Original Article

Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension

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In the current study, we screened 7 clonal lines from single seed phenotypes of Lamiaceae family for the inhibition of α-amylase, α-glucosidase and angiotensin converting enzyme (ACE) inhibitory activity. Water extracts of oregano had the highest α-glucosidase inhibition activity (93.7%), followed by chocolate mint (85.9%) and lemon balm (83.9%). Sage (78.4%), and three different clonal lines of rosemary: rosemary LA (71.4 %), rosemary 6 (68.4%) and rosemary K-2 (67.8%) also showed significant α-glucosidase inhibitory activity. The α-glucosidase inhibitory activity of the extracts was compared to selected specific phenolics detected in the extracts using HPLC. Catechin had the highest α-glucosidase inhibitiory activity (99.6%) followed by caffeic acid (91.3%), rosmarinic acid (85.1%) and resveratrol (71.1%). Catechol (64.4%), protocatechuic acid (55.7%) and quercetin (36.9%) also exhibited significant α-glucosidase inhibitory activity. Results suggested that α-glucosidase inhibitory activity of the clonal extracts correlated to the phenolic content, antioxidant activity and phenolic profile of the extracts. The clonal extracts of the herbs and standard phenolics tested in this study did not have any effect on the α-amylase activity. We also investigated the ability of the clonal extracts to inhibit rabbit lung angiotensin I-converting enzyme (ACE). The water extracts of rosemary, rosemary LA had the highest ACE inhibitory activity (90.5%), followed by lemon balm (81.9%) and oregano (37.4%). Lower levels of ACE inhibition were observed with ethanol extracts of oregano (18.5%) and lemon balm (0.5%). Among the standard phenolics only resveratrol (24.1%), hydroxybenzoic acid (19.3%) and coumaric acid (2.3%) had ACE inhibitory activity.

Key Words: phenolic phytochemicals, antioxidants, Lamiaceae, amylase, glucosidase, angiotensin converting enzyme, enzyme inhibitors, resveratrol, rosmarinic acid, diabetes, hypertension.

Introduction

Hyperglycemia, a condition characterized by an abnormal postprandial increase of blood glucose level, has been linked to the onset of type II insulin-independent diabetes mellitus and associated cardiovascular complications including hypertension.[1,2] Hydrolysis of dietary carbohydrates such as starches are the major source of glucose in the blood glucose. The enterocytes of the small intestine can only absorb monosaccharides such as glucose and fructose from our diet.[3-5] Therefore, the dietary polysaccharides need to be broken down to monosaccharides before they can be absorbed. This hydrolysis is carried out by a group of hydrolytic enzymes called α-glucosidases which includes sucrase, maltase, glucoamylase, dextrinase and the pancreatic α-amylase.[3-5] It is now believed that inhibition of these enzymes involved in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet and therefore can be an important startegy in the management of type-II diabetes.[6] Several α-glucosidase inhibitors such as acarbose[7], trestatin[8], amylostatin[9] and valiolamine[10] have been isolated from microorganisms. Acarbose isolated from *Actinoplanes sp* is now used in the management of type II diabetes. A main drawback of using drugs such as acarbose is side effects such as abdominal distention, flatulence, meteorism and possibly diarrhea.[11,12] It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon.[10,12]

Natural α-amylase and α-glucosidase inhibitors from food-grade plant sources offer an attractive strategy to control of post-prandial hyperglycemia. Natural 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 used as effective therapy for postprandial hyperglycemia with minimal side effects.[10]

One of the long-term complications of diabetes is hypertension, or high blood pressure. Angiotensin I-Converting

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Enzyme (ACE) is an important enzyme involved in maintaining vascular tension. ACE activates a histidyl-leucine dipeptide called angiotensin I, into a potent vasoconstrictor called angiotensin II.[13] Angiotensin II also stimulates the synthesis and release of aldosterone, which increase blood pressure by promoting sodium retention in the distal tubules.[14] Inhibition of Angiotensin I-Converting Enzyme (ACE) is considered a useful therapeutic approach in the treatment of high blood pressure in both diabetic and non-diabetic patients.[15,16] Antihypertensive drugs have been isolated from a number of plant species.[17] It is now believed that screening plant extracts for inhibition of ACE will be an effective method to search for new anti-hypertensive agents.[18]

Plants belonging to Lamiaceae family (Mint Family) include -sage, rosemary, lavenders, oregano- and have long been used in food preservation, culinary flavors and aromas and for the treatment of common aliments as traditional medicine. Recent research has now indicated that these herbs are a rich source of phenolic phytochemicals having high antioxidant activity. Further it has shown that these phenolic phytochemicals posses specific therapeutic properties and may be responsible for their beneficial effect on human health.[19,20] Phenolic phytochemicals are now implicated to have potential for management of many chronic oxidation-linked diseases such as diabetes and CVD.[21-26] Recently it has been shown that phenolics from the Lamiaceae family of herbs such as rosmarinic acid from *Rosmarinus officinalis*, and galanigin from *Origanum vulgare* have anti-inflammatory[27] and cancer chemopreventive[28] activity.

Large variations in the phenolic content and phenolic profile in plants have limited their success as therapeutic agents in spite of their many beneficial effects. These variations are a result of heterogeneous plants arising due to cross-pollination. Plants which originate from different heterozygous seeds are phenotypically variable causing, substantial phytochemical inconsistency, which translate into unreliable clinical effects as well as inconsistent health benefits. Other factors, such as cultivar, variety, maturity, processing, and storage, also influence the content of plant phenolics.[29] We have developed several clonal lines of herbs from Lamiaceae family having consistent phenoic content, phenolic profile and anti-oxidant activity.[30] Therefore, the objective of this research was to screen several clonal lines of different species belonging to the Lamiaceae family for α-amylase, α-glucosidase and ACE inhibition activity. We also compared the inhibition activity with the individual phenolics found in the phenolic profile of the targeted herbs and correlated them to total phenolic and antioxidant activity.

Materials and Methods

Clonal lines of Rosemary; *Rosmarinus officinalis* (Rosemary LA, Rosemary RoK-2 and Rosemary Ro-6), Lemon balm; *Melissa officinalis*, Sage; *Salvia officinalis*, Chocolate mint; *Mentha piperata*, and oregano; *Origanum vulgare* (Oregano Go-19-2) were generated by Dr. Kalidas Shetty at the National Institute of Agro-biological Sciences (Tsukuba Science City, JAPAN), at CC Botanicals(Barford, Warwick, United Kingdom) and Harekal Gardens(Mangalore, India). 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 extraction

Water extracts

A total of 5g of dried herb powders were added to 100-ml of distilled water and refluxed for 1 hour and cooled. The extract was then filtered through a Whatman # 2 filter and centrifuged at 10,000 x g for 10 min.

