocactin, bougainvillein	*Bacillus cereus* ATCC 11778	—	—	15.60 ± 0.20 μg ml^{-1}	PEN: 7.50 ± 0.00 μg ml^{-1} TET: Resistant CTAX: Resistant	Tenore et al. (2012)
		Staphylococcus aureus ATCC 13709	—	—	15.60 ± 0.20 μg ml^{-1}	PEN: 0.03 ± 0.00 μg ml^{-1} TET: 2.00 ± 0.30 μg ml^{-1} CTAX: 2.00 ± 0.40 μg ml^{-1}	Tenore et al. (2012)
		Enterococcus faecalis ATCC 14428	—	—	15.60 ± 0.20 μg ml^{-1}	PEN: 8.00 ± 0.00 μg ml^{-1} TET: 2.00 ± 0.60 μg ml^{-1} CTAX: Resistant	Tenore et al. (2012)
		Listeria monocytogenes ATCC 15313	—	-	15.60 ± 0.20 μg ml^{-1}	PEN: Resistant TET: Resistant CTAX: 16.00 ± 0.00 μg ml^{-1}	Tenore et al. (2012)

(Continues)

TABLE 1 (Continued)

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion	Broth dilution MIC	Control MIC	Reference
Red spinach (leaves)	Betanin, amaranthine, decarboxy-amaranthine	*Staphylococcus aureus* ATCC 6538P	—	—	3.13 μg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
		Staphylococcus aureus ATCC 29213	—	—	1.56 μg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
		MRSA ATCC 700699	—	-	1.56 μg ml^{-1}	CAM: 0.013 mg ml^{-1}	Yong et al. (2017)
		MRSA ATCC 33591	—	—	1.56 μg ml^{-1}	CAM: 0.050 mg ml^{-1}	Yong et al. (2017)
		MRSA ATCC 43300	—	—	3.13 μg ml^{-1}	CAM: 0.013 mg ml^{-1}	Yong et al. (2017)
		Enterococcus faecalis ATCC 29212	—	—	1.56 μg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
		Enterococcus faecium ATCC 19434	—	—	0.78 μg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
		VRE ATCC 700802	—	—	1.56 μg ml^{-1}	CAM: 0.050 mg ml^{-1}	Yong et al. (2017)
		Bacillus cereus ATCC 14579	—	—	1.56 μg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
		Bacillus subtilis ATCC 8188	—	—	1.56 μg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)

Abbreviations: CAM, chloramphenicol; CTAX, cefotaxime; nd, not detected; PEN, penicillin; TET, tetracycline.

TABLE 2 In vitro antibacterial activity of betalains from different sources against Gram-negative bacteria

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion	Broth dilution MIC	Control MIC	Reference
Xoconostle pear (0, 4, 6, 8, 10%)	Not determined	*Escherichia coli* O157:H7 H1730, 944, F4546, 43,895 (in combination)	—	1.40 ± 0.30 to 9.80 ± 1.01	—	—	Hayek and Ibrahim (2012)
Beetroot pomace extract (100 mg ml^{-1})	Betanin, vulgaxanthin	*Escherichia coli* ATCC 10536	—	13.33 ± 0.58	—	—	Vulic et al. (2013)
			nd	13.33 ± 0.58	—	—	Velićanski et al. (2011)
		Salmonella Typhimurium ATCC 14028	—	25.00 ± 1.00	—	—	Vulic et al. (2013)
			8.00 ± 0.00	16.00 ± 0.00, 25.00 ± 1.00	—	—	Velićanski et al. (2011)
		Pseudomonas aeruginosa	—	13.33 ± 0.58	—	-	Vulic et al. (2013)
			nd	13.33 ± 0.58	—	—	Velićanski et al. (2011)
		Citrobacter freundii	—	20.33 ± 0.58	—	—	Vulic et al. (2013)
			nd	20.33 ± 0.58	—	—	Velićanski et al. (2011)
		Citrobacter youngae	—	10.67 ± 0.60	—	—	Vulic et al. (2013)
		Enterobacter cloacae	—	12.00 ± 0.00	—	—	Vulic et al. (2013)
			nd	12.00 ± 0.00			Velićanski et al. (2011)
Beetroot pomace extract (10, 20, 50 mg ml^{-1})	Betanin, vulgaxanthin	*Escherichia coli* ATCC 10526	8.00 ± 0.00	—	1.50 mg ml^{-1}	CAM: 5.00 μg ml^{-1}	Čanadanović-Brunet et al. (2011)
		Pseudomonas aeruginosa ATCC 27853	nd	—	4.50 mg ml^{-1}	CAM: 50.00 μg ml^{-1}	
Red beetroot (1, 2, 4, 8 mg/100 g)	Not determined	*Escherichia coli*	—	8.30 ± 0.47 to 11.00 ± 0.81	—	—	Vijaya and Thangaraj (2019)
		Klebsiella pneumoniae	—	10.30 ± 0.47 to 14.60 ± 0.47	—	—	
		Proteus spp.	—	nd	—	—	
		Acinetobacter baumannii	—	8.30 ± 0.47, 11.00 ± 0.81	—	—	
		Pseudomonas aeruginosa	—	8.30 ± 0.47	—	—	
		Salmonella spp.	—	11.00 ± 0.81, 12.00 ± 0.81	—	—	
Red pitahaya (pulp)	Betanin, isobetanin, phyllocactin, bougainvillein	*Escherichia coli* ATCC 25922	—	—	13.30 ± 0.00 μg ml^{-1}	PEN: 64.0 ± 0.40 μg ml^{-1} TET: 32.0 ± 0.20 μg ml^{-1} CTAX: 32.0 ± 0.10 μg ml^{-1}	Tenore et al. (2012)
		Proteus mirabilis ATCC 7002	—	—	15.60 ± 0.00 μg ml^{-1}	PEN: 4.00 ± 0.00 μg ml^{-1} TET: 32.0 ± 0.10 μg ml^{-1} CTAX: 0.03 ± 0.00 μg ml^{-1}	

(Continues)

TABLE 2 (Continued)

