Original Article

Potential of cranberry-based herbal synergies for diabetes and hypertension management

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Water soluble cranberry–based phytochemical combinations with oregano, rosemary, and *Rhodiola rosea* were evaluated for total phenolic content, related antioxidant activity and inhibition of diabetes management– related α-glucosidase, pancreatic α-amylase inhibition, and hypertension - related ACE – I inhibitory activities. Water extracts of oregano had 114.9 mg/g DW of phenolics which was highest among all the extracts tested, whereas the 75% cranberry with 25% oregano combinations had the highest phenolics (38.9 mg/g DW) among all the combinations tested. The water extracts of oregano had the highest DPPH radical inhibition activity (73.6 %), whereas among combinations the 75% cranberry and 25% oregano had the highest DPPH radical inhibition activity (50.8 %). These results indicated a correlation between total phenolic content and antioxidant activity. The water extracts of pure *Rhodiola rosea* had the highest α-glucosidase inhibition, whereas the 75% cranberry and 25% *Rhodiola rosea* combination had the highest inhibition among the combinations. In the case of α-amylase inhibition the water extracts of *Rhodiola rosea* had the highest inhibition, whereas the 75% cranberry with 25% *Rhodiola rosea* combination had the highest inhibition among the combinations. All the water extracts tested indicated that they had anti–ACE-I inhibitory activity. More specifically, among the water extracts 100% cranberry had the highest ACE-I inhibitory activity and among the combination the 75% cranberry with 25% rosemary had the highest ACE–I inhibitory activity. The analysis of α-glucosidase, α - amylase, and ACE-I inhibitory activities suggested that inhibition depend on the phenolic profile of each unique extract and by bringing together synergistic combinations to cranberry, health beneficial functionality was enhanced. This enhanced functionality in terms of high α-glucosidase and α-amylase inhibitory activities indicate the potential for diabetes management, and high ACE – I inhibitory activity indicates the potential for hypertension management.

Key Words: Water soluble phenolics, antioxidants, α-amylase, α-glucosidase, angiotensin converting- I enzyme, inhibitor, type 2 diabetes, hypertension, synergies, cranberry, rosemary, *Rhodiola rosea*, oregano

Introduction

Diabetes mellitus is a metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Specifically chronic hyperglycaemia of type 2 diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels, and has been shown to be also linked with hypertension.[1] Hyperglycaemia is a condition characterized of high levels of blood glucose. The major source of blood glucose is the hydrolysed dietary carbohydrates, such as starch. The dietary carbohydrates are hydrolysed by pancreatic α–amylase with absorption aided by α–glucosidases in order to be absorbed by the small intestine.[2] It is believed that inhibition of these enzymes can be important strategies for management of type 2 diabetes.[3]

α–Glucosidase is an enzyme that catalyses the final step of glucose absorption in the intestine during the digestive process of carbohydrates, and hence α–glucosidase inhibitors could retard the rapid utilization of dietary carbohydrates and suppress postprandial hyperglycemia.[4] The possibility of clinical use of such inhibitors for diabetic or obese patients has been attempted by acarbose, which has been shown to effectively reduce the intestinal absorption of sugars in humans.[5,6]

α–Amylase acts upon large polysaccharides (starch) at internal bonds. Natural α–amylase inhibitors offer an attractive therapeutic approach to the treatment of postprandial hyperglycaemia, by ultimately decreasing glucose release from starch. Recently it has been shown that phenolics play a role in mediating α-amylase inhibition and therefore have potential to contribute to the management of type 2 diabetes.[7]

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A problem with using α–amylase inhibitors that are highly active, is the occurrence of certain side effects, such as abdominal distention, flatulence, meteorism and possibly diarrhea. Previous reports have shown that such effects are possibly caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon.[8] Natural α-amylase and α-glucosidase inhibitors from plants have shown to have lower inhibitory effect against α-amylase activity and a stronger inhibition activity against α-glucosidase and therefore can be potentially used as effective therapy for postprandial hyperglycaemia with minimal side effects.[9]

High blood pressure (hypertension) is a common complication of diabetes. Recent statistical data show that hypertension occurs in approximately 30% of patients with type 1 diabetes and from 50 to 80% of patients with type 2 diabetes.[10] Hypertension is a multifactorial process and the main cause of illness in industrialized countries. One of the most important intermediary factors for controlling hypertension is the action of the Angiotensin–converting enzyme (ACE).[11] Angiotensin I converting enzyme (ACE) is a glycoprotein peptidylpeptide hydrolase, whose main known functions are to cleave histidyl–leucine from Angiotensin I forming the potent vasoconstrictor Angiotensin II, and to degrade bradykinin to inactive peptides.[12] Recent results indicating that certain flavonoid–rich foods can induce reductions in blood pressure and inhibit ACE–I activity, both *in vivo* and *in vitro*,[13] opens the possibility that consumption of select flavonoid rich foods may mimic synthetic ACE inhibitors and provide health benefits, but without adverse side effects.[13]

