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Antioxidant Capacity and Anti-diabetic Activity of Wild Berry Stem Infusions

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Authors' contributions

Author HPVR is the principal investigator who designed the study. Author IP performed the experiments, statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The potential health benefits of green tea sand herbal teas have led to an increased demand of specialty tea products in the food market.
Aims: To determine the proximate composition, phytochemical compositions and hypoglycemic potentials of the methanol and hot water extracts of the stems of wild berries from Eastern Canada.
Study Design: Extraction of the air-dried stems of wild berries with both methanol and hot water, and testing the various extracts for the proximate composition, phytochemical composition and hypoglycemic potentials in comparison to green tea and rose-hip fruit.
Place and Duration of Study: The stems of wild blueberry, raspberry and blackberry along with rose-hip fruits were collected from the wild habitats of Windsor area of Nova Scotia, Canada in the year 2013; while the commercial green tea was purchased from a local store (Bulk Barn, Truro, Nova Scotia).
Methodology: Phenolic composition and characterization was done using spectrophotometric analyses and UPLC-ESI-MS. Carotenoid and caffeine content were measured using HPLC-PDA. The antioxidant capacities were evaluated using ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl(DPPH)and oxygen radical absorbance capacity (ORAC) assays. The

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anti-diabetic properties were investigated by α-amylase, α-glucosidase and advanced glycation products (AGEs) inhibition assays *in vitro*.
Results: The extracts consisted of phenolic acids, carotenoids, dihydrochalcones and flavonoids. The highest total phenolic content was observed in the stem extracts of blueberry (3019 and 4267 mg catech in equivalents (CE)/L for methanol and hot water extraction, respectively). The total antioxidant capacity was also the highest in blueberry stem, followed by blackberry stem. The blackberry stem and raspberry stem exhibited the greatest inhibition of α-amylase and α-glucosidase activities (IC_{50}=20-68 and 10-28 mg/L, respectively). Raspberry stem extract exhibited the highest anti-AGE activity with IC_{50} of 1.0-3.1 mg/L.
Conclusion: The present findings demonstrate a potential use of wild berry stems as a value-added ingredients in herbal tea blends.

Keywords: Herbal tea; phytochemicals; antioxidant; diabetes; amylase; glucosidase; glycation; berry.

1. INTRODUCTION

Diabetes mellitus (type 2 diabetes) is a chronic disorder marked by hyperglycemia and is related with imbalance and disturbance of carbohydrate, fat and protein metabolism emerging from imperfections in insulin secretion, action, or both [1]. This disease accounts for almost 90–95% of all identified cases of diabetes in adults [2]. According to the World Health Organization [3], at least 220 million people have diabetes globally and this number is estimated to double by 2030. Among therapeutic approaches for treating diabetes, inhibition of carbohydrate hydrolyzing enzymes such as α-glucosidase and α-amylase are the most commonly used to decrease postprandial hyperglycemia. These inhibitors are the drug-design targets in the advancement of formulae for the treatment of diabetes, obesity and hyperlipidemia [4]. Also, protein glycation, a reaction between carbonyl groups of reducing sugars and amino groups of protein, has been demonstrated to be a major pathogenic factor in diabetes. Advanced glycation end products (AGEs) are a group of complex and heterogeneous compounds generated during protein glycation and through a complex series of reactions cause structural and functional modifications to proteins and lipids, thereby giving rise to oxidative stress and vascular changes [5]. For centuries, plant-based medicines have been used for the treatment of diabetes, particularly in developing countries. Not only the fruit and leaves, other plant parts such as rhizomes and barks have also been used in health care applications.

There is a plethora of evidence suggesting that oxidative stress due to reactive oxygen species (ROS) plays a critical role in the tissue damage and pathological events in living organisms. The harmful effects of ROS are limited through a high-performance antioxidant system, consisting of enzymes, proteins, vitamins (A, C, E), carotenoids, polyphenols, glutathione and trace elements(for review, see ref [6]). Therefore, antioxidant activity may be of vital importance in attenuating the onset and/or the progression of oxidative pathologies. Plant polyphenols have been recognized to many health promoting properties such as anti-cancer, anti-inflammatory, antioxidant, anti-diabetic, and anti-aging. For instance, green tea polyphenols have been reported to ameliorate adipose insulin resistance by combatting oxidative stress *in vivo* [7].

Recently, the increased health consciousness among consumers has resulted in increased consumption of beverages that are healthy but tasty and flavorful. In lieu of this, tea and herbal infusions are some of the most demanding beverages as they contribute to the major source of polyphenols in our diet [8]. A report from the Government of Canada [9] showed that specialty teas such as herbal, black, green and flavored fruit tea currently make up 60% of total tea market share in Canada, which shows the high consumer interests in natural health beverages. Therefore, identification of plant-based value-added ingredients with potential therapeutic benefits will substantiate incorporation of them in herbal tea blends.

Wild berries such as blueberry, raspberry and blackberry contain high contents of phytochemicals such as anthocyanins, flavonols, phenolic acids and proanthocyanidins with several health enhancing properties such as anti-carcinogenic, anti-microbial, anti-inflammatory, anti-diabetic, anti-aging, among others (for review, see ref [10]). Previously, most of the literature on phytochemical analyses and biological activity has been limited to the berry

fruit, and little attention is given to the phenolic characterization and biological activity of vegetative byproducts such as stems. There have been some studies focusing on the phenolic composition of berry leaves, flowers and rhizomes; however, no report is available about the potential use of stems of major wild berry crops. Except for the berries, other plant parts cannot be consumed in their original form but can be incorporated in nutraceuticals, food supplements or blended tea preparations. For instance, many biologically active polyphenols, found in the leaves of berry crops such as blackcurrants and sea-buckthorns, have long been used in a wide variety of applications [11-13]. In addition, wild rose fruit (rosehips) is known for the high content of vitamin C and other dietary constituents including polyphenols which have demonstrated modulatory effects on cell proliferation and oxidative reactions in cellular systems [14].

Several studies have been conducted for the presence and the activity of antioxidants in tea and herbs but emphasis has been given to organic solvent extracts isolated from dried leaves. Studies describing the phenolic profiles, antioxidant activity and biological activity of herbal tea infusions are limited. Therefore, the aim of this study was to evaluate phenolic composition, antioxidant capacity, anti-diabetic activity of four different kinds of herbal tea infusions from stems of wild berry crops and wild rose fruit found in Eastern Canada and to compare them with corresponding solvent extracts as well as commercial green tea. In addition, the relationship between antioxidant capacity and bioactive composition was determined to present new information on these extracts for nutritionists and the general public.

2. MATERIALS AND METHODS

2.1 Chemicals

HPLC grade methanol, acetonitrile and formic acid were purchased from (Sigma Aldrich, Oakville, ON, Canada). The liquid chromatography standards were purchased as follows: Phloridzin, phloretin, chlorogenic acid, ferulic acid and caffeic acid from Sigma Aldrich (Oakville, ON, Canada); catechin, epicatechin, quercetin, quercetin-*3*-*O*-galactoside and quercitin-*3*-*O*-glucoside from Chroma Dex, Inc. (Santa Ana, CA, USA); quercitin-*3*-*O*-rhamnoside, quercitin-*3*-*O*-galactoside and anthocyanin standards from Indofine Chemical Company (Hillsborough, NJ, USA) and2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals (Richmond, VA, USA). Unless and otherwise stated, all other chemicals, reagents and enzymes used for different assays were obtained from Sigma Aldrich, Canada.

