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Cranberry synergies for dietary management of *Helicobacter pylori* infections

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Abstract

Cranberry and its products are important components of the cranberry processing industry and have historically been associated with positive health benefits such as preventing urinary tract infections. These health benefits are associated with phenolic phytochemicals in the juice which are now known to have potential for inhibition of development and progression of cancer and cardiovascular diseases. *Helicobacter pylori* is an important human pathogen linked to peptic ulcer and now to cardiovascular diseases. Control of this pathogen using synthetic antimicrobials such as currently approved antibiotics has limitations due to potential development of resistance and low compliance. We believe a profile of antimicrobials compared to a single compound could be potentially more effective in managing *H. pylori* infections. We have investigated the effect of cranberry, blueberry and grape seed extracts on inhibiting *H. pylori* have been investigated. The ability of blueberry, grape seed and oregano extract on enhancing the antioxidant and anti-*H. pylori* activity of cranberry powder in a mixture was also investigated. The anti-*H. pylori* activity of the cranberry fruit extracts and their synergies correlated with antioxidant activity and the presence of biphenyls as well as polyphenolic phytochemicals. The anti-*H. pylori* activity of cranberry juice extract was significantly improved by its synergistic blending with blueberry, grape seed and oregano extract. The lower efficacy of purified phenolics in inhibiting *H. pylori* compared with fruit powder at similar dosage levels suggests a synergistic mode of functionality of these individual phenolics in whole food background. Consumption of blends of fruit juices with other fruit as well as herb extracts can impart unique functional attributes and could be an effective strategy in developing diet-based management of *H. pylori* infections as well as other oxidation linked diseases.

Keywords: Cranberry juice extract; Wild blueberry extract; Grape seed extract; Oregano extract; Phenolic antioxidants; 1-Diphenyl-2-picrylhydrazyl radical; *Helicobacter pylori*; Antimicrobial activity

1. Introduction

Helicobacter pylori is a spiral, ‘‘Gram negative, acid tolerant, microaerophilic bacterium that lives in the stomach and duodenum [1,2]. Infections related to *H. pylori* are generally recognized as one of the etiological agents of gastritis, peptic ulcer, gastric cancer, mucosa-associated lymphoid tissue lymphoma [3–5] and now to cardiovascular diseases [6]. *H. pylori* have co-evolved with humans and epidemiological studies indicates that *Helicobacter* species are indigenous to stomachs of more than 50% population and reaching 80% in some countries [7,8]. Most chronic infections of *H. pylori* are asymptomatic and if colonization of the bacteria persists symptoms appear in 15–20% of the infected populations [9]. The current treatment options available for infected patients include a combinational therapy of antibiotics and proton-pump inhibitors such as bismuth sulphate and nitroimidazole [10,11]. An important drawback of this approach is the development of antibiotic resistant colonizing strains of *H. pylori* against commonly used antibiotics such as tetracycline, amoxicillin, metronidazole leading to relapse of the infection [12,13]. Also, low compliance related to rigorous treatment regimens and associated side effects has led to increased demand for the development of more effective and simpler therapeutic

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strategies for managing *H. pylori* infections with fewer side effects [14].

Epidemiological studies have indicated that populations consuming diets rich in fruits and vegetables have lower incidences of certain types of cancer including gastric cancer [15,16]. This has led to the initial evidence that these populations may be protected by the anti-bacterial compounds (phytochemicals) in plant based extracts [17].

Many biologically active components having anti-*H. pylori* activity have since then been isolated from fruits, vegetables and herbs [17–20]. Recent research with purified compounds from peppers, wine and many other natural products have shown that phenolic phytochemicals such as cinnamic acids, cinnamaldehydes, coumarins, phenolic acids, capsaicin, flavanoids and tannins have high anti-*H. pylori* activity [21–27]. However, it is now believed that whole foods are more effective when compared to single compounds in the dietary management of many diseases [28–30]. This could possibly be due to the many modes of action of the different bioactive components in whole foods and their possible synergistic mode of action which may be contributing to their higher effectiveness [31,32]. Cranberry, blueberry and grape are important commercial crops of North America and are consumed in many popular foods [33]. Recent research has linked the consumption of these fruits to many health benefits such as inhibition of carcinogenesis, protection against cardiovascular diseases and other oxidation linked diseases [34–36]. It is now believed that these positive health benefits are a result of the constituent phenolic phytochemicals such as phenolic acids, flavanoids and proanthocyanidins [37,38]. In addition to these health benefits, emerging research has indicated that extracts from these fruits have antimicrobial activity against several foodborne as well as human pathogens [39]. Even though extracts from cranberry, blueberry and grape have been shown to inhibit the adhesion of *H. pylori* to the gastric mucus, very limited literature exists on the actual anti-*H. pylori* activity of these fruits [39–41]. Dietary management of *H. pylori* infection by consuming fruits and their products could be an effective strategy due to likelyhood of high compliance and absence of side effects. Also, many bioactive components in these fruits can be potential hurdles to counter the development of resistance strains because of their different modes of action. Therefore, in this research we have investigated the anti-*H. pylori* activity of cranberry, wild blueberry and grape seed extracts. We have also investigated the effect of synergies of cranberry extract with blueberry and grape seed extracts on enhancing *H. pylori* inhibition. We have further investigated the effect of cranberry and oregano synergies on *H. pylori* inhibition due to the documented high antioxidant and anti-*H. pylori* activity of oregano [19,20].

2. Materials and methods

2.1. Fruit extracts

Cranberry juice extract, wild blueberry extract and grape seed extract used in this research were kindly provided by Decas Cranberry Products Inc. (Carver, MA): The fruit juice powders and synergy mixtures were prepared as described in Table 1. Nutricran–AO extract which was a pre-formulated mixture of cranberry juice extract and oregano extract was obtained directly from Decas Cranberry Products Inc.

2.1.1. Water extraction

Fifty milliliters of distilled water was added to 5 g of the juice extracts mixed thoroughly and then centrifuged at 15,000 × *g* at 4 °C for 20 min.

2.1.2. Total soluble phenolics assay [42]

One milliliter of extract was transferred into a test tube and mixed with 1 ml of 95% ethanol and 5 ml of distilled water. To each sample, 0.5 ml of 50% (v/v) Folin–Ciocalteu reagent was added and mixed. After 5 min, 1 ml of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 725 nm. The absorbance values were converted to total phenolics and were expressed in milligrams equivalents of gallic acid per gram dry weight (DW). Standard curves were established using various concentrations of gallic acid in 95% ethanol.

2.1.3. Determination of antioxidant activity

2.1.3.1. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) inhibition system [43]. To 3 ml of 60 μM DPPH in ethanol, 500 μl of extracts were added, the decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The readings were compared with the controls, which contained 500 μl of 95% ethanol instead of the

Table 1
Fruit juice extracts and their synergy mixtures (w/w)

Name	Description
NC	Cranberry juice extract
WBB	Wild blueberry extract
GSP	Grape seed extract
NC–AO	Cranberry juice extract enriched with oregano extract pre-formulated
SYN-1	Cranberry juice extract (75%) + wild blueberry extract (20%) + grape seed extract (5%)
SYN-2	Cranberry juice extract (75%) + wild blueberry extract (15%) + grape seed extract (10%)
SYN-3	Cranberry juice extract (75%) + wild blueberry extract (10%) + grape seed extract (15%)
SYN-4	Cranberry juice extract (75%) + wild blueberry extract (25%)