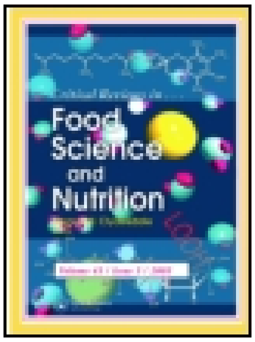

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REVIEW

A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health

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ABSTRACT
This review discusses the available literature concerning the bioactive compounds of beet (*Beta vulgaris* L.) and their ability to modulate the gut microbiota and parameters indicative of gastrointestinal health. Data of published literature characterize beet as a source of a variety of bioactive compounds (e.g. diet fiber, pectic-oligosaccharides, betalains and phenolics) with proven beneficial effects on human health. Beet extracts and pectin and pectic-oligosaccharides from beet have shown able to modulate positively gut microbiota composition and activity, with noticeable bifidogenic effects, in addition to stimulate the growth and metabolism of probiotics. Beet betalains and phenolics seem to increase the production of metabolites (e.g. short chain fatty acids) by gut microbiota and probiotics, which are linked with different beneficial effects on host health. The outstanding contents of betalains and phenolics with antioxidant, anti-inflammatory and anti-carcinogenic properties have been linked to the positive effects of beet on gastrointestinal health. Beet should be a healthy choice for use in domestic meal preparations and a source of ingredients to formulate added-value functionalized food products.

KEYWORDS
Beta vulgaris L; bioactive compounds; gut health; modulation

Introduction

Beet (*Beta vulgaris* L. subsp. v*ulgaris*), Chenopodiaceae family, is a plant originated from southern and eastern Europe and northern Africa. The world beet root production in 2017 reached 301 million tons with a harvested area of 4.89 million hectares. Europe ranks the first in beet root production (207.9 tons), representing 69% of world beet production, followed by Asia (42.7 tons), America (34.3 tons) and Africa (15.9 tons) (FAOSTAT, 2019).

Beet subspecies are formed by four main biotypes cataloged considering their specific morphological characteristics and end-use, to cite: leaf (leaf beet group), sugar (sugar beet group), forage (fodder beet group) and vegetable beet (garden beet group). In leaf beet, popularly known as Mangold, spinach beet and Swiss chard, the leaves and petioles are the most valued parts because of the typical small dimeter of the roots. In sugar beet, the roots have high sucrose contents, being usually used for sugar extraction and ethanol production, while the leaves are destined commonly for animal feed. Roots, stems and leaves of horticultural beet, popularly known as red beet or table beet, are used for human consumption and roots are the most commercially valued part of the plant because of the outstanding nutritional composition (Lange, Brandenburg, and Bock 1999; Batista et al. 2016).

Beet anatomical division consists of roots (hypocotyl), sprout (composed by semi-rigid red to purple stems, also named petiole) and leaves (leafy limb), which are tender and have varied color and shape depending on beet cultivar (Kumar 2015; Ninfali and Angelino 2013). The most valued and considered usually edible part of beet is the root, which has received increasing interest for use in food formulation because of the attractive color and presence of different nutrients and bioactive compounds, being recognized as a super food because of their unusually high contents of antioxidants, vitamins and other constituents able to exert health promoting properties (Chawla et al. 2016; Kumar 2015; Maheshwari, Parmar, and Joseph 2013). There has been a variety of beet-based preparations available for human consumption, to cite: in raw or cooked form as ingredient to salads, cooked as stews, minimally processed, crunch slices and pickles. Beet have been also used to formulate functionalized products, such as juices, gels, fermented beverages, dried powders (tablets and capsules) and crunchy beet root slices. Recently, beet products have attracted increased attention because of their proven blood pressure-lowering properties and ability to induce improvements in endothelial function (Chhikara et al. 2019; Wiczkowski et al. 2018).

Extracts and beet powders have been also considered potential alternatives to replace synthetic colorants in food

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industry. Betalains are well-known natural pigments with red-violet and yellow-orange tonalities and stability over a wide pH range, besides to be considered safe for human consumption. However, the practical application of betalains as food pigments has been limited due to their sensitivity to degradation when exposed to light and high temperatures during food processing and storage (Esatbeyoglu et al. 2015; Halwani, Sindi, and Jambi 2018; Polturak and Aharoni 2018).

In recent years, there has been a growing interest on the biological activities of beet, notably of red beet, including their positive effects on gastrointestinal health (Babbar et al. 2017; Baião et al. 2017; Clifford et al. 2015; Chhikara et al. 2019; Ninfali et al. 2017). Additionally, beet consumption has been considered an adjuvant therapeutic option in a range of pathologies associated with oxidative stress and inflammation. These possible properties and applications have been primarily associated with the presence of betalains in beet, which have displayed antioxidant, anti-inflammatory and chemo-preventive activities in in vitro and in vivo experiments (Clifford et al. 2015; Lechner and Stoner 2019; Rahimi et al. 2019).

Some investigations have shown that carbohydrates present in beet, primarily pectin, could influence the gastric emptying, nutrients absorption in small intestine and fermentation in large intestine (Prandi et al. 2018; Tian et al. 2016). Metabolization of oligo and polysaccharides from beet by bacteria forming the gut microbiota has indicated the ability of these components to modulate positively gut microbial communities and stimulate the production of specific metabolites, which are indicative of potential prebiotic properties (Gómez et al. 2016; Holck et al. 2011; Leijdekkers et al. 2014; Vigsnaes et al. 2011). Prebiotic effects of oligo and polysaccharides and phenolics have received increasing attention because of the evidence that gut microbial communities play an important role in human health, with impacts on host metabolism, physiology, nutrition and immune functions (Danneskiold-Samsøe et al. 2019; Guinane and Cotter 2013). Compositional and functional disruption of gut microbiota has been linked to different pathological intestinal conditions (e.g. obesity and malnutrition), systematic diseases (e.g. diabetes and hypertension) and chronic inflammatory diseases (e.g. inflammatory bowel diseases) (Cavalcanti Neto et al. 2018; Cui et al. 2019; Shamoon, Martin, and O'Brien 2019).