Phenolic phytochemicals are secondary metabolites of plant origin which constitute one of the most abundant groups of natural metabolites and form an important part of both human and animal diets.[14-16] Recent studies have shown that phenolic phytochemicals have high antioxidant activity and certain therapeutic properties.[17] Cranberry is a traditionally and widely consumed fruit in the United States, containing a wide range of phenolic phytochemicals, and has been historically associated with positive health benefits.[18] Recent results indicate that plants belonging to Lamiaceae family (Mint Family), including oregano and rosemary, have potential for management of many chronic oxidation-linked diseases such as diabetes and CVD.[19-21] *Rhodiola rosea* L., also known as "golden root" or "roseroot", belongs to the plant family Crassulaceae.[22] Recently it has been shown that in *Rhodiola rosea* L the biologically active substances salidroside, rosin, rosavin, rosarin and tyrosol, which are mainly found in plant rhizomes, demonstrate therapeutic effect.[23] These active components affect the central nervous system by increasing the ability to concentrate, improve mental and physical power and improve general resistance of the cells against outside infections. They also prevent the circulatory system from stress and arrhythmias, and posses some antioxidant activity. Some data confirm that the *Rhodiola rosea* L. preparations stop the growth of the malignant tumors and metastases in the liver.[23]

The aim of this investigation is to determine whether cranberry alone compared to combinations with oregano, rosemary and *Rhodiola rosea* have anti-type 2 diabetic and anti-hypertension functionality and to explore the possibility of cranberry-based combinations to synergistically enhance these health beneficial effects.

Materials and Methods

Dried cranberry powder was supplied by Decas Cranberry Products Inc. Dried oregano, *Rhodiola rosea*, and rosemary in powder form was supplied by Barrington Nutritionals. α-Amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1. 20) and angiotensin converting enzyme (EC 3.4.15.1) were purchased from Sigma Chemical Co. (St. Louis, MO). Unless noted, all chemicals also were purchased from Sigma Chemical Co. (St. Louis, MO).

Sample preparation

1 gram of powder was dissolved in 10 ml cold water. In the case of combinations for investigating synergistic functional benefits 0.75 grams of cranberry and 0.25 grams of the selected powder were mixed based on maximum saturation of antioxidant potential from combinations. Samples were mixed vigorously for 1 minute and then centrifuged two times at 10,000 x g for 10 minutes and the supernatant was collected.

Total phenolics assay

The total phenolics was determined by an assay modified from Shetty *et al*., (1995).[24] Briefly, one millilitre of extract was transferred into a test tube and mixed with 1ml of 95% ethanol and 5ml of distilled water. To each sample 0.5ml of 50% (v/v) Folin-Ciocalteu reagent was added and mixed. After 5 min, 1ml 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 grams dry weight (DW) of the sample. Standard curves were established using various concentrations of gallic acid in water.

Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

To 3ml of 60 μM DPPH in ethanol, 250 μl of each extract was 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 250 μl of water instead of the extract. The % inhibition was calculated by:

$$\%\ \text{inhibition} = \left(\left[\frac{A_{517}^{Control} - A_{517}^{Extract}}{\left[A_{517}^{Control} \right]} \right] \right) x100$$

α-Amylase inhibition assay

Porcine pancreatic α-amylase (EC 3.2.1.1) was purchased from Sigma Chemical Co. 500μl of extract and 500μl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing amylase solution (0.5 mg/ml) were incubated at 25°C for 10 minutes. After preincubation, 500μl of a 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at timed intervals. The

reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with 1.0ml of dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540 nm.

$$\%\ \text{inhibition} = \left(\left[\frac{A_{540}^{Control} - A_{540}^{Extract}}{\left[A_{540}^{Control} \right]} \right] \right) x100$$

This assay was modified compared to previous methods used in the laboratory that had 24 hour incubation time (McCue *et al*., 2004),[25] which in light of physico-biological mode of starch breakdown is too long. Therefore, we developed an assay with 10 min incubation time.