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion	Broth dilution MIC	Control MIC	Reference
		Proteus vulgaris ATCC 12454	—	—	15.60 ± 0.10 μg ml^{-1}	PEN: 4.00 ± 0.30 μg ml^{-1} TET: Resistant CTAX: 2.00 ± 0.10 μg ml^{-1}	
		Pseudomonas aeruginosa ATCC 27853	—	—	62.50 ± 0.10 μg ml^{-1}	PEN: Resistant TET: 32.00 ± 0.10 μg ml^{-1} CTAX: 16.00 ± 0.00 μg ml^{-1}	
		Salmonella typhi Ty2 ATCC 19430	—	—	31.50 ± 0.20 μg ml^{-1}	PEN: 4.00 ± 0.00 μg ml^{-1} TET: 1.00 ± 0.30 μg ml^{-1} CTAX: 0.50 ± 0.10 μg ml^{-1}	
		Enterobacter cloacae ATCC 10699	—	—	31.30 ± 0.00 μg ml^{-1}	PEN: 4.00 ± 0.00 μg ml^{-1} TET: Resistant CTAX: Resistant	
		Enterobacter aerogenes ATCC 13048	—	—	31.30 ± 0.40 μg ml^{-1}	PEN: 4.00 ± 0.00 μg ml^{-1} TET: Resistant CTAX: Resistant	
		Yersinia enterocolitica ATCC 23715	—	—	62.50 ± 0.10 μg ml^{-1}	PEN: 18.00 ± 0.60 μg ml^{-1} TET: 8.00 ± 0.00 μg ml^{-1} CTAX: 0.10 ± 0.00 μg ml^{-1}	
		Klebsiella pneumoniae ATCC 27736	—	—	62.50 ± 0.30 μg ml^{-1}	PEN: Resistant TET: 16.00 ± 0.10 μg ml^{-1} CTAX: 0.10 ± 0.00 μg ml^{-1}	
	Betanin, isobetanin, phyllocactin, hylocerenin	*Shigella flexneri* ATCC 12022	—	—	6.25 mg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 3.000 μg ml^{-1}	Yong et al. (2018)
		Salmonella Typhimurium ATCC 14028	—	—	3.13 mg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
					3130 μg ml^{-1}	CAM: 3.000 μg ml^{-1}	Yong et al. (2018)
		Klebsiella pneumoniae ATCC 10031	—	—	3.13 mg ml^{-1}	CAM: 0.006 mg ml^{-1}	Yong et al. (2017)
			—	—	6250 μg ml^{-1}	CAM: 6.000 μg ml^{-1}	Yong et al. (2018)
		Escherichia coli ATCC 25922	—	—	6.25 mg ml^{-1}	CAM: 0.002 mg ml^{-1}	Yong et al. (2017)
					6250 μg ml^{-1}	CAM: 2.000 μg ml^{-1}	Yong et al. (2018)
		Pseudomonas aeruginosa ATCC 10145	—	—	6.25 mg ml^{-1}	CAM: 0.100 mg ml^{-1}	Yong et al. (2017)

TABLE 2 (Continued)

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion Zone of inhibition (mm)	Broth dilution MIC	Control MIC	Reference
					$6250\,\mu g\,ml^{-1}$	CAM: $100.000\,\mu g\,ml^{-1}$	Yong et al. (2018)
		Pseudomonas aeruginosa ATCC BAA-47	—	—	$6.25\,mg\,ml^{-1}$	CAM: $0.025\,mg\,ml^{-1}$	Yong et al. (2017)
					$6250\,\mu g\,ml^{-1}$	CAM: $25.000\,\mu g\,ml^{-1}$	Yong et al. (2018)
Red pitahaya (peel)	Betanin, isobetanin, phyllocactin, bougainvillein	*Escherichia coli* ATCC 25922	—	—	$13.30 \pm 0.40\,\mu g\,ml^{-1}$	PEN: $64.0 \pm 0.40\,\mu g\,ml^{-1}$ TET: $32.0 \pm 0.20\,\mu g\,ml^{-1}$ CTAX: $32.0 \pm 0.10\,\mu g\,ml^{-1}$	Tenore et al. (2012)
		Proteus mirabilis ATCC 7002	—	—	$62.50 \pm 0.40\,\mu g\,ml^{-1}$	PEN: $4.00 \pm 0.00\,\mu g\,ml^{-1}$ TET: $32.0 \pm 0.10\,\mu g\,ml^{-1}$ CTAX: $0.03 \pm 0.00\,\mu g\,ml^{-1}$	
		Proteus vulgaris ATCC 12454	—	—	$31.30 \pm 0.20\,\mu g\,ml^{-1}$	PEN: $4.00 \pm 0.30\,\mu g\,ml^{-1}$ TET: Resistant CTAX: $2.00 \pm 0.10\,\mu g\,ml^{-1}$	
		Pseudomonas aeruginosa ATCC 27853	—	—	$62.50 \pm 0.00\,\mu g\,ml^{-1}$	PEN: Resistant TET: $32.00 \pm 0.10\,\mu g\,ml^{-1}$ CTAX: $16.00 \pm 0.00\,\mu g\,ml^{-1}$	
		Salmonella typhi Ty2 ATCC 19430	—	—	$62.50 \pm 0.00\,\mu g\,ml^{-1}$	PEN: $4.00 \pm 0.00\,\mu g\,ml^{-1}$ TET: $1.00 \pm 0.30\,\mu g\,ml^{-1}$ CTAX: $0.50 \pm 0.10\,\mu g\,ml^{-1}$	Tenore et al. (2012)
		Enterobacter cloacae ATCC 10699	—	—	$31.30 \pm 0.40\,\mu g\,ml^{-1}$	PEN: $4.00 \pm 0.00\,\mu g\,ml^{-1}$ TET: Resistant CTAX: Resistant	
		Enterobacter aerogenes ATCC 13048	—	—	$62.50 \pm 0.40\,\mu g\,ml^{-1}$	PEN: $4.00 \pm 0.00\,\mu g\,ml^{-1}$ TET: Resistant CTAX: Resistant	
		Yersinia enterocolitica ATCC 23715	—	—	$62.50 \pm 0.00\,\mu g\,ml^{-1}$	PEN: $18.00 \pm 0.60\,\mu g\,ml^{-1}$ TET: $8.00 \pm 0.00\,\mu g\,ml^{-1}$ CTAX: $0.10 \pm 0.00\,\mu g\,ml^{-1}$	
		Klebsiella pneumoniae ATCC 27736	—	—	$125.00 \pm 0.20\,\mu g\,ml^{-1}$	PEN: Resistant TET: $16.00 \pm 0.10\,\mu g\,ml^{-1}$ CTAX: $0.10 \pm 0.00\,\mu g\,ml^{-1}$	

(Continues)

TABLE 2 (Continued)

Source of betalains	Betalain composition	Species	Disc diffusion Zone of inhibition (mm)	Agar-well diffusion	Broth dilution MIC	Control MIC	Reference
Red spinach (leaves)	Betanin, amaranthine, decarboxy-amaranthine	*Shigella flexneri* ATCC 12022	—	—	3.13 mg ml^{-1}	CAM: 0.003 mg ml^{-1}	Yong et al. (2017)
		Salmonella Typhimurium ATCC 14028	—	—	3.13 mg ml^{-1}	CAM: 0.003 mg ml^{-1}	
		Klebsiella pneumoniae ATCC 10031	—	—	1.56 mg ml^{-1}	CAM: 0.006 mg ml^{-1}	
		Escherichia coli ATCC 25922	—	—	3.13 mg ml^{-1}	CAM: 0.002 mg ml^{-1}	
		Pseudomonas aeruginosa ATCC 10145	—	—	3.13 mg ml^{-1}	CAM: 0.100 mg ml^{-1}	
		Pseudomonas aeruginosa ATCC BAA-47	—	—	3.13 mg ml^{-1}	CAM: 0.025 mg ml^{-1}	

Abbreviations: CAM, chloramphenicol; CTAX, cefotaxime; nd, not detected; PEN, penicillin; TET, tetracycline.

spectrum beta-lactamase (ESBL); an enzyme produced by some bacteria that can hydrolyse extended spectrum cephalosporin antibiotics (Ghafourian et al., 2015). All bacteria were tested for ESBL activity prior to determining inhibitory zones. ESBL activity was observed in one of the three *E. faecalis* isolates (Vijaya & Thangaraj, 2019). Despite the fact that *E. faecalis* contributes to a relatively small fraction of vancomycin-resistant Enterococci (VRE), it is one of the leading causes of nosocomial Enterococcal infections such as catheter-associated UTIs, bacteraemia, neonatal sepsis, endocarditis and surgical and burn wound infections (Ramos et al., 2020). Although *E. faecalis* is sensitive to vancomycin, it exhibits significant resistance to popular antibiotics such as cephalosporins and macrolides. Clinical isolates from UTIs have also shown resistance to tetracyclines, minocycline and erythromycin (Ma, Zhang, et al., 2021).