2.2 Experimental Material

The samples were collected from the wild habitats of Windsor area of Nova Scotia, Canada in the year 2013. After harvesting a bulk sample of approximately one kg from multiple growing locations, the samples were sub-divided into three replicates. The stems were cut into small chunks (1 cm) and were dried at 45°C in a cabinet drier(Model RS45H, The Edward Milner Co. Ltd, Agincourt, ON, Canada)until constant weight was observed. Three commercial green tea powders were purchased from Bulk Barn (Truro, Nova Scotia, Canada). Later, the stems, wild rose fruit and green tea samples were ground in a coffee grinder (Model 6378-33, Sunbeam, China) to fine powder and were stored at -20°C until use.

2.3 Chemical Compositions

Crude protein, crude fat and ash of oven dried berry samples and green tea were determined according to AOAC Methods 955.04, 920.39 and 930.05, respectively. Iron was determined as outlined in AOAC Methods 921.01. Analysis for iron was carried out using atomic absorption spectroscopy set at 422.7 nm. All the chemical composition analyses were carried out in triplicates.

2.4. Preparation of Hot Water Extracts

Five grams of each sample were infused into 100 ml of boiling water (equivalent to a small tea cup) for 5 min, filtered through Whatman No. 4 paper and then centrifuged to separate supernatant from residue. The supernatants were used for phenolic composition analyses using UPLC-ESI-MS and were stored at -20°C until further use.

2.5 Preparation of Methanol Extracts

Each sample powder (5 g) was extracted with 100 mL of 100% methanol with sonication conditions as determined previously [15]. Briefly, the tubes containing samples were subjected to sonication for 15 min at 30°C thrice with 10 min intervals. Contents were filtered through centrifugation at 4900 rpm for 10 min. The

methanol extracts were then stored at −20°C until further use.

2.6 Total Phenolic Content: Folin-Ciocalteu Assay

The Folin-Ciocalteu assay was used to measure the total phenolic content as described by Singleton and Rossi [16] and modified by Rupasinghe et al. [17]. Calibration curves were plotted using standards at concentration range of 10-750 mg/L. The results were expressed as mgcatechin equivalents (CE)/L.

2.7 UPLC-ESI-MS Analysis

An ultra-high performance liquid chromatography (UPLC) (Model H-class system, Waters, Milford, MA, USA) equipped with an Aquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 µm) (Waters, Milford, MA, USA) was used for the characterization of phenolic profile of extracts. For the analysis of non-anthocyanin phenolics, gradient elution was carried out with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with the flow rate of 0.2 mL/min and injection volume of 2.0 µL. A linear gradient profile was used with the following proportions of solvent A applied at time t (min); (t, A%): (0, 94%), (2, 83.5%), (2.61, 83%), (2.17, 82.5%), (3.63, 82.5%), (4.08, 81.5%), (4.76, 80%), (6.75, 20%), (8.75, 94%), (12, 94%). The analysis of anthocyanins was performed as described below; the mobile phases were 5% (v/v) formic acid in water (solvent A) and 5% (v/v) formic acid in methanol (solvent B). The linear gradients used were as follows; (t, A %): (0, 10%), (8, 30%), (17, 40%), (19, 40%), (20, 10%), (22, 10%).

MS analysis was performed with a Micromass Quattro micro API MS/MS system, which is controlled by MassLynx V4.1 data analysis system (Micromass, Cary, NC, USA). Electrospray ionization in negative ion mode (ESI-) was used for the ionization of the flavonols, flavan-3-ols and phenolic acids. The mass spectrometry conditions included capillary voltage of 3000 V with nebulizing gas (N_2) at a temperature of 375°C. Electrospray ionization in positive mode (ESI+) was used for the analysis of anthocyanins. The mass spectroscopy conditions used were capillary voltage of 3500 V with nebulizer gas at 375°C and a flow rate of 0.35 mL/min. The cone voltage (25-50 V) was optimized for each individual compound. Individual samples were identified using a multiple reactions monitoring mode and specific precursor-product ion with quantified calibration curves generated by external standards.

2.8 Proanthocyanin Content

For total proanthocyanidins content, dimethylcinnamaldehyde (DMAC) method was used. Briefly, DMAC reagent (0.1%) was prepared fresh in methanol acidified in 6N sulfuric acid. Calibration curves of standard were plotted using catechin at 10-500 mg/L. The micro plates were prepared by adding 50 µL of the samples and 150 µL of the DMAC reagent. The plates were incubated in the dark at 25°C for 30 min using a BMG Fluostar Optima micro-plate reader (Mandel, QC, Canada). The visible absorption maximum of the reaction (λ_{max}) was monitored at 640 nm.

2.9 Total Carotenoid Content

Total carotenoids were extracted under reduced light conditions using a modified method of Rivera and Canela [18]. To a 10 mL of methanol/hot water extracts, 10 mL hexane-diethyl ether (9:1 v/v) was added and the mixture was shaken vigorously. Then, 20 mL saturated NaCl solution was added and the mixture was vortexed for 1 min. A separation funnel was used to separate the aqueous and organic layers prior to nitrogen evaporation of organic phase and the dried extract was dissolved in 5 mL methanol. Then the absorbance reading was taken using a spectrophotometer at 470 nm. The samples were diluted with methanol, if necessary. Appropriate blank was used to set the baseline absorbance of the spectrophotometer at 470 nm. The samples were diluted with methanol, if necessary. The total carotenoids were calculated according to Gross [19] using the equation:

$$\text{Total carotenoids} = (AV\times10^6)/(A^{1\%}\times100\ G)$$

Where A is the absorbance at 470 nm, Vis the total volume of extract, $A^{1\%}$ is the extinction coefficient for a mixture of solvents arbitrarily set at 2500, Gis the sample weight in grams.

2.10 Caffeine Content

Caffeine in the extracts was determined using a Waters Alliance 2695 HPLC system connected to a Waters 996 photodiode array (PDA) detector. A reverse phase C_{18} column (250×4.6 mm; 8 µm; Phenomenex, Torrence, CA, USA) was used and the analysis conditions were: PDA at 277 nm;

temperature of 25°C for column; mobile phase of methanol and deionized water (70:30); run time 15 min and isocratic flow rate 0.6 mL/min. An injector volume of 2 µL was used for each run. Four point calibration curves were obtained by using caffeine standard at concentration range between 20 and 200 mg/L. The results are expressed in mg/L.

2.11 Total Antioxidant Capacity

2.11.1 FRAP assay

The ferric reducing antioxidant power (FRAP) assay was used to determine the electron donating potential of the bioactive compounds present in the samples based on the assay described by Benzie and Strain [20] and modified by Rupasinghe et al. [17] and the results were expressed as mmol Trolox equivalents (TE)/L using a standard curve of Trolox with a concentration range of 10-500 µM.

2.11.2 DPPH•radical scavenging assay

The free radical scavenging activity of the extracts was measured using DPPH method. To a 0.1 mL of 0.2 mM solution of DPPH• (1, 1-diphenyl-2-picrylhydrazyl) in methanol, 0.1 mL of a phenolic extract in methanol was added at different concentrations. The decrease in the absorbance at 517 nm was measured at 0, 5 and then every 15 min until the reaction reached a plateau using the above described micro plate reader. The percentage of DPPH• remaining at the steady-state was calculated as a function of the molar ratio of antioxidant to DPPH•, against methanol without DPPH• as the blank reference. Each sample was tested three times and the values were averaged. The IC_{50} value (mg/L), defined as the amount of the sample to scavenge 50% of the DPPH•, was obtained by interpolation from linear regression analysis using Excel.

