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Colonic fermentation of polyphenolics from Sea buckthorn (*Hippophae rhamnoides*) berries: Assessment of effects on microbial diversity by Principal Component Analysis

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ABSTRACT

The present study investigates the stability of polyphenolic in Sea buckthorn berries juice (SBJ) during different phases of digestion and its effect on colonic microbial diversity. At each stage, the Total polyphenolic content (TPC), Total antioxidant activity (TAA) and polyphenolic profile was determined. A 1.64 and 2.20 folds increase in TPC with 4.88 and 9.61 folds increase in TAA were observed during gastric and small intestine digestion ($p < 0.05$) with the release of quercetin from food matrix. The digestion resulted in deformation of intact crystalline structure as indicated by scanning electron micrographs. The colonic fermentation resulted in an increase in quercetin, caffeic acid with decrease in rutin and chlorogenic acid after 36 h of fermentation ($p < 0.05$). The Shannon diversity index (H) of beneficial groups including Lactic acid bacteria (LAB), Bacteroides/Prevotella and Bifidobacteria was increased by 35%, 71% and 17%, respectively ($p < 0.05$). The PCA analysis indicated that the presence and digestion of polyphenolics promote the proliferation of Bacteroides/Prevotella group as well as Lactic acid bacteria and Bifidobacteria. The results suggest that SBJ is good source of prebiotic substrate in terms of the proliferation of beneficial gut microbiota.

1. Introduction

Polyphenols form a major proportion of the human diet as these are present in a broad range of commonly consumed berries, fruits, vegetables, and plant-derived products. The intake of polyphenol rich diets is reported to have beneficial effects by decreasing the risk of various chronic diseases, such as coronary heart disease, specific cancers, and neurodegenerative disorders (Cueva et al., 2017; Xie et al., 2017). Also, plant based polyphenols assert prebiotic properties which can enhance the gut ecology, leading to host health benefits (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013). In view of the new perception of food products by consumers, increasing consumer awareness about the effect of diet on the incidence of risk of chronic diseases promotes the development and production of functional foods. However, the suggested health benefits of these polyphenolic rich foods depend on their bioavailability, which is measured as an amount of nutrients that are digested, absorbed and metabolized through regular metabolic pathways (Dueik & Bouchon, 2016; McGhie & Walton, 2007). Therefore, it is important to understand the metabolic profile of these food ingredients in terms of absorption, metabolism, and elimination from the body, in order to ascertain there *in vivo* actions.

Sea buckthorn (*Hippophae rhamnoides*), a plant of the Elaeagnaceae family has been reported to possess high nutraceutical and therapeutic values (Patil, 2017). SB berries grown in the trans-Himalayan regions of India, with an elevation of 3000–4000 m are being consumed locally for their health benefits. The polyphenolic rich berries and their products have been reported to inhibit the low-density lipoprotein (LDL) cholesterol oxidation and platelet aggregation, reduction of atopic dermatitis, immunomodulation, cytoprotective effects and protection from gastric ulcers (Gasparrini et al., 2017; Guo, Guo, Li, Fu, & Liu, 2017; Suryakumar & Gupta, 2011). The ripened berries of sea buckthorn are orange-red and are rich source of organic acids, polyphenols (gallic acid, catechin, epicatechin, *p*-coumaric acid, caeffic acid, ferulic acid, rutin, quercetin, resveratol, myricetin *etc.*), carbohydrates, carotenoids, proteins, minerals and fatty acids (Bal, Meda, Naik, & Satya, 2011; Bittová, Krejzová, Roblová, Kubáň, & Kubáň, 2014; Chauhan & Varshneya, 2012; Guo et al., 2017; Pop et al., 2014). Despite their potential biological activities, the effect of digestion on bioaccessibility of the active ingredients of SBJ and their effect on gut microbiota is not known more specifically certain beneficial bacterial species belonging to the genera Lactobacillus, Bacteroides/Prevotella and Bifidobacterium. These bacterial groups are considered beneficial

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microorganisms as these contribute to health benefits by inhibiting a wide range of pathogens, improvement of lactose digestion, reduction of serum cholesterol, stimulation of the immune system through cytokine stimulus, increased mucus secretion and reinforcement of intestinal epithelial cell tight junctions (Duggan, Gannon, & Walker, 2002; Gotteland et al., 2008; Vitali et al., 2010).