α-Glucosidase inhibition assay

α-Glucosidase assay was done by using 50µl of sample solution and 100µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0U/ml) and incubated in 96 well plates at 25°C for 10 minutes. After pre-incubation, 50µl of 5mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 minutes. Before and after incubation, absorbance readings were recorded at 405nm by microplate reader, Thermomax (Molecular device Co., Virginia, USA) and compared to a control which had 50 µl of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\%\ \text{inhibition} = \left(\left[\frac{\Delta A_{405}^{Control} - \Delta A_{405}^{Extract}}{\left[\Delta A_{405}^{Control} \right]} \right] \right) x100$$

Angiotensin converting enzyme inhibition assay

ACE inhibition was assayed by modifying a method developed by Cheung and Cushman (1973).[26] The substrate, hippuryl-histidyl-leucine (HHL) and angiotensin I-converting enzyme (ACE) from rabbit lung (EC 3.4.15.1) was used. Fifty microliters of extracts were incubated with 100µl of 1.0M NaCl-borate buffer (pH 8.3) containing 2.0 mU ACE-I solution at 37°C for 10 minutes. After pre-incubation, 100µl of a 5.0 mU substrate (HHL) solution was added to reaction mixture. Test solutions were incubated at 37°C for 1 hour. The reaction was stopped with 150 µl of 0.5 N HCl. The hippuric acid formed was detected and quantified by HPLC method. 5µl of sample was injected using Agilent ALS 1100 autosampler into an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with DAD 1100 diode array detector. The solvents used for gradient were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for the 5 min, then decreased to 0% for next 5 min (total run time, 18 min). The analytical column used was Nucleosil 100-5C18, 250x4.6 mm i.d., with packing material of 5 µm particle size at a flow rate 1ml/min at ambient temperature. During each run the chromatogram was recorded at 228 nm and integrated using Agilent Chemstation enhanced integrator for detection of liberated hippuric acid. Pure hippuric acid (purchased from Sigma Chemical Co., St. Louis, MO) was used to calibrate the standard curve and retention time. The % inhibition was calculated by:

$$\%\ \text{inhibition} = \left(\left[\frac{E^{Control} - E^{Sample}}{\left[E^{Control} - E^{Blank} \right]} \right] \right) x100$$

HPLC analysis of phenolic profiles

Two ml of phytochemical extracts were filtered through a 0.2 µm filter. 5 µl of sample was injected using Agilent ALS 1100 autosampler into Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with DAD 1100 diode array detector. The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was in-creased to 60% for the first 8 min and to 100% over the next 7 min, then decreased to 0% for the next 3 min and was maintained for the next 7 min (total run time, 25 min). The analytical column used was Agilent Zorbax SB-C18, 250x4.6 mm i.d., with packing material of 5µm particle size at a flow rate of 1 ml/min at ambient temperature. During each run the chromatogram was recorded at 306 nm and 333 nm and integrated using Agilent Chemstation enhanced integrator. Pure standards of protocatechuic acid, chlorogenic acid, caffeic acid, ellagic acid, resveratrol and rosmarinic acid (purchased from Sigma Chemical Co., St. Louis, MO) in 100% methanol were used to calibrate the standard curve and retention times.

Statistical analysis

All experiments were performed at least in triplicates. Analysis at every time point from each experiment was carried out in triplicates. Means, standard errors and standard deviations were calculated from replicates with in the experiments and analyses using Microsoft Excel XP. IC values were calculated using ED50plus vol.1. software, developed by Mario H. Vargas, MD.

Results and Discussion

Total phenolics

The total phenolic content in water extracts was analysed by the Folin-Ciocalteu method. In this study 3 different combinations of cranberry were used for synergy studies. One combination was with rosemary, which has lower total water soluble phenolic content than cranberry, and two combinations were with *Rhodiola rosea* and oregano, both of which have higher concentration of total water soluble phenolics than cranberry. Water extracts of oregano had 114.9 mg/g DW of phenolics which was highest among all the extracts tested, whereas combination of 75% cranberry with 25% oregano had the highest phenolics (38.9 mg/g DW) among all the combinations tested (Fig. 1). The results in Figure 1 show that there is a synergistic effect from combinations on the total water soluble phenolic content of the extracts.