Ethanol extracts [12%]

A total of 5g of dried herb powder were stirred in 100ml of 12% concentration of ethanol at 40°C for 2hrs and cooled. The extract was then filtered through a Whatman # 2 filter and centrifuged at 10,000 x g for 10 -min.

Total phenolics assay

The total phenolics was determined by an assay modified from Shetty *et al*., (1995).[31] Briefly, one milliliter 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 5min, 1ml of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 725nm. 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 95% ethanol.

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

To 3ml of 60μM DPPH in ethanol, 250μl of each extract was added, the decrease in absorbance was monitored at 517nm until a constant reading was obtained. The readings were compared with the controls, which contained 250μl of 95% ethanol instead of the extract. The % inhibition was calculated by:

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

β-Carotene oxidation model system

One milliliter of 200μg/ml of *β*-carotene in chloroform was pipetted into a round-bottomed flask. Chloroform was evaporated using a rotary evaporator under vacuum at 40°C for 5min. The *β*-carotene adhered to the sides of the flask were scraped and dissolved with 20μl of purified linoleic acid and 184μl of Tween 40 emulsifier. To this, 50ml of 50mM H_2O_2 was added and shaken vigorously until a uniform emulsion was obtained. Aliquots (5ml) of this emulsion were transferred to each test tube containing 100μl of extract. The samples were vortexed for 1 min and incubated at 50°C for 30 min. Subsequently, absorbance readings were recorded at 470 nm and compared to a control which had 100μl of ethanol in place of the extract. The antioxidant activity was expressed as

protection factor (PF) and was calculated as follows:

$$\text{Antioxidant protection factor (APF)} = \left(\frac{A_{470}^{Sample}}{A_{470}^{Control}} \right)$$

α-amylase inhibition assay

Porcine pancreatic *α*-amylase (EC 3.2.1.1) was purchased from Sigma Chemical Co. A total of 500µl of extract and 500µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing *α*-amylase solution (0.5mg/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 0.006M 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 color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm.

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

This assay was modified compared to previous methods used in the laboratory that had 24 hour incubation time (*McCue et al.*, 2004),[32] which in light of physico-biological mode of starch breakdown is long. Therefore, we developed an improved and more sensitive assay with 10 min incubation time.

α -glucosidase inhibition assay

α-Glucosidase (EC 3.2.1.20) was purchased from Sigma Chemical Co. 50µl of sample solution and 100µl of 0.1M phosphate buffer (pH 6.9) containing *α*-glucosidase solution (1.0U/ml) were incubated in 96 well plates at 25°C for 10 minutes. After preincubation, 50µl of 5mM p-nitrophenyl-*α*-D-glucopyranoside solution in 0.1M 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 and compared to a control which had 50µl of buffer solution in place of the extract by micro-array reader (Thermomax, Molecular device Co., Virginia, USA). 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).[33] The substrate, hippuryl-histidyl-leucine (HHL) and angiotensin I-converting enzyme (ACE) from rabbit lung (EC 3.4.15.1) were purchased from Sigma. Fifty microliters of extracts were incubated with 100µl of 1.0–M NaCl-borate buffer (pH 8.3) containing 2.0mU ACE-I solution at 37°C for 10 minutes. After pre-incubation, 100µl of a 5.0mM substrate (HHL) solution was added to reaction mixture. Test solutions were incubated at 37°C for 1 hour. The reaction was stopped with 150µl of 0.5N 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) 10mM 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 phytochemicals

Two ml of dried herb extracts were filtered through a 0.2 µm filter. 5µl 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) 10mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased 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 1ml/min at ambient temperature. During each run the chromatogram was recorded at 306nm and 333nm 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 duplicates. Analysis at every time point from each experiment was carried out in duplicates or triplicates. Means, standard errors and standard deviations were calculated from replicates with in the experiments and analyses using Microsoft Excel XP.

Results and Discussion

Total phenolics and HPLC analysis of extract

The total phenolic content in water extracts and all the ethanol extracts was analysed by the Folin-Ciocalteu method. Water extracts of oregano (Go-19-2) had 62.6 mg/g dw of phenolics which was highest among all the extracts tested (Fig. 1). The chocolate mint extract and lemon balm extract had 36.2 mg/g dw and 28.3 mg/g dw of soluble phenolics, respectively (Fig. 1). The 12% ethanol extracts of the herbs had approximately 50% lower total soluble phenolics than water extracts.

Five major phenolics were identified in the extracts using HPLC and these were; rosmarinic acid, caffeic acid, coumaric acid, protocatechuic acid and quercetin (Table 1). In general, water extracts had higher content of these phenolics than 12% ethanol extracts samples. In both the water and ethanol extract samples, rosmarinic acid was the found to be a major phenolic. Water extracts of oregano had high concentrations of rosmarinic acid (16.56 mg/g dw), coumaric acid (0.16mg/g dw), protocatechuic acid (0.12mg/g dw) and caffeic acid (0.10mg/g dw) (Table 1). Water extracts of lemon balm was high in quercetin content (5.54mg/g dw) compared to 3.61mg/g dw in 12% ethanol extracts. The water extracts also contained significant amount of rosmarinic acid (4.84 mg/g dw), protocatechuic acid (0.38 mg/g dw), caffeic acid (0.34 mg/g dw) and coumaric acid (0.27 mg/g dw) (Table 1).

The rosmarinic acid contents of different clonal lines of rosemary (rosemary LA, rosemary RoK-2 and rosemary Ro-6) were 3.75, 1.44, and 1.50mg/g dw for water extract and 0.66, 0.59 and 0.42 mg/g dw for 12% ethanol extract, respectively. The phenolic phytochemical found at next highest concentration was quercetin. In sage extracts quercetin was not detected in water extract and 12% ethanol extracts. Another major phenolic of significance that was observed in the lemon balm extracts was protocatechuic acid which were found at levels of 0.38 mg/g dw in water extracts and 0.27 mg/g dw in 12% ethanol extracts. Other phenolic such as coumaric acid was generally found in lower concentrations in the 12% ethanol extracts than water extracts (Table 1).

Antioxidant activity by DPPH and ß-carotene assay

The antioxidant activity of the extracts was monitored using the DPPH radical inhibition (DRI) assay and by the β-carotene antioxidant protection factor (APF) measurement. The ability of phenolics to inhibit the DPPH radical formation was measured both in water and 12 % ethanol extracts. The water extracts of oregano had the highest DPPH radical inhibition activity (77.7%) followed by lemon balm (67.6%) and chocolate mint (58.7%) (Fig. 2). The results indicated that the DPPH scavenging activity of rosemary, sage, chocolate mint and oregano extracts was directly proportional to the total soluble phenolic content in them (Fig. 2). However, lemon balm showed high DPPH scavenging activity with lower total phenolic content than chocolate mint and oregano extracts, this could be due to high content of protocatechuic acid (Table 1). This suggests that the profile of individual phenolics in the extracts may be more important in contributing to the antioxidant activity than the total phenolics content.