During gastrointestinal (GI) absorption, most of the easily digestible food components are generally metabolized or absorbed in the upper GI tract. Remaining complex carbohydrates like dietary fiber, oligosaccharides, arabinogalactan, cellulose, xylan, pectin and polyphenols remain indigestible in the upper GI tract and are utilized by gut microbiota in the lower gut (Hooper, Midtvedt, & Gordon, 2002; Kamiloglu et al., 2017). Among these ingredients, the bioaccessibility of polyphenols is reported to be influenced by factors such as chemical structure and food matrix. It has been estimated that 5–10% of the polyphenols are absorbed in the small intestine and remaining accumulates in the colon where the gut microbiota plays important role in the breakdown of large polyphenolic compounds into low molecular weight absorbable polyphenols for their beneficial effects (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2009). Indeed, a range of these potential benefits of polyphenols have been demonstrated through *in vitro*, *ex vivo* and animal assays (Perez-Vizcaino & Duarte, 2010; Spencer, Vafeiadou, Williams, & Vauzour, 2012). However, human trials often are constrained by ethical considerations while animal model often gives observations that are not applicable in humans due to differences in microbial gut composition between those of humans and animals (Nguyen, Vieira-Silva, Liston, & Raes, 2015; Venema & van den Abbeele, 2013). On the other side *in vitro* studies offer many advantages such as simplicity, ease of application and low cost so they are preferred to *in vivo* studies (Yi, Akoh, Fischer, & Krewer, 2006).

This study aimed to assess the stability of polyphenolic compounds in the SBJ during an *in vitro* simulated gastric and small intestinal phase. Following the small intestine digestion, the impact of the digested fraction of SBJ on specific gut microbial communities was assessed under colonic batch-culture fermentation.

2. Material and methods

2.1. Sea buckthorn berries collection and processing

The sea buckthorn berries were collected from the Spiti region (North latitude 31°44′57″ & 33° 42′54″ and East longitude 76°56′29″ & 78°41′34″) of Himachal Pradesh and were washed with distilled water followed by treatment with potassium metabisulphite (1.5 g/kg). Berries juice was prepared as described by Bump (1989) and yield of juice was 1 L/2.5 kg berries. The heat treatment to 1 L of SBJ was given in closed contained (2 L) at 80 °C for 30 min to prevent spoilage of the juice and it was stored at 4 °C until further use.

2.2. In vitro gastric and small intestine digestion

The SBJ was subjected to initial HCl/pepsin digestion followed by small intestinal pancreatin/bile digestion (Attri, Singh, Singh, & Goel, 2017; Boyer, Brown, & Liu, 2005). A 50 mL of juice was diluted with 200 ml HCl (Merck, Mumbai, India) containing 8.0 g/L NaCl (Merck, Mumbai, India) and 1.2 g/L porcine pepsin (MP Biomedicals, California, USA) adjusted to pH of 1.2 for gastric phase digestion. The digestion was done for 1 h at 37 °C at an agitation of 120 rpm. The gastric phase (50 ml) treated samples were diluted with 80 ml phosphate buffer (0.1 M, pH 7.5) containing 0.175 g/L oxgall bile (MP Biomedicals, California, USA) and 1.1 g/L porcine pancreatin (Sigma-Aldrich, Missouri, USA) for small intestine phase digestion and was further incubated for 1 h at 37 °C at 120 rpm. Before and after the gastric and small intestinal digestion phases, the digested SBJ aliquots were stored at − 20 °C until further analysis. A blank was also prepared with same processing conditions without the SBJ. After the small intestinal pancreatic digestion, the digested sample was lyophilized and used as substrate for fermentation under colonic fermentation.