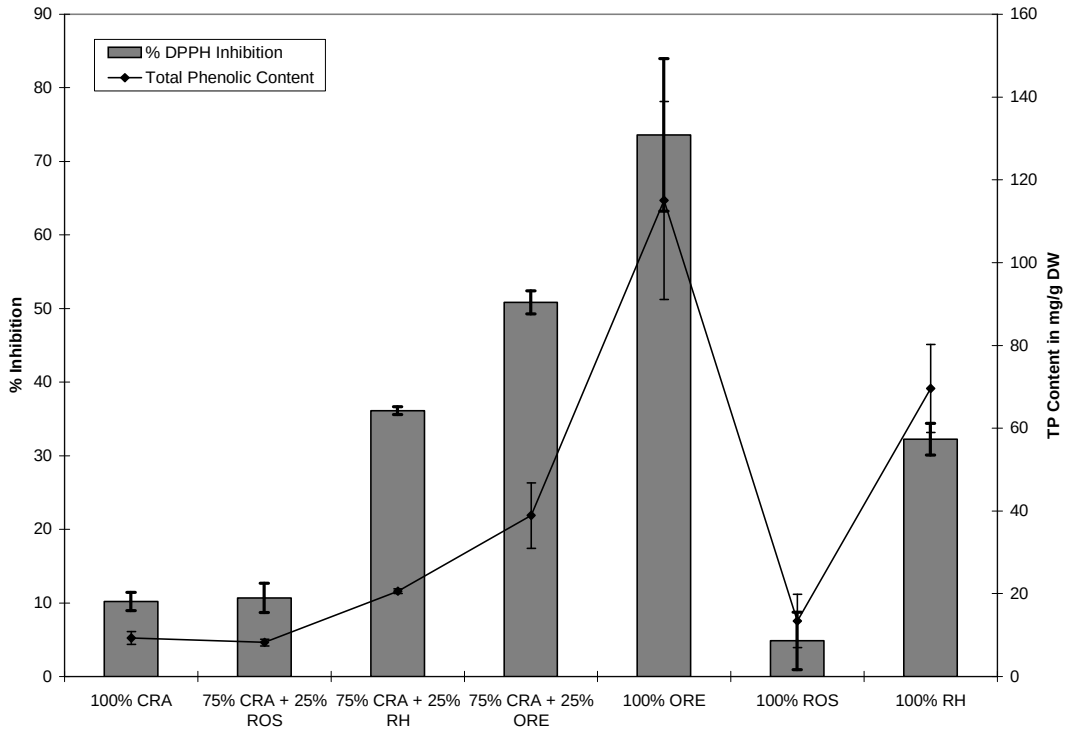

Figure 1. Summarized results of Total Phenolic concentration and DPPH scavenging activity.

Antioxidant activity by DPPH assay

The antioxidant activity on weight basis (1 gram) of extracts and synergistic enhancement from combinations were monitored using the DPPH radical inhibition (DRI) assay. The water extracts of oregano had the highest DPPH radical inhibition activity (73.6%), whereas among the combinations, 75% cranberry with 25% oregano had the highest DPPH radical inhibition activity (50.8%) (Fig. 1). These results indicate that there is a synergistic effect on the DPPH radical inhibition activity from cranberry with various phytochemical combinations. Further, there is a correlation between antioxidant activity with the total water soluble phenolic content (Fig. 1).

α-Glucosidase and α-Amylase Inhibition

The analysis of α-glucosidase and α-amylase inhibition were undertaken to investigate the type 2 diabetes management potential of cranberry based combinations. The α-glucosidase and α-amylase inhibitory activities were measured in three different total phenolic contents of the extracts and their combinations. α-Glucosidase activity was measured at 50, 100, and 200 µg/ml samples and inhibition was observed at all concentrations (Fig. 2). The water extracts of *Rhodiola rosea* had the highest inhibition, whereas the 75% cranberry with 25% *Rhodiola rosea* had the highest inhibition among the combinations. There is a clear synergistic effect with all the cranberry-based combinations.