The antioxidant protection factor (APF) measured by the ß-carotene assay was similar for both water and ethanol extracts (Fig. 3). However, this was not the case for the 12% ethanol extracts of chocolate mint had higher APF suggesting that they were rich in lipophilic

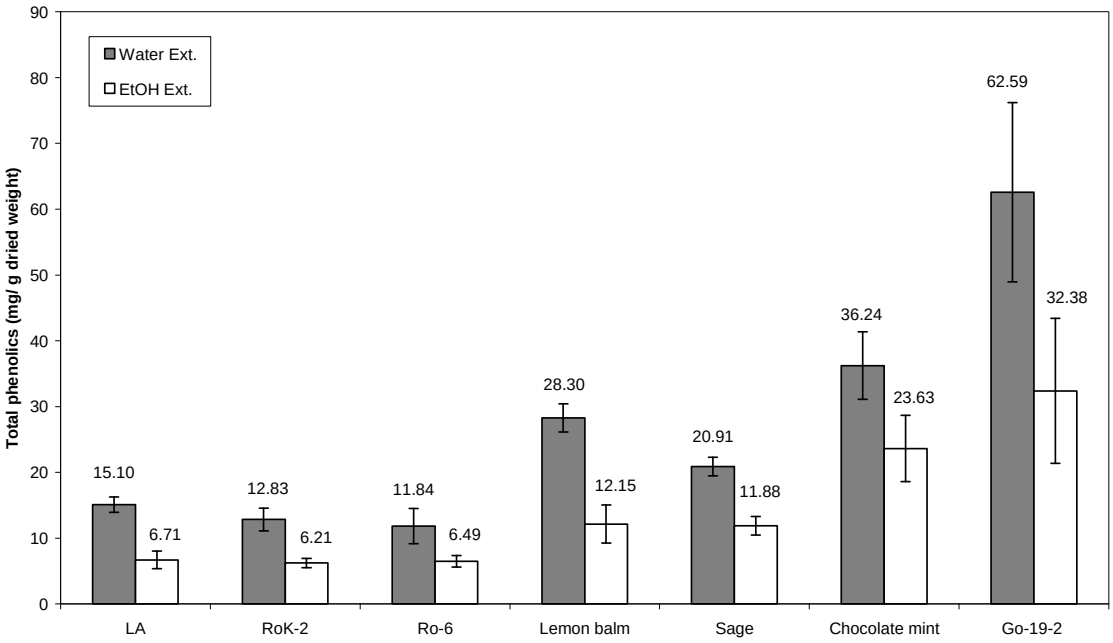

Figure 1. Total soluble phenolics in water and 12% ethanol extracts of clonal herbs of Lamiaceae family (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano)

Table 1. Individual phenolic compounds analyzed by HPLC in water extracts of clonal herbs of Lamiaceae

Phenolics (mg/g dw)	Lamiaceae Family						
	Rosemary LA	Rosemary RoK-2	Rosemary Ro-6	Lemon Balm	Sage	Chocolate mint	Oregano Go-19-2
Procatechuic acid	0.12 ± 0.03	0.11 ± 0.04	0.09 ± 0.03	0.38 ± 0.03	0.10 ± 0.03	0.29 ± 0.11	0.12 ± 0.01
Caffeic acid	0.20 ± 0.01	0.24 ± 0.03	0.21 ± 0.05	0.34 ± 0.04	0.34 ± 0.07	0.23 ± 0.07	0.10 ± 0.02
Coumaric acid	0.19 ± 0.03	0.20 ± 0.01	0.22 ± 0.01	0.27 ± 0.04	0.13 ± 0.01	0.11 ± 0.09	0.16 ± 0.12
Rosmarinic acid	3.75 ± 0.21	1.44 ± 0.22	1.50 ± 0.14	4.84 ± 1.11	3.16 ± 0.10	6.90 ± 2.56	16.5 ± 3.53
Quercetin	3.97 ± 0.10	3.55 ± 0.03	3.48 ± 0.08	5.54 ± 1.11	ND*	2.34 ± 0.07	ND
Total	8.23	5.54	5.50	11.37	4.53	9.87	16.93

Table 2. Individual phenolic compounds analyzed by HPLC in 12 % ethanol extracts of clonal herbs of Lamiaceae family.

Phenolics (mg/g dw)	Lamiaceae Family						
	Rosemary LA	Rosemary RoK-2	Rosemary Ro-6	Lemon Balm	Sage	Chocolate mint	Oregano Go-19-2
Procatechuic acid	0.05 ± 0.03	0.06 ± 0.02	0.06 ± 0.03	0.27 ± 0.03	0.03 ± 0.01	0.18 ± 0.08	0.03 ± 0.01
Caffeic acid	0.12 ± 0.00	0.13 ± 0.03	0.12 ± 0.04	0.05 ± 0.01	0.11 ± 0.03	0.14 ± 0.04	0.13 ± 0.04
Coumaric acid	0.10 ± 0.01	0.10 ± 0.00	0.07 ± 0.01	0.01 ± 0.00	0.08 ± 0.01	0.18 ± 0.01	0.42 ± 0.01
Rosmarinic acid	0.66 ± 0.02	0.59 ± 0.02	0.42 ± 0.07	0.23 ± 0.01	0.72 ± 0.01	1.32 ± 0.13	3.78 ± 0.56
Quercetin	1.41 ± 0.01	1.75 ± 0.01	2.23 ± 0.07	3.61 ± 0.08	ND*	0.06 ± 0.01	0.10 ± 0.07
Total	2.34	2.63	2.90	4.17	0.94	1.88	4.46