2.11.3 ORAC assay

The ORAC assay was performed as described elsewhere [21]. Briefly, the standards and samples were prepared using 75 mM phosphate buffer (pH 7). Peroxy radical was generated using AAPH which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: Excitation at 490 nm and emission at 510 nm using the above mentioned micro-plate reader. The antioxidant capacity of the samples was calculated as mmol Trolox equivalents (TE)/L using a quadratic relation developed from area under the florescence decay curves for standards made to 10-1000 µM concentrations, relative to blank.

2.12 α-Amylase Inhibitory Assay

The pancreatic porcine α-amylase assay was adapted from Sudha et al. [22] with modifications. Briefly, both methanol and hot water extracts were dried to obtain a completely dry powder. The test extracts, enzyme and soluble starch were dissolved in 20 mM sodium phosphate buffer containing 6 mMNaCl (pH 6.9). The test extracts at different concentrations were dissolved in buffer solution. To a test tube, added 250 µL of pancreatic porcine α-amylase (1 U/mL, dissolved in the buffer (pH 6.9) and 100 µL of test extract at concentration ranging from 15.6-250 mg/L were added. The mixture was pre-incubated at 37°C for 15 min, prior to the addition of 250 µL of 0.5% starch. The mixture was then vortexed and incubated again at 37°C for 15 min followed by the reaction termination using 1 mL of dinitrosalicylic acid color reagent. The tubes were placed in boiling water bath for 5 min, cooled to room temperature and diluted. Two hundred microliters of the reaction mixture was taken into a 96 well clear plate and the absorbance was read at 540 nm using the micro-plate readeras described above. The control α-amylase at 1 U/mL without any inhibitor represented 100% enzyme activity. Appropriate test extract controls containing the reaction mixture except the enzyme were used to correct for the color interference. A knownα-amylase inhibitor, acarbose was used for comparison studies. The percentage inhibition of test sample on α-amylase was calculated as:

$$\text{Inhibition (\%)} = 100 \times (A_S - A_{SB})/(A_C - A_{CB})$$

Where A_S, A_{SB}, A_C, and A_{CB} are the absorbance of sample, sample blank, control, and control blank, respectively. The results were expressed in terms of IC_{50} and IC_{90}, representing the amount of test extracts required to cause the enzyme inhibition by 50% and 90%, respectively.

2.13 α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory assay was adapted from Li et al. [23] with modifications. Briefly, both methanol and hot water extracts were freeze dried to obtain a completely dry powder. From this powder, various concentrations of each extract were prepared in 10 mM potassium

phosphate buffer (pH 6.8). To a 96-well clear plate, a reaction mixture containing 20 µL extract at different concentrations, 20 µL α-glucosidase (0.5 U/mL) and 60 µL of 10 mM potassium phosphate buffer (pH 6.8) was pre-incubated at 37°C for 15 min prior to adding 20 µL of 5 mM*p*-nitrophenol-α-D-glucopyranoside substrate. The mixture was then incubated at 37°C for the reaction to take place. After 15 min, 80 µL of stop solution containing 200 mM sodium carbonate was added. Then the absorbance at 405 nm was recorded using micro-plate reader. The positive control sample was the mixture of the enzyme and substrate without inhibitors. The sample controls and blanks were the mixtures of sample and control, respectively, except α-glucosidase was instead with buffer, respectively. The inhibition (%) of test sample on α-glucosidase was calculated same way as with α-amylase assay. Acarbose, a prescribed drug for α-glucosidase inhibition, was also used for comparison purpose.

2.14 Inhibition of Advanced Glycation Products (AGEs)

This method was modified based on human serum albumin (HSA)-glucose assay described by Liu et al. [24]. HSA and glucose were dissolved in phosphate buffer (PBS; 50 mM, pH 7.4) to a concentration of 35 mg/mL and 16.5 mM, respectively. The herbal extracts were dissolved in the same PBS buffer. One mL of the HAS solution was mixed with 1 mL of glucose solution and 1 mL of herbal extracts at different concentrations between 15-500 mg/L. The mixtures were incubated at 37°C for a week. Sodium azide (0.2 g/L) was used as an antimicrobial agent. PBS buffer was used as blank, HSA-glucose without any inhibitory agent was used as control and aminoguanidine and metformin were used for comparative purpose. After seven days of incubation, fluorescence of samples was measured using an excitation of 330 nm and an emission of 410 nm, respectively. The percentage inhibition of AGEs formation = [1-(fluorescence of the test group/fluorescence of the control group)] × 100%.

2.15 Statistical Analysis

The experiments were performed in completely randomized design in triplicates and data is expressed as mean±standard deviation. The significant difference between extraction methods, total phenolic content, proanthocyanidins, carotenoids, caffeine, FRAP, ORAC, DPPH, α-amylase, α-glucosidase inhibition values were tested using one-way analysis of variance (ANOVA) and the multiple mean comparison was performed in General Linear Model (SAS V8, Cary, NC, USA) using Tukey's test. In order to evaluate the relationship between the different results on assays, the Pearson correlation analyses were performed using MINITAB 16 (State College, PA, USA). Significant levels were defined as probabilities of 0.05 or less.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The general composition and the mineral content of the selected berry stems, wild rose fruit and green tea powder are presented in Table 1. All samples contained very low amount of moisture (3.4-6.6%), as reflected from their dry matter content. Crude protein content of berry stems was 5.8–8.2 g/100 g DM, which was significantly lower than commercial green tea powder (23.4 g/100 g DM), suggesting that green tea is a good source of protein. This was in line with previous reports showing that green tea and its by-products contained protein in the range of 24-31g/100 g [25-26]. However, the protein content of wild rose fruit (7.5 g/100 g DM) in the present study was more than two-fold higher than reported by others [27]. The proximate compositions were similar for the stems of blueberry, blackberry and raspberry, except significantly higher crude fat for blueberry stem. Among all the samples, wild rose fruit was found to contain the highest crude fat content of 3.8 g/100 g DM, which was approximately six times higher than reported earlier [27]. The concentration of mineral elements varied greatly among the given samples. Overall, the blackberry stem was found to possess the highest elemental composition of 0.72, 0.24, 0.12, 0.11 and 0.06 g/100 g DM for Ca, K, P, Na and Mn, respectively. However, the Fe content of green tea powder was significantly higher than selected wild berry stems and wild rose fruit. Raspberry stem contained the highest Fe content among all the three wild berry stems. On the other hand, wild rose fruit displayed remarkably high concentrations of K (1.23 g/100 g DM), Mg (0.31 g/100 g DM) and P (0.20 g/100 g DM).

3.2 Characterization of Polyphenols

3.2.1 Qualitative analysis for polyphenols

Hot water extraction and sonication-assisted methanol extraction methods were compared for total phenolic content (TPC) as measured by Folin Ciocalteu method. The results clearly showed that the TPC of hot water extract of wild berry stems were higher than extracts produced by methanol extraction (Table 2). This was true for green tea as well as for wild rose fruit extracts. A comparative study [28] also supported this fact that the TPC of wild rose fruit extracts were better extracted by water as compared to organic solvents such as ethanol and methanol. Among all stem extracts, blueberry stem contained the highest content of TPC (3019-4267 mg CE/L), irrespective of the method used for extraction. In comparison to blueberry stem, green tea extracts were found to contain 38% and 32% higher TPC in methanol and hot water extracts, respectively. On the other hand, raspberry stem was observed to contain the lowest TPC with only 1219 and 3222 mg CE/L for methanol and hot water extraction, respectively.