Tenore et al. (2012) found that betacyanin-rich fractions of red pitahaya had antimicrobial effects against *E. faecalis*, *B. cereus*, *L. monocytogenes* and *S. aureus*. Betacyanin was extracted with 70% methanol and fractionated, and the betacyanin composition was bougainvillein, betanin, isobetanin and phyllocactin. The betacyanin fraction from red pitahaya flesh had higher inhibitory effects (MIC value of 7.8 μg ml^{-1}) than the betacyanin fraction from red pitahaya peel (MIC value of 15.6 μg ml^{-1}). As a result, both of these fractionated samples had higher antibacterial activity than their whole extracts. Both Tenore et al. (2012) and Vijaya and Thangaraj (2019) demonstrated the potential of betacyanin to inhibit *E. faecalis* and *L. monocytogenes* which was not observed by Vulic et al. (2013) and Velićanski et al. (2011). Possible explanations include: (a) no fractionation of the sample extracts, therefore the amount of betalain in BPE was insufficient to inhibit these bacteria; (b) a variation in the bacterial strains utilized and (c) the source from which betalains was extracted.

Betacyanin in red spinach and red pitahaya exhibited antibacterial activity against 10 Gram-positive bacterial species (Yong et al., 2017). Following betacyanin analysis, betanin was detected in both plants. Amaranthine and decarboxy-amaranthine were found in red spinach while isobetanin, phyllocactin and hylocerenin were found in red pitahaya. Two strains of *S. aureus*, three strains of methicillin-resistant *S. aureus* (MRSA), *E. faecalis*, *E. faecium*, VRE, *B. cereus* and *Bacillus subtilis* were among the micro-organisms studied. Methanol extracts of both plants inhibited each of the bacteria; however, sub-fractionated samples of each plant exhibited considerably lower MIC values, indicating greater inhibitory effects. The greater betacyanin concentration in sub-fractionated samples was thought to be responsible for the enhanced antibacterial activity when compared with corresponding methanol extracts of red spinach and red pitahaya.

Sub-fractionated red spinach outperformed red pitahaya in terms of antibacterial activity against all tested bacteria, with the exception of one MRSA strain, where there was no difference between the MIC values. The presence of amaranthine may have contributed to the higher inhibitory effects of red spinach (Yong et al., 2017). Overall, sub-fractionated red spinach had the greatest antibacterial activity (MIC of 0.78 mg ml^{-1}) against *E. faecium*, a leading nosocomial Enterococcus (Zhou et al., 2020). Though *E. faecium* is less virulent than *E. faecalis*, it is responsible for the majority of VRE (Fiore et al., 2019) and has thus been designated an ESKAPE pathogen and a high-priority Gram-positive bacterium for which new antibiotics are urgently needed (Tacconelli et al., 2018).

Yong et al. (2018) observed that betacyanin-rich extracts from the pulp of red pitahaya (including betanin, hylocerenin and phyllocactin) displayed higher antibacterial activity against 10 Gram-positive bacteria after 6 days of refrigerated storage at 4°C compared to that of freshly harvested fruits. Among the bacteria studied were two strains of *S. aureus*, three strains of MRSA, *E. faecalis*, *E. faecium*, VRE, *B. cereus* and *B. subtilis*. Betacyanin fractions from freshly harvested fruits demonstrated weak antimicrobial activity against each of the bacteria with MIC values of 50,000 μg ml^{-1} or greater. In contrast, refrigerated betacyanin samples exhibited stronger inhibitory effects with lower MIC values (3130 μg ml^{-1}) for *S. aureus* (ATCC 29213), MRSA (ATCC 43300), *E. faecalis* and *E. faecium* compared to the rest of the Gram-positive bacteria (MIC of 6250 μg ml^{-1}). After 6 days of refrigerated storage, the betacyanin content rose by 57.2% from 0.38 to 0.63 g kg^{-1}, which may have enhanced the antibacterial action. It was suggested that the rise in betacyanin content was caused by the presence of water in the whole fruit, which caused hydrolysis and the synthesis of betalamic acid, which then recondensed with *cyclo*-DOPA to produce more betacyanin. Furthermore, the enzymes involved in betacyanin formation (glucosyltransferases and acyltransferases) may have worked at a slower pace at lower temperatures, yielding more betacyanin (Yong et al., 2018).

Betanin from beetroot demonstrated anti-infective effects against MRSA (ATCC 33591) in vivo using a *Caenorhabditis elegans* infection model (Choo et al., 2020). The infection assay was initiated by adding overnight grown MRSA culture together with worm M9 buffer into a 24-well plate before the worms were transferred. Following this, betanin concentrations of 100, 200, 300 and 400 μg ml^{-1} based on the MIC value of betanin against MRSA (>20 mg ml^{-1}) were added to the wells, after which the worms were then transferred and incubated at 25°C. The survival of *C. elegans* was then monitored every 24 h. The mean time to death (TD_{mean}) of infected *C. elegans* was prolonged following betanin treatment at all concentrations compared to the control (without treatment). The greatest effect of betanin treatment on the survival of infected worms occurred at 200 μg ml^{-1}, with a prolonged lifespan lasting up to 76 h. However, the TD_{mean} began to decrease after 200 μg ml^{-1}, indicating that the anti-infective effects did not occur in a dose-dependent manner. Nonetheless, the fact that betalain treatment did demonstrate anti-infective activity against MRSA-infected worms at all doses highlights the potential of betanin as an antimicrobial agent. Anti-infective substances affect the virulent properties of bacteria, allowing the host immune system to clear the infection (Choo et al., 2020). Future research should focus on establishing the underlying mechanism of the anti-infective properties of betanin.

Gram-negative bacteria

Betalain-containing extracts of *Opuntia matudae*, also known as xoconostle pear, suppressed the growth of four strains of *Escherichia coli* O157:H7 (H1730, 944, F4546, 43,895) (Hayek & Ibrahim, 2012). Though the specific composition of betalain was not determined, it has previously been proven that xoconostle pears are mostly composed of betanin, isobetanin and betanidin (Sanchez-Gonzalez et al., 2013). The bacteria were treated with varying concentrations (0, 4, 6, 8, 10%) of xoconostle extract and incubated in brain heart infusion (BHI) broth for 8 h at 37°C before the evaluation of changes in bacterial growth via growth over time assay. Optical density was measured at 2 h intervals in order to monitor bacterial growth and the final populations were then determined by the end of the 8 h incubation period. This study showed a reduction in the final population (log CFU ml^{-1}) of all *E. coli* O157:H7 strains after treatment. Xoconostle extract concentrations of 4, 6 and 8% reduced bacterial growth in a dose-dependent manner, whereas 10% inhibited bacterial growth in all strains. In the absence of xoconostle extract, *E. coli* O157:H7 growth persisted and reached the stationary phase. The inhibition zones of all *E. coli* O157:H7 strains (as a combination) against xoconostle extract were determined using the agar well diffusion technique after a 12 h incubation at 37°C. To produce varied concentrations, different amounts of xoconostle extract (from 200 to 1000 μl with 25 μl unit increments) were prepared to 1 ml with distilled water. The data revealed that as extract concentration grew, so did the inhibition zones. During the 12 h incubation, the lowest volume concentration that had a significant inhibitory effect was 400 μl ml^{-1}. The minimum lethal volume (lowest volume concentration that had the strongest inhibitory effects 3 days after incubation) was determined to be 650 μl ml^{-1}, resulting in an inhibition zone of 2.8 ± 0.25 mm. The results of the agar