*N.D. Not detected

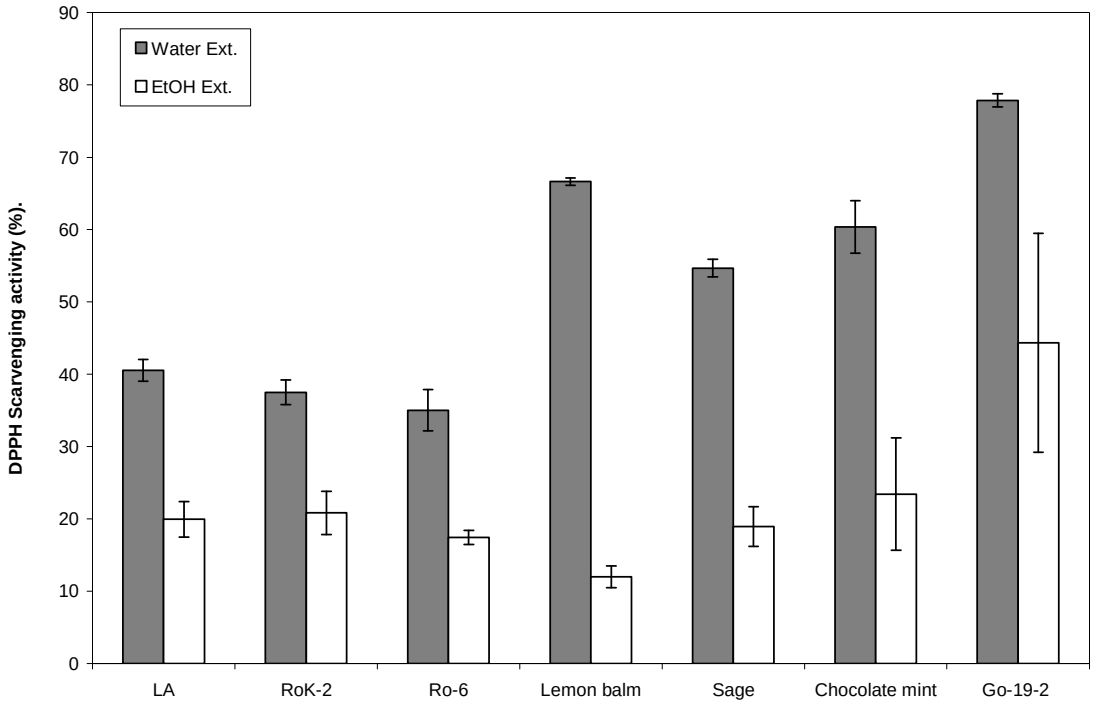

Figure 2. DPPH radical scavenging activity of water and 12% ethanol extracts of clonal herbs of Lamiaceae family (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano).

antioxidants which were more efficient in protecting β-carotene oxidation.

Even though the water extracts had higher total soluble phenolics compared to the 12% ethanol extracts, similar antioxidant protection of the both extracts in the ß-carotene assay may suggest the possible higher levels of lipophilic phenolic antioxidants in 12% ethanol extracts compared to the water extracts. ß-carotene assay measures the ability of the antioxidant in preventing the oxidative deterioration of lipids and fatty acids at the lipid-water interface. Therefore, higher antioxidant activity of the phenolic antioxidants from the water and 12% ethanol extract in these two assays suggest a possible biological functionality in preventing the oxidative degradation of membrane lipids.

Amylase/glucosidase inhibition

Previous research with clonal herbal extracts reported an association between antioxidant activity and amylase inhibition activity (McCue *et al*., 2004).[27] Therefore, we compared the α-glucosidase and α-amylase inhibitory activies with standard phenolics (Fig. 4). Pure catechin on a constant weight and constant pH basis had the highest α-glucosidase inhibitory activity (99.6%) followed by caffeic acid (91.3%), rosmarinic acid (85.1%), resveratrol (71.1%). Catechol (64.4%), protocatechuic acid (55.7%) and quercetin (36.9%) also showed significant α-glucosidase inhibitory activity (Fig. 4). However, when the pH of the sample was not adjusted, caffeic acid had the lowest α-glucosidase inhibitory activity (15.6%) followed by rosmarinic acid (25.9%) and catechin (75.0%) (Fig. 4). Correlation of the α-glucosidase inhibitory activities of the standard phenolics samples with pH may suggest the solubility of each phenolic compound at that pH may be important factor in contributing to the α-glucosidase inhibitory activity. Many tested phenolics showed a comparable inhibition of the α-glucosidase activity, but did not have any inhibitory activity against porcine pancreatic α -amylase.

The ability of the herb extracts to inhibit the α-glucosidase and α-amylase was measured in two different extracts, the first extract was prepared on constant weight basis (5g in 100 ml) and the second extract had the same amount of phenolics (1000 µg/ml). The α-glucosidase inhibitory activity of water and 12% ethanol extracts was directly proportional to the concentration of the total soluble phenolics, rosmarinic acid and caffeic acid (Fig. 5, Table 1). Water extracts of oregano had the highest α-glucosidase inhibitory activity (93.7%) followed by chocolate mint (85.9%), lemon balm (83.9%), sage (78.4%). Water extracts of rosemary LA (71.4%), rosemary Ro-6 (68.4%) and rosemary RoK-2 (67.8%) also showed significant inhibition. Similar inhibition in α-glucosidase inhibition was seen when the water extracts were compared at the same phenolic level (1000µg/ml) (Fig. 5). Among the water extracts, the extracts from oregano had the most potent α-glucosidase inhibitory activity which also had the highest content of rosmarinic acid (Table 1). This was true even for the 12% ethanol extracts standardized on phenolic basis (1000µg/ml). All the extracts showed similar α-glucosidase inhibition except oregano extract which had higher inhibitory activity had higher inhibition activity. This may suggest that real content of each phenolic compounds which have high α-glucosidase inhibitory activity was increased during the readjustment of phenolic content to 1000µg/ml. The α-glucosidase inhibitory of activity of the 12% ethanol extracts correlated with rosmarinic acid, caffeic acid and quercetin content which individually showed high α-glucosidase inhibitory activity (Fig.4, 6). Among all the extracts, the 12% ethanol extract of oregano which had the highest rosmarinic acid content also had the most potent α-glucosidase inhibitory activity (Table 1). This was followed by ethanol extract of lemon balm which contained high protocatechuic acid and quercetin (Fig. 6). All the extracts showed a comparable inhibition of the α-glucosidase but did not have any inhibitory activity against porcine pancreatic α-amylase. This was surprising in light of previous results, showing the ability of the herbs to inhibit α-amylase activity. It is likely previous assay results for α-amylase was likely reflection of long 24 hour incubation time, in which clonal extracts may be effective (McCue et al., 2004).

Based on the results above, Lamiaceae family which has high content of rosmarinic acid, caffeic acid, protocatechuic acid and quercetin could be used for the management of glycemic response in diabetes mellitus pateints. This strategy would likely have lower abdominal side effects arising from excessive inhibition of pancreatic α-amylase, which results in the abnormal bacterial fermentation of undigested carbohydrates in the colon.

ACE Inhibition

Hypertension is a risk factor for many cardiovascular diseases is also associated with long term diabetes. Control of hypertension via modulation of angiotensin I-converting enzyme (ACE) by dietary anti-hypertensive agents is an important strategy to manage this risk factor. Here, we investigated the ability of the clonal herb extracts and standard phenolics on inhibiting the activity of rabbit lung ACE. The results indicated that resveratrol had the highest ACE inhibitory activity (24.1 %) followed by hydroxybenzoic acid (19.3%) and coumaric acid (2.3 %) (Fig. 7).