This review discusses the available literature concerning the bioactive compounds in beet and their ability to modulate the composition and function of gut microbiota and different parameters indicative of gastrointestinal health.

Nutrients and bioactive compounds in beet

Results of investigations with focus on beet composition have shown the presence of a variety of nutrients, including high amounts of dietary fiber, minerals (e.g. potassium, sodium, iron, copper, magnesium, calcium, phosphorus and zinc), vitamins (e.g. vitamin A, thiamin, riboflavin, niacin, ascorbic acid and B complex vitamins), essential amino acids, phytosterols, phenolics and betalains (Kumar 2015; Kushwaha et al. 2018; Nemzer et al. 2011; Wang, Beltranena, and Zijlstra 2016). Data of nutritional composition of different fresh beet samples have been variable according to beet cultivar, edaphoclimatic characteristics and harvest and postharvest conditions. Red beet grown under organic agricultural practices have shown typically improved nutritional characteristics, higher contents of total polyphenols and betalains, as well as higher antioxidant capacity when compared to conventionally grown red beet (Carrillo et al. 2017; Chhikara et al. 2019).

A study assessing the nutritional and betalainic profile of red beet dried extracts found that contents of nutrients and betalains depended on the type of drying technique (e.g. air-dried, freeze-dried and spray-dried). Contents of nutrients in beet dried extracts were in the range of 2.17–6.14% for ashes, $<$0.007–1.52% for lipids, 3.81–12.4% for proteins, $<$1–557% for vitamin C and 75.4–89% for carbohydrates, including fibers ($>$1–21.8%) and sugars (30.8–62.5%). Contents of fructose, glucose and sucrose in dried red beet extracts were in the range of 0.8–1.9, 1.1–2 and 30.8–62.5%, respectively. Potassium, calcium and magnesium were the minerals found in highest contents in beet extracts and glutamic acid was the most prevalent amino acid (Nemzer et al. 2011).

High contents of bioactive compounds have been purified from beet samples, including carotenoids (mainly β-carotene and lutein), saponins (e.g. oleanolic acid and several betavulgarosides), flavonoids (rutin, kaempferol, rhamnetin, rhamnocitrin and astragalin), phenolic acids (e.g. ferulic acid, caffeic acid, p-coumaric acid, syringic acid and vanillic acid) and nitrate (644–1800 mg/kg) (Lechner and Stoner 2019; Lidder and Webb 2013).

Betalains have been the most studied bioactive compounds in beet. Betalains are water soluble indole-derived pigments found in plants belonging to Caryophyllales family. Predominant forms of betalains are betacyanin (red-violet) and betaxanthin (yellow-orange) (Ninfali and Angelino 2013). Red beet root is the primary source of betalains in western diets (Esatbeyoglu et al. 2015). Natural betalain can serve not only as safe additives to add natural color or antioxidant properties to food, pharmaceutical and cosmetic formulations, but also with the end of promoting beneficial effects on health, preventing the occurrence of chronic-degenerative diseases, such as hypertension, dyslipidemia, cancer and cardiovascular diseases (Rahimi et al. 2019). Beet is an allowed source of betalains approved for use as food additives in USA (Title 1 of Code of Federal Regulations, 21 DFR 73, 40) and European Union (E-162) (Martins et al. 2017).

Total betalain contents in a range of 0.8 and 1.3 g/L were found in fresh juice prepared with seven different beet varieties, being approximately 60 and 40% of betacyanins and betaxanthins, respectively. Hydroxycinnamic acids were also detected in beet fresh juices (Wruss et al. 2015). Phenolic compounds identified in extracts from *B. vulgaris* cv. Detroit Dark Red were 4-hydroxybenzoic acid (0.012 mg/g), chlorogenic acid (0.018 mg/g), caffeic acid (0.037 mg/g), catechin

(0.047 mg/g) and epicatechin (0.032 mg/g) (Georgiev et al. 2010).

Consumption of beet juice and beet booster shots has been an attracting niche market, being recognized as adjuvants for health and physical performance (Domínguez et al. 2018). Red beet juices were previously evaluated, including sixteen commercial beet root juices and five commercial beet powders. Contents of different compounds (e.g. minerals, betalains, oxalic acid, phenolic acids, nitrates and sugars) in juices prepared with seven red beet varieties, sixteen commercial beet root juices and five commercial beet powders were measured. Highest variations were found in contents of nitrates (565–4626 mg/L) in commercial beet juice samples, which were mostly associated with typical variation in levels of this ion in beet samples. High nitrate contents have been the main reason for use of beet juice as a high-sport performance booster (Wruss et al. 2015).

Beet leaves have been considered good sources of some nutrients. Dehydrated red beet leaves have shown presence of important polyunsaturated fatty acids, such as ω-6 and ω-3 (ΣPUFAs 48.3 mg/g) and minerals, such as calcium (1864.85 mg/kg), iron (256.30 mg/kg), magnesium (1.79 mg/kg), potassium (20,784.90 mg/kg) and sodium (256.30 mg/kg) (Biondo et al. 2014). Beet fiber has been described as a material derived from all plants belonging to the species *B. vulgaris* and comprises typically cellulose (22–24%), hemicelluloses (30%) and pectin (25%), with a ratio of insoluble to soluble fiber of approximately 2:1. Sugar beet fiber has a fiber content varying from 70 to 80 g/100 g and a limited content of protein (5%), ash (3%) and moisture (7%) (Harland 2018; Sivapragasam et al. 2014). Sugar beet pulp has higher contents of pectin than other lignocellulosic biomass, in addition to have an average 3–4% of sucrose, being considered a good sugar source (Panagiotopoulos et al. 2010).