well diffusion experiment agree with those of the growth over time assay, demonstrating that xoconostle pears have antibacterial properties against *E. coli* O157:H7. *E. coli* is an Enterobacteriaceae; a Gram-negative bacterial family that the World Health Organization (WHO) has identified as a critical priority in the continuing antibiotic resistance challenge (Tacconelli et al., 2018). Most UTIs and hospital-acquired pneumonia are caused by pathogenic *E. coli*. Other infections include myositis, meningitis, osteomyelitis, and, in extreme situations, bloodstream infections (Shebl & Gulick, 2021; Vila et al., 2016). Intestinal *E. coli* pathotypes are strongly linked to diarrheal diseases as well as other clinical manifestations such as haemolytic uraemic syndrome and haemorrhagic colitis (Ameer et al., 2021). In some areas, the proportion of resistant *E. coli* isolates from bloodstream infections to routinely used antibiotics such as fluoroquinolones and third-generation cephalosporins (3GC) might be as high as 60% (Kern & Rieg, 2020). Resistance to ciprofloxacin, trimethoprim-sulphamethoxazole and fluoroquinolone has also been identified in *E. coli* urine isolates (Kaye et al., 2021).

Vulic et al. (2013) discovered that 100 mg ml^{-1} BPE containing betanin and vulgaxanthin inhibited the growth of six Gram-negative bacteria. Inhibitory effects were observed against all tested bacteria: *E. coli*, *P. aeruginosa*, *Enterobacter cloacae* and *Citrobacter youngae*, with the highest inhibition zones reported in *Salmonella* Typhimurium and *Citrobacter freundii* at 25.0 ± 1.0 mm and 20.33 ± 0.58 mm respectively. Similarly, Velićanski et al. (2011) found that 100 µl of BPE enhanced its inhibitory effects against *E. coli*, *P. aeruginosa*, *E. cloacae*, with the largest inhibitory effect reported against *S*. Typhimurium followed by *C. freundii*. *S*. Typhimurium was the most susceptible Gram-negative bacteria to BPE with inhibitory effects observed against all tested volumes (15, 50 and 100 µl) in a dose-dependent manner (8.0 ± 0.0, 16.0 ± 0.0 and 25.0 ± 1.0 mm respectively). Vulic et al. (2013) and Velićanski et al. (2011) observed larger inhibitory zones produced by the control (cefotaxime/clavulanic acid) than those formed after treatment with BPE for each of the bacteria. *S*. Typhimurium is one of the most predominant serotypes of Salmonella to cause gastroenteritis, whereas *C. freundii* causes UTIs and is linked to a variety of infections including the respiratory tract, wounds, meninges, liver, peritoneum and the bloodstream (Liu et al., 2021; Xiang et al., 2020). Multi-drug resistance (MDR) is becoming more common in *Citrobacter* spp. and *S*. Typhimurium. Resistance to piperacillin, broad-spectrum cephalosporins such as ceftazidime, ceftriaxone and piperacillin/tazobactam has been seen in 39–48% of *C. freundii* isolates—popular medications used to treat *C. freundii* infections. Fluoroquinolone-resistant isolates have also been reported (Liu et al., 2021). Most *S*. Typhimurium outbreaks have been caused by MDR strains—those that are resistant to a variety of commonly used antibiotics such as ampicillin, streptomycin, sulphonamides, tetracycline and chloramphenicol. This makes clinical treatment of *S*. Typhimurium challenging, and as a result, morbidity and mortality rates have increased; hence novel or alternative treatment options are required (Wang et al., 2019; Xiang et al., 2020).

At BPE doses of 10, 20 and 50 mg ml^{-1}, Čanadanović-Brunet et al. (2011) evaluated the antibacterial properties of BPE ethanol extract against *E. coli* and *P. aeruginosa*. Both pathogens showed only slight susceptibility to the extract's antibacterial action. The zone of inhibition and MIC obtained for *E. coli* were 8 mm and 1.5 mg ml^{-1}, respectively, but *P. aeruginosa* had no zone of inhibition and a MIC of 4.5 mg ml^{-1} was acquired to yield findings.

A minimum concentration of 50 and 5.0 µg ml^{-1} of chloramphenicol antibiotic (control) was required to inhibit *P. aeruginosa* and *E. coli* respectively. Similar to *E. coli*, *P. aeruginosa* is an ESKAPE pathogen that has also been recognized as a critical priority bacterium for which new antibiotics are needed (Tacconelli et al., 2018). Vulic et al. (2013) and Velićanski et al. (2011) obtained inhibition zones of 13.33 ± 0.58 mm for both *E. coli* and *P. aeruginosa* after BPE treatment. The higher inhibitory effects shown in these two investigations compared to Čanadanović-Brunet et al. (2011) might be attributed to changes in extraction procedures, concentrations and volume of extract employed, which may have influenced the composition of components in beetroot pomace.

Red beetroot extracts containing betanin, isobetanin and vulgaxanthin inhibited five of six ESBL-producing Gram-negative bacterial species: *A. baumannii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Salmonella* spp., except *Proteus* spp. (Vijaya & Thangaraj, 2019). This could have been because the bioactive components of red beetroot were of insufficient quantity to generate inhibitory effects. The strongest antibacterial activity was recorded against *K. pneumoniae*, followed by *E. coli*. Both showed no impact at 1 mg of extract, but higher doses of 2, 4 and 8 mg caused inhibitory zones of 10.3 ± 0.47, 12.6 ± 0.47 and 14.6 ± 0.47 mm in *K. pneumoniae*, respectively, and 8.3 ± 0.47, 9.3 ± 0.47 and 11 ± 0.81 mm in *E. coli* respectively. In *A. baumannii* and *Salmonella* spp., a minimum of 4 mg betalain was required to induce inhibitory effects, while *P. aeruginosa* demonstrated the least susceptibility, with an observable inhibitory effect at only 8 mg betalain. *K. pneumoniae* is an ESKAPE pathogen that causes about one-third of Gram-negative infections such as UTI, pneumonia, wound infections, cystitis, endocarditis and septicaemia (Effah et al., 2020). Being a member of the Enterobacteriaceae family, *K. pneumoniae* contributes significantly to the drug-resistant bacterial population

(Tacconelli et al., 2018). In addition to ESBLs, certain *K. pneumoniae* isolates possess carbapenemase genes, allowing them to survive carbapenem medicines—a last-resort antibiotic normally used to treat MDR strain infections (Effah et al., 2020). WHO has also designated both the ESKAPE pathogens *A. baumannii* and *P. aeruginosa* as critical priority bacteria in the antimicrobial resistance problem due to their increasing carbapenem-resistant characteristic (Tacconelli et al., 2018).