Among the water extracts, rosemary LA had the highest ACE inhibitory activity (90.5%) followed by lemon balm (81.9%), oregano (37.4%) and rosemary RoK-2 (2.0 %) (Fig. 8). In the ethanol extracts, oregano had lower ACE inhibitory activity (18.5%) followed by lemon balm (0.5%) (Fig. 8). ACE inhibitory activity of the extracts did not correlate well with the total soluble phenolic content, antioxidant activity or the concentration of individual phenolics in the extracts such as coumaric acid (Fig. 8). This was especially true for rosemary LA and lemon balm extracts which had high ACE-inhibitory activity but did not correlate with antioxidant activity and total soluble phenolic content. Within the same rosemary species, different clonal lines had different activities. However the ACE-inhibition activity of the extracts increased with increase with the total phenolic dosage (Fig. 9) which may be a reflection of increase in overall extract dosage rather than phenolics. When ACE-inhibition activity of the extracts from rosemary LA and lemon balm were compared at the same phenolic level,

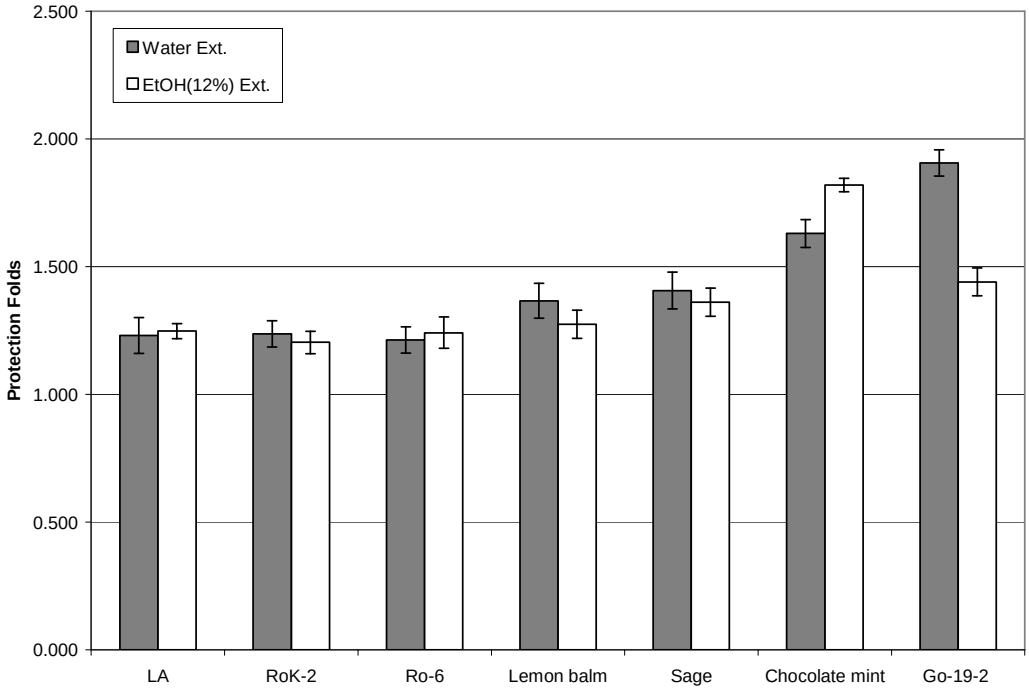

Figure 3. Antioxidant protection factor of water and 12% ethanol extracts of clonal herbs of Lamiaceae family measured by β-carotene-linoleic acid assay (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano).

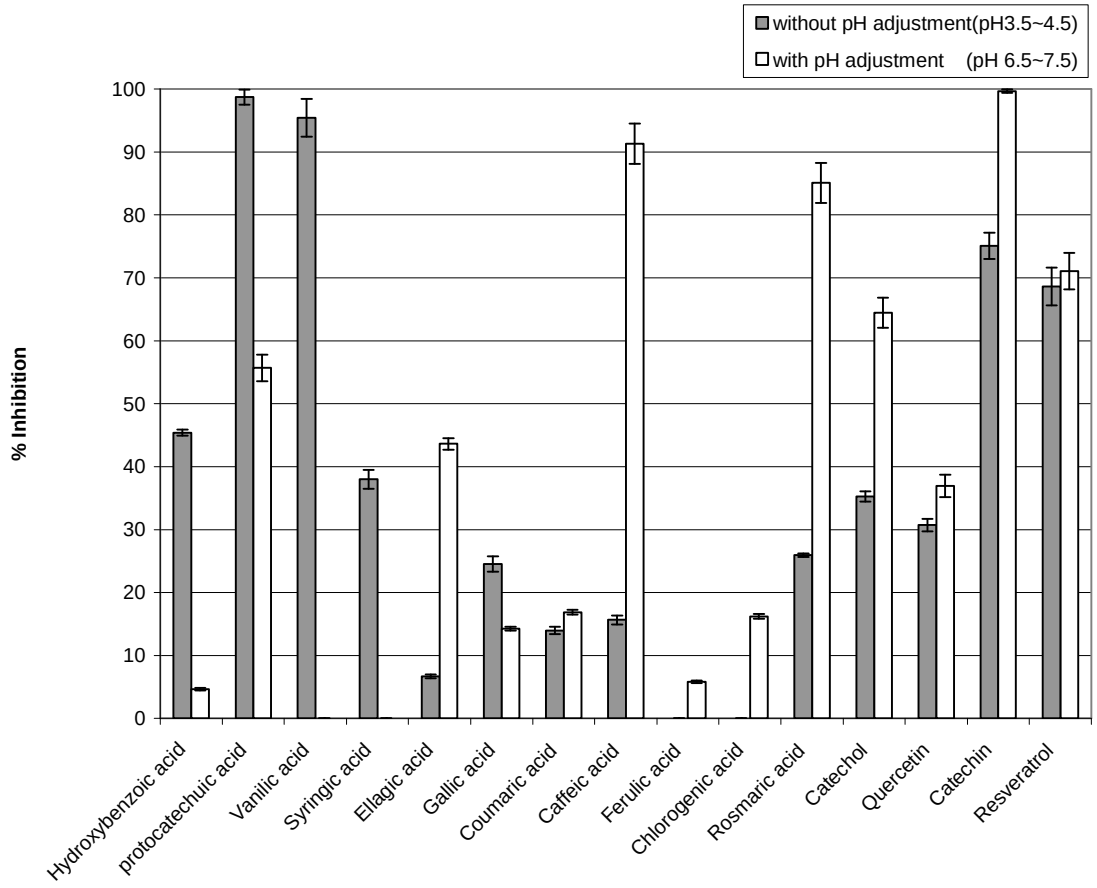

Figure 4. α-Glucosidase inhibitory activity of standard phenolics at different pH (at constant phenolic 1000 μg/ml)

