Medicinal and Aromatic Plant Science and Biotechnology ©2008 Global Science Books

Antihyperglycemic and Hypoglycemic Effects of Aqueous and Hydroethanolic Extracts of *Pentaclethra macrophylla* Benth on Wistar Rats: Inhibition of α-Amylase

Gilles Ines Dongmo Fomekong* • **Claudia Edwedje Noumessing Momo** • **Julius Enyong Oben**

Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, Faculty of Science, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon

Corresponding author: * **gillesfomekong@yahoo.fr**

ABSTRACT

This study reports on the antihyperglycemic and hypoglycemic properties of *Pentaclethra macrophylla* Benth on Wistar rats. The inhibition of α-amylase was also determined using 0.25, 0.5, 1, 1.5, and 2 mg/mL of plant extracts. The aqueous and hydroethanolic extract showed a pronounced antihyperglycemic activity ($P<0.001$) from 75 mg/kg to 300 mg/kg body weight (BW) after 60 min. The improved regulation of blood glucose was obtained at 150 mg/kg BW with 23.75% and 28.85% reduction, respectively for aqueous and hydroethanolic extracts after 2 h 30 min of treatment. In the hypoglycemic assay on normoglycemic rats, we observed a slight decrease of blood glucose from 38 mg/kg to 75 mg/kg BW during the experimental period. Between the 5th and the 8th hour post-treatment, we observed a significant ($P<0.001$) decrease of blood glucose at 150 mg/kg and 300 mg/kg BW, respectively. On the order hand, the *in vitro* experiments showed that amylase activity was inhibited in the presence of these plant extracts. The extent of amylase inhibition was correlated with an increase in the concentration of extracts. Also the hydroethanolic extract showed better amylase inhibition activity. We obtained a 50% inhibitory concentration (IC_{50}) of 0.292 mg/mL with the hydroethanolic extract while the aqueous extract showed an IC_{50} of 3.19 mg/mL. The significance of plant-based amylase inhibitors for modulation of diabetes mellitus and other oxidation-linked diseases is hypothesized and discussed.

Keywords: blood glucose, diabetes, enzyme, plant extracts
Abbreviations: BW, body weight; **OGTT**, oral glucose tolerance test

INTRODUCTION

Ethnopharmacological literature has shown some medicinal plants in the diet of local populations of wild animals (gorillas and chimpanzees) in the forest and the same medicinal plants are used by local human populations for various parasitic infections, inflammations, pain and related illnesses (Cousins and Huffman 2002). *Pentaclethra macrophylla* Benth: (Mimosaceae) is one of the plants in Africa used in traditional herbal practice for the treatment of disorders of both domestic and wild animals and human diseases (Akah *et al*. 1999). *P. macrophylla* is also known as the African oil bean tree (Oliver 1960). The plant is most found in the forests of Eastern, Western and Central Africa (Keay *et al*. 1969). All the parts of the plant are used for various human ailments. The bark, fruits, seeds and the leaves are used as anthelmintics, for gonorrhea, convulsion and as analgesic (Githens 1948; Bouquet *et al*. 1971; Iwu 1993) while whole leaves are always given to domestic and wild animals and ruminants (Rogers *et al*. 1990), while the aqueous extract of the leaves is orally administered to people (Akah *et al*. 1999). The antimicrobial property of the fixed oil extracted from seeds is used in the preparation of a formulation against pruritus, worms and dysentery (Singha 1963; Gugnani and Ezenwanze 1985; Kamanzi Atindehou *et al*. 2002). Others studies have demonstrated various applications of this plant in Cameroon including its use as an adjunct to lower blood glucose response in non-insulin-dependent diabetics and in other applications to lower the blood serum cholesterol level (FAO 2001).

Long-term hyperglycemia can lead to damage in cells that cannot block sugar from entering. In these cells (especially those lining blood vessels), as mitochondria utilize entering sugars, harmful by-products accumulate. Much research has focused on glycosidase inhibitors to control hyperglycemia, but many forms of starch are also digested for rapidly glucose absorption (Fujita *et al*. 2001; Ghavami *et al*. 2001; Jonston *et al*. 2002; Notkins 2002). Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes (Fujita *et al*. 2001; Ghavami *et al*. 2001; Notkins 2002). Inhibitory activity against amylase by flavonoids and anthocyanins has been reported (Kim *et al*. 2000; Matsui *et al*. 2001).