Tenore et al. (2012) discovered antimicrobial effects of betacyanin-rich fractions derived from red pitahaya against nine Gram-negative bacteria: *E. cloacae*, *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhi* and *Klebsiella aerogenes* (formerly known as *Enterobacter aerogenes*), *Yersinia enterocolitica* and *K. pneumoniae*. There was no difference in the inhibitory effect of betacyanin fraction from red pitahaya flesh and that of the peel against *E. cloacae*, *E. coli*, *P. aeruginosa* and *Y. enterocolitica*. The betacyanin fraction from the flesh was shown to be more effective (by at least 50%) than the peel against all other Gram-negative bacteria tested. Nonetheless, fractionated samples from both the flesh and peel had significantly higher inhibitory effects than the whole extracts.

Betacyanins in red spinach and red pitahaya demonstrated antimicrobial effects against six Gram-negative bacteria; *E. coli*, *K. pneumoniae*, *Shigella flexneri*, *S.* Typhimurium and two strains of *P. aeruginosa* (Yong et al., 2017). Similar to the results against Gram-positive bacteria, lower MIC values were obtained for the sub-fractionated samples of red spinach and red pitahaya against the tested Gram-negative bacteria. It is likely that the increased betacyanin content in sub-fractionated samples induced a stronger inhibitory effect against the bacteria. Sub-fractionated red spinach exhibited stronger antimicrobial activity compared to that of red pitahaya, possibly due to the difference in betalain composition—particularly, the presence of amaranthine in red spinach. The highest antimicrobial activity from sub-fractionated red spinach was observed against *K. pneumoniae* with a MIC of 1.56 mg ml^{-1}, while that of its methanol extract was 12.5 mg ml^{-1}. Similarly, the highest antimicrobial effect of sub-fractionated red pitahaya was 3.13 mg ml^{-1} for both *K. pneumoniae* and *S.* Typhimurium compared to its methanol extract which exhibited a MIC of 12.5 mg ml^{-1} for both.

Betacyanin fractions from red pitahaya pulp containing betanin, isobetanin, hylocerenin and phyllocactin displayed antibacterial activity against six Gram-negative bacteria: *S. flexneri*, *S.* Typhimurium, *K. pneumoniae*, *E. coli* and two strains of *P. aeruginosa* (Yong et al., 2018). The betacyanin fractions from freshly harvested fruits demonstrated lower antimicrobial activity (MIC values of 50,000 μg ml^{-1} or above) when compared to that of fruits stored for 6 days at 4°C. Among these bacteria, betacyanin fraction from fruits stored for 6 days at 4°C inhibited *S.* Typhimurium the most (MIC value of 3130 μg ml^{-1}) compared to the rest of the Gram-negative bacteria (MIC value of 6250 μg ml^{-1}).

Anti-biofilm activity

Biofilms are complex communities of microorganisms grown in multicellular aggregates and encapsulated in a self-produced extracellular matrix that adheres to a variety of biotic or abiotic surfaces (Lopez et al., 2010). Generally, biofilm-producing bacteria are around 1000 times more resistant to antimicrobials than planktonic bacteria (Thi et al., 2020). There are various differences in the drug resistance mechanisms of biofilm-producing bacteria compared to that of planktonic bacteria, even towards the same antimicrobial agents. However, in some cases these resistance mechanisms may combine, leading to an increase in drug resistance of the biofilm-producing bacteria (Yong, Dykes, & Choo, 2019). Research on the antibiofilm activity of betalains is fairly scarce and should be evaluated further.

Betacyanin fractions from red spinach and red pitahaya demonstrated anti-biofilm activity against five *S. aureus* strains and four *P. aeruginosa* strains (Yong, Dykes, Lee, & Choo, 2019). The anti-biofilm activity of betacyanin fractions from each plant was determined using a broth microdilution assay, and the minimum biofilm inhibitory concentration (MBIC) was determined. Anti-biofilm activity was detected against all *S. aureus* strains from both plants, with the betacyanin fraction from red spinach exhibiting stronger inhibitory effects than that of red pitahaya. The MBIC of betacyanin from red spinach was 0.313 mg ml^{-1} for the majority of the strains, with the exception of one MRSA strain (*S. aureus* ATCC 700699), which required an MBIC of 1.5 mg ml^{-1} to inhibit biofilm production, but it was also reported that this particular strain was a weak biofilm producer. Compared to red spinach, the betacyanin fraction from red pitahaya had greater anti-biofilm effects against *P. aeruginosa*; a dosage less than 1 mg ml^{-1} was able to suppress biofilm formation in all four strains. The anti-biofilm action of each plant may have varied owing to its unique betacyanin structure. In red spinach, the greater biofilm inhibitory activity of betacyanin against *S. aureus* may be attributed to amaranthine, which is more polar than other betacyanins from both plants (betanin, hylocerenin, phyllocactin) (Yong, Dykes, Lee, & Choo, 2019). This was validated by testing the anti-biofilm activity of isolated amaranthine against all *S. aureus* and *P. aeruginosa* strains. Similar inhibitory effects were observed, thus the hydrophilicity

of amaranthine may have interfered with bacterial surface charge, affecting bacterial adhesion. However, red pitahaya betacyanin demonstrated superior anti-biofilm activity against *P. aeruginosa*, presumably owing to phyllocactin. The acyl groups may have reacted with the bacterium, lowering its surface hydrophobicity and therefore preventing it from adhering to the surface. Both plant betacyanin fractions had anti-biofilm effects against *P. aeruginosa* and *S. aureus* on polystyrene and glass surfaces. The red spinach betacyanin fraction demonstrated greater benefits than the red pitahaya betacyanin fraction against bacterial adhesion on the polystyrene surface. The capacity of betacyanins to lower bacterial cell surface hydrophobicity may have reduced bacterial adherence to the polystyrene surface, reducing bacterial biofilm development (Yong, Dykes, Lee, & Choo, 2019).

Yong et al. (2021) exhibited anti-biofilm activity of mixed betacyanin fractions from red spinach and red pitahaya on diverse polymer surfaces, including polyvinyl chloride, polyethylene, polypropylene and silicone rubber, against a co-culture of *S. aureus* and *P. aeruginosa*. Following up from Yong, Dykes, Lee, and Choo (2019), betacyanin concentrations (0.313 to 2.5 mg ml^{-1}) from both plants were tested as a combination against 5 *S. aureus* strains and 4 *P. aeruginosa* strains. A concentration of 0.625 mg ml^{-1} of each betacyanin fraction was shown to have anti-biofilm capabilities against all tested strains of *S. aureus* and *P. aeruginosa*. In the absence of the mixed betacyanin fractions, dual-species biofilm formation and cell attachment of *S. aureus* and *P. aeruginosa* were greater across the different polymer surfaces. The combined betacyanin fractions (0.625 mg ml^{-1}) were found to include 29.6 ± 0.6% amaranthine, 25.5 ± 0.4% phyllocactin, 23.6 ± 0.1% betanin, 8.4 ± 0.2% decarboxy-amaranthine, 6.7 ± 0.4% hylocerenin and 6.4 ± 0.1% isobetanin. Biofilm formation was achieved using a co-culture of *S. aureus* (ATCC 6538P) and *P. aeruginosa* (ATCC 27853), as both strains are efficient biofilm producers, and the chosen betacyanin formulation fraction suppressed biofilm formation of these strains in co-culture more than that of single betacyanin fractions. The betacyanin formulation fraction reduced the growth of dual-species biofilms on all polymer surfaces tested. This was thought to be accomplished by obstructing the interaction between the bacteria and the surfaces, hence reducing bacterial attachment. With the exception of *P. aeruginosa* on polyvinyl chloride surfaces, the quantity of *S. aureus* and *P. aeruginosa* on all polymer surfaces decreased after treatment with the mixed betacyanin fraction. Silicone rubber, which has the most hydrophobic surface of the polymers investigated, showed the greatest biofilm suppression and cell attachment decreased after betacyanin treatment.