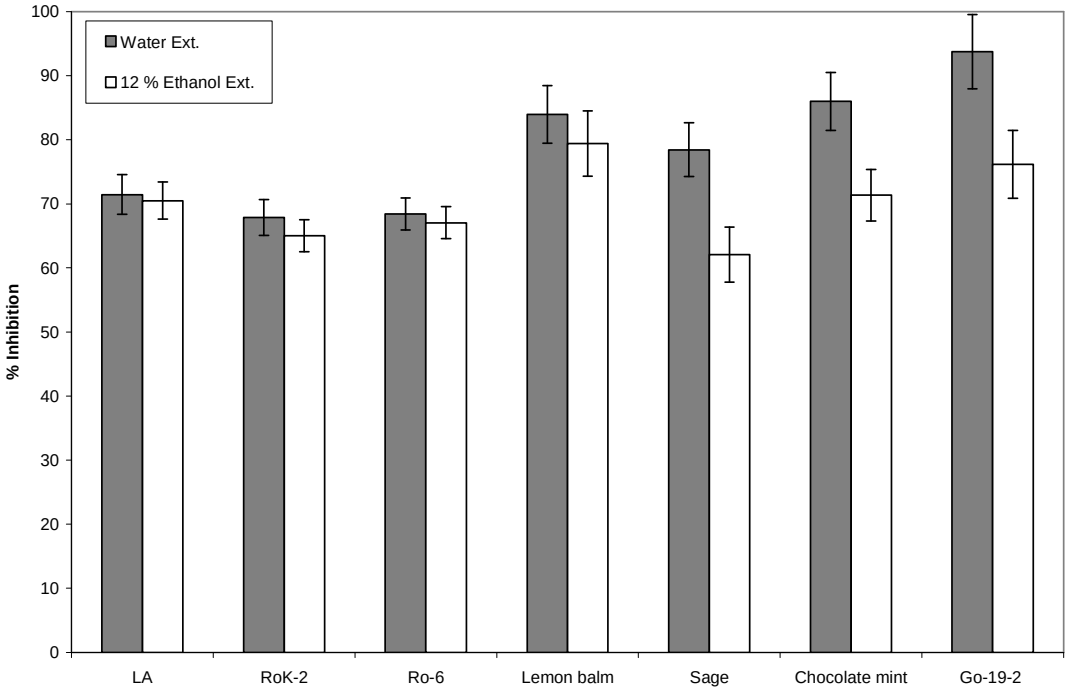

Figure 5. α-Glucosidase inhibitory activities of water and 12% ethanol extracts of clonal herbs of Lamiaceae family (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano).

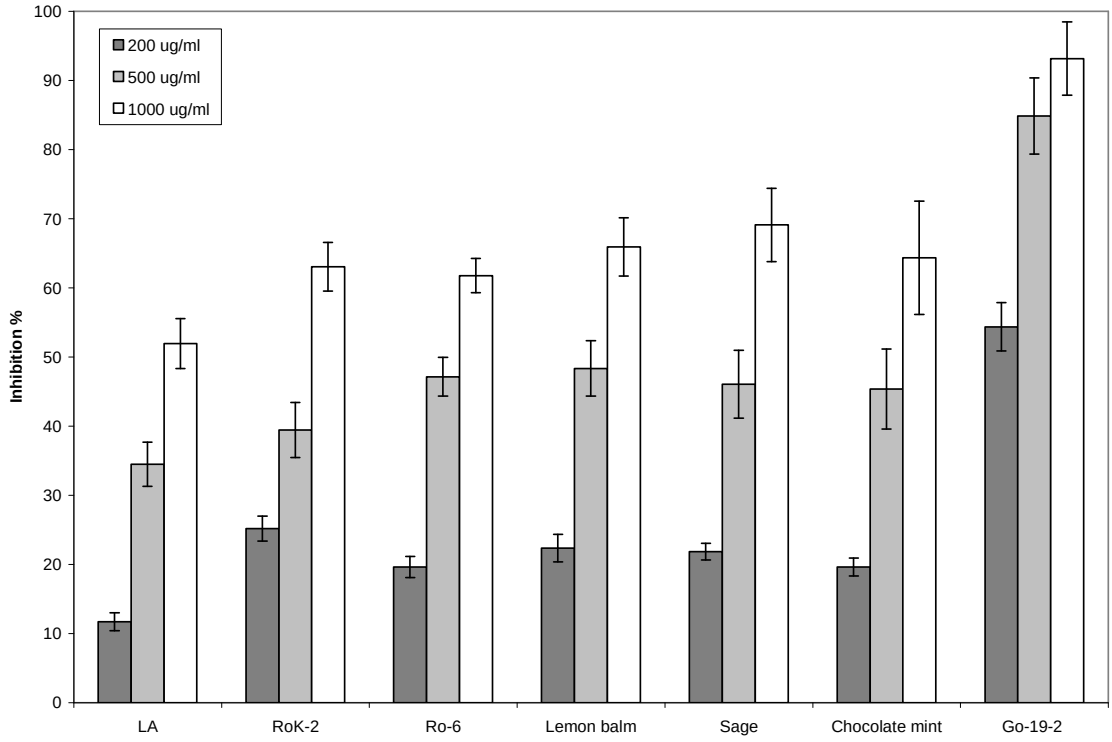

Figure 6. Dose dependent changes iṇ α-Glucosidase inhibitory activity of water extracts of clonal herbs of Lamiaceae family (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano, μg/ml total phenolics).