Although not used commonly for human consumption, sugar beet pulp is an important by-product from beet sugar industry. Due to the high pectin content, sugar beet pulp has become an important source of pectins with good emulsifying properties (Ai et al. 2019). Beet pectin is mainly composed of homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II regions and nanostructures, such as galacturonic acid, rhamnose, arabinose, galactose, glucose, xylose, neutral sugars and ferulic acid (Larsen et al. 2019; Liu et al. 2019). Sugar beet pectin has usually a high content of rhamnogalacturonan-I (RG-I) region. RG-I-rich pectins with a high number of side chains have been indicated as potential sources of a new class of prebiotics named pectin-derived oligosaccharides (Babbar et al. 2017; Gullón et al. 2013; Mao et al. 2019).

Researchers have investigated methods for extraction and characterization of oligosaccharides from beet pectin, involving chemical (Mao et al. 2019; Martínez et al. 2009), enzymatic (Babbar et al. 2017; Concha and Zúñiga 2012; Leijdekkers et al. 2013) and combined methods (Chen, Meng, et al. 2015; Guo et al. 2017). Oligosaccharides obtained with beet pectin hydrolysis have been indicated for use as prebiotics, which should add value to an important sugar beet agro-industrial by-product (Babbar et al. 2017).

Biotransformation of beet betalains

Biotransformation of bioactive compounds naturally found in foods is influenced by their physicochemical properties, food matrix constitution and food processing conditions (Oliveira and Bastos 2011). Studies on the kinetics and absorption of bioactive compounds with measurements of plasma levels and/or urinary excretion have shown that metabolites found in blood resulting from digestive and hepatic activity differ generally from native dietary compounds, indicating that the most abundant compounds in a food are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach et al. 2005). Effects of these active metabolites on living tissues could be also affected by their specific chemical structure, metabolism and composition and function of host gut microbiota (Chhikara et al. 2019; Sawicki et al. 2018).

Betalains are hydrophilic pigments formed by betalamic acid and subdivided into two groups based on their chemical composition and structure, to cite: betacyanins (red-violet), derived from condensation of betalamic acid with cyclo-3,4-dihydroxyphenylalanine (cyclo-DOPA); and betaxanthins (yellow-orange), derived from condensation of betalamic acid immonium with amines and distinct amino acids. Betacyanins are classified into four groups: betanin-type, amaranth-type, gomfrenine-type and type-2-decarboxybetanine (Esatbeyoglu et al. 2015).

Tyrosine is the precursor of betalains and its biosynthesis occurs in plant cytoplasm, involving three main enzymes: tyrosinase, 4,5-DOPA-extradiol dioxygenase and betanidin-glucosyltransferase (Esatbeyoglu et al. 2015). Betalains biosynthesis begins with the hydroxylation of tyrosine with molecular oxygen through the action of tyrosinase (Gandía-Herrero and García-Carmona 2012). Formed betalamic acid is the intermediate point in synthesis of all betalains, being responsible for bioactive properties of plant pigments (Esatbeyoglu et al. 2015).

Orally administered betanin, the major pigment in red beet, is poorly absorbed in small intestine, being mostly metabolized in large intestine (Khan 2016). Results of a clinical trial with healthy individuals showed that, after supplementation with a single dose of a commercial red beet juice, the amount of intact betalains (betanin and isobetanin) recovered in urine was 1001 ± 273 mg, which corresponded to 0.28 ± 0.08% of total orally administered dose. Maximum excretion rate (91.7 ± 30.1 mg/h) of betalains was found after 3 h of oral administration. Terminal elimination rate constant (kz), half-life and expected total betalain amount excreted in urine were 0.097 ± 0.021/h, 7.43 ± 1.47/h and 1228 ± 291 mg, respectively. Urinary excretion of non-metabolized betalains seemed to be fast and mono-exponential, indicating a one-compartment model, as well as that bioavailability of betalains was low because the minor portion of systemic elimination occurs via renal clearance. These results indicated that measurements of non-metabolized compounds and their metabolites in plasma, urine and bile should be investigated to a better comprehension of betalain bioavailability (Frank et al. 2005).

Effects of supplementation with fermented beet juice for six weeks were studied in 24 healthy volunteers. The experiment was done in two periods: during the first period

(1 week), volunteers were deprived of products with betalain pigments; and during the second period (7 weeks), volunteers had their diet enriched with industrialized fermented beet juice (dose of 200 mL/60 kg of body weight). Twelve betalain derivatives were found in blood plasma and urine of healthy volunteers after beet juice consumption. Highest betalain levels in blood plasma (87.65 ± 15.71 nmol/L) and urine (1.14 ± 0.12 μmol) were found after the first and second week of beet root juice consumption, respectively. Long-term and regular consumption of beet juice caused stabilization of betalain profile in physiological fluids, which included native betalains and their decarboxylated and dehydrogenated metabolites (Sawicki et al. 2018).

In addition, phenolic compounds found in red beet, such as epicatechin, catechin hydrate, 4-hydroxybenzoic acid and caffeic acid, could exert synergistic effects with betalains to increase mutually their antioxidant effects. Hairy root cultures of red beet (*B. vulgaris* cv. Detroit Dark Red) were collected and used for betalain extraction in order to compare the antioxidant activities of betalain extracts from hairy roots and intact red beet. Hairy root extract had higher antioxidant activity than intact beet extract, which was mostly associated with the 20-fold higher total phenolic content found in hairy root extract (944 ± 22 mg FAE/g DE) when compared to intact beet extract (47 ± 09 mg FAE/g DE). Although to a lesser extent, betalain content in hairy root extract (47.11 ± 1.27 mg/g) were also increased when compared to intact beet extract (39.76 ± 0.98 mg/g), which could have also contributed to the higher antioxidant activity found in hairy root extract. These results suggested that phenolic compounds could exert synergistic effects with betalains to enhance the antioxidant properties (Georgiev et al. 2010). Antioxidant activity of betalains depends on their chemical structure and electron donor capacity. Results of investigations on structure-activity relationship in betalains have indicated that the antiradical activity for the simplest pigments is enhanced by the connection of betalain characteristic electron resonance system with an aromatic ring. Betalamic acid, the central structure of betalains, has strong antiradical and antioxidant activities (Khan 2016; Gandía-Herrero, Escribano, and García-Carmona 2016; Slimen, Najar, and Abderrabba 2017).