Diabetes is a major risk factor for premature atherosclerosis, and oxidative stress plays an important role. In fact, diabetic monocytes produce increased superoxide anion (O^{-}_{2}). Over the centuries, herbal drugs have served as a major source of medicines for prevention and treatment of diseases including diabetes mellitus. It is estimated that more than 200 species of plants exhibit hypoglycemic properties, including many common plants, such as pumpkin, wheat, celery, wax gourd, lotus root and bitter melon but the basis of this activity is frequently not investigated (Lamer 1985; Yeh *et al.* 2003; Mentreddy 2007).

Synthetic hypoglycemic agents can produce serious side effects and are not suitable for use during pregnancy. Therefore, the search for more effective and safer hypoglycemic compounds has continued to be an important area of active research, and after the recommendations made by WHO on diabetes mellitus (Lamer 1985; Ndam 2005) research on hypoglycemic compounds from medicinal plants has become an important aspect of this project.

In the present study the biological activities of *P. macrophylla* were investigated, in particular the effects on blood glucose and inhibition of porcine α-amylase.

Received: 7 November, 2007. Accepted: 13 January, 2008.

Original Research Paper

MATERIALS AND METHODS

Plant materials

The stem-bark of *P. macrophylla* was sun-dried until constant weight, and ground to powder. Plant powder (1500 g) was used for both aqueous and hydroethanolic extraction. Five hundred g of this plant powder was decocted in 4 L of distilled water for 15-20 min. This was repeated four times, until the resulting extract gave no further coloration. When cooled to room temperature, the preparation was sieved through four layers of cotton fabric gauze. The filtrate was allowed to stand for 90 to 120 minutes after which the supernatant was filtered through Whatman filter paper N°1. The decoction obtained was evaporated at 40°C till total dryness using a convection air oven. The dry solid material obtained (yield: 9.15% w/w) was used immediately or stored at 4°C. The remaining powder (1000 g) was soaked in 5 L of a mixture of distilled water/ethanol (1:1) for 48 h, and for a further 24 h in the same solvent. This was filtered and concentrated to a small volume to remove all water and ethanol using a Biichi RE 120 rotary evaporator (TECHNIK AG Lab, Switzerland). The remaining liquid was later further dried in an oven at 40°C, to obtain an extract of 120 g (12% yield).

Experimental animals

Three-month-old male Wistar Albinos rats weighing 180-200 g were obtained from the animal house of the LNNB (Laboratory of Nutrition and Nutritional Biochemistry), Department of Biochemistry, University of Yaounde I, Cameroon. All animals were kept in an environmentally controlled room with a natural light-dark cycle. The animals had free access to water and were fed with standard laboratory diet. The study was approved by institutional animal ethical committee.

Effect of oral administration of *P. macrophylla* extracts on normoglycemic rats

The animals were divided into 6 groups, each of which was treated as follows (**Table 1**). The control group was treated with distilled water while the reference group was treated with tolbutamide (80 mg/kg BW). The test groups received 38, 75, 150 or 300 mg/kg BW of plant extracts.

The aqueous and hydroethanolic extracts were dissolved respectively in distilled water. The rats were fed with the test material (*P. macrophylla* extracts or tolbutamide which were dissolved in water) orally by gastric intubation after 12 h of fasting (only water available *ad libitum*).

Glucose level was monitored at 0, 1, 2.5, 5 and 8 h after feeding with the plant extracts or tolbutamide.

Effect of *P. macrophylla* extracts on glucose tolerance on normoglycemic rats

The oral glucose tolerance test was performed in overnight fasted (16 h) rats. They received either test sample (aqueous and hydroethanolic extracts) at the following doses (38, 75, 150 or 300 mg/kg BW), while the tree other groups were fed with distilled water (negative control), glucose (positive control) and tolbutamide (reference group) respectively (**Table 2**).

These groups of rats, except for the control groups received plant extracts 30 min prior to the oral glucose load of 1 g/kg body weight. Glucose concentration was measured before administration and subsequently at 0.5, 1, 2 and 2.5 h after the glucose load.