With regards to the proanthocyanidin content of the hot water extracts of wild berry stems, it ranged between 289-513 mg CE/L, with blackberry stem having the highest proanthocyanidin content (Table 2). However, the green tea extracts were found to have proanthocyanidins of 1681 mg CE/100 mL, which is 75% higher than that of blueberry stem. Since catechin was used as standard, therefore, the monomeric catechins present in green tea are expected to interfere in the results. On the other hand, the methanol extracts of wild berry stems showed a greater range of proanthocyanins between 350-621 mg CE/L, with blueberry being the highest. Similar to hot water extracts, green tea showed remarkably high values of proanthocyanidin content of 2657 mg CE/L in methanol extracts, owing to its monomeric flavan-3-ols, as reflected by the UPLC-ESI-MS analysis.

3.2.2 UPLC-ESI/MS characterization

Characterization of major common phenolic compounds by UPLC-ESI-MS methods for different berry samples and green tea are presented in Table 3. A total of 19 common polyphenols were identified and quantified in herbal extracts. It was interesting to note that the berry stems contained similar phenolic compounds as found in green tea extracts, with varying concentrations. The most predominant phenolic sub-groups found in wild berry stem extracts were flavonol glycosides and dihydrochalcones, with varying concentrations of phenolic acids and flavan-3-ols. While the concentration of most flavan-3-ols was the highest in green tea samples as expected, the berry stems contained remarkably high amounts of epicatechins (70-100 mg/L).

Table 1. Proximate composition and mineral analysis (g/100 g DW) of green tea, wild rosehip fruit and stems of wild berries

Analysis	Green tea	Wild raspberry stem	Wild blueberry stem	Wild blackberry stem	Wild rose fruit
Dry matter (%)	93.4±0.4^{a}	95.3±0.1^{c}	96.6±0.0^{a}	95.9±0.1^{b}	94.8±0.0^{c}
Crude protein	23.4±2.3^{a}	6.1±0.1^{b}	5.8±0.1^{b}	8.2±0.5^{b}	7.5±0.6^{b}
Crude fat	2.0±0.4^{bc}	1.6±0.1^{c}	2.6±0.3^{b}	1.9±0.2^{c}	3.8±0.2^{a}
Ash	6.5±0.1^{a}	1.6±0.1^{c}	1.9±0.0^{c}	2.2±0.3^{c}	4.4±0.3^{b}
Iron	0.1±0.0^{a}	ND^{b}	ND^{b}	ND^{b}	ND^{b}
Calcium	0.7±0.2^{a}	0.5±0.0^{b}	0.4±0.0^{b}	0.7±0.1^{a}	0.4±0.0^{b}
Potassium	1.4±0.3^{a}	0.1±0.0^{b}	0.2±0.0^{b}	0.2±0.0^{b}	1.2±0.1^{a}
Magnesium	0.2±0.0^{b}	0.1±0.0^{c}	0.1±0.0^{c}	0.1±0.0^{c}	0.3±0.0^{a}
Phosphorus	0.3±0.0^{a}	0.1±0.0^{c}	0.1±0.0^{c}	0.1±0.0^{c}	0.2±0.0^{b}
Sodium	ND^{b}	ND^{b}	ND^{b}	0.1±0.0^{a}	0.1±0.0^{a}
Manganese	0.1±0.0^{a}	ND^{b}	0.1±0.0^{a}	0.1±0.0^{a}	ND^{b}

ND, note detected: amounts lesser than 0.01 g/100 g DW, $^{a-c}$Different letters within rows denote significant differences between values as determined by one way ANOVA analysis

Table 2. Total phenolic content, total proanthocyanidins, total carotenoids and caffeine content of wild rose fruit and wild berry stem extracts in comparison to green tea

Extraction method	Sample	Totalphenolic content (mg CE/L)	Total proanthocyanidins (mg CE/L)	Total carotenoids (mg/L)	Caffeine content (mg/L)
Methanol	Greentea	4876±243[a]	2657±81.4[a]	410±37[a]	1356±330[a]
	Wild raspberry stem	1219±87[b]	350±31.2[b]	137±16[b]	277±19[b]
	Wild blueberry stem	3019±232[ab]	621±13.2[b]	197±19[b]	31±1[c]
	Wild blackberry stem	1887±58[ab]	391±10.2[b]	320±8[ab]	20±4[c]
	Wild rose fruit	3309±174[ab]	528±21.1[b]	206±14[ab]	15±1[c]
Hot water	Greentea	6299±197[a]	1681±70[a]	1332±125[a]	1558±250[a]
	Wild raspberry stem	3222±261[b]	289±16[b]	275±13[b]	335±30[b]
	Wild blueberry stem	4267±725[ab]	325±28[b]	416±11[b]	287±15[b]
	Wild blackberry stem	3802±203[ab]	513±14[b]	332±15[b]	307±14[b]
	Wild rose fruit	4557±116[ab]	686±35[b]	776±32[ab]	268±8[b]

[a-c]*Different letters across columns denote significant differences between values as determined by one way ANOVA analysis. CE: catechin equivalents*

Among the hot water extracts, dihydrochalcone concentration in berry stem samples was 67-81% higher than obtained in wild rose fruit and up to 45% higher than found in green tea. Furthermore, for phenolic acid and total flavonol concentration, the wild blackberry stem ranked the highest with 66.4 mg/100 mL and 45.2 mg/L, respectively. As compared to green tea and wild rose fruit, the wild blackberry stem contained phenolic acid concentration significantly higher by 87% and 99%, respectively; while 18% and 95.5% higher yields were observed for total flavonol concentration. The wild rose fruit extracts were found to contain the lowest concentrations of all the selected phenolic compounds studied.

Similarly, among the methanol extracts, the berry stems were found to have significantly higher ($P<0.05$) concentrations of dihydrochalcones, phenolic acids and flavonol derivatives, with the exception of flavan-3-ols. Unlike the hot water extracts, blueberry stem exhibited the highest chlorogenic acid and quercetin rutinoside content. The green tea extracts contained 84% and 60% lower concentration of phenolic acids as compared to blueberry and blackberry, respectively. Similarly, for flavonols, the blueberry stem contained the highest concentrations of 51 mg/L, a value 23% higher than found in green tea and 86% higher than found in wild rose fruit. The flavonol concentration of water extract of blackberry stem was 18% higher than green tea extracts. In addition, the blueberry stem also had the highest concentrations of all the four anthocyanins detected (Table 3) as compared to all other extracts, irrespective of the method used for extraction. The blackberry stem ranked second to blueberry stem in terms of anthocyanin concentration. Overall, the berry stem extracts showed promising results in terms of their phenolic concentrations and the variety of flavonoids present in them as compared to green tea and wild rose fruit extracts. Using these wild berry ingredients in herbal tea blends, well-balanced flavonoid-rich infusions can be developed.