Although the bioavailability of beet betalains has not been individually and clearly determined, the achievement of these data could be valuable to give a better understanding of their potential for use in health-promoting dietary interventions (Clifford et al. 2015).

Modulatory effects of beet on gut microbiota

Retrieved studies assessing the in vitro effects of beet and beet bioactive compounds on intestinal microbiota and probiotics with information on tested beet part, bioactive compounds, examined doses, experimental models, measured parameters and main results are shown in Table 1. Non-sucrose polysaccharides from sugar beet pulp, composed of cellulose, hemicelluloses and pectin, have shown resistant to simulated human gastrointestinal conditions and able to reach the colon where could be fermented by gut microbiota (Sivapragasam et al. 2014). Pectin-derived oligosaccharides have shown also resistant to enzymes present in foregut (stomach and small intestine), but they can be fermented by microorganisms found in large intestine, resulting in production of short-chain fatty acids (SCFA) and stimulation of growth and/or activity of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* species (Gullón et al. 2011; Prandi et al. 2018).

Some studies have investigated the effects of beet consumption on gut microbiota composition, which may induce a prebiotic-like effect. Prebiotics are substrates utilized selectively by microorganisms forming the gut microbiota, conferring a variety of health benefits to the host (Gibson et al. 2017). Pectin and pectin-derived oligosaccharides were cited as prebiotics due to their capacity of modulating the gut microbiota composition, particularly increasing the population of the bacterial species *Faecalibacterium prausnitzii* and *Roseburia intestinalis*. These positive effects on gut microbiota reported to pectin-derived oligosaccharides were similar or even higher than those caused by the commercial prebiotics fructooligosaccharides and galactooligosaccharides (Gullón et al. 2013).

Sugar beet pectin has high methoxyl pectins with relatively higher fractions of rhamnogalacturonan and neutral sugars (galactose, arabinose and rhamnose). After an in vitro fermentation using a TIM-2 colon model, the production of propionic acid was relatively high (43.3 mmol) in media with sugar beet pectin, which correlated positively with a relative high abundance of *Prevotella copri* and *Ruminococcus* spp., indicating that these bacterial species were able to produce propionic acid from sugar beet pectin fermentation. Additionally, abundance of *Oscillospira, Blautia, Dorea, Ruminococcus, Coprococcus, R. torques, Lachnospiraceae* and *Clostridiales*, within the phylum Firmicutes, and *Paraprevotella*, *B. uniformis*, *B. ovatus*, *P. distasonis* and *Prevotella*, within the phylum Bacteroidetes, were increased during sugar beet pectin fermentation. These results indicated that microbial gut communities could be specifically modulated by sugar beet pectin (Larsen et al. 2019).

Administration of a standard diet (RMH-B) with 3% (w/w) of sugar beet pectin, mainly composed of uronic acid (63 mol%), galactose (18 mol%) and arabinose (13 mol%), for seven weeks increased the cecal population of *Lactobacillus* spp. and *Lachnospiraceae* spp. in Wistar rats. Stimulatory effects of sugar beet pectin on these two microbial groups were higher than those caused by a soy pectin enriched diet. These results indicated that dietary supplementation with sugar beet pectin could modulate positively microbiota composition and stimulate SCFA production in large intestine (Tian et al. 2016).

Some studies have focused on production of sugar beet pectic oligosaccharides with application of different methods, including enzymatic and acid hydrolysis, being also suggested the ability of these compounds to stimulate the growth of specific gut bacterial populations (Concha and Zúñiga 2012; Goméz et al. 2016; Gómez et al. 2019). Pectic oligosaccharides obtained with sugar beet pectin autohydrolysis and purified with membrane filtration induced a shift in

Table 1. Retrieved studies assessing the in vitro effects of beet and beet bioactive compounds on intestinal microbiota and probiotics.

