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Original Article

α-Amylase Inhibitory Activities of Six *Salvia* Species

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Abstract

α-Amylase inhibitory activities of ethanol extracts from six selected Iranian *Salvia* species (*S. hydrangea* DC., *S. hypoleuca* Benth., *S. officinalis* L., *S. reuterana* Boiss., *S. verticillata* L. and *S. virgata* Jacq.) were examined, using in vitro model. In addition, antioxidant capacities (DPPH• radical scavenging) and total flavonoid cotents of the extracts were also determined. The results showed that the extracts of *S. verticillata* and *S. virgata,* significantly and concentration dependently, inhibited α-amylase activity. All the extracts exhibited antioxidant activities, among which *S. verticillata*, *S. virgata*, *S. officinalis*, *S. hypoleuca* and *S. hydrangea* were found to be the most active in terms of assay. *S. hypoleuca* and *S. reuterana* extracts had the highest total flavonoid contents. However, a favorable correlation was not found between α-amylase inhibitory effects or antioxidant activities and the total flavonoid contents of the extracts.

Keywords: α-Amylase inhibitory activity; Antioxidant activity; Flavonoid content; *Salvia* species.

Introduction

Diabetes mellitus is a metabolic disorder characterized by a congenital (type I insulin-dependent diabetes mellitus/IDDM) or acquired (type II noninsulin-dependent diabetes mellitus/ NIDDM) inability to transport glucose from the bloodstream into cells (1). The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (2). At the present time it is estimated that 150 million people,worldwide, have diabetes and that this will increase to 220 million by 2010 and 300 million by 2050 (3). Globally, type II diabetes afflicts approximately 90% of all diabetes (1).

Various pharmacological approaches are used to improve diabetes via different modes of action such as stimulation of insulin release, increase, the number of glucose transporters, inhibition of gluconeogenesis and reduction of absorption of glucose from the intestine (4). One of the most beneficial therapies for type II diabetes is said to be the control of postprandial hyperglycemia after a meal (5). Stabilization of blood glucose is important for diabetic patients, because it prevents hyperglycemia and the complications associated with diabetes (6). The best therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes in the digestive organs (5). The enzymes are responsible for the breakdown of oligo- and disaccharides to monosaccharides. α-Amylase is one the enzymes that catalyses the breakdown of starch to maltose and finally to glucose, which is the only sugar that can be utilized by the body (7). The inhibition of these enzymes leads to a decrease in blood

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glucose level, since monosaccharides are a form of carbohydrates which are absorbed through the small intestine (2). There are several reports of established or screened and developed enzyme inhibitors and its effects on blood glucose levels after food uptake (5).

In recent years, plants and their constitutions have received much attention in the treatment of diabetes for various reasons and many researchers have focused on hypoglycemic agents from medicinal plants (4). Based on the existing studies, it is found that polyphenols and flavonoids are among the natural active antidiabetic agents (8). These compounds have been reported to exert various biological effects, including carbohydrate hydrolyzing enzyme inhibition and antioxidant activity. Polyphenolic compounds are able to inhibit the activities of digestive enzymes due to their ability to bind with proteins. The inhibitory activities of plant phytochemicals, including polyphenols, against carbohydrate hydrolyzing enzymes contribute to the lowering of postprandial hyperglycemia in the management of diabetes, as observed in vivo (9). On the other hand, phenolic compounds and flavonoids constitute one of the major groups of herbal compounds acting as free radical scavengers and antioxidants. Free radicals and reactive oxygen species (ROS) can react with biological molecules, leading to cell and tissue injuries and pathological events. The role of free radicals and ROS in the etiology of many chronic diseases has been well-known. Therefore, free radical scavengers and antioxidants, especially phenolic compounds, are important for human health and have been proposed as health-promoting natural products (9, 10).