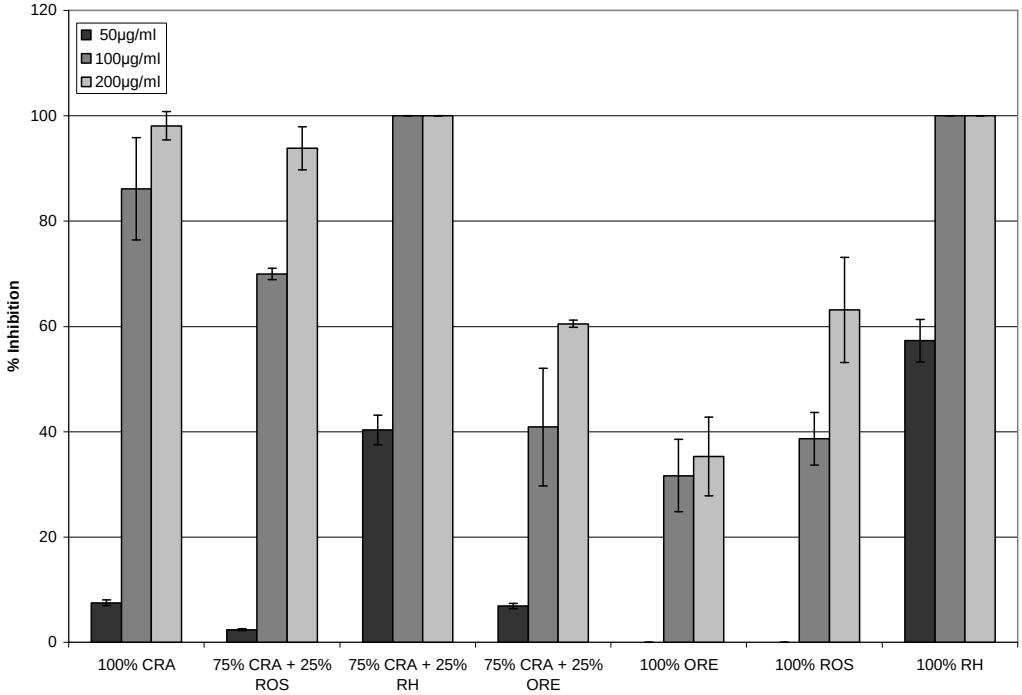

Figure 2. α– Glucosidase inhibition at three different total phenolic contents.

Table 1. IC80, IC50, and IC20 values for strong, mild and weak (respectively) α - glucosidase inhibitors.

Samples	IC80 µg/ml	IC50 µg/ml	IC20 µg/ml
100% RH	93		
75% CRA + 25% RH	116		
100% CRA		90	
75% CRA + 25% ROS		106	
75% CRA + 25% ORE			68
100% ROS			81
100% ORE			105

Table 2. IC80, IC50, and IC20 values for strong, mild, and weak (respectively) α -amylase inhibitors.

Samples	IC80 µg/ml	IC50 µg/ml	IC20 µg/ml
100% RH	100		
75% CRA + 25% RH	104		
100% CRA		110	
75% CRA + 25% ROS		164	
75% CRA + 25% ORE			142
100% ORE			164
100% ROS			198

α-Amylase activity was measured at 50, 100 and 200 µg/ml samples and inhibition was observed at all concentrations (Fig. 3). Water extracts of *Rhodiola rosea* had the highest inhibition, whereas the combination of 75% cranberry with 25% *Rhodiola rosea* had the highest inhibition among the combinations (Fig. 3). In order to compare the two enzyme inhibitory activities, we introduced the concepts of IC 80 for the strong inhibitors (phenolic content needed for 80% inhibition), IC 50 for the mild inhibitors (phenolic content needed for 50% inhibition), and IC 20 for the weak inhibitors (phenolic content needed for 20% inhibition) (Tables 1 & 2). The results indicate that the cranberry with *Rhodiola rosea* combinations had the highest α-glucosidase and α-amylase inhibitory activities.

ACE I Inhibition

Hypertension is a common complication of diabetes.[10] Control of hypertension via modulation of angiotensin I-converting enzyme (ACE –I) by dietary agents could be an important strategy to manage this risk factor.[12] In this investigation the ability of cranberry-based combinations to inhibit ACE–I at three different total phenolic contents (100, 200, and 500 µg/ ml) was undertaken. The results indicated that all the water extracts had ACE- I inhibitory activity. More specifically, the water extracts of pure cranberry had the highest ACE-I inhibitory activity and among the combinations 75% cranberry with 25% rosemary had the highest ACE – I inhibitory activity (Fig. 4).

HPLC analysis of phenolic profiles

The HPLC phenolic analysis showed that in cranberry alone (Fig. 5, 6) chlorogenic and ellagic acid were significant, and they could be important for ACE-I inhibitory activity, and to some extend to α-glucosidase and α-amylase inhibition. Cranberry with oregano combinations had higher rosmarinic acid (Fig. 5), which also likely contributed to the high antioxidant activity and total phenolic content. More likely rosmarinic acid contributed to the ACE–I as well as to α-glucosidase and α-amylase