References	Tested beet part or product	Bioactive compounds	Experimental models/ measured parameters	Main results
Holck et al. 2011	Sugar beet pectin (3%, w/w)	Homogalacturonan (HG) and rhamnogalacturonan I (RGI) (1.4%, w/w) by enzymatic cleavage.	Fermentation with fecal samples from patients with ulcerative colitis (UC)	Pectic-oligosaccharides with slightly different structures had different effects on Bacteroidetes and Firmicutes
Holck et al. 2010	Sugar beet pulp	Feruloylated and Nonferuloylated Arabino-oligosaccharides from sugar beet pectin (5 g/L)	Fermentation with health human fecal samples	Selective stimulation of bifidobacteria by feruloylated and nonferuloylated long-chain arabino-oligosaccharides
Klewicka and Czyżowska 2011	Beet juice (0.7 L/kg)	Not informed	Fermentation of beet juice with *Lactobacillus brevis* and *L. paracasei*	*L. brevis* and *L. paracasei* had high counts in fermented beet juice up to 30 days of refrigerated storage. Fermented beet juice had anti-mutagenic activity
Onumpai et al. 2011	Sugar beet pectin	Beet arabinan (1%, w/v)	Fermentation with human fecal inoculum	Stimulation of *Bifidobacterium* and *Lactobacillus* growth species and increased abundance of Bacteroides-Prevotella group
Gómez et al. 2016	Sugar beet pulp	Pectic oligosaccharides (5 and 10 g/L)	Fermentation with human fecal cultures	Increased populations of different beneficial bacterial species, with remarkable bifidogenic effects
Tian et al. 2016	Sugar beet	Pectin (3%, w/w)	Wistar rats fed a standard diet chow (RMH-B) with 3% (w/w) sugar beet pectin for 7 weeks	Increased cecal population of *Lactobacillus* spp. and *Lachnospiraceae* spp.
Zhang, Lin, and Zhong 2016	Sugar beet	Pectin (1- 4%, w/v)	Used to prepare solid/oil/ water emulsions to encapsulate *L. salivarius*	Improvement in survival rates of *L. salivarius* under different environmental stresses, including a simulated gastrointestinal digestion
Chung et al. 2017	Sugar beet pectin (3%, w/w)	Homogalacturonan oligomer (degrees of polymerization (DP) DP4 (45 mg/g) and DP5 (37 mg/g)	Utilization as carbon source by *Faecalibacterium prausnitzii, Eubacterium eligens* and *Bacteroides thetaiotaomicron*	*E. eligens* was the most effective to degrade diet-derived pectins, whit ability to grow on purified pectic-oligosaccharides. Purified pectic-oligosaccharides promoted the growth of beneficial Firmicutes species
Henning et al. 2017	Beet root	Not informed	Determination of fecal microbiota composition of healthy adults at phylum level after four days of beet juice consumption	Consumption of mixed juice caused weight loss, increased plasma levels of nitric oxide and decreased lipid oxidation. These effects were primarily associated with the high contents of fiber and nitrate in beet root pulp
Panghal et al. 2017	Fresh beet juice (700 mL)	Not informed	Fermentation of pasteurized beet juice	*L. plantarum, L. rhamnnosus* and *L. delbrueckii* increased antioxidant activity and contents of total phenols and flavonoid in fermented juice
Mohsen, Alsaman, and Mahrous 2018	Fresh red beet (200 g/L)	Not informed	Fermentation with *L. plantarum* P108, *L. acidophilus* P110	Fermented beet root had increased total phenolic content and antioxidant activity. Beet root fermented with *L. acidophilus* P110 had highest total phenolic content and antioxidant activity
Münnich et al. 2017	Molasse sugar beet pulp (0–400 g/kg)	Not informed	Determination of ruminal microbial communities and fermentation profile of molasse sugar beet pulp added of ruminal fluid and solid cow digesta	Beet pulp stimulated ruminal acetate production and propionate fermentation. High replacement rates of beet pulp caused decreased utilization of ammonia and higher ruminal methane production
Prandi et al. 2018	Sugar beet pulp	Pectin and Pectin-oligo saccharide mixtures (1%, w/w)	Fermentation with *Lactococcus lactis, L. amylovorus, L. casei, L. plantarum, L. rhamnosus, L. curvatus, L. fermentum, L. acidophilus* and *L. delbrueckii*	Different fractions stimulated the growth of *Lactobacillus* species, with the exception of *L. lactis*. Pectin oligosaccharides with low polymerization degree arabinans and little or no free galacturonic acid were the most effective to stimulate *Lactobacillus* species
Gomez et al. 2019	Sugar beet pulp by-product	Pectic oligosaccharides (10 g/L)	Substitute of glucose (10 g/L) as a carbon source in laboratory medium	Stimulation of the growth of probiotic *Lactobacillus* isolates, especially of *L. reuteri, L. plantarum* and *L. rhamnosus*

(*continued*)

Table 1. Continued.

References	Tested beet part or product	Bioactive compounds	Experimental models/ measured parameters	Main results
Larsen et al. 2019	Sugar beet	Pectin with higher fractions of rhamnogalacturonan and neutral sugars (7.5 g)	Fermentation using a TIM-2 colon model	Increases in population of *Oscillospira, Blautia, Dorea, Ruminococcus, Coprococcus, R. torques, Lachnospiraceae* and *Clostridiales,* within phylum Firmicutes, as well as of *Paraprevotella, B. uniformis, B. ovatus, P. distasonis and Prevotella,* within phylum Bacteroidetes
Mladenović et al. 2019	Sugar beet molasses (5 - 25%, w/v)	Not informed	Adaptation of *L. paracasei* NRRL B-4564 to sugar beet molasses to ensure substrate utilization and enhanced lactic acid production on molasses-enriched distillery stillage	Adapted *L. paracasei* NRRL B-4564 enhanced the lactic acid and biomass production. Sugar beet molasses was shown a source of sugars molasses, nitrogen and minerals required for growth of lactic acid bacteria and increased lactic acid production

abundance of populations of different beneficial bacterial species, with a remarkable bifidogenic effect revealed by increases in *Bifidobacterium* spp. counts from 11.8% to 23.4% during fermentation. Additionally, the results of this study indicated that sugar beet pectic oligosaccharides induced a more remarkable prebiotic effect than pectin, besides to act similarly to or better than fructooligosaccharides (Goméz et al. 2016). Structure of oligosaccharides fractionated from beet pectins (beet arabinan) were reported to increase the populations of *Bifidobacterium* spp. and *Lactobacillus* spp. during fermentation with a human fecal inoculum, with an outstanding bifidogenic effect (Onumpai et al. 2011).

Pectic oligosaccharides obtained through enzymatic hydrolysis of sugar beet pulp derived from industrial beet by-products were also capable of stimulating the growth of probiotic *Lactobacillus* isolates, especially of *L. reuteri*, *L. plantarum* and *L. rhamnosus*, in laboratory media. These results indicated that sugar beet-derived pectic oligosaccharides could confer beneficial effects on gut microbiota by modulating the growth of probiotic bacteria (Gómez et al. 2019).

