Journal of Applied Pharmaceutical Science Vol. 5 (05), pp. 089-093, May, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50517
SSN 2231-3354

Inhibition of alpha-amylase by "insulin plant" (*Myrcia sphaerocarpa* DC) extracts: an alternative for the treatment of diabetes mellitus?

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ARTICLE INFO

Article history:
Received on: 20/02/2015
Revised on: 17/03/2015
Accepted on: 09/04/2015
Available online: 27/05/2015

Key words:
diabetes; insulin plant; alpha-amylase.

ABSTRACT

Diabetes mellitus is an important medical-economical-social problem requiring up search for less costly alternatives for treatment, such as use of medicinal plants. Of the species cited in ethnobotanical studies, the "insulin plant" (*Myrcia sphaerocarpa* DC) appears among those used by diabetics. The objective of this work was to evaluate the inhibitory activity of alpha-amylase by aqueous extracts of "insulin plant", prepared by different methods at a proportion of 1:200 (w:v), for information about the alleged hypoglycemic effect. For this, five samples of commercially teas "insulin plant" were obtained; aqueous extracts were prepared; and thereafter, they were tested for inhibition of alpha-amylase, before and after exposure to simulated gastric fluid. Results demonstrated before exposure to gastric fluid, a significant inhibition of enzyme activity (greater than 50%) in the samples A and C, using the decoction and sample E in all methods of extraction. Comparing methods of extraction, the decoction was more effective. After exposure to gastric fluid, there was a reduction in the activity of enzyme inhibition, suggesting negative influence of low pH on the properties of the inhibitor. These results do not elucidate the mechanism of the hypoglycemic action reported in folk medicine and literature, but indicate a promising path.

INTRODUCTION

The Chronic Noncommunicable Diseases (CNCD) are currently the main causes of death in the world. The increased prevalence is accompanied by a high number of premature deaths, loss in life quality due high limitation for work and leisure activities and economic impacts for families, communities and society at large (Alwan *et al*., 2010; Malta *et al*.,2011; Ministério da Saúde, 2012; Schmidt *et al*.,2011; Sociedade Brasileira de Diabetes, 2007). Among the CNCD diabetes mellitus has been highlighted as a problem of medical and socio-economic importance in the context of public health. Once, there was a rapid increase in the risk group and the long-term metabolic control and the treatment of complications, result in high costs for individuals, families and health systems (Defani *et al*., 2011; Malta *et al*., 2011; Ministério da Saúde, 2007; Sociedade Brasileira de Diabetes, 2003). In the search for cheaper and more accessible alternative treatment for diabetes, there is a growing interest in the use of medicinal plants and herbal medicines (Borges *et al*, 2008; Defani *et al*., 2011; Rosa *et al*., 2012; Santos *et al*., 2012). Of the species cited in ethnobotanical studies in different regions of Brazil, the "insulin plant" appears among those used by diabetics. Under this popular nomenclature, several species are reported in the literature, especially *Myrcia sphaerocarpa* DC. (Rosa *et al*., 2012; Santos, 2005), *Cissus sicyoides L.* (Defani *et al*., 2011; Pepato *et al*.,2003; Santos *et al*., 2008; Santos *et al*., 2012; Viana *et al*., 2004), *Cissus verticillata* L.(Pereira da Silva and Proença, 2008), *Myrcia guianensis, Myrcia uniflora, Myrcia salicifolia e Myrcia speciosa* (Santos, 2005) e *Myrcia multiflora* (Matsuda *et al*., 2002; Santos, 2005). Despite the reported hypoglycemic effect, these species have not been validated in the mechanism of action, toxicity and usage, being rejected as ethical drug free prescription. Thereby, this study proposes: a) obtaining of **"**insulin plant**"** tea commercial samples, as well as the identification of the used scientific name, b) preparing of aqueous extracts by different methods (decoction, infusion, maceration and tisane), and c) conducting tests of α-amylase inhibition, aiming to obtain information about the supposed hypoglycemic effect.

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MATERIALS AND METHODS

Sample Collection and Preparation

Five tea samples (A, B, C, D and E) of **"**insulin plant**"** were acquired in the form of dried leaves in drugstores and specialized stores of phytoterapy, at the cities of Lavras, Uberaba and Juiz de Fora, all of them at Minas Gerais state – through convenience sampling. The samples were taken to the Laboratory of Biochemistry ICBN, archived and identified with data on the species at the label and/or technical report, manufacturing dates and expiration dates, and informations about the provider.