S. aureus biofilms are responsible for endocarditis, ventilator-associated pneumonia, implant-related infections, central line-associated infections and surgical site infections (Dauros-Singorenko et al., 2020) whereas *P. aeruginosa* biofilms are notoriously known for their lethal association with cystic fibrosis (Olivares et al., 2020). *S. aureus* and *P. aeruginosa* can coexist in different types of infections, for example chronic wound infections. Dual-species biofilms produced by these bacteria increase their virulence and tolerance, making them more resistant to antibiotics (Reigada et al., 2021). The findings from Yong et al. (2019) and Yong et al. (2021) show the potential of betacyanin to be used as an anti-biofilm agent and should therefore be explored further in future investigations.

Antiviral activity

Dengue virus

Dengue is a vector-borne viral infection that predominantly affects tropical and subtropical countries, but has the potential to spread to other geographical regions. There are four distinct serotypes of the dengue virus (DENV-1 to DENV-4), each of which is primarily transmitted to humans by the *Aedes aegypti* mosquito (Guzman et al., 2017), though DENV-2 is considered the most lethal (Abd Kadir et al., 2013). Chang et al. (2020) reported antiviral activity of betacyanin fractions from red spinach and red pitahaya against dengue virus serotype 2 (DENV-2). Phyllocactin (51.3%) was the most common betacyanin identified in the betacyanin fraction from red pitahaya, followed by betanin (28.98%), hylocerenin (14.12%) and isobetanin (5.57%). The most common betacyanin found in the betacyanin fraction from red spinach was amaranthine (70.27%), followed by decarboxy-amaranthine (21.57%) and betanin (8.15%). Both betacyanin fractions displayed dose-dependent antiviral activity against DENV-2 in infected Vero cells via virus yield inhibition assay. The lower half minimum inhibitory concentration (IC_{50}) of betacyanin fraction from red spinach (14.62 μg ml^{-1}) and higher selectivity index of 28.51 compared to that of red pitahaya (125.8 μg ml^{-1} and 5.28 respectively) indicates that betacyanin from red spinach may be more effective, safer and capable of inhibiting DENV-2 at a lower dose. A maximal non-toxic betacyanin concentration from red pitahaya and red spinach of 379.5 μg ml^{-1} and 172.6 μg ml^{-1} displayed direct virucidal effects against DENV-2 with an IC_{50} of 126.70 μg ml^{-1} and 106.8 μg ml^{-1}, resulting in 95% and 65.9% viral inhibition respectively. This suggests that betacyanins may have direct antiviral effects on extracellular DENV-2 particles by binding to a non-structural

protein, namely the envelope (E) protein, preventing the virus from initiating virus infection by interfering with the ability of DENV-2 to attach to host cells.

Further research on assessing the cellular processes of DENV-2 impacted by betacyanin treatment should be conducted in the future to discover how betacyanins affect DENV-2 cellular activities throughout its life cycle. Following these studies, in vivo tests should be conducted to evaluate the safety, efficacy and drug delivery of betacyanin. Even though 80% of the 100–400 million annual DENV infections are mild and asymptomatic, approximately 96 million (67–136 million) infections result in clinical manifestations each year and severe dengue could be fatal (World Health Organization, 2022). As there is no antiviral medication available yet for DENV infections, more research in discovering therapeutics for DENV infections are needed.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Molecular docking simulations revealed that betacyanin from super red dragon fruit (*Hylocereus costaricensis*) has a high binding affinity for four key proteins from SARS-CoV-2: Main Protease (M^{pro}), Spike Glycoprotein (S), spike ectodomain structure and receptor binding domain (Tallei et al., 2021). Betacyanin had the strongest binding affinity with the receptor-binding domain ($-9.5\,kcal\,mol^{-1}$), followed by Spike Glycoprotein ($-9.2\,kcal\,mol^{-1}$), spike ectodomain structure ($-8.6\,kcal\,mol^{-1}$) and Main Protease ($-7.4\,kcal\,mol^{-1}$). The binding affinities of nelfinavir and hydroxychloroquine sulphate against the same receptors were examined for comparison. Betacyanin was discovered to have a higher affinity for Spike Glycoprotein, spike ectodomain structure and receptor binding domain than nelfinavir and hydroxychloroquine sulphate. It is important to note that hydroxychloroquine sulphate has been discontinued from COVID-19 drug trials (World Health Organization, 2020) though nelfinavir remains a potential candidate (Ohashi et al., 2021). Despite having higher binding affinities, betacyanin did not meet the Lipinski's Rule of Five (RO5) requirement for optimal drug-likeness of a lead compound as its molecular weight is more than 500 Dalton and there were more hydrogen bond donors and hydrogen bond acceptors than recommended. Nonetheless, the octanol–water partition coefficient (Log P) of betacyanin was within the RO5 range (<5), with a value of −2735, indicating that betacyanin has a strong polarity, allowing it to readily pass through permeable cell membranes. Overall, betacyanin exhibits the potential to act as an inhibitor against SARS-CoV-2 despite violating three of the four RO5 criteria.

Lucas-Gómez et al. (2020) reported comparable interactions between betalain and the SARS-CoV-2 nsp12 protein using in silico approaches, albeit the authors stated that any possible inhibitory effects would be of small magnitude. It must be noted that the specific betalain molecule studied was not stated in the research. In future investigations, lead structure optimization of betacyanin could be performed in such a manner that any drug-like physicochemical features are preserved while increasing its bioactivity and selectivity. The ongoing COVID-19 pandemic, caused by SARS-CoV-2, highlights the critical need for effective antiviral medications. This research lays the groundwork for further investigation of the potential antiviral effects of betacyanin against SARS-CoV-2.

Antifungal activity

Moulds

Beetroot pomace extract was tested against plant pathogens *Aspergillus niger* and *Penicillium aurantiogriseum*, though no antimicrobial activity was detected (Vulic et al., 2013). Betacyanin-rich fractions of red pitahaya flesh and peel composed of bougainvillein, betanin, isobetanin and phyllocactin exhibited antifungal activity against *Aspergillus flavus*, *Botrytis cinerea*, *Cladosporium herbarum* and *Fusarium oxysporum* with MIC values of 500 $\mu g\,ml^{-1}$, while the control (econasol) MIC values ranged from 3 to 4 $\mu g\,ml^{-1}$ (Tenore et al., 2012). Unfractionated samples of the flesh and peel of red pitahaya had no inhibitory effects against any of the tested moulds. This suggests that betacyanin needs to be isolated in future studies in order to test its full antifungal potential; other constituents in whole extracts may not work well with betacyanin to suppress the growth of these moulds.