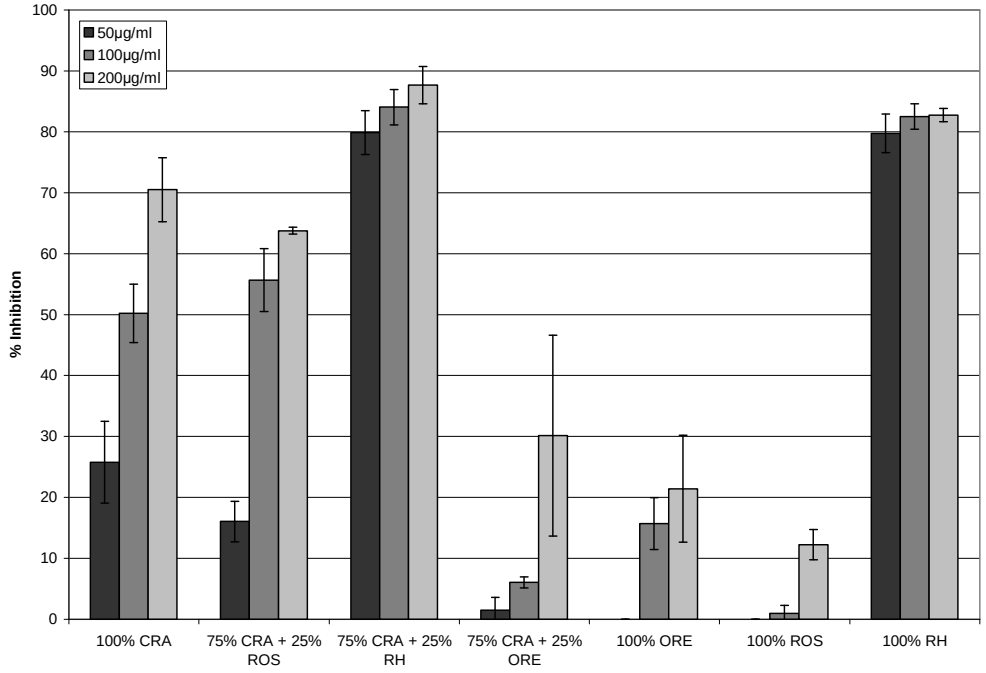

Figure 3. α-Amylase inhibition at different total phenolic contents

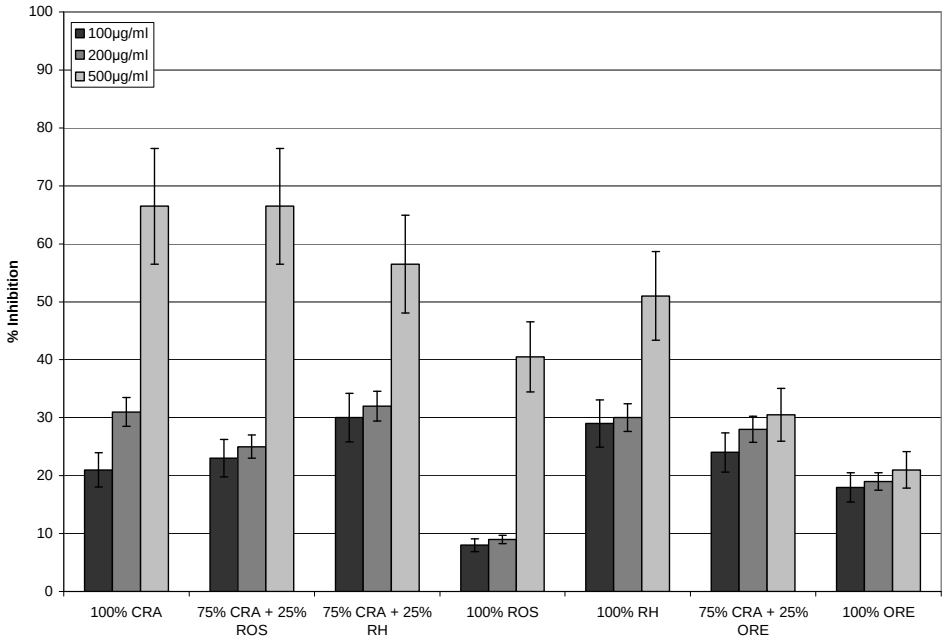

Figure 4. ACE I inhibition at different total phenolic contents

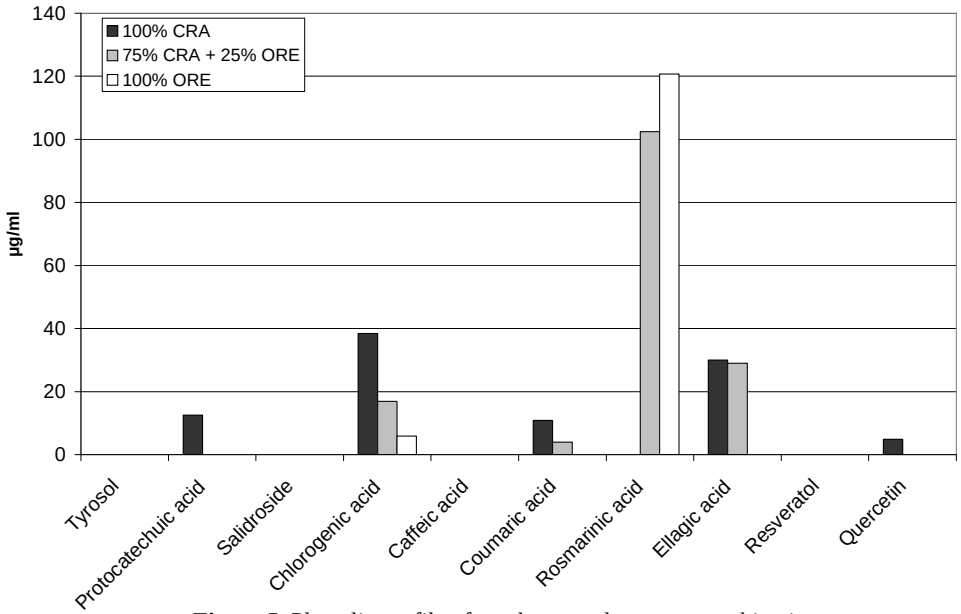

Figure 5. Phenolic profile of cranberry and oregano combination

inhibitory activities observed with cranberry and oregano combinations. However, these inhibitory activities were less when compared to cranberry alone. Rosmarinic acid therefore had less effect on ACE – I, α-glucosidase and α-amylase activities than cranberry alone or cranberry combinations with *Rhodiola rosea*. Rosemary combinations with cranberry had less phenolics than other combinations and this reflected in the reduced functionality responses (data not shown).

Cranberry and *Rhodiola rosea* combinations had slightly higher total phenolic content than cranberry alone and had very significant α-glucosidase and α-amylase inhibitory activities, but similar ACE – I inhibitory activity. Therefore, α-glucosidase and α-amylase inhibitory activities are linked to the increased phenolics from *Rhodiola rosea*, and particular tyrosol (Fig. 6).