The reduction of brute samples for to laboratory samples was performed manually, randomly, with the weighing carried out in a semi-analytical balance. The preparation of aqueous extracts was performed at the time leading up to the inhibition assays, using the different methods described below.

Decoction

The sample and water at a proportion of 1:200 (w:v) were put in heating in a closed vessel, after getting to ebullition point it was kept for 10 minutes. Following, the decoction was put in room temperature for 30 minutes and was filtered on tissue (organza), until the obtaining of a clear liquid used in the inhibition tests.

Infusion

Boiled water was added to the sample (in a vessel) at the proportion of 1:200 (w:v). The infusion was kept covered and put in room temperature for 10 minutes, after was filtered on tissue (organza), until the obtaining of a clear liquid used in the inhibition tests.

Maceration

The sample and water at a proportion of 1:200 (w:v) were put in a closed vessel and left resting for 10 days. After that, the macerated was filtered on tissue (organza), until the obtaining of a clear liquid used in the inhibition tests.

Tisane

The sample was immersed in boiling water at a proportion of 1:200 (w:v) and remained boiling for 5 minutes in a closed vessel. Removed from the heating, it was kept under room temperature for 10 minutes and filtered on tissue (organza), until the obtaining of a clear liquid used in the inhibition tests.

Enzyme Inhibition Test

The pancreatic enzyme α-amylase type VIB porcine (Sigma ®) was used for the inhibition test. The identification of the α-amylase activity was realized according to the methodology proposed by Noelting and Bernfeld (1948) using as substrate 1% starch prepared in Tris buffer 0,05 mol L^{-1} (pH 7.0) plus 38 mmol NaCl L^{-1} and $CaCl_2$ 0.1 mmol L^{-1}. The plant extract and α-amylase were pre incubated for 20 minutes in a water bath at 37°C. Thereafter, added substrate and the mixture was incubated for four time periods (10, 20, 30 and 40 minutes). The reaction was stopped with adding 3,5 dinitro salicylic acid and the product was read in a spectrophotometer at 540 nm.

Additionally, to simulate the digestion process, were also conducted tests on enzyme activities in the presence of simulated gastric fluid prepared according to The United States Pharmacopeia - USP (1995). The extract was incubated in the presence of fluid for 1 hour in a water bath at 37°C, neutralized with sodium bicarbonate (pH 6.8 -7.0) and then carried out activity tests.

The enzyme inhibition was obtained by determining the slopes of the lines (absorbance vs. time) - testing the activity control (without extract) and sample (with extract). The slope of the line is due to the rate of product formation per minute of reaction and the presence of inhibitor causes a decrease in this gradient, expressed in percentage terms. All analyzes were performed in triplicate.

Experimental Design and Statistical Analysis

The slopes of the lines and the inhibition rates were obtained from the insertion of data into Excel spreadsheet. The results of the three replicates were analyzed by ANOVA - Scott-Knott test using the software SISVAR® (Ferreira, 2000).

RESULTS AND DISCUSSION

In literature, the popular name "insulin plant" is cited for different species, however in our study all samples corresponded to *Myrcia sphaerocarpa* DC. The results of enzyme inhibition tests, expressed as average percentage before and after exposure to simulated gastric fluid, are shown in Table 1.

Statistical analysis revealed significant differences between the samples and/or extraction methods. Despite the fact that the five samples are marketed as "insulin plant ", is important to consider that such variations in inhibition percentage can be attributed to factors edaphoclimatic, preparation methods and possible contamination with other species. Gobbo-Neto and Lopes (2007) report that the harvest is one of the most important factors, since the concentration and nature of the active constituents are not constant throughout the year.

Souza (2011) cites that significant inhibition of the alpha-amylase is greater than 50%; value shown by samples A and C, using the decoction, and sample E in all extraction methods. Souza (2011) also mentions that values exceeding the 80% - values presented for samples A and E of this study - represent high enzymatic inhibition. Comparing the extraction methods used in this study, the decoction was more effective in inhibiting alpha-amylase in relation to others, with values significantly higher for samples A, C and D. There were exceptions the sample B in which was not observed inhibition and E where the value of inhibition was statistically similar to tisane. Also in relation to sample E, high percentages of inhibition were observed in all methods, suggesting an influence on the presentation, unlike the others, had the dried leaves, fragmented for commercialization.