Several medicinal plants including *Salvia* species have been advocated in Traditional Iranian Medicine for their hypoglycemic effects (11). The genus *Salvi*a commonly called sage is one the largest members of the family Lamiaceae that contains nearly 900 species spread throughout the world (12). In the Flora of Iran, the genus is represented by about 58 species of which 17 species are endemic (13). They are well known among people and widely used as flavoring agents or fragrances and for various medicinal purposes (10, 14). *Salvia* species are rich sources of polyphenolic flavonoids and phenolic acids (10, 12). The plants and their isolated constituents possess significant antioxidant activity in the enzyme dependent and enzyme independent systems (10, 15).

The aim of the present study was to evaluate the anti-α-amylase potential of six Iranian *Salvia* species (*S. hydrangea* DC., *S. hypoleuca* Benth., *S. officinalis* L., *S. reuterana* Boiss., *S. verticillata* L. and *S. virgata* Jacq.). To the best of our knowledge, there is no scientific published data on the inhibitory effects of the selected plants on carbohydrate hydrolyzing enzymes, especially α-amylase. Recently, the free radical scavenging activities of five *Salvia* species have been reported by us (10). This investigation was also designed to determine the antioxidant capacities of the aforementioned *Salvia* species by the 2,2′-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging method and estimate the total flavonoid contents of the selected species by the $AlCl_3$ assay and study the correlation between flavonoid contents and α-amylase inhibitory effects and/or antioxidant activities.

Experimental

Chemicals

All the chemicals used were purchased from Sigma-Aldrich Chemie Gmbh (Germany) and Merck (Germany) companies. The chemicals were of analytical grade.

Plant materials

The plants (*S. hydrangea*, *S. hypoleuca*, *S. officinalis*, *S. reuterana*, *S. verticillata*, *S. virgata*) were collected in Tehran province during the flowering period in summer 2004 and 2005 and authenticated by M. Kamalinejad. A voucher specimen for each plant was deposited at the Herbarium of the Department of Pharmacognosy, Shaheed Beheshti University (M. C.), Tehran, Iran. The plants were dried in a drying room, with active ventilation at ambient temperature. Only the aerial parts of the plants were used for investigation.

Extraction

The dried and fine plant parts (100 g) were extracted with a 90% ethanolic solution (500 ml)

at ambient temperature for 48 h. The extracts were filtered and concentrated under reduced pressure at approximately 40 °C.

α-Amylase inhibition

The α-amylase inhibition assay was adapted and modified from Giancarlo et al. (16). The Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium choride. The extracts were dissolved in DMSO to give concentrations from 14.7 to 36 mg/ml (14.7, 18.4, 23, 28.8, 36 mg/ml). The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml).

1 ml of each plant extract and 1 ml enzyme solution were mixed in a tube and incubated at 25 °C for 30 min. To 1 ml of this mixture was added 1 ml of starch solution and the tube incubated at 25 °C for 3 min. Then, 1 ml of the color reagent was added and the closed tube placed into an 85 °C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm in a Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). Individual blanks were prepared for correcting the background absorbance. In this case,the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing plant extracts with 1 ml DMSO. Acarbose solution (at the concentrations of 0.0094, 0.0184, 0.036, 0.07, 0.11, 0.21 μg/ml) was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$$I_{\alpha\text{-amylase}}\% = 100 \times (\Delta A_{Control} - \Delta A_{Sample}) / \Delta A_{Control}$$

$$\Delta A_{Control} = A_{Test} - A_{Blank}$$

$$\Delta A_{Sample} = A_{Test} - A_{Blank}$$

The $I_{\alpha\text{-amylase}}$% was plotted against the sample concentration and a logarithmic regression curve established in order to calculated the IC_{50} value (inhibitory concentration). This would represent the concentration of sample (μg/ml) necessary to decrease the absorbance of α-amylase by 50%.