3.3 Total Carotenoid Content

Since blueberry, blackberry and raspberry are known to contain carotenoids including β-carotene, lutein, zeaxanthin, beta-cryptoxanthin and alpha-cryptoxanthin [29], the present study demonstrated the presence of total carotenoids in the wild berry stem extracts as well (Table 2). The total carotenoid content of berry stems ranged between 137-320 mg/L in methanol extracts, with blackberry stem containing the highest value. In hot water extracts, the berry stems contained the carotenoid content in the range of 275-417 mg/L in hot water extracts, with blueberry stem with the highest value. In line with

previous studies [30-31], the present findings showed that presence of high carotenoid content in commercial green tea with mean values of 410 and 1332 mg/L in methanol and hot water extracts, respectively.

3.4 Caffeine Content

Our study determined the caffeine content of the novel herbal extracts between 269-335 mg/L, a range 5-6 times lower than found in the green tea (Table 2). The hot water extracts showed higher amounts of caffeine than found in methanol extracts, owing its greater solubility in water. A previous study has shown the caffeine content of different green teas in the range of 100-300 mg/L based on a 2 g tea powder per 200 mL extraction solution [32]; while the present study based on a 5 g green tea/100 mL extract showed the caffeine content range of 1356-1558 mg/L. Low caffeine content found in herbal extracts is interesting due to the fact that there is an increasing demand for decaffeinated beverages due to potential harmful health effects of caffeine. While caffeine was the only methylxanthine evaluated in the present study, green tea and herbal teas have been reported to contain negligible amounts of other types including theobromine and theophyline [33].

3.5 Antioxidant Capacity

The antioxidant scavenging potency of berry stem extracts in comparison with green tea and wild rose fruit is presented in Fig. 1. The scavenging effect on a 0.02 mM DPPH solution exerted by various concentrations of berry stem extracts ranged from 3-99%. Among methanol extracts, the IC_{50} (antioxidant concentration required to quench 50% of the initial DPPH) ranged from 200-617 mg/L (Table 3), with blueberry stem extract being the one with lowest IC_{50} value. The lower the IC_{50} value, the higher the antioxidant capacity. Owing to its high flavan-3-ol content, the green tea had the highest hydrogen donating capacity with a low IC_{50} value of 67.2 mg/L, followed by blueberry stem. A similar trend was noticed for the FRAP assay, which showed blueberry stem has the greatest antioxidant capacity with a Trolox equivalence (TE) of 8.7 mmol/L, only second to green tea values of 11.4 mmol TE/L. The wild rose fruit extract was not significantly different from the blueberry extract, both for FRAP and DPPH values. On the other hand, the raspberry stem gave the lowest FRAP and DPPH values of 3.5 mmol TE/L and 616.5 mg/L, respectively, most probably due to the relatively low TPC content.

For hot water extracts, blueberry and blackberry stems ranked the highest in terms of FRAP values of 6.7 and 6.5 mM, respectively. The IC_{50} values of DPPH scavenging activity were found to be the lowest in blackberry stem extracts (211 mg/L), though not significantly different from raspberry stem extract. However, the stem extracts had higher IC_{50} values than the tested green tea and wild rose fruit extracts, which did not show any significant difference in their values.

The hot water extracts showed blueberry and blackberry stem exhibiting the strongest ORAC values of 53.5 and 55.7 mmol TE/L, respectively, followed closely by raspberry stem extract (Fig. 1). Interestingly, the berry stem extracts displayed very close values to green tea extract, thereby showing antioxidant equivalence. This was true for the methanol extracts as well, with the wild blueberry stem extract having the highest antioxidant capacity. The wild berry extracts presented antioxidant capacities greater than exhibited by wild rose fruit extracts.

3.6 Correlation Analysis of Total Phenolic Content and Antioxidant Activity

Pearson correlation was applied to TPC, UPLC-ESI-MS phenolic content (LC-PC), UPLC-ESI-MSanthocyanins (LC-AN), proanthocyanidins, carotenoids, FRAP, ORAC and $DPPH^{\bullet}$ scavenging activity (Table 4). Pearson correlation coefficient showed a significant linear relationship between TPC and FRAP values ($r = 0.71$, $p = 0.0002$; $r = 0.75$, $p = 0.0002$) in both hot water and methanol extraction, respectively. The LC-PC found were linearly correlated with FRAP ($r = 0.75$, $p = 0.0002$) for methanol extraction; while no significant correlation was observed for the same in hot-water extraction. A negative correlation existed between $DPPH^{\bullet}$ scavenging activity, TPC and LC-PC in both hot water ($r = -0.49$, $p = 0.06$; $r=-0.78$, $p=0.0002$, respectively) and methanol extraction ($r = -0.75$, $p = 0.0001$; $r=-0.73$, $p= 0.0001$, respectively).A significantly strong negative correlation was also found between $DPPH^{\bullet}$ scavenging activity and FRAP ($r= -0.75$, $p=0.00$; $r=-0.97$, $p=0.01$) in hot water and methanol extraction, respectively. The $DPPH^{\bullet}$ scavenging activity as IC_{50} values was significantly correlated with ORAC ($r = 0.65$, $p= 0.01$; $r = 0.72$, $p = 0.00$) in methanol extraction and hot water extraction, respectively. On the

other hand, a strong positive correlation existed between FRAP and ORAC values in methanol extraction; which did not prove true in hot water extraction. ORAC values, TPC and LC-PC were correlated linearly in hot water (r = 0.52, p= 0.04; r = 0.72, p = 0.0001) and methanol extraction (r=0.68, p=0.03; r= 0.55, p= 0.03), respectively. Also, a negative correlation was calculated between the IC_{50} values of $DPPH^{\bullet}$ scavenging activity and ORAC (r = -0.32, p= 0.06; r = -0.65, p=0.03) in both hot water and methanol extractions. Besides, a significantly strong positive correlation was exhibited between proanthocyanidin content, TPC (r = 0.87, p= 0.0001), LC-PC (r = 0.93, p = 0.0001), ORAC (r = 0.60, p = 0.04), FRAP (r = 0.64, p = 0.04) and $DPPH^{\bullet}$ (r = -0.66, p = 0.03).

3.7 Anti-diabetic Potential of Wild Berry Extracts

Results of the anti-diabetic potential of the herbal extracts showed a dose-dependent inhibition of both the carbohydralases and AGE (Figs. 2, 3 and 4). The results also showed that higher concentrations of the tested extracts were required for α-amylase inhibition than that of α-glucosidase inhibition.

3.7.1 α-Amylase activity

The inhibitory potencies of the tested extracts against α-amylaseare summarized in Table 5. Among methanol extracts, wild raspberry stem followed by wild blackberry stem extract exhibited the highest inhibition of α-amylase with IC_{50} value of 20 and 24.5 mg/mL, respectively. These values were significantly different from the data obtained for wild blueberry, green tea and wild rose fruit extracts (152, 131, and 96 mg/mL, respectively). In agreement with previous study [34], the IC_{50} value of acarbose was lower than that of the green tea. However, acarbose was found to exhibit 5-6 times lower α-amylase inhibition than the wild blackberry and raspberry extracts. Even at the highest concentration tested (500 mg/L), 28% of α-glucosidase activity remained. However, 100% enzyme inhibition was achieved at 30-40 mg/L for wild raspberry and blueberry stem extracts. On the other hand, wild blueberry stem, wild rose fruit and green tea extracts demonstrated moderate inhibition for both the enzymes. In hot water extracts, the α-amylase inhibition potential of the tested extracts was as follows: Wild blackberry stem>wild raspberry stem>wild blackberry stem>green tea >wild rose fruit (IC_{50} = 40, 68, 87, 136, 159 mg/L, respectively). The IC_{90} values of wild blackberry and raspberry stems were significantly lower (3-6 and 1.5-1.6 times for methanol and hot water extracts, respectively) than that of acarbose (Table 5). Since the tested extracts contain mainly flavonoids, previous reports have presented structural attributes required for flavonoids for α-amylase and α-glucosidase inhibition [35-36].