Table 1: Percent inhibition of alpha-amylase by aqueous extracts of *Myrcia sphaerocarpa* DC in proportion 1:200 (w:v) obtained by different extraction methods (infusion, decoction, maceration and tisane).

AMOSTRA		Infusion	Decoction	Maceration	Tisane
A	Before the fluid	0 [a]	86,12 ± 2,6 [h]	6,95 ± 6,02 [b]	3,92 ±0,61 [b]
	After the fluid	4,19±1,87 [b]	5,45 ±4,81 [b]	0,63 ±1,09 [a]	0 [a]
B	Before the fluid	18,02 ± 1,75 [c]	0 [a]	0 [a]	21,78 ± 3,08 [d]
	After the fluid	4,10±1,54 [b]	0 [a]	1,04 ±1,08 [a]	0,54 ±0,93 [a]
C	Before the fluid	0,60 ±1,03 [a]	70,18 ± 4,18 [g]	24,25 ± 7,95 [d]	10,00 ± 3,08 [b]
	After the fluid	0 [a]	1,33 ± 2,31 [a]	3,56 ±0,38 [b]	1,92 ± 3,33 [a]
D	Before the fluid	0 [a]	48,78 ± 9,44 [f]	0 [a]	0 [a]
	After the fluid	21,86± 3,79 [d]	0 [a]	0 [a]	15,80 ± 1,38 [c]
E	Before the fluid	85,6 ± 3,29 [h]	91,61 ± 2,76 [i]	67,68 ± 4,63 [g]	93,07 ± 3,47 [i]
	After the fluid	14,91± 2,78 [c]	31,06 ± 7,30 [e]	11,25 ± 8,39 [c]	0,85 ±1,48 [a]

*[I:] Average percentage obtained from three replicates ± standard deviation.
*a - i: Statistical variation between the results of the sample according to Scott-Knott ($p < 0.05$). Same letter represent statistically identical results.

The results were similar to those obtained by Martins *et al.*,(2014) in his work with *Origanum vulgare L.* leaves, in which the decoction was more effective to have higher antioxidant activity compared with infusion and hydroalcoholic extract. Funke and Melzig (2006) working with alpha-amylase inhibitors in 16 species, including *Galega officinalis*, also observed influence of the extraction method. The extract obtained at room temperature showed little effect on the enzyme, while that obtained with boiling buffer showed a 35% inhibition of enzyme activity.

Our results demonstrate a reduction in the activity of enzyme inhibition after exposure to simulated gastric fluid, except for sample D. These results corroborate Pereira *et al.*,(2010) who worked with green and black teas, observed inhibition of alpha-amylase 42.19 and 73.44%, respectively, and absence of inhibition after simulation with gastric fluid.

Freitas *et al.*,(2014) assessed the activity of enzyme inhibition of extracts of commercial teas *Syzygium sp* front of the alpha-amylase by different extraction methods (infusion, decoction and maceration) and different proportions (1:20, 1:50 and 1 : 100) obtained significant inhibition of the enzyme before exposure to fluid, with an average of 92.84%. However, the authors reported that the samples showed a reduction in the percentage of inhibition after exposure to gastric fluid. And this reduction may be related to the negative influence of low pH on the properties of the inhibitor. Unlike, Pereira *et al.*,(2011) assessed the crude extract of white bean flour found that the percentages of inhibition of α-amylase was 79.1% and 81.8% with and without gastric fluid, respectively, concluding that the inhibitor was stable. By comparing the results of this study to the literature, it can be inferred the presence of different inhibitors between species, requiring determination of these as a way to provide more data on the use of the same.