Antioxidant and DPPH radical scavenging activity

The antioxidant activities of the plant extracts were assessed by their ability to bleach the stable DPPH radical (10). To 2.5 ml of each sample solution at different concentrations (5, 10, 25, 50, 100, 250, 500, 1000 μg/ml, in ethanol 90%), 1 ml of 0.3 mM $DPPH^{\bullet}$ solution in ethanol was added. Test tubes were vortexed and incubated for 30 min at room temperature in the dark. The absorbance values were then determined at 518 nm. The inhibition percentage of DPPH radical was assessed, using the following formula:

$$I_{DPPH\bullet}\% = 100 \times [A_{Control} - (A_{Sample} - A_{Blank})] / A_{Control}$$

Ethanol (1 ml) plus each sample solution (2.5 ml) was used as the blank. $DPPH^{\bullet}$ solution (1 ml) plus ethanol (2.5 ml) was used as the negative control. Also,a rutin solution (at the concentrations of 2.5, 5, 10, 25, 50, 100 μg/ml) was used as the positive control.

The $I_{DPPH\bullet}$% was plotted against the sample concentration and a logarithmic regression curve established in order to calculated the IC_{50} value (inhibitory concentration), which is the concentration of sample (μg/ml) necessary to decrease the absorbance of $DPPH^{\bullet}$ by 50%.

Determination of total flavonoid content

The amount of the total flavonoid content for each extract was determined,based on the colorimetric assay, using rutin as the reference compound (10). 2.5 ml of a rutin solution (at the concentrations of 75, 50, 37.5, 25 μg/ml, in ethanol 90%) was mixed with 2.5 ml of the $AlCl_3$ reagent in ethanol 90% (20 mg/ml). After 40 min, the absorbance was measured at 415 nm. A combination of ethanol 90% (2.5 ml) and the rutin solution (2.5 ml) was used as the blank. Then, a linear calibration curve (absorbance versus concentration) was constructed. The same procedure was carried out on 2.5 ml of

Table 1. IC_{50} values of antioxidant ($DPPH^{\bullet}$ scavenging) activities and total flavonoid contents of the tested *Salvia* species extracts.

Plant species	IC_{50} (μg/ml)[a,b]	Total flavonoid content (μg/mg)[b,c]
S. hydrangea	44.57 (40.63 – 48.89)[e]	53.30 ± 1.20[e]
S. hypoleuca	36.81 (33.63 – 40.29)[d,e]	53.16 ± 1.95[e]
S. officinalis	30.67 (27.88 – 33.73)[d]	17.24 ± 1.31[b,c]
S. reuterana	125.10 (109.68 – 142.70)[f]	46.97 ± 4.43[d]
S. verticillata	23.53 (20.56 – 26.93)[d]	10.81 ± 1.98[b,c]
S. virgata	27.01 (24.08 – 30.29)[d]	8.54 ± 0.99[a]

[a]Note: The IC_{50} value of the positive control, rutin, was measured as 6.69 (6.46 – 6.91) μg/ml.
[b]The IC_{50} values have been presented along with their respective 95% confidence limits (n = 3).
[c]The total flavonoid content values are means ± SEM (n = 3).
[d]Letters (a-f) denote homogeneous subsets at $p < 0.05$ (Tukey's post test).

each sample in ethanol 90%, instead of the rutin solution. The total flavonoid content for each extract [as μg rutin equivalents (RE)/mg of extract] was determined on the basis of the standard curve.

Statistical analysis

The IC_{50} values were estimated by non- linear curve-fitting and presented as their respective 95% confidence limits. Total flavonoid contents have been shown as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey's post test was used to assess the presence of significant differences ($p < 0.05$) between the extracts. All the statistical analyses were accomplished, using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

Results and Discussion

α-Amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars thus, the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently, postprandial rise in blood glucose is decreased. Hence, they have long been though to improve glucose tolerance in diabetic patients (17, 18). On the other hand, Natural polyphenols have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes (9). Besides, phenolic compounds are currently regarded natural antioxidants and considered to be important ingredients for human health (10). Therefore, in this study, α-amylase inhibitory activities, antioxidant capacities (based on the DPPH radical scavenging method) and total flavonoid contents (based on the $AlCl_3$ assay) of the ethanol extracts obtained from six Iranian *Salvia* species (*S. hydrangea*, *S. hypoleuca*, *S. officinalis*, *S. reuterana*, *S. verticillata*, *S. virgata*) were determined and compared to each other.