Determination of α-amylase inhibitory activity by iodine-starch assay

The inhibitory activity various concentrations of the extracts against α-amylase was assayed with an iodine-starch method (Komaki *et al*. 2003) with some modifications. Five different volumes of extract standard solution (2 mg/mL) were mixed with 100 µl (0.1%) of soluble starch substrate in phosphate buffer (0.25 M, pH 7.0). After 5 min of incubation at 37°C, 20 µl of 30 µg/mL solution of α-amylase in phosphate buffer, pH 7.0, was added to the mixture. After the mixture was further incubated for 10 min, 2 mL of 0.01 N iodine solution was added, followed by measurement of the absorbance at 660 nm. The inhibition activity (%) was calculated as [(A-B)/A] x 100, where A is a decrease in the absorbance in the absence of the extract and B is that in its presence.

Table 1 Repartition of animals during the effect of oral administration of *Pentaclethra macrophylla*.

Groups	Description
1	Normal/untreated rats (control group)
2	Normal rats treated with 80 mg/kg body weight tolbutamide
3	Normal rats treated with 38 mg/kg body weight of plant extract
4	Normal rats treated with 75 mg/kg body weight of plant extract
5	Normal rats treated with 150 mg/kg body weight of plant extract
6	Normal rats treated with 300 mg/kg body weight of plant extract

Table 2 Repartition of animals during the oral glucose tolerance test.

Groups	Description
1	Normal/untreated rats (control group)
2	Normal rats treated only with glucose
3	Normal rats treated with 80 mg/kg body weight tolbutamide
4	Normal rats treated with 38 mg/kg body weight of plant extract
5	Normal rats treated with 75 mg/kg body weight of plant extract
6	Normal rats treated with 150 mg/kg body weight of plant extract
7	Normal rats treated with 300 mg/kg body weight of plant extract

Collection of blood samples and determination of blood glucose level

Blood samples from the control and experimental rats were collected from the tail vein of each rat and determined using the glucose oxidase method (Trinder 1969).

Statistical analysis

The results are presented as mean ± SEM. Differences between means were assessed by one-way analysis of variance (ANOVA) followed by Holm-Sidak's test using Sigma-stat 3.1 software. Values of $P<0.05$ imply statistical significance. The inhibitory concentration 50% (IC_{50}) was calculated from the Prism dose-response curve (statistical programme) obtained by plotting the percentage of inhibition *versus* the concentrations.

RESULTS

Effect of oral administration of *P. macrophylla* extracts on normoglycemic rats

These results are shown in **Figs. 1, 2**.

From these results, we observed that 1 hour after the administration of plant extracts the tests groups had a slight increase of blood glucose, but which was not significant. From this moment onwards until the 8th hour of the experiment, we observed a slight but significant ($P<0.001$) decrease of blood glucose at 150 mg/kg and 300 mg/kg BW, respectively, corresponding to the 5th and 8th hour.

Oral glucose tolerance test (OGTT) on normoglycemic rats using *P. macrophylla* extracts

These results are showed in **Figs. 3, 4**.

The aqueous and hydroethanolic extract showed a pronounced antihyperglycemic activity ($P<0.001$) from 75 mg/kg to 300 mg/kg BW after 60 min. Better regulation of blood glucose was obtained at 150 mg/kg BW with a 23.75% and 28.85% reduction, respectively for aqueous and hydroethanolic extracts at the end of the treatment period.

Effect of *P. macrophylla* on inhibition of α-amylase

Table 3 shows the results obtained during the α-amylase inhibition assay.

This experiment showed that amylase activity was in-

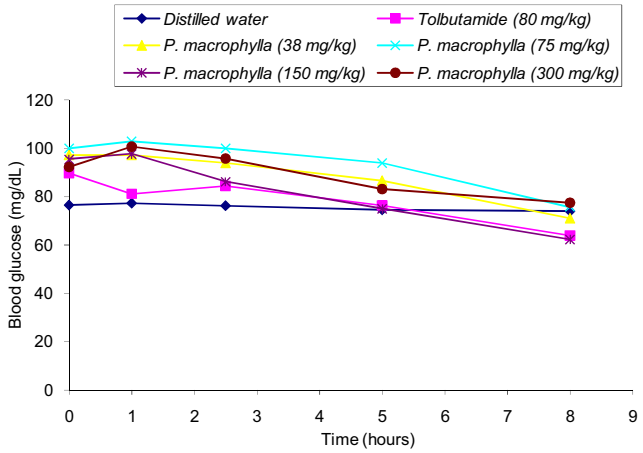

Fig. 1 Effect of oral administration of aqueous extract of *P. macrophylla* on normoglycemic rats.