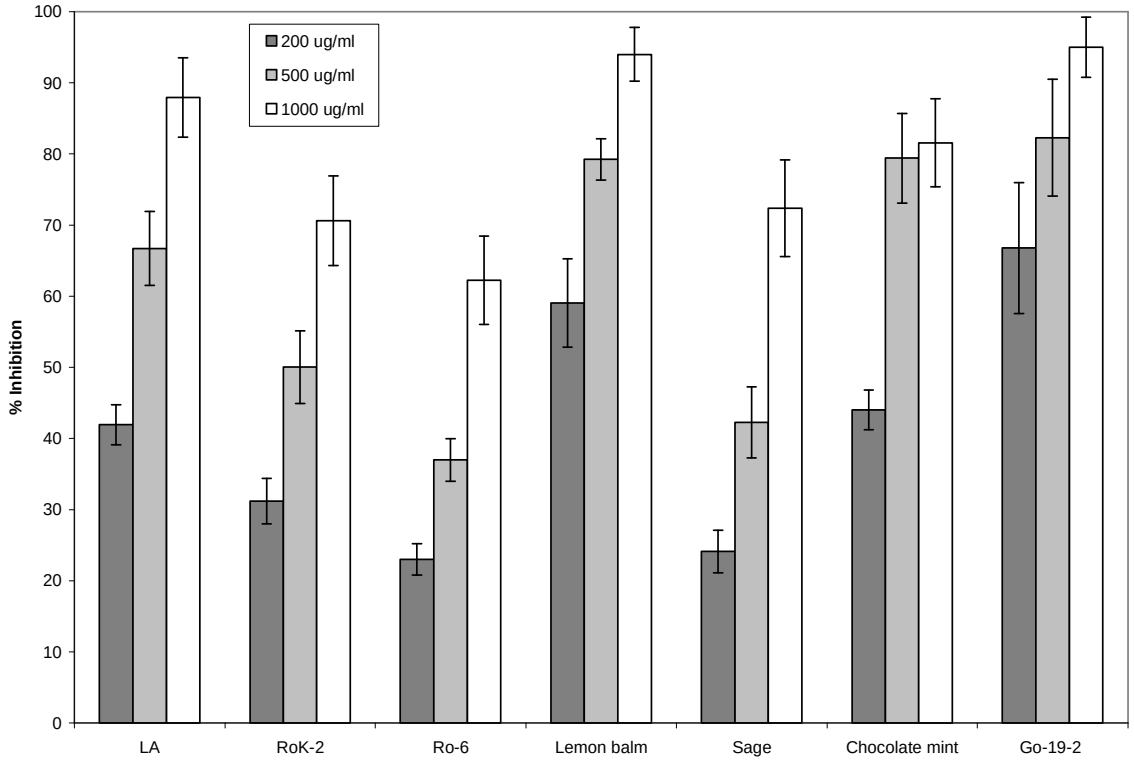

Figure 7. Dose dependent changes in α-Glucosidase inhibitory activity of 12% ethanol extracts of clonal herbs of Lamiaceae family (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano, µg/ml total phenolics).

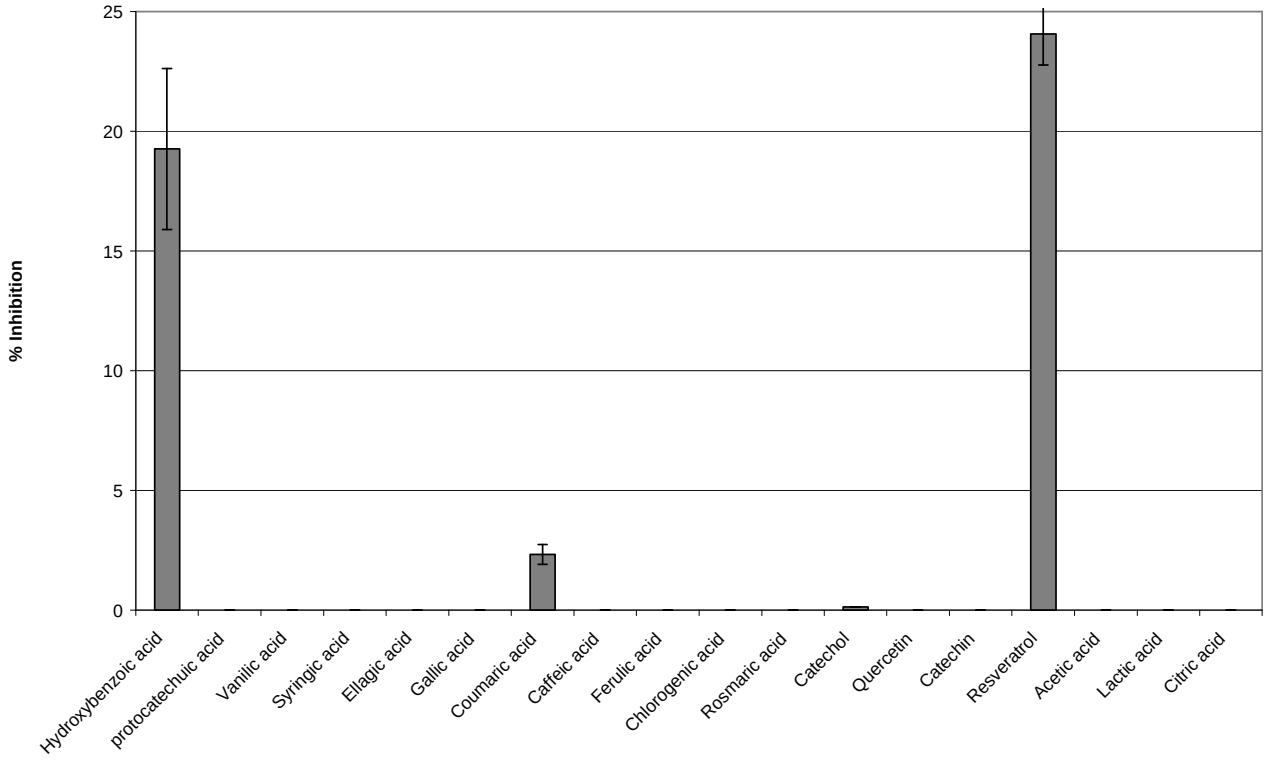

Figure 8. ACE-I inhibitory activity of standard phenolics at different pH (at constant phenolic 1000 µg/ml)

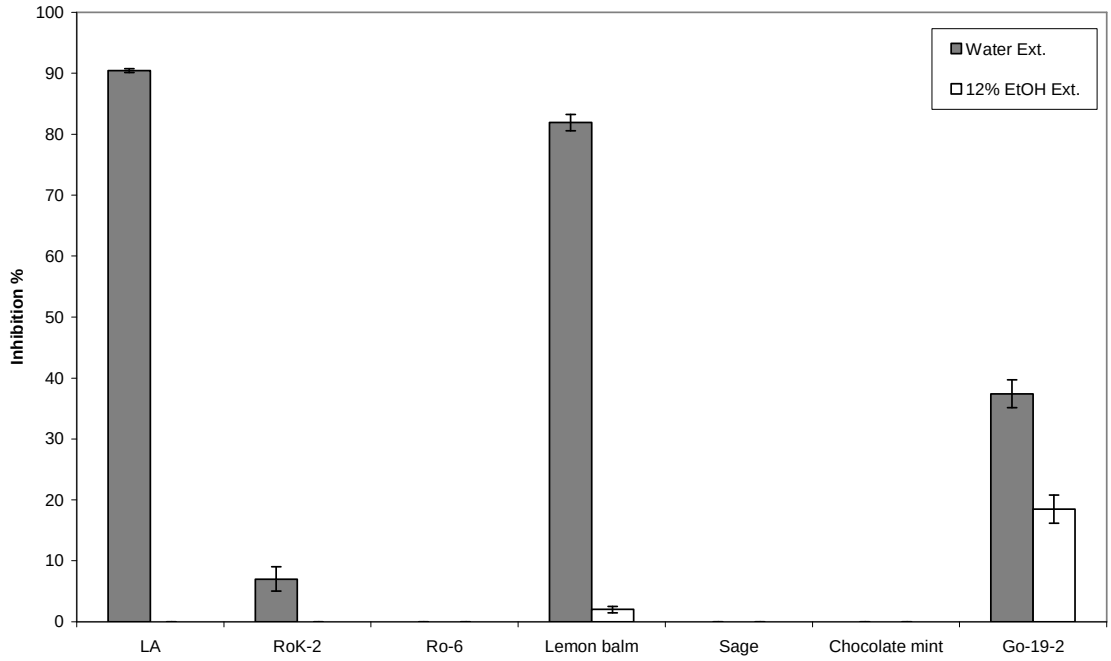

Figure 9. ACE-I inhibitory activity of water and 12% ethanol extracts of clonal herbs of Lamiaceae family (LA; rosemary, RoK-2; rosemary, Ro-6; rosemary, Go-19-2; oregano, each samples; 1.0 mg/ml total phenolics, Oregano; 4.0 mg/ml)