3.7.2 α-glucosidase activity

Extracts of varying concentrations were used to assess their inhibitory potential against α-glucosidase. A dose-dependent enzyme inhibition was observed for all the tested extracts (Fig. 3). Among the hot water extracts, wild blackberry stem (IC_{50}=28.2 mg/L) followed by wild raspberry stem (IC_{50}=11.3 mg/L) were the most active α-glucosidase inhibitors (Table 5). The same trend was followed by methanol extracts where wild blackberry stem (IC_{50}=10.3 mg/L) and wild raspberry stem (IC_{50}=11 mg/L) showed the statistically highest inhibitory activities. Interestingly, their inhibitory activities were 13-38 times higher than that of clinically used acarbose (IC_{50} = 383mg/L).On the other hand, green tea and wild rose fruit were moderate inhibitors of α-glucosidase with IC_{50} values of 78 and 64 mg/mL, which were still lower than the acarbose. The IC_{90} values of wild blackberry and wild raspberry stems were significantly lower (28-31 and 6-16 times for methanol and hot water extracts, respectively) than that of acarbose (Table 5). Previous studies have also shown the α-glucosidase inhibitory effects of green tea to be more potent than acarbose [34,37]. Similar to the methanol extracts, IC_{50} values of the hot water extracts varied between 11-140 mg/L for α-glucosidase inhibition, with wild blackberry and wild raspberry stems having the lowest IC_{50} values.

Overall, our data suggests that extracts of wild berry stems show promise as natural α-glucosidase and α-amylase inhibitors that may limit the digestion of dietary starches. Little is known about the mechanism by which these extracts inhibit carbohydrate hydrolases, although the interaction of various flavonoids-rich plant extracts with several digestive enzymes have been reported. These natural herbal extracts can be used as ingredients for the manufacturing of value-added food, blended tea and nutraceuticals.

Table 3. UPLC-ESI-MS characterization of phenolic compounds (mg/L) present in wild berry stem and fruit extracts in comparison to green tea

Extraction method	Phenolic group	Phenolic compound	Green tea	Wild raspberry stem	Wild blueberry stem	Wild blackberry stem	Wild rose fruit
Methanol							
	Dihydrochalcones	Phloridzin	0.9±0.0	1.3±0.1	1.3±0.1	1.4±0.1	0.7±0.0
	Phenolic acids	Chlorogenic acid	4.7±0.3	0.5±0.0	63.7±6.4	0.2±0.0	0.2±0.0
		Caffeic acid	0.2±0.0	1.6±0.0	0.1±0.0	0.1±0.0	0.1±0.0
		Isoferulic acid	7.2±0.1	4.6±0.1	14.4±1.2	30.0±1.0	0.7±0.2
	Flavonols	Q. galactoside+glucoside	50.0±0.9	5.2±0.2	24.7±0.4	25.4±0.6	18.0±0.4
		Q.-3-*O*-rhamnoside	7.3±1.0	0.9±0.2	3.6±0.3	3.0±0.3	3.0±0.2
		Q.	2.1±0.1	0.2±0.0	0.6±0.1	2.0±0.1	2.0±0.0
		Q.-3-*O*-rutinoside	24.7±2.1	0.7±0.1	22.1±1.9	43.0±0.5	0.2±0.0
	Flavan-3-ols	EGC	549.9±71.0	0.4±0.0	17.0±1.5	0.1±0.0	0.1±0.0
		Catechin	25.5±16.6	4.7±1.8	33.6±2.3	17.3±1.3	16.5±0.4
		Epicatechin	64.8±10.4	69.5±14.9	78.1±4.1	99.6±3.7	28.0±1.4
		EGCG	430.2±9.2	0.2±0.0	0.1±0.0	ND	ND
		ECG	88.4±12.3	1.2±0.2	ND	0.5±0.0	0.3±0.0
	Anthocyanins	Cyanidin-3-*O*-galactoside	0.1±0.1	ND	8.0±1.0	ND	ND
		Cyanidin-3-*O*-glucoside	ND	0.1±0.0	0.6±0.0	0.6±0.0	ND
		Petunidin-3-*O*-glucoside	0.2±0.1	0.2±0.0	0.1±0.0	ND	ND
		Delphinidin-3-*O*-glucoside	ND	0.1±0.0	0.7±0.0	ND	0.1±0.0
		Total by UPLC-ESI-MS	1256.1[a]	91.4[c]	268.7[b]	223.2[b]	69.9[d]
Hot water							
	Dihydrochalcones	Phloridzin	0.6±0.0	1.1±0.0	0.5±0.1	1.0±0.1	0.2±0.0
		Phloritin	ND	ND	0.1±0.0	ND	ND
	Phenolic acids	Chlorogenic acid	6.5±0.3	0.7±±0.2	0.4±0.0	65.4±4.0	0.2±0.0
		Caffeic acid	0.2±0.0	2.9±0.2	5.1±0.7	0.2±0.0	ND
		Ferulic acid	0.1±0.0	0.1±0.0	0.2±0.0	ND±	0.2±0.0
		Isoferulic acid	1.7±0.2	1.1±0.1	0.7±0.1	0.8±0.0	0.1±0.0
	Flavonols	Q.galactoside+glucoside	5.4±0.6	3.9±0.2	20.5±0.4	21.1±0.6	0.4±0.0
		Q.-*3*-*O*-rhamnoside	6.2±0.2	1.0±0.0	6.2±0.3	2.6±0.2	1.3±0.3
		Q.	0.3±0.1	ND	1.1±0.1	0.1±0.0	0.1±0.0
		Q.-*3*-*O*-rutinoside	24.9±0.2	0.9±0.2	4.1±0.1	21.4±0.4	0.2±0.0
	Flavan-3-ols	EGC	516.1±165.6	0.5±0.1	0.5±0.1	14.8±0.2	0.2±0.0

Extraction method	Phenolic group	Phenolic compound	Green tea	Wild raspberry stem	Wild blueberry stem	Wild blackberry stem	Wild rose fruit
		Catechin	27.5±1.9	4.7±0.2	14.2±0.1	24.1±0.4	3.8±0.0
		Epicatechin	58.7±23.6	65.7±2.6	84.1±6.0	64.0±4.0	0.2±0.0
		EGCG	266.0±170.0	0.1±0.0	0.2±0.0	0.4±0.0	0.2±0.0
		ECG	36.3±3.5	1.8±0.1	0.4±0.0	ND	0.2±0.0
	Anthocyanins	Cyanidin-3-*O*-galactoside	ND	ND	0.5±0.0	ND	ND
		Cyanidin-3-*O*-glucoside	ND	1.1±0.0	8.7±1.0	10.0±0.7	0.2±0.0
		Petunidin-3-*O*-glucoside	ND	0.1±0.0	0.1±0.0	ND	ND
		Delphinidin-3-*O*-glucoside	ND	ND	0.5±0.1	ND	ND
		Total by UPLC-ESI-MS	950.5[a]	85.7[d]	148.1[c]	225.9[b]	7.5[e]

Q, Quercetin; EGC, (-)-Epigallocatechin; EGCG, (-)-Epigallocatechin gallate; ECG, (-)-epicatechin gallate, [a-c]*Different letters across rows denote significant differences between values as determined by one way ANOVA analysis*