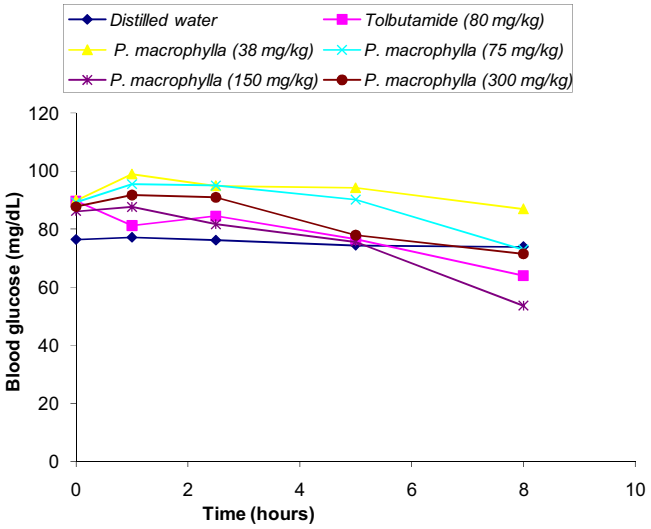

Fig. 2 Effect of oral administration of hydroethanolic extract of *P. macrophylla* on normoglycemic rats.

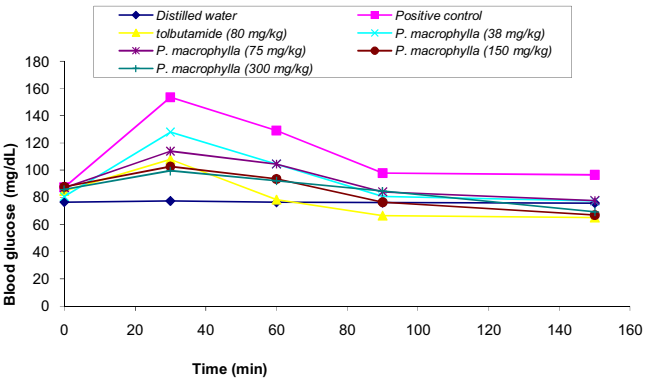

Fig. 3 Variation of blood glucose during OGTT on normoglycemic rats using aqueous extracts of *P. macrophylla*.

hibited in the presence of these plant extracts. The extent of amylase inhibition was correlated with increased concentration of extracts. Also the hydroethanolic extract showed better amylase inhibition activity. We obtained a 50% IC_{50} of 0.292 mg/mL with the hydroethanolic extract while the aqueous extract showed an IC_{50} of 3.19 mg/mL.

DISCUSSION

From the results obtained we observed that 75, 150, and 300 mg/kg BW of these extracts produced a significant

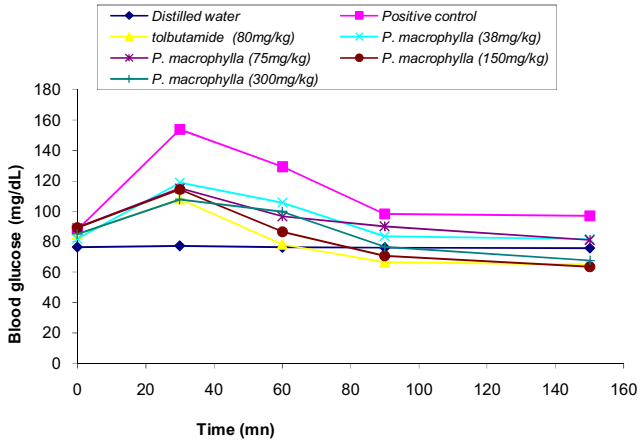

Fig. 4 Variation of blood glucose during OGTT on normoglycemic rats using hydroethanolic extracts of *P. macrophylla*.