2.3. Scanning electron microscopy

The scanning electron microscopy (SEM) was performed with Field emission scanning electron microscope (FE, SEM, Quanta 200 FEG) for morphological characterization of lyophilized undigested, gastric and small intestine phase digested SBJ.

2.4. Colonic fermentation

2.4.1. Faecal sample preparation

Faecal samples were collected in sterile vials from five healthy individuals who have no history of antibiotic treatment three months prior to the study (Table S1). These stool samples were stored at − 20 °C, were pooled together and processed within 3 h of collection. A 10 g of the pooled faecal matter was homogenized in anaerobic sterile 100 mL phosphate buffer (0.1 M; pH 7.5) containing 0.1% sodium thioglycolate (Himedia, Mumbai, India) as a reducing agent. The faecal slurry obtained was used as inoculum for colonic fermentation.

2.4.2. Colonic batch fermentation

Batch culture fermentation was carried out in glass serum bottles with rubber top (Volume 150 mL) in triplicates. Briefly, 45 mL of carbohydrate free basal sterile medium previously described by Valdés-Varela, Ruas-Madiedo, and Gueimonde (2017), containing 2 g/L yeast extract (Himedia, Mumbai, India), 2 g/L peptone (Himedia, Mumbai, India), 0.1 g/L NaCl (Merck, Mumbai, India), 0.04 g/L K_2HPO_4 (Fisher Scientific, Waltham, MA, USA), 0.04 g/L KH_2PO_4 (Fisher Scientific, Waltham, MA, USA), 2 g/L $NaCO_3$ (Merck, Mumbai, India), 0.01 g/L $MgSO_4 \cdot 7H_2O$ (Fisher Scientific, Waltham, MA, USA), 0.01 g/L $CaCl_2 \cdot 6H_2O$ (Fisher Scientific, Waltham, MA, USA), 2 ml/L Tween 80 (Fisher Scientific, Waltham, MA, USA), 0.05 g/L haemin (Himedia, Mumbai, India), 10 mg/L vitamin K_1 (MP Biomedicals, California, USA), 0.5 g/L L-cysteine hydrochloride monohydrate (MP Biomedicals, California, USA), 0.5 g/L ox gall bile (MP Biomedicals, California, USA), 1 mg/L resazurin (Himedia, Mumbai, India) was dispensed into the serum bottles. The pH of the medium was adjusted to 7.0 and anaerobic conditions were maintained by flushing O_2 free N_2 gas. The serum bottles containing media were inoculated with 5 mL of faecal slurry (1:10 w/v). To check the effect of SBJ on gut microbiota, 250 mg lyophilized fraction of small intestine digested SBJ was added to media, whereas SBJ control containing 250 mg of lyophilized fraction of undigested juice. A serum bottle without any SBJ was used as experimental blank. In order to mimic the conditions of the large intestine serum bottles were fluxed with O_2 free N_2 gas for 15 min two times a day and were incubated at 37 °C for 72 h. For analysis of Total polyphenolic content, changes in gut microbial population and polyphenolics, 1 mL aliquot was drawn from each bottle at an interval of 12 h up to 72 h and the samples were stored at − 80 °C until analyzed.

2.5. Total polyphenol content (TPC) analysis

Total polyphenol content was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965). Briefly, a 250 µL sample of predigested, gastric, small intestinal phase and colonic fermented sea buckthorn were diluted with 15 mL distilled water. To this mixture 1.25 mL of Folin-Ciocalteu reagent (2N, Merck, Mumbai, India) and 3.75 mL sodium carbonate (20% w/v, Fisher Scientific, Waltham, MA, USA) were added and final volume was made up to 25 mL. After incubation at room temperature in dark for 2 h, the absorbance at 765 nm was measured. Total polyphenol contents were expressed as milligram of gallic acid equivalents per litre (mg GAE/L).