Administration of a mixed juice prepared with beet, apple, lemon and ginger to healthy adults that consumed only the tested mixed juice for three days caused a decrease in relative fecal abundance of Firmicutes and Proteobacteria, besides to an increase in relative fecal abundance of Bacteroidetes and Cyanobacteria. Relative fecal abundance of some bacterial genera was also increased, to cite: *Halospirulina* (1467%), *Paraprevotella* (348%), *Barnesiella* (200%), *Odoribacter* (200%) and *Bacteroides* (144%). Otherwise, relative fecal abundance of other bacterial genera was decreased, to cite: *Streptococcus* (8%), *Subdoligranulum* (30%), *Eisenbergiella* (40%), *Ruminiclostridium* (50%) and *Dialister* (67%). These data indicated that administration of mixed juice with beet root was capable of inducing alterations in human gut microbiota composition. Consumption of mixed juice also caused weight loss, increased plasma levels of nitric oxide (vasodilator) and decreased lipid oxidation, which were associated primarily with high contents of fiber and nitrate in beet pulp used to prepare the juice (Henning et al. 2017).

Capability of probiotic bacteria of boosting the health promoting properties of beet juice have also been reported. Fermentation of pasteurized beet juice by probiotic *Lactobacillus plantarum*, *L. rhamnnosus* and *Lactobacillus delbrueckii* resulted in increased contents of total phenolics and flavonoids and antioxidant activity in fermented juice. Fermentation of beet juice by probiotics caused probably structural disintegration of cell wall in beet root cells with the release or synthesis of different antioxidant compounds (Panghal et al. 2017).

Fermentation of beet root juice by probiotic *Lactobacillus brevis* and *L. paracasei* resulted in increased contents of betanin, isobetanin and neobetanin, as well as of betanidine and isobetanidine, which were not found in unfermented beet root juices. Betanidine and isobetanidine are aglycones with ability to neutralize free radicals. Betanidin and its isomer isobetanidin were formed in fermented beet root juice possibly as a result of the activity of β-glucosidase produced by tested probiotics, which catalyzes the transformation of betanin into betanidine. *L. brevis* and *L. paracasei* had high counts (approximately 8.15 log CFU/mL) in fermented beet juice during 30 days of refrigerated storage (Klewicka and Czyżowska 2011).

Beet juice fermented by probiotic *Lactobacillus casei* and *L. brevis* for 48 h at 30 °C was administered in three different volumes (1.5, 3 and 6 mL daily) for Wistar rats fed a basic casein diet for four weeks. Consumption of fermented beet root juice (6 mL daily) modulated positively cecal microflora and metabolic parameters of rats. Fecal counts of *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteriodes* spp. and *Enterococcus* spp. were in the range of 8.2–8.6, 6.2–7.5, 8.0–8.3 and 7.3–7.7 log CFU/g, respectively, during fermentation. Fecal counts of *Clostridium* spp. increased by 1.1–1.6 log CFU/g, while fecal counts of Enterobacteriaceae decreased by 0.8–2.1 log CFU/g in rats. Furthermore, administration of beet root fermented juice increased the fecal contents of SCFA in rats, besides to decrease the activity of α-glucosidase and β-glucuronidase, which were indicative of reduced post-prandial hyperglycemia and enterohepatic circulation of toxic compounds, respectively. These results indicated that consumption of beet juice fermented by probiotic *L. casei* and *L. brevis* induced positive effects on cecal microbiota and metabolic parameters in rats (Klewicka, Zduńczyk, and Juśkiewicz 2009).

Effects of beet on gastrointestinal diseases

Retrieved studies assessing the effects of beet and beet bioactive compounds on parameters indicative of gastrointestinal health with information on tested beet part or product, bioactive compounds, examined doses, experimental models, measured parameters and main results are shown in Table 2. Beet exert a variety of beneficial effects to human health, which are mediated by different naturally occurring bioactive compounds, most notably betalains, phenolics, soluble fiber and pectin (Chhikara et al. 2019; Clifford et al. 2015; Martinez et al. 2015). Inflammation is a biological response of immune system triggered by different factors, such as pathogens, damaged cells and toxic compounds, which may induce acute and/or chronic inflammatory responses in gastrointestinal tract, leading to tissue damage and disease (Chen et al. 2018). There has been evidence that betalains and betaine can exert anti-inflammatory effects by interfering with pro-inflammatory signaling cascades. Nuclear Factor-Kappa B (NF-κB) cascade is the most important cascade in inflammatory processes, being involved in activation and transcription of most target genes that regulate and amplify inflammatory response (i.e. cytokines, chemokines, apoptotic and phagocytic cells). Betalains and betaine can reduce the levels of pro-inflammatory cytokines (e.g. TNF-α, IL-6, IL-8 and IL-1β), reactive oxygen and nitrogen species, as well as the activity of cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) (Clifford et al. 2015; Lechner and Stoner 2019).

A study evaluated the free-radical scavenging activity of betanin in human hepatoma cell lines (HT-29) and confirmed protective effects of betanin to avoid DNA-damage. Beet betalains (15 μmol/L) decreased DNA-damage caused by H_2O_2 in HT-29 enterocytes (measured by the so-called Comet-assay), as well as increased the transcription of nuclear factor erythroid 2-related factor 2 (Nrf2), which induces endogenous cellular antioxidant defense mechanisms. These data indicate that betanin can act as both a free radical scavenger and inducer of antioxidant defense mechanisms in cells (Esatbeyoglu et al. 2014).

Different mechanisms could be involved in hepatoprotective and anticarcinogenic effects of betanin. Hepatoprotective and anticarcinogenic mechanisms of betanin were evaluated considering its influence on activation of Nrf2 and expression of Glutathione S-transferase and NAD(P)H:quinone oxidoreductase in two cell lines (non-tumor human hepatocytes THLE-2 and hepatocellular carcinoma cells HepG2). Betanin (2, 10 and 20 μM) activated the Nrf2-ARE binding sequence in non-tumor human hepatic cell lines (translocation of Nrf2 from the cytosol to the nucleus) and increased the expression of mRNA of phase II detoxifying enzymes, including NAD(P)H:quinone oxidoreductase 1 and Glutathione S-transferase activity (Krajka-Kuźniak et al. 2013).