The growth of *B. cinerea*, responsible for grey mould disease, was suppressed in betalain-producing tobacco flowers (Polturak et al., 2017). The tobacco plants were introduced with a vector (pX11) containing three betalain-related genes (CYP76AD1, BvDODA1 and cDOPA5GT) that predominantly made betacyanins (betanin and isobetanin). Antifungal activity of betalains was assessed by determining plant resistance toward *B. cinerea* in wild-type against pX11-expressing tobacco plants. The magnitude of *B. cinerea* infection was determined by measuring lesions formed in plant leaves after being exposed to droplets of *B. cinerea* spore suspension. Relative to the wild-type plants, the infected

pX11-expressing plants displayed a 90% reduction in average lesion area 2 days after infection, and only 4% of leaves reached a state of advanced necrosis 3 days post-infection. Between the second and third day post-infection, the size of lesions increased in both pX11 and wild-type plants though they were still considerably smaller (around 60%) in pX11 leaves. Evidently, betalain-producing tobacco plants have higher survival chances and increased resistance towards plant pathogen *B. cinerea* compared to control plants.

Polturak et al. (2017) reported that the occurrence of red depigmentation around infection sites on pX11 leaves indicates a possible fungal resistance mechanism. Because of their ability to scavenge reactive oxygen species (ROS), betalains were degraded (causing depigmentation), delaying plant cell death and necrotrophic fungal development. Over 1000 plant species worldwide are affected by *B. cinerea*; the second most prevalent pathogenic plant fungus (Reboledo et al., 2020). Antifungal resistance, including MDR, has become a growing problem with *B. cinerea* infections (Chatzidimopoulos et al., 2016), necessitating the development of novel antifungal agents to manage these disease outbreaks which affect a large variety of crops, for example tomato, lettuce, beans, strawberry, grape, etc.

Yeasts

To the authors' best knowledge, there is only one study that demonstrated the antifungal activity of betalain against yeasts. Betalains have been proven to inhibit a diverse range of microorganisms, though research into their mechanism of antimicrobial activity is limited. Forthcoming studies should examine the precise cellular and molecular mechanism of microbial inhibition (Gengatharan et al., 2015).

Tenore et al. (2012) discovered the antifungal activity of betacyanin against yeasts *Candida albicans*; an opportunistic fungal pathogen of humans, and *Rhizoctonia solani*; a necrotrophic plant pathogen (Abdelghany et al., 2022; Hernday et al., 2010). Betacyanin-rich fractions of red pitahaya flesh and peel exhibited antifungal activity against both yeasts, although whole extracts did not. The flesh fraction inhibited *C. albicans* more effectively (MIC value of 250 $\mu g\,ml^{-1}$) than the peel fraction (MIC value of 500 $\mu g\,ml^{-1}$), whereas both fractions inhibited *R. solani* at 125 $\mu g\,ml^{-1}$. The MICs of the control (amphotericin) for *C. albicans* and *R. solani* were 1.00 $\mu g\,ml^{-1}$. Beetroot pomace extract was tested against *C. albicans* and *Saccharomyces cerevisiae*; an emerging fungal pathogen, however, inhibitory effects were not observed against either of these yeasts (Vulic et al., 2013).

Antimalarial activity

Stembark extracts of spiny amaranth (*Amaranthus spinosus*) containing amaranthine and erect spiderling (*Boerhaavia erecta*) containing betanin demonstrated antimalarial effects against mice inoculated with erythrocytes parasitized with *Plasmodium berghei* (Hilou et al., 2006). Different doses of each plant extract were tested against infected mice based on the relative toxicity (LD_{50}) of spiny amaranth and erect spiderling (1450 $mg\,kg^{-1}$ and 2150 $mg\,kg^{-1}$ respectively). Results from the in vivo mouse model assay showed that all doses of erect spiderling (50 to 1000 $mg\,kg^{-1}$) and spiny amaranth (100 to 900 $mg\,kg^{-1}$) inhibited *P. berghei* in a dose-dependent manner, while maximum doses suppressed parasitaemia at $55.21 \pm 0.52\%$ and $53.10 \pm 0.79\%$ respectively. Chloroquine, an antimalarial medication, inhibited parasitaemia by $56 \pm 2.20\%$ at a dose as low as 2.5 $mg\,kg^{-1}$. The effective dose for 50% inhibition (ED_{50}) was found to be $564.95 \pm 6.23\,mg\,kg^{-1}$, $789.36 \pm 7.19\,mg\,kg^{-1}$ and $14.59 \pm 3.2\ mg\,kg^{-1}$ for extracts erect spiderling, spiny amaranth and chloroquine drug respectively. These data reveal that the plant stem bark extracts do have antimalarial effects, however, their efficacy is quite modest when compared to the standard chloroquine treatment. The ED_{50} values of the two plant extracts show that erect spiderling has stronger antimalarial effects than spiny amaranth. Based on the chemical analysis, Hilou et al. (2006) reported that the antimalarial effects were likely due to betacyanins and phenolics present in the extracts. The lower ED_{50} of erect spiderling may be related to its higher betacyanin content (210.99 mg betanin equivalents/100 g) compared to spiny amaranth (23.87 mg amaranthine equivalents/100 g), which may explain the stronger antimalarial effects. A possible mechanism of action may be due to the ability of betacyanins to chelate inner cations necessary for parasite growth (Ca^{2+}, Fe^{2+} and Mg^{2+}) and block intracellular transport of choline in parasites (Hilou et al., 2006). Malaria morbidity and mortality have risen as antimalarial medication resistance has developed (Centers for Disease Control and Prevention, 2018). In 2020 alone, there were approximately 241 million cases of malaria worldwide. Most infections are caused by *Plasmodium falciparum* and *Plasmodium vivax*—both of which represent the biggest dangers and are the most resistant to existing antimalarial medications (World Health Organization, 2021), therefore newer drugs are required. With the foundation laid by Hilou et al. (2006), future research might focus on isolating betacyanin from these plant extracts in order to investigate its parasitaemia inhibiting action in more depth.