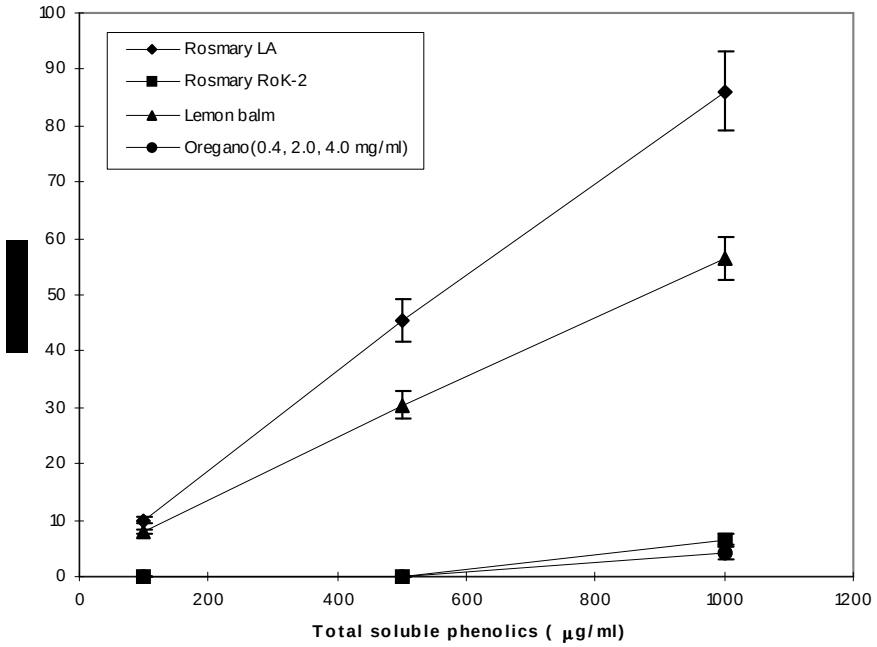

Figure 10. Dose dependent changes in ACE-I inhibitory activity of water extracts of clonal herbs of Lamiaceae family (µg/ml total phenolics).

both extracts inhibited the ACE activity at a total soluble phenolics concentration 100µg/ml (Fig. 9).

Dose dependent analysis showed that rosemary LA had the most potent ACE inhibitory activity (Fig.10). The ACE inhibitory activities of rosemary RoK-2 and oregano extracts were similar at a total soluble phenolic concentration of 100, 500 and 1000µg/ml which indicates that there may be other soluble factors linked to ACE inhibitory activity. The lack of correlation of the ACE inhibitory activity of the samples with phenolic content in this assay may suggest these differences could potentially be due to phenolics that were not detected by HPLC or by non phenolic compounds which may be important factor in contributing to the total ACE inhibitory activity.

Conclusion

The major phenolic components of the Lamiaceae family of herbs: rosmarinic acid, caffeic acid, protocatechuic acid, resveratrol, catechin, catechol, coumaric acid and quercetin had strong α-glucosidase (EC 3.2.1.20) inhibitory activity. Resveratrol and coumaric acid also had significant ACE inhibitory activity. Lamiaceae family has good inhibitory profile on carbohydrate degrading enzyme such as α-glucosidase related to carbohydrate digestion. Water and 12% ethanol extracts from Lamiaceae family had no inhibitory effect against to α-amylase. Strong inhibition of α-glucosidase could be potentially used as effective therapy for postprandial hyperglycemia with less side-effects such as abdominal distention, flatulence, meteorism and possibly diarrhea caused by the excessive inhibition of pancreatic α-amylase, which results

in the abnormal bacterial fermentation of undigested carbohydrates in the colon. Among all the extracts, water extracts from rosemary LA and lemon balm showed strong ACE-inhibitory activity. Control of hypertension via modulation of angiotensin I-converting enzyme (ACE) by dietary anti-hypertensive agents could be an important strategy to manage hypertension which is a risk factor for CVD and often results from long-term diabetes mellitus. Based on these results two clonal herbs from Lamiaceae family, rosemary LA and lemon balm have the potential development of effective dietary strategy for postprandial hyperglycemia and hypertension linked to diabetes mellitus and associated cardiovascular diseases.

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Original Article

Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension

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唇形科植物克隆株系对糖尿病和高血压影响的研究

本次研究中，我们观察了 7 株来自于单一唇形科种子表现型的克隆株系品种对 α–淀粉酶、α–葡萄糖苷酶和血管紧张素转化酶(ACE)的抑制作用。牛至株系的水提取物有最高的 α–葡萄糖苷酶抑制活性(93.7%),其次为巧克力薄荷(85.9%)和柠檬香蜂草(83.9%)；另外，3 种不同的迷迭香克隆植株：迷迭香 LA(71.4%),迷迭香 6(68.4%)和迷迭香 K2(67.8%)也表现出显著的 α–葡萄糖苷酶抑制活性。把这些提取物中的 α–葡萄糖苷酶抑制活性与通过 HPLC 检测到的提取物中的单一酚类物质相比较，儿茶素显示出最高的 α–葡萄糖苷酶抑制活性(99.6%)，其次为咖啡酸(91.3%),迷迭香酸(85.1%)和白藜芦醇(71.1%)。另外，儿茶酚(64.4%)、原儿茶酸(55.7%)和槲皮素(36.9%)也显示出显著的 α–葡萄糖苷酶抑制活性。结果显示，克隆株系提取物中的 α–葡萄糖苷酶抑制活性与其酚含量、抗氧化剂活性和提取物酚物质组成相关。克隆株系提取物和所使用的标准酚物质并不表现对 α–淀粉酶的抑制活性，我们也观察了各克隆株系提取物对兔肺血管紧张素转化酶 I(ACE)的抑制作用。结果表明，迷迭香 LA 的水提取物有最高的 ACE 抑制活性(90.5%)，其次为柠檬香蜂草(81.9%)和牛至(37.4%)。而牛至和柠檬香蜂草的醇提取物其 ACE 抑制活性较低，分别为 18.5%和 0.5%。在酚标准品中，只有白藜芦醇、水扬酸和香豆酸显示 ACE 抑制活性，其活性分别为 24.1%、19.3%和 2.3%。

关键词：酚类植物化学因子、抗氧化剂、唇形科、淀粉酶、葡萄糖苷酶、血管紧张素转化酶、酶抑制因子、白藜芦醇、迷迭香酸、糖尿病、高血压。