Effects of phenolics of beet stalks and leaves on liver oxidative damage in mice fed a high-fat (HF) diet were investigated. HF diet mice groups were supplemented during eight weeks with dehydrated beet stalks and leaves or beet stalks and leaf ethanol extract. Dehydrated beet stalks and leaves attenuated the deleterious effects of a HF diet on lipid metabolism, reduced fasting blood glucose levels, ameliorated cholesterol levels and reduced Glutathione-peroxidase and Glutathione-reductase activities in mice. However, ethanolic extract from beet stalks and leaves did not prevent liver damage caused by HF diet. Presence of flavonoids, such as vitexin derivatives, in beet stalks and leaves were reported to protect the liver from damage induced by HF diet. These results indicated that dehydrated beet leaves were more biologically active than ethanolic beet leaves extract, probably because of the interactions of phenolics with other components in dehydrated leaves, especially proteins. Phenolics can interact with proteins leading to formation of soluble and insoluble complexes, which could affect their absorption and biological activities (Lorizola et al. 2018).

Protective effects of long-term feeding (28 days) with beet juice (8 mL/kg body weight) on phase I and phase II enzymes, DNA damage and liver injury induced by hepatocarcinogenic *N*-nitrosodiethylamine (NDEA) was evaluated in rats. Beet juice consumption conferred hepatic protection against a range of inflammatory markers induced by NDEA administration, such as alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, lactate dehydrogenase, gamma glutamyl transferase, albumin, bilirubin, creatinine and blood urea nitrogen. Long term feeding with beet juice had protective effects against oxidative liver damage induced by *N*-nitrosodiethylamine in rats (Krajka-Kuźniak et al. 2012).

Chemoprevention of cancer with bioactive foods or their extracted/purified components has been associated with normalization of expression of different genes (Lechner and Stoner 2019). Identification of plant compounds (e.g. polyphenols) capable of inducing the killing of tumors through apoptosis has been recognized as a promising strategy to avoid proliferation of cancer cells (Chen, Zhao, and Yu 2016). The relationship between chemical structure and anti-cancer activity for a range of sugar beet pectins extracted and modified with different methods was evaluated by measuring cell viability and apoptosis detection in HT29 and DLD1 colon cancer cells. Pectin extracted from sugar beet pulp differed considerably from pectin extracted from citrus peel. Obtained sugar beet pectin had a higher neutral sugar and lower GalA content, as well as a lower degree of esterification (DE) and higher degree of acetylation (DAc) when compared to citrus peel pectin. Alkali treatment increased the ratio of rhamnogalacturonan I (RGI) to homogalacturonan and increased the anti-cancer effects of pectin, indicating an important role of neutral sugar side chains, such as galatan and arabinan, for pectin bioactivity (Maxwell et al. 2016).

Phenolics, such as gallic acid (GA), cyanidin-3-O-glucoside chloride (CGC) and epicatechin (EP), were extracted from sugar beet molasses and screened for antioxidant and cytotoxicity effects on human colon (CaCO-2) and hepatocellular (HepG2) cancer cell lines. GA had the strongest antioxidant activities and its antitumor activities increased in a dose-dependent manner. In particular, CGC (400 μg/

Table 2. Retrieved studies assessing the effects of beet and beet bioactive compounds on parameters indicative of gastrointestinal health.

References	Tested beet part or product	Bioactive compounds	Experimental models/ measured parameters	Main results
Klewicka et al. 2012	Beet root	Beet root juice fermented by *Lactobacillus brevis* 0944 and *Lactobacillus paracasei* 0920 (0.8 L/Kg)	Effects against aberrant crypt foci formation and genotoxicity of fecal water in rats	Beet root reduced the number of aberrant crypt foci in rats treated with N-Nitroso-N-methylurea, in addition to increase the counts of *Lactobacillus/ Enterococcus* adhered to colonic epithelium
Krajka-Kuźniak et al. 2012	Beet root juice (8 mL/Kg)	Not informed	Protective action of beet root juice on hepatocarcinogenic N-nitrosodiethylamine-induced liver injury in rats	Administration of beet juice for 28 days had a protective effect against oxidative damage to liver
Kapadia et al. 2013	Red beet root extracts	Not informed	Potential antiproliferative synergistic activity of red beet extract with the drug doxorubicin against pancreatic tumor cells	Extracts from red beet root in combined concentrations attenuated the efficacy of the chemotherapeutic drug doxorubicin
Krajka-Kuźniak et al. 2013	Beet root	Betanin (2, 10 and 20 μM)	Evaluation of the hepatoprotective and anticarcinogenic effect of betanin in non-tumor hepatocytes and hepatocellular carcinoma cells	Betanin induced the expression of detoxifying enzymes, indicating that it is partly responsible for the beet hepatoprotective activity
Esatbeyoglu et al., 2014	Red beet extract diluted with dextrin	Betanin (10 μL)	Evaluation the free-radical scavenging activity of betanin on counteract hydrogen peroxide induced DNA damage in human liver hepatoma cells	Betanin acted as a free radical scavenger and n inducer of endogenous cellular enzymatic antioxidant defense mechanisms
Chen, Meng, et al. 2015	Sugar beet molasses	Gallic acid, cyanidin-3-O-glucoside chloride and epicatechin extracted from sugar beet molasses (400 μg/mL)	The phenolics were screened for antioxidant activity and cytotoxicity effects measured by methyl thiazolyl tetrazolium assay against human colon (CaCO-2) and hepatocellular cancer cell lines	Gallic acid had the strongest antioxidant activities and its antitumor activities increased in a dose-dependent manner. Cyanidin-3-O-glucoside chloride caused inhibition of the proliferation of human colon and hepatocellular carcinoma cell lines
Nowacki et al. 2015	Fresh red beet root extracts, with a mix of betanin (64%, w/v) and isobetanin (36%, w/v)	Betanin and isobetanin	Human colorectal cell lines (HT-29 ATCC® HTB-38). All cells were cultured as monolayer (2D) and as aggregates (3D) and treated with betanin-enriched beet root extract for 48 h	Betanin concentrate inhibited the proliferation of cancer cells. HT-29 cells were not sensitive to betanin concentrate at 40 μM and cell proliferation was not decreased
Maxwell et al. 2016	Not informed	Sugar beet pectin (1 mg/mL)	Relationship between pectin structure and anti-cancer activity for a range of sugar beet pectins, extracted and modified in a variety of ways	Modified sugar beet pectin (by alkali treatment) increased the ratio of rhamnogalacturonan I to homogalacturonan and apoptosis induction
Farabegoli et al. 2017	Not informed	Betaxanthins (0.35 ng/mL) and betacyanins (0.25 ng/mL) from red beet root	Investigated in CaCo-2 colon cancer cell lines	Betaxanthins and betacyanins from red beet root reduced the expression of pro-inflammatory markers and caused down regulation of anti-apoptotic protein Bcl-2
Lorizola et al. 2018	Beet stalks and leaves (0.5%, w/w)	Not informed	Liver oxidative damage in mice fed a high-fat diet and supplemented during 8 weeks with dehydrated beet stalks and leaves	Beet stalks and leaves attenuated the deleterious effects of a HF diet on lipid metabolism, reduced fasting blood glucose levels, ameliorated cholesterol levels and reduced Glutathione-peroxidase and Glutathione-reductase activities