LIMITATIONS AND FUTURE WORK

Based on these studies, it is not entirely clear whether betalain acts on its own or with other components in plant extracts to induce antibacterial effects. Certain Gram-positive and Gram-negative bacteria were not inhibited after treatment with beetroot pomace and red beetroot ethanol extract. This, therefore, raises the question; do the other components in these extracts improve or hamper the antibacterial activity of betalains? In future studies, betacyanin/betaxanthin from beetroot should undergo separation and purification protocols (e.g. using thin-layer chromatography [TLC], and column chromatography methods) prior to conducting antimicrobial assays. The other components in these extracts may have either (a) obstructed the inhibitory potential of betalains against bacteria, resulting in the lack of or minimal inhibitory effects observed, or (b) had a synergistic effect, resulting in the higher antibacterial effects observed against most bacteria. However, these discrepancies could have also been due to differences in the strains of bacteria and the size of inoculum used. Vulic et al. (2013) and Velićanski et al. (2011) tested BPE against *Bacillus* sp. and *Bacillus* spp., respectively, and no inhibitory effects were observed though it was able to inhibit *B. cereus* in both studies. This disparity could mean that not all *Bacillus* species are susceptible to betalains, however, the underlying reason behind this remains unclear as the exact species studied were not identified. Furthermore, the inconsistencies between all antibacterial studies could have also been due to the differences in extraction parameters—particularly the type of solvent used, solvent concentration, material-solvent ratio, temperature and time. Extraction under the following parameters has been proven to yield the highest betalain content from red beetroot: 20% ethanol (diluted with water), material-to-solvent ratio 1/22.96 (w/v), 47.71°C and 183.65 min (Nhon & Hang, 2020). Future studies could therefore use similar parameters for optimal extraction, though this does vary according to the source of betalain being used. Certain studies did not report the total betacyanin/betaxanthin content, nor was molecular characterization carried out, therefore it is not clear which betalain compounds or how much of them (their mass) were involved in the antibacterial activity. The ratio of betacyanin/betaxanthin in different plants varies—cacti fruits, for example *Opuntia* spp., and *Hylocereus* spp. are reportedly good sources of betalains (Sadowska-Bartosz & Bartosz, 2021). Geographical factors should also be considered as betalain-containing fruits in certain regions could be richer in these pigments compared to others due to differences in environmental factors, for example soil and climate, and how they are cultivated. For example betacyanin content in red pitahaya juice from Malaysia was found to be roughly four times greater compared to that of Australia (Ramli & Asmah, 2014). In most studies, betacyanin seemed to inhibit Gram-positive bacteria more than Gram-negative bacteria. This is most likely due to structural variations; Gram-negative bacteria have an outer membrane, but Gram-positive bacteria have not, functioning as an additional barrier for substances to enter the cells (Jones, 2017). Gram-positive bacteria have been found to be more sensitive to betalains derived from red beetroot (Yong et al., 2018).

The mechanism of antimicrobial activity has not yet been determined, so there still remains a significant research gap. Betacyanins are thought to cause cell death due to their effects on cellular membranes, which result in a loss of the cellular pH gradient, lower ATP levels and a loss of the proton motive force (Yong et al., 2018). The ability of betalains to disrupt membrane integrity could be assessed by detecting which components are released from bacterial cells after betalain treatment. The loss of ATP and cellular ions, for example could be detected using an ATP bioluminescence kit and atomic emission spectroscopy respectively. UV spectroscopy and gel electrophoresis could be used to determine if betalains disrupt the genetic component of bacteria (Raheem & Straus, 2019). To date, the most popular notion has been that their ability to chelate interior cations (Ca^{2+}, Fe^{2+} and Mg^{2+}) and operate as modulators of adhesive molecule expression in endothelial cells is what accounts for their antimicrobial activity (Madadi et al., 2020; Sadowska-Bartosz & Bartosz, 2021). Future studies should incorporate protocols such as time-kill assays and flow cytofluorometry as they could determine whether the inhibitory effect is microbicidal or microbiostatic, time-dependent or concentration-dependent, and can provide insight into the nature of cell damage inflicted to the subject micro-organisms (Balouiri et al., 2016).

The exact mechanism of antiviral activity of betacyanin against DENV-2 needs to be studied at the cellular level. Current research suggests that betacyanin affects extracellular DENV particles; possibly by binding to the non-structural envelope (E) protein, however, this is yet to be validated. Time-of-addition studies should be performed to determine at which stage of the viral life cycle can be affected (Aoki-Utsubo et al., 2018). This would give insight into the mechanism of antiviral effects. The interaction between betacyanin and DENV envelope protein could be validated through thermal shift assays (Jafari et al., 2014) and in silico approaches, for example molecular docking (Pinzi & Rastelli, 2019). Future studies should include other dengue virus serotypes as

well (DENV-1, −3 and −4). The effect of betacyanin on viral RNA synthesis could be assessed with the use of quantitative-PCR (qPCR). The in silico study of binding affinity of betacyanin for SARS-CoV-2 proteins revealed that the Lipinski's Rule of Five (RO5) criteria were not satisfied, though this is not uncommon in the case of natural products (Ntie-Kang et al., 2019). Even with the violations, betacyanin has the potential to be made into an antiviral agent; nonetheless, it could be suggested that combining it with a synthetic molecule may boost its chances of being developed as a viable drug. Tallei et al. (2021) and Kaur et al. (2018) asserted that betalains have previously been claimed to have limited bioavailability; however, Madadi et al. (2020) reported otherwise—based on a series of studies, it was concluded that betacyanin and betaxanthin both have good availability in the human body, but with betaxanthin having better bioavailability. Nevertheless, enhancement of betalain bioavailability is required in future studies.

The antimicrobial properties of betalains on bacteria, fungi and viruses will need to be investigated further utilizing in vivo experiments. Albeit in vitro data suggest that betalains might be a promising drug candidate, this should be validated in living organisms. The use of animal models will allow the safety, efficacy, toxicity, metabolism and delivery of betalain to be determined before continuing with human subjects. It must be noted, however, that 90% of clinical drug development fails (Sun et al., 2022), indicating that existing in vitro and animal testing methodologies do not reliably anticipate what will happen in clinical trials. The variations between animal and human systems generate inaccuracies in terms of the efficacy of drug candidates and allow for unforeseen toxicity. Bearing this in mind, forthcoming studies could look into other approaches to replace animal testing, such as advanced computational models and organ-on-a-chip technologies that mimic the in vivo microstructural and functional traits of human tissues and organs (Ma, Peng, et al., 2021; Wilkinson, 2019). Nanotechnology could be used to provide more effective modes of drug delivery, and to increase safety and efficacy. Nanoparticles reportedly reduce the systemic toxicity of drugs, enhance drug solubility, improve stability and reduce drug resistance. Incorporating this type of technology in the drug development process could therefore improve the antimicrobial properties of betalains. This approach could also be used to address the issue of low bioavailability betalains (Li et al., 2022).

CONCLUSION

Betalains exhibit potential to be developed into antimicrobial drugs, but much more research is needed. Antibacterial inhibitory effects were observed over a diverse range of bacteria, including multi-drug resistant ESKAPE pathogens and biofilm-producing species, indicating that betalains could be used in conjunction with broad-spectrum antibiotics in clinical settings in the future. However, to reach this point, current research gaps need to be addressed and this applies to not just antibacterial activity of betalains, but all antimicrobial aspects. To begin, betalain extraction and purification methods need to be optimized in order to yield pure samples. The molecular basis of antimicrobial activity must be understood at the cellular level, as only theories have been proposed so far. This would bring us a step closer to deciding how betalain compounds should be developed to treat certain infections in the latter stages of drug development. Findings from in vitro tests must be reproduced and validated through in vivo tests, and pharmacokinetic profile (absorption, distribution, metabolism, excretion) and toxicology of betalains must be extensively investigated. Alternatively, due to the inaccuracies of animal testing, emerging in vitro techniques with improved reproducibility and accuracy could be used to assess the pharmacokinetic profile of betalains directly within human systems before proceeding to human trials. Currently, there are no studies that looked into the synergistic effects of betalains with existing antimicrobial drugs. Therefore, an interesting area to explore in future studies would be the use of betalains alongside conventional antimicrobials (which microbes are known to be resistant to) to see whether they could increase susceptibility, that is lessen microbial resistance to the treatment.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Wee Sim Choo https://orcid.org/0000-0003-0495-3303

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