The quantification of total flavonoid content was developed, using the $AlCl_3$ reagent and rutin as standards. Table 1 presents the total flavonoid content, in rutin equivalent (RE), calculated for each extract. It varied from 53.16 to 8.54 μg RE/mg of the extract. When the flavonoid content of each extract was compared with the others, the *S. hypoleuca* (53.16 ±1.95 μg/mg) and *S. reuterana* (46.97±4.43 μg/mg) extracts were found to have significantly higher contents, while *S. virgata* (8.54 ± 0.99), *S. verticillata* (10.81±1.98) and *S. officinalis* (17.24 ±1.31) contained remarkably lower amounts of these compounds. The order obtained in terms of the total flavonoid content for the tested extracts was *S. hypoleuca* ≥ S. *reuterana* > *S. hydrangea* > *S. officinalis* ≥ *S. verticillata* ≥ *S. virgata*.

The antioxidant activity of each extract was monitored, using the DPPH radical assay. All the tested plants exhibited favorable concentration dependent radical scavenging and antioxidant activities and the IC_{50} values ranged from 23.53 to 125.10 μg/ml (Table 1 and Figure 1). *S. verticillata* [23.53 (20.56–26.93) μg/ml], *S. virgata* [27.01 (24.08–30.29) μg/ml], *S. officinalis* [30.67 (27.88 – 33.73) μg/ml], *S. hypoleuca* [36.81 (33.63 – 40.29) μg/ml] and *S. hydrangea* [44.57 (40.63 – 48.89) μg/ml] showed the highest antioxidant capacities, with no significant difference among them. The

Table 2. α-Amylase inhibitory activities and IC_{50} values of the tested *Salvia* species extracts.

Plant species	Concentrations (mg/ml)	Inhibition (%)[a]	IC_{50} (mg/ml)[b]
S. hydrangea	14.70	-13.43 ± 1.20	-
	18.40	2.65 ± 0.57	
	23.00	16.27 ± 1.64	
	28.80	32.24 ± 0.64	
	36.00	20.35 ± 1.84	
S. hypoleuca	14.70	14.27 ± 0.99	-
	18.40	28.31 ± 0.99	
	23.00	45.83 ± 0.63	
	28.80	53.36 ± 1.89	
	36.00	34.47 ± 2.77	
S. officinalis	14.70	-3.91 ± 1.29	-
	18.40	3.26 ± 0.88	
	23.00	24.95 ± 1.96	
	28.80	9.72 ± 0.84	
	36.00	2.76 ± 0.41	
S. reuterana	14.70	1.19 ± 0.45	-
	18.40	6.66 ± 0.57	
	23.00	11.01 ± 0.92	
	28.80	14.39 ± 1.18	
	36.00	25.01 ± 1.68	
S. verticillata	14.70	26.86 ± 0.93	18.34 (18.07 – 18.61)
	18.40	46.29 ± 0.58	
	23.00	63.96 ± 1.86	
	28.80	95.20 ± 0.66	
	36.00	99.82 ± 0.18	
S. virgata	14.70	11.37 ± 0.61	19.73 (19.30 – 20.17)
	18.40	23.83 ± 1.18	
	23.00	63.92 ± 0.74	
	28.80	67.28 ± 0.49	
	36.00	76.69 ± 1.15	

[a]Note: The IC_{50} value of the positive control, acarbose, was measured as 0.851 (0.028 – 0.013) mg/ml.
[b] α-Amylase inhibitory activities values are means ± SEM (n = 3).
[c] The IC_{50} values have been presented along with their respective 95% confidence limits (n = 3).

extract with the weakest scavenging potency was *S. reuterana* [125.10 (109.60 –142.70) µg/ml], which had significantly lower activity than the other tested extracts. Therefore, the scavenging activity of the extracts in decreasing order was *S. verticillata* ≥ *S. virgata* ≥ *S. officinalis* ≥ *S. hypoleuca* ≥ *S. hydrangea* > *S. reuterana*.