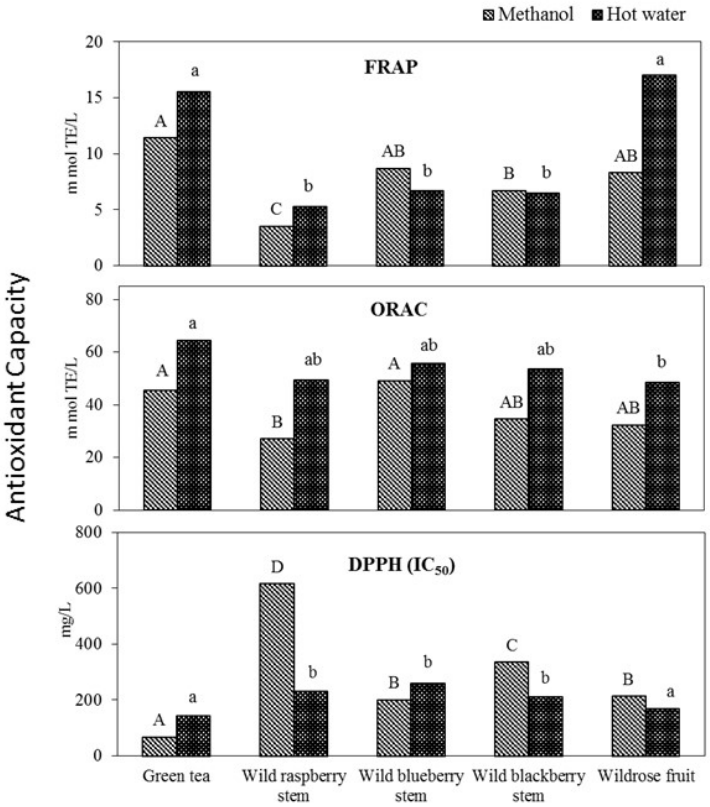

Fig. 1. FRAP, ORAC and DPPH for wild rose fruit and wild berry stem extracts in comparison to green tea

[A-C]Denote significant differences between methanol extract sat p≤0.05, [a-b]Denote significant differences between hot water extracts at p≤0.05

Table 4. Pearson correlation coefficients to exhibit linear relationship among the total phenolic content and antioxidant capacity measures (FRAP, DPPH and ORAC) in wild berry extracts in comparison to green tea

Extraction method		TPC	FRAP	ORAC	DPPH	LC-PC	Proanthocyanidins
Methanol							
	FRAP	0.74*					
	ORAC	0.68*	0.71*				
	DPPH	-0.75*	-0.97*	-0.65*			
	LC-PC	0.73*	0.75*	0.56*	-0.62*		
	Proanthocyanidins	0.84*	0.73*	0.57*	-0.63*	0.97*	
	Carotenoids	0.37	0.72*	0.17	-0.65*	0.62*	0.58*
Hot water							
	FRAP	0.71*					
	ORAC	0.52*	0.23				
	DPPH	-0.49	-0.75*	-0.32			
	LC-PC	0.78*	0.38	0.72*	-0.62*		
	Proanthocyanidins	0.87*	0.64*	0.60*	-0.66*	0.93*	
	Carotenoids	0.51	0.35	0.44	-0.52	0.68*	0.65*

**Significant correlations are shown (p≤0.05). TPC, total phenolic content; FRAP, ferric reducing antioxidant power; DPPH, 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity (reported as IC_{50}); ORAC, oxygen radical absorbance capacity; LC-PC, phenolic content measured by UPLC-ESI-MS*

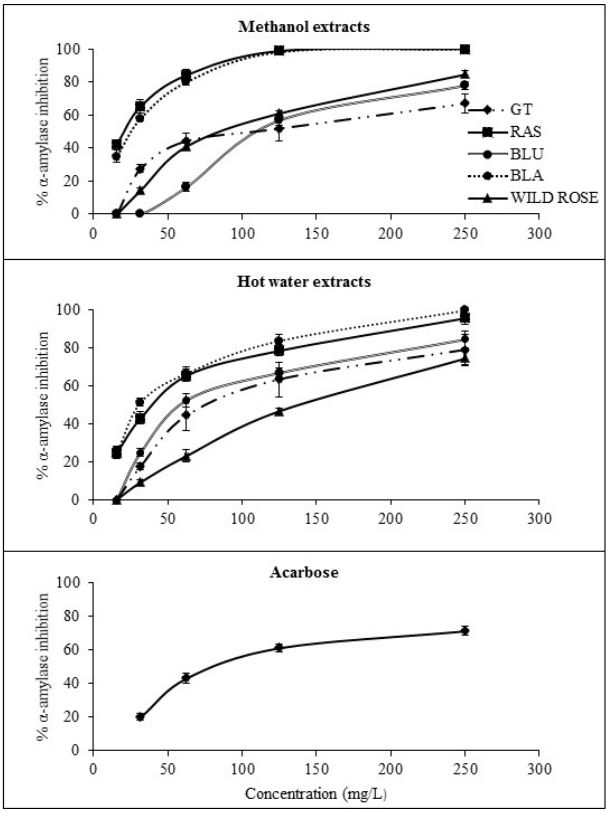

Fig. 2. Inhibitory activities of α-amylase by the wild berry extracts in comparison with commercial green tea and drug acarbose

GT, green tea; RAS, wild raspberry stem; BLU, wild blueberry stem; BLA, wild blackberry stem

3.7.3 Advanced glycation products (AGE) inhibition

The herbal extracts, particularly the wild raspberry stem extract significantly inhibited protein glycation mediated by glucose and the inhibitory effects increased with concentrations (Fig. 4). The IC_{50} concentrations of wild raspberry stem extract were calculated as 3.1 and 1.0 mg/L for hot water and methanol extracts, respectively (Table 5), which was very less than obtained by the common anti-glycation agents, aminoguanidine (177.6 mg/L) and metformin (417 mg/L) under the given experimental conditions. At 125 mg/L, the raspberry stem inhibited 93.5% of the fluorescence associated with AGE product formation. Both in hot water and methanol extracts, green tea equivalently exhibited anti-AGE property with IC_{50} of 5.9 and 3.1 mg/L, respectively. The low IC_{50} values for green tea could be explained by the fact that epigallocatechin-gallate (EGCG) present in it inhibit the glycation process far better than the antiglycation agent, aminoguanidine [38], which is why green tea was used for comparison purpose in the current study. The wild blueberry stem extract had the highest IC_{50}, hence, were least effective than other herbal extracts (Table 5). As reflected from the IC_{50} and IC_{90} values, all herbal extracts, except blueberry stem extract, were greater than or as effective as aminoguanidine in a dose-dependent fashion. Stronger anti-AGE effect of plant polyphenol extracts in comparison to aminoguanidine have been previously documented [24]. Our results on wild berry stems and fruit phytochemicals were consistent with a previous research in which flavonoids (catechin, epicatechin, epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), quercertin) demonstrated inhibition of glucose mediated protein glycation [38]. Leaves and stems of low bush blueberries (*Vaccinium angustifolium*) in different seasons have been shown to have anti-glycation effects [39]. In addition, a good correlation between the free radical scavenging activity and the inhibitory effect on AGE generation for pure phenolic compounds and polyphenols-rich plant extracts has been described [38].