Table 3 Effect of *Pentaclethra macrophylla* on inhibition of α-amylase.

Sample	Concentration (mg/mL)	% inhibition	IC_{50}
Aqueous extract	0.25	NI	3.19
	0.5	NI	
	1	1.35	
	1.5	18.65	
	2	22.51	
Hydroethanolic	0.25	49.18	0.292
	0.5	55.46	
	1	55.94	
	1.5	55.17	
	2	71.60	

NI: No inhibition

antihyperglycemic activity (P<0.001) after 1 h of treatment. However, the better regulation of blood glucose was obtained at 150 and 300 mg/kg BW at the end of the treatment period. Sugars, saponins, alkaloids, flavonoids and others phenolic compounds were obtained after the qualitative phytochemical screening and antioxidant potential of both extracts (Fomekong *et al*. 2007) are known to be bioactive antidiabetics principles (Stanley *et al*. 2004). Phenolic compounds and sterols are also found to be effective antihyperglycemic agents (Ivora *et al*. 1989). The antihyperglycemic activity of aqueous and hydroethanolic extracts may be due to the presence of more than one bioactive principle and their synergistic properties. These results are different from those obtained by Osadebe *et al.* (2004) who, after evaluating the antihyperglycemic and hypoglycemic activities of 200 mg/kg BW of crude methanolic extract of dried leaves of *P. macrophylla* in normoglycemic and alloxan-induced diabetic albino rats, obtained a non-significant variation of blood glucose. During the hypoglycemic assay, we observed that the test groups 1 h after the administration of plant extracts had a slight increase of blood glucose which was not significant. From this moment until the 8th hour of experiment, we observed a slight decrease of blood glucose which was significant (P<0.001) in 150 and 300 mg/kg BW, respectively at the 5th and the 8th hour. The slight increase of blood glucose during the 1st hour of treatment could be related at the metabolism of sugar contained in these extracts as previously described (Fomekong *et al*. 2007).

We also observed during this experiment that amylase activity was inhibited in the presence of these plant extracts. The same results were obtained with Gordon *et al*. (2005) who studied the effect of different polyphenolic components of soft fruits on α-amylase inhibition. Tannins, flavonoids and anthocyanin compounds which are found in these plants (Fomekong *et al*. 2007) have already been reported to be effective inhibitors of α-amylase activity (Gordon *et al*. 2005). The inhibition of α-amylase activity by our plant extracts could be due to the presence of these bioactive principles.

CONCLUDING REMARKS

The present work indicated that both aqueous and hydro-ethanolic extracts showed a pronounced antihyperglycemic, hypoglycemic and α-amylase inhibitory activity and can be useful for the regulation of blood glucose. Further investigations to find out the active principle(s) and to elucidate the exact mechanism of action are, therefore, required to be undertaken. Longer duration studies on *P. macrophylla* and its isolated compounds on diabetic models are necessary to develop a potent antidiabetic drug.

REFERENCES

Akah PA, Aguwa CN, Agu RU (1999) Studies on the antidiarrhoeal properties of *Pentaclethra. macrophylla* leaf extracts. *Phytotherapy Research* **13**, 292-295

Bouquet A, Cave A, Paris R (1971) Plantes medicinales du Congo – Brazzaville (III). Plantes medicinales et phytotherapiques. *Tome* **5 (2)**, 154-158

Cousins D, Huffman MA (2002) Medicinal properties in the diet of gorillas: An ethnopharmacological evaluation. *African Studies Monoghaphs* **23 (2)**, 65-89

Fomekong DGI, Oben EJ, Momo NEC, Enyegue MD (2007) Phytochemical constituents and antioxidant potential of some Cameroonian medicinal plants. *Pharmacology Online* **2**, 436-452

Food and Agriculture Organization (FAO) (2001) Collecte et analyse de données pour l'aménagement durable des forêts - joindre les efforts nationaux et internationaux. Programme de partenariat CE-FAO (1998-2001). Données statistiques des produits forestiers non-ligneux du Cameroun, 36 pp

Fujita H, Yamagami T, Ohshima K (2001) Fermented soybean-derived water-soluble touchi extract inhibits α-glucosidase and is antiglycemic in rats and humans after single oral treatments. *Journal of Nutrition* **131**, 1211-1213