The α-amylase inhibitory activities varied widely among the tested plants. As can be observed in Table 2 and Figure 2, *S. verticillata* [IC_{50}=18.34 (18.07–18.61) mg/ml)] and *S. virgata* [IC_{50}=19.73 (19.30–20.17) mg/ml] exhibited noticeable concentration dependent effects and did not show any significant differences in their IC_{50} values ($p > 0.05$). An increase in graded concentrations of the *S. reuterana* extract resulted in a decrease in α-amylase activity from 1.19 ± 0.45% to 25.01 ± 1.68% (Table 2). However, the inhibitory activity of *S. reuterana* on α-amylase was weak and it did not reach the

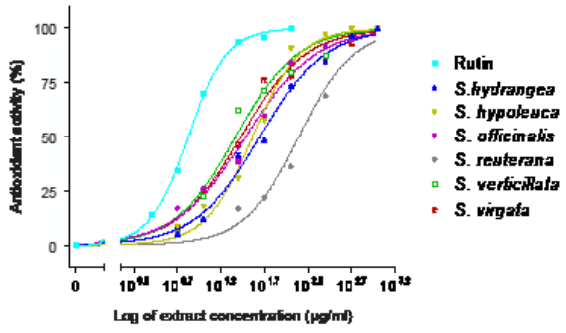

Figure 1. Antioxidant activities of the studied *Salvia* species extracts using DPPH• (Each point represents the mean of three experiments and the vertical bars represent the SEM.).

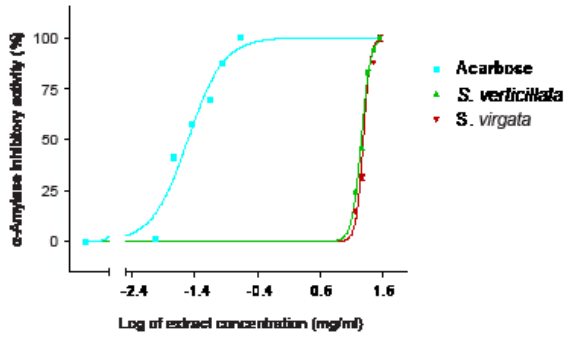

Figure 2. α-Amylase inhibitory activities of the studied *Salvia* species extracts (Each point represents the mean of five experiments and the vertical bars represent the SEM.).

50% inhibition level of enzyme activity. Two negative inhibition values were observed for *S. hydrangea* (-13.42±1.20%) and *S. officinalis* (-3.91±1.29%) at a concentration of 14.7 mg/ml (Table 2). This could indicate that α-amylase is activated rather being inhibited. This finding is in agreement with other published data (17, 19, 20). Besides, a concentration dependent inhibition was not observed,when the concentrations of *S. hydrangea*, *S. hypoleuca* and *S. officinalis* extracts were increased. The highest inhibitory activities for these extracts were found to be 52.24±0.64% (at 28.8 mg/ml concentration), 53.38±1.09% (at 28.8 mg/ml concentration) and 24.95±1.96% (at 23 mg/ml concentration), respectively (Table 2). It is probably due to the fact that at high extract concentrations, there is a conformational change derived from binding of compounds to the enzyme (8, 20).

Among the analyzed extracts, a favorable correlation between α-amylase inhibitory effects and flavonoid contents and/or antioxidant activities and flavonoid contents was not found. This was especially true for *S. verticillata* and *S. virgata,* which had high enzyme inhibitory and antioxidant effects, despite having the lowest total flavonoid contents. In general, the antioxidant capacity and the enzyme inhibitory activity of plant extracts not only depend on the quantity of polyphenols but also might depend on the quality of polyphenols. Other researchers have also reported that biological activities of polyphenols depend on the extent of hydroxylation and conjugation (18, 21, 22).