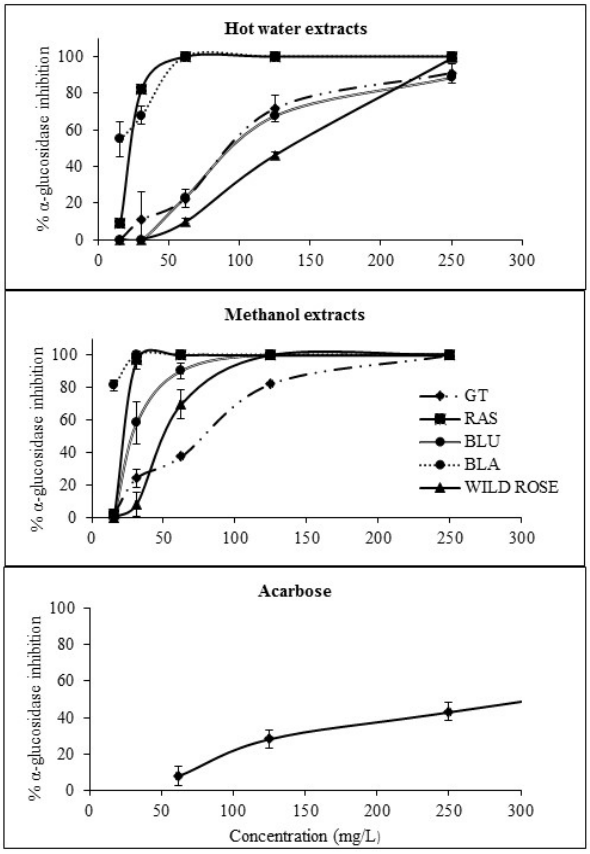

Fig. 3. Inhibitory activities of α-glucosidase by the herbal extracts in comparison with commercial green tea and drug acarbose

GT, green tea; RAS, wild raspberry stem; BLU, wild blueberry stem; BLA, wild blackberry stem

Table 5. IC_{50} and IC_{90} values of the natural herbal extracts against pancreatic porcine α-amylase, α-glucosidase and advanced glycation end products (AGE)

Extraction method	Samples	α-Amylase inhibition (mg/L)		α-Glucosidase inhibition (mg/L)		AGE inhibition (mg/L)	
		IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Methanol	Green tea	131.4^{c}	380.6^{e}	78.0^{d}	135.9^{d}	3.1^{a}	105.3^{b}
	Wild raspberry stem	20.1^{a}	95.6^{b}	11.5^{a}	29.2^{a}	1.0^{a}	75.0^{a}
	Wild blueberry stem	152.2^{d}	264.3^{d}	36.5^{b}	59.2^{b}	154.6^{c}	887.7^{d}
	Wild blackberry stem	24.0^{a}	67.1^{a}	10.4^{a}	25.6^{a}	121.5^{b}	783.7^{c}
	Wild rose fruit	96.8^{b}	170.2^{c}	64.4^{c}	106.3^{c}	206.7^{d}	$>2000^{e}$
Hot water	Green tea	135.9^{d}	258.4^{c}	123.7^{c}	224.2^{c}	5.9^{a}	196.5^{b}
	Wild raspberry stem	68.2^{b}	207.8^{b}	28.2^{b}	50.7^{a}	3.2^{a}	135.0^{a}
	Wild blueberry stem	87.1^{c}	253.0^{c}	131.7^{d}	231.0^{c}	$>2000^{d}$	$>2000^{d}$
	Wild blackberry stem	40.5^{a}	186.5^{a}	11.3^{a}	130.0^{b}	358.2^{c}	$>2000^{d}$
	Wild rose fruit	158.5^{e}	286.3^{d}	139.5^{e}	229.5^{c}	341.1^{b}	601.5^{c}
Acarbose		121.1	312.6*	383.5^{*}	819.3*		
Aminoguanidine		-	-	-	-	177.6*	>2000
Metformin		-	-	-	-	417.02*	>2000

*$^{a-e}$Different letters across columns denote significant differences between IC_{50} and IC_{90} values as determined by one way ANOVA analysis. *Signifies the significant difference between results obtained for commonly used drugs in comparison to the tested plant extracts*

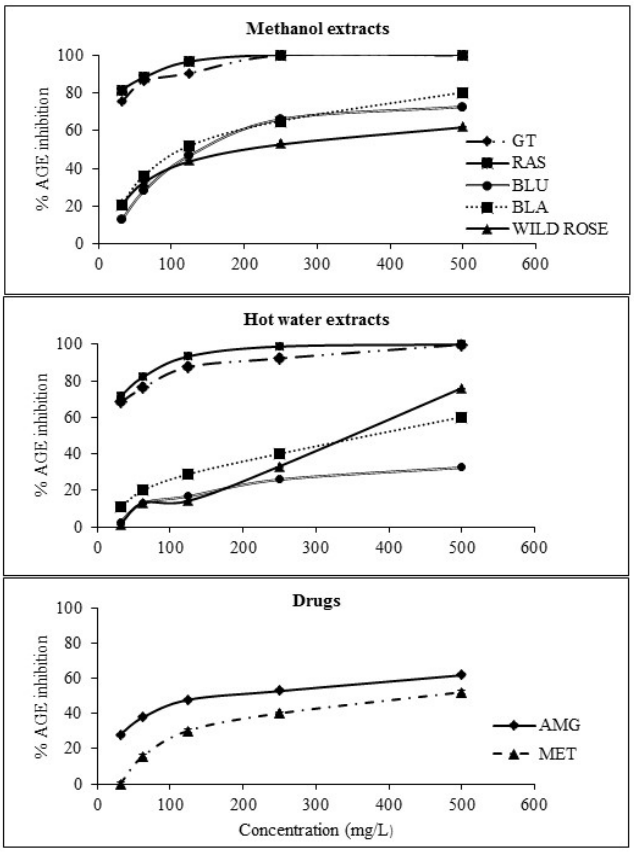

Fig. 4. Inhibitory activity of advanced glycation products by the herbal extracts in comparison with commercial green tea, aminoguanidine (AMG) and metformin (MET)

GT, green tea; RAS, wild raspberry stem; BLU, wild blueberry stem; BLA, wild blackberry stem

4. CONCLUSION

The present study was undertaken to evaluate the proximate composition, polyphenol concentration, antioxidant capacity and anti-diabetic potential of wild berry extracts including wild blueberry, wild blackberry, wild raspberry stems and wild rose fruit. The results showed that irrespective of the extraction method used, the wild blueberry stem contained remarkably high content of total phenolic content and antioxidant capacity (FRAP, ORAC and ORAC), followed by the wild blackberry stem. In addition, the wild blueberry and wild blackberry stems contained high total carotenoid content. A dose-dependent α-amylase, α-glucosidase and AGE inhibition was observed for all the tested extracts. Extracts of wild blackberry and wild raspberry stems showed the highest inhibition of both the carbohydrate hydrolysis enzymes at tested concentrations, the present results suggest that extracts of wild berry stems show promise as natural glucosidase inhibitors that may limit the digestion of dietary starches. Little is known about the mechanism by which these extracts inhibit glucosidases, although the interaction of various flavonoid-rich plant extracts with several digestive enzymes have been reported. Also, wild raspberry exhibited significantly higher anti-AGE activity as compared to the prescribed drugs aminoguanidine and metformin. In addition, the herbal extracts contained relatively very less amounts of caffeine, as compared to green tea. Thus, herbal extracts can be used as value-added ingredients for the manufacturing of specialty tea blends, functional foods and nutraceuticals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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