Ghavami A, Johnston BD, Jensen MT, Svensson B, Pinto BM (2001) Synthesis of nitrogen analogues of salacinol and their evaluation as glycosidase inhibitors. *Journal of the American Chemical Society* **123**, 6268-6271

Githens TS (1948) *African Handbooks – Drug Plants of Africa*, University of Pennsylvania Press, 64 pp

Gordon JMc, Faina S, Dobson P, Smith P, Blake A, Stewart D (2005) Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agriculture and Food Chemistry* **53**, 2760-2766

Gugnani HC, Ezenwanze EC (1985) Antibacterial activity of extracts of ginger (*Zingiber officinale*) and African oil bean seed (*Pentaclethra macrophylla*). *Journal of Communicable Diseases* **17**, 233-236

Ivora MD, Paya M, Villar A (1989) A review of natural products and plants as potential antidiabetic drugs. *Journal of ethnopharmacology* **27**, 243-275

Iwu MM (1993) *Handbook of African Medicinal Plants*, CRC Press, Florida, USA, 941 pp

Johnston BD, Ghavami A, Jensen MT, Svensson B, Pinto BM (2002) Synthesis of selenium analogues of the naturally occurring glycosidase inhibitor salacinol and their evaluation as glycosidase inhibitors. *Journal of the American Chemical Society* **124**, 8245-8250

Kamanzi AK, Koné M, Terreaux C, Traore D, Hostettmann K, Dosso M (2002) Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. *Phytotherapy Research* **16**, 497-502

Keay RWJ, Onochie CEA, Stanfield DP (1969) *Nigerian Trees*, Department of Forest Research, Ibadan, pp 11, 119-120

Kim JS, Kwon CS, Son KH (2000) Inhibition of alphaglucosidase and amylase by luteolin, a flavonoid. *Bioscience, Biotechnology and Biochemistry* **64**, 2458-2461

Komaki E, Yamaguchi S, Maru I, Kinoshita M, Kakehi K, Ohta Y, Tsukada Y (2003) Identification of anti-α-amylase components from olive leaf extracts. *Food Science and Technology Research* **9**, 35-39

Lamer J (1985) *The Pharmacological Basis of Therapeutics* (7th Ed), MacMillan, New York, 1692 pp

Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K (2001) α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. *Journal of Agriculture and Food Chemistry* **49**, 1948-1951

Mentreddy SR (2007) Medicinal plant species with potential antidiabetic properties. *Journal of the Science of Food and Agriculture* **87**, 743-750

Ndam NEC (2005) Traditional medicine and modern medicine: shades and lights (Advocacy for a cultural anthropology of medicine in Africa today). *Acta Endoscopica* **35**, 219-226

Notkins AL (2002) Immunologic and genetic factors in type 1 diabetes. *Journal of Biological Chemistry* **277**, 43545-43548

Oliver BEP (1960) *Medicinal Plants of Nigeria*, Nigerian College of Arts Science and Technology, 760 pp

Osadebe PO, Okide GB, Akabogu IC (2004) Study on anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn.) sourced from five different host trees. *Journal of Ethnopharmacology* **95**, 133-138

Rogers ME, Maisels F, Williamson EA, Fernandez M, Tutin CEG (1990) The diet of gorillas in the Lope Reserve, Gabon: A nutritional analysis. *Oecologia* **84**, 326-339

Singha SC (1963) *Medicinal Plants of Nigeria*, Nigerian College of Arts Science and Technology, 36 pp

Stanley MPP, Kamalakkannan N, Menon PV (2004) Antidiabetic and anti-hyperlimidaemic effect of alcoholic *syzigium cumini* seeds in alloxan induced diabetic albino rats. *Journal of Ethnopharmacology* **91**, 209-213

Trinder P (1969) Determination of blood glucose using an oxydase-peroxidase system with a non-carcinogenic chromogem. *Journal of Clinical pathology* **21**, 158-161

Yeh GY, Kaptchuk TH, Eisenberg DM, Phillips RS (2003) Systematic review of herbs and supplements for glycemic control in diabetes. *Diabetes Care* **26**, 1277-1294