The findings of in the present study not only clarify the α-amylase inhibitory effect, the antioxidant activity and the total flavonoid contents of six Iranian *Salvia* specie, but also introduce novel sources for the prevention of non-communicable diseases in Iran and in other countries. However, further in vitro and in vivo studies are needed to confirm the present observations.

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References

(1) Abesundara KJ, Matsui T and Matsumoto K. alpha-Glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, *Cassia auriculata*, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose. *J. Agric. Food Chem.* (2004) 52: 2541-1545

(2) Funke I and Melzig MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of α-amylase activity. *Braz. J. Pharmacogn.* (2006) 16: 1-5

(3) Li Y, Wen S, Kota BP, Peng G, Li GQ, Yamahara J and Roufogalis BD. *Punica granatum* flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J. Ethnopharmacol.* (2005) 99: 239-244

(4) Youn JY, Park HY and Cho KH. Anti-hyperglycemic activity of *Commelina communis* L.: inhibition of

alpha-glucosidase. *Diabetes Res. Clin. Pract.* (2004) 66S: S149-S155
(5) Kim YM, Jeong YK, Wang MH, Lee WY and Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition* (2005) 21: 756-761
(6) Mai TT and Chuyen NV. Anti-hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr and Perry. *Biosci. Biotechnol. Biochem.* (2007) 71: 69-76
(7) Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A and Subratty AH. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects in vitro. *Phytother. Res.* (2006) 20: 228-231
(8) Andrade-Cetto A, Becerra-Jiménez J and Cárdenas-Vázquez R. Alpha-glucosidase inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. *J. Ethnopharmacol.* (2008) 116: 27-32
(9) Mai TT, Thu NN, Tien PG and Van Chuyen N. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *J. Nutr. Sci. Vitaminol.* (2007) 53: 267-276
(10) Nickavar B, Kamalinejad M and Izadpanah H. In vitro free radical scavenging activity of five *Salvia* species. *Pakistan J.Pharm. Sci.* (2007) 20: 291-294
(11) Amin G. *Popular Medicinal Plants of Iran*. Vice-chancellorship of Research, Tehran University of Medical Sciences, Tehran (2005) 250
(12) Lu Y and Foo LY. Polyphenolics of *Salvia*. *Phytochemistry* (2002) 59: 117-140
(13) Mozaffarian V. *A Dictionary of Iranian Plant Names.* Farhang Moaser Publishers, Tehran (1996) 477-480
(14) Nickavar B, Mojab F and Asgarpanah J. Volatile composition of the essential oil of *Salvia hypoleuca* Benthh. *Int. J. Aromather.* (2005) 15: 51-53
(15) Perry NS, Bollen C, Perry EK and Ballard C. *Salvia* for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. *Pharmacol. Biochem. Behav.* (2003) 75: 651-659
(16) Giancarlo S, Rosa LM, Nadjafi F and Francesco M. Hypoglycaemic activity of two spices extracts: *Rhus coriaria* L. and *Bunium persicum* Boiss. *Nat. Prod. Res.* (2006) 20: 882-886
(17) Ali H, Houghton PJ and Soumyanath A. Alpha-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *J. Ethnopharmacol.* . (2006) 107: 449-455
(18) Kwon YI, Jang HD and Shetty K. Evaluation of *Rhodiola crenulata* and *Rhodiola rosea* for management of type II diabetes and hypertension. *Asia Pac. J. Clin. Nutr.* (2006) 15: 425-432
(19) Gowri PM, Tiwari AK, Ali AZ and Rao JM. Inhibition of alpha-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa* Roxb. seeds. *Phytother. Res.* (2007) 21: 796-799
(20) Kim JS, Kwon CS and Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.* (2000) 64: 2458-2461
(21) Gálvez M, Martín-Cordero C, Houghton PJ and Ayuso MJ. Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food Chem.* (2005) 23: 1927-1933
(22) Pulido R, Bravo L and Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* (2000) 48: 3396-3402

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