Conclusions and implications

Diabetes mellitus is an emerging health concern in many parts of the world. For example, recent statistics indicate that from 1980 through 2003 in USA alone the diabetes patients more than doubled (from 5.8 million to 13.8 million).[27] More specifically, people aged 65 years or older account for almost 40% of the population with diabetes.[27] This research shows the positive potential of cranberry and cranberry – based phytochemical synergies for type 2 diabetes and hypertension management.

Initial studies indicated that antioxidant activity and water soluble phenolic content of cranberry and cranberry-based combinations were correlated. The most effective combination for increased antioxidant activity was 75% cranberry with 25% oregano, while 100%

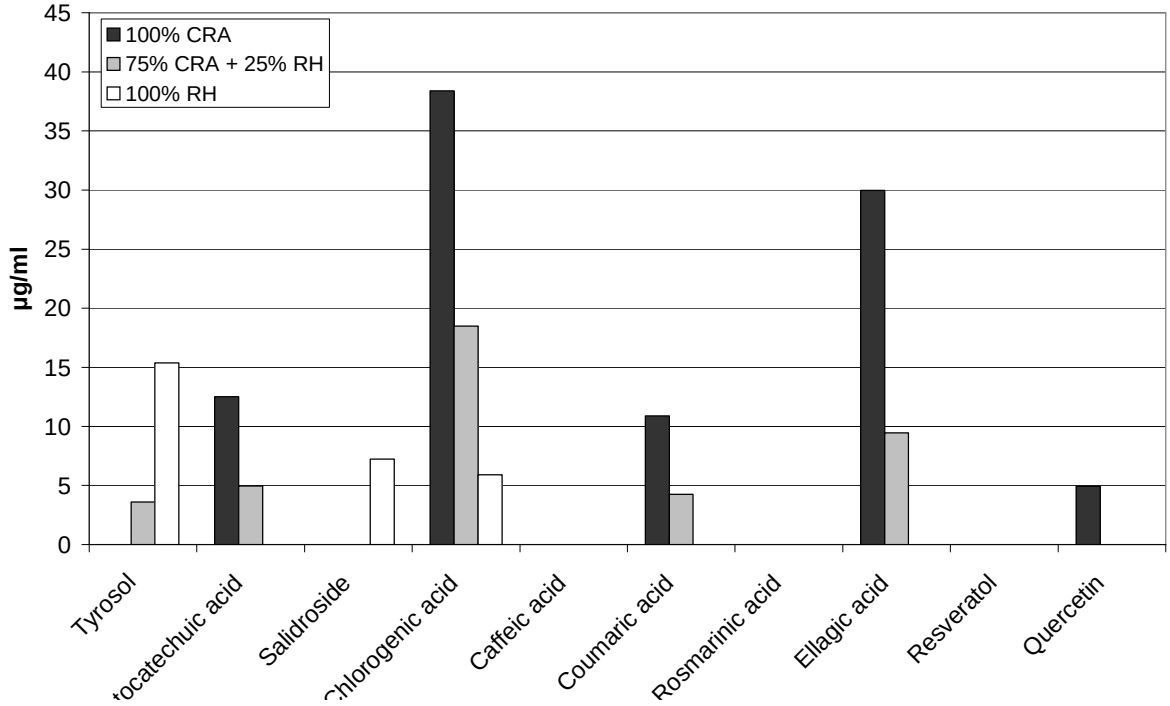

Figure 6. Phenolic profile of cranberry and *Rhodiola rosea* combination.