mL) caused inhibition of 94.9 and 87.3% in proliferation of human colon and hepatocellular carcinoma cell lines, respectively (Chen, Meng, et al. 2015).

Cytotoxic activity of betaxanthins (R1) and betacyanins (R2) from red beet (cv. *Detroit*) were investigated in CaCo-2 colon cancer cell lines when these compounds were tested individually or in combination with vitexin-2-O-xyloside (the main cytotoxic flavonoid from beet seeds). Betalains tested individually even in low concentrations (0.25–0.35 μg/mL) had toxic effects on CaCo-2 cell lines. Combinations of vitexin-2-O-xyloside + betaxanthins, vitexin-2-O-xyloside + betacyanin and vitexin-2-O-xyloside + betaxanthins + betacyanins had synergistic toxic effects on CaCo-2 cell lines, being the highest toxic effects found after a 72 h-exposure. These data indicated the use of a cocktail of betaxanthins, betacyanins and vitexin-2-O-xyloside as a chemopreventive alternative against colon cancer due to the capability of betalains of reducing the expression of pro-inflammatory markers and causing a down regulation of anti-apoptotic protein Bcl-2 (Farabegoli et al. 2017).

A study evaluated the effects of extracts from fresh red beet containing a mix of betanin and its stereoisomer isobetanin (64% and 34%, respectively) on human colorectal cell lines (HT-29 ATCC® HTB-38). Cells were cultured as monolayer (2D) and aggregates (3D) and treated with betanin-enriched beet extract for 48 h. Betanin concentrate inhibited the proliferation of cancer cells, which was associated with induction of apoptotic cell death and autophagic activity for cancer cells. However, HT-29 cells were not sensitive to betanin concentrate at 40 μM and cancer cell proliferation was not decreased (Nowacki et al. 2015).

Potential antiproliferative synergistic activity of red beet extract with the drug doxorubicin was evaluated against pancreatic tumor cells (PaCa) in exponential growth phase. Different concentrations of beet extract and doxorubicin (0.29–290 μg/mL) in different combinations were tested for cytotoxic effects with measurements of viability of PaCa after 72 h of incubation. Results indicated a reduction in effective dose of doxorubicin (a chemotherapeutic drug) when combined (1:5 ratio) with beet extract (IC_{50}, IC_{75} and IC_{90}) to decrease the viability of PaCa. The authors stated that red beet extract in selected combined concentrations could enhance the therapeutic efficacy of chemotherapeutic drugs to reduce toxic side-effects as a consequence of the reduction of drug effective dose, in addition to prevent development of decreased cancer cell sensitivity to drug treatment (Kapadia et al. 2013).

Protective effects of beet juice fermented by probiotic *L. brevis* and *L. paracasei* against aberrant crypt foci formation and genotoxicity of fecal water in rats was reported. Beet juice reduced the number of aberrant crypt foci and extensive aberrations in rats treated with *N*-Nitroso-*N*-methylurea (used as carcinogen). Fecal water obtained from rats fed with N-Nitroso-N-methylurea-containing diet induced pronounced cytotoxic and genotoxic effects in Caco-2 cell lines, but administration of fermented beet juice abolished these effects. Betalains in fermented beet root juice was reported as a potential biologically active agent inhibiting the development of cancer cells. Additionally, the beet juice administrations increased the number of bacterial cells, including *Lactobacillus*/*Enterococcus*, adhered to colonic epithelium. These results indicated that fermented beet juice could be a functional food with capability of preventing precancerous alteration induced by carcinogens, in addition to decrease the cyto- and genotoxicity of fecal water (Klewicka et al. 2012).

Conclusion and future perspectives

Available literature has demonstrated conversely that beet has outstanding nutritional value and presence of a variety of bioactive compounds. There has been consistent evidence that non-sucrose polysaccharides, namely pectin and pectic oligosaccharides, from beet can modulate positively the gut microbiota composition and function, in addition to exert stimulatory effects on growth and metabolism of probiotic bacteria, indicating that beet could exert prebiotic properties. Positive effects of beet betalains and phenolics on gut microbiota and probiotics have also resulted in the production of bacterial metabolites, namely SCFA, which have been linked to an array of beneficial effects induced by prebiotics on host health. High contents of betalains and some phenolics with anti-inflammatory, antioxidant and anti-carcinogenic properties have been associated with positive effects induced by beet on parameters indicative of gastrointestinal health. Fermentation of beet products by probiotics seems to impact positively on the beneficial biological effects of these products on gastrointestinal health. Beet should be considered a healthy food option for use by consumers in different domestic meal preparations as well as by food industry as an ingredient to formulated added-value functionalized food products.

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