oregano had the highest antioxidant activity of all samples. These findings support previous investigations indicating that phenolic phytochemicals with antioxidant properties have additional health beneficial properties.[19]

In the case of α-amylase and α-glucosidase inhibitory activities the most effective combination was 75% cranberry with 25% *Rhodiola rosea*, indicating synergistic enhancement of functionality. Although pure cranberry appeared to be a mild inhibitor (Tables 1,2) the combination with *Rhodiola rosea* had a strong synergistic inhibition (Tables 1,2). It is also important to emphasize that all the extracts and their combinations had higher α-glucosidase inhibitory activity and lower α-amylase inhibitory activity. However the exception was cranberry combination with *Rhodiola rosea*, where the effect was reversed with a slightly higher α-amylase inhibitory than α-glucosidase inhibitory activity. This is potentially an important finding to reduce the side effects of excessive high α-amylase inhibition of drug treatments.[8] It is also an important finding that all *Rhodiola rosea* extracts appeared to be potentially more effective for type 2 diabetes management, since all the *Rhodiola rosea* treatments, in combinations and alone, gave the highest α-amylase and α-glucosidase inhibitory activities. In the case of ACE-I inhibition, 75% cranberry with 25% rosemary combination and 100% cranberry had the highest inhibitory activities.

Finally, the results indicate that all the inhibitory activities increased as we increased the phenolic dose, indicating dose dependence of water soluble phenolics for potential type 2 diabetes and hypertension management. Phenolics have been shown to have antioxidant and antimicrobial activities[19,28] and this investigation indicates that properly optimized phenolic diets could be an important strategy for type 2 diabetes and hypertension management.

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以酸果蔓为基础的草药间协同作用在糖尿病和高血压治疗方面具有潜在功效

本研究检验了水溶性酸果蔓与牛至、迷迭香和红景天混合后总酚的含量。酚类物质具有抗氧化活性，并能抑制与糖尿病治疗相关的 α-葡萄糖苷酶、胰腺 α-淀粉酶的活性，与高血压相关的血管紧张素转化酶的活性。在所有受测单一中药水提物中，以牛至水提物的总酚含量最高，占干物质总量的 11.49%；而在所有受测中药混合物中，75%酸果蔓与 25%牛至的总酚含量最高，占干物质总量的 3.89%。牛至水提物具有最高的二苯基 β 苦基肼游离基抑制活性（73.6）；而在所有混合物中，75%酸果蔓与 25%牛至混合物的二苯基 β 苦基肼游离基抑制活性最高（50.8%）。上述结果表明水提物的总酚含量与其抗氧化活性具有相关性。纯红景天水提物具有最高的 α-葡萄糖苷酶抑制活性；而在所有混合物中，75%酸果蔓与 25%红景天混合物具有最高的 α-葡萄糖苷酶抑制活性。纯红景天水提物还具有最高的 α-淀粉酶抑制活性；而在所有混合物中，75%酸果蔓与 25%红景天混合物具有最高的 α-淀粉酶抑制活性。所有受测水提物均具抑制血管紧张素转化酶的功效。确切地说，在所有单一中药水提物中，纯酸果蔓水提物具有最高的抑制血管紧张素转化酶的活性；在所有中药混合物中，75%酸果蔓与 25%迷迭香具有最高的抑制血管紧张素转化酶的活性。对 α-葡萄糖苷酶、α-淀粉酶和血管紧张素转化酶抑制活性的分析表明，单一中药水提物的抑制活性依赖于其总酚含量；其它中药与酸果蔓混合后出现协同效应，对健康有利的功效得到增强。在高的 α-葡萄糖苷酶、α-淀粉酶抑制活性方面增强的功效表明其在糖尿病治疗方面具有潜在功效；而高血管紧张素转化酶的抑制活性表明其在高血压治疗方面具有潜在功效。

关键词：水溶性酚、抗氧化剂、α-淀粉酶、α-葡萄糖苷酶、血管紧张素转化酶、糖尿病、高血压、协同作用、酸果蔓、迷迭香、红景天、牛至。