According Salunkhe *et al.*, (1990) the inhibition of alpha-amylase by teas can be associated with the presence of phenolic compound (non-protein inhibitors), because there interaction between the aromatic ring hydroxylated and aminoacids enzyme. Ali *et al.,* (2006), Kandra *et al.,* (2004) and Nickavar and Abolhasani (2013) discuss the association between inhibition by plant extracts and the presence of phenolic compounds, tannins and triterpenoids. Ali *et al.,* (2006) worked with six species Malaysia's plants, including *Phyllanthus amarus* hexane extract, identified and isolated a triterpenoid mixture (oleanolic acid and ursolic acid - 2: 1) that showed potent inhibition of alpha-amylase. It is noteworthy that, although authors as Sivakumar *et al.,* (2006) and Wang *et al.*,(2006) cited Antunes (2008) affirm that the amylase inhibitors are produced by plants especially in seeds and tubers, this work shows the presence of inhibitors in the leaves showing the importance of further studies.

Among the species receiving designation **"**insulin plant**"**, some studies have assessed their ability to enzyme inhibition. Matsuda *et al.,* (2002) in their studies, found that the methanol extracts of *Myrcia multiflora* leaves show potent aldose reductase inhibitory activity-which catalyzes the reduction of glucose to sorbitol in rats, associating the use of the beneficial effects in diabetic patients, because the accumulation sorbitol has been implicated in chronic complications of diabetes, such as neuropathy, retinopathy and cataracts.

Khalil *et al.*,(2008) worked with aqueous extract (decoction) of *Cissus sicyoides* and *Bauhinia forficata* to identify antioxidant activity related to prevention of diabetes complications found for the first, 48 % inhibition (100 µg/ml) of myeloperoxidase, an enzyme present in all stages of development of atherosclerosis and other diseases. According to the authors, the results support the use of the species in the prevention of diabetes.

Ferreira *et al.,* (2006) evaluated the effects of aqueous extract of *Myrcia uniflora* on thyroid function, isolating two flavonoids (mearnsitrin and myricitrin) of the species. The authors observed that a solution of these inhibited 50% of the peroxidase activity, which participates in the synthesis of thyroxine and triiodothyronine hormones, concluding that the indiscriminate use with nutritional iodine deficiency may contribute to the development of hypothyroidism.

Ferreira (2010) conducted tests on samples of 2,4,6-trihidroxiacetifenona (THA) isolated from *Myrcia multiflora*, in order to assess hypolipidemic potential of the compound in rats. There was a reduction in cholesterol, triglyceride and lipase levels of treated animals with THA higher than treated with available drugs in the market (lovastatin and orlistat), possibly by blocking the absorption of triglycerides due to an inhibitory effect on lipase, and induction of cholesterol 7α-hydroxylase.

CONCLUSION

All samples obtained commercially, with designation "insulin plant" correspond *Myrcia sphaerocarpa* DC. Aqueous extracts of "insulin plant" at a proportion of 1:200 (w:v), exhibit a high inhibition of α-amylase before to exposure to simulated gastric fluid, especially when used the decoction as extraction method. However, after exposure to the fluid, there is a significant reduction of the inhibitory activity, possibly related to the effect of low pH on the inhibitor.

These results do not elucidate the mechanism (hypoglycemic action) reported in folk medicine and literature, but indicate a promising path. There is an evident need for further studies to determine the bioactive compounds present as well as any toxic effects, and other possible mechanisms of action.

ACKNOWLEDGEMENTS

We are grateful to FAPEMIG for financial support in the form of scientific initiation scholarship (BIC/Fapemig).

REFERENCES

Ali H, Houghton PJ, Soumyanath A. α-amylase inhibitory activity of some Malaysian plants used to treat diabetes: with particular reference to *Phyllanthus amarus*. J Ethnopharmacol, 2006; 107(3):449-455.

Alwan A, Maclean DR, Riley LM, Tursan D'espaignet E, Mathers CD, Stevens GA, Bettcher D. Monitoring and surveillance of chronic non-communicable diseases: progress and capacity in high-burden countries. The Lancet, 2010; 376:1861–1868.

Antunes AF. 2008. Atividade inibitória de extratos vegetais do cerrado sobre alfa-amilases. [ONLINE] Available at: http://bdtd.bce.unb.br/tedesimplificado/tde_busca/arquivo.php?codArquivo=3856. [Accessed 8 november 2013].

Borges KB, Bautista HB, Guilera S. Diabetes – utilização de plantas medicinais como forma opcional de tratamento. Revista Eletrônica de Farmácia, 2008; 5(2):12-20.

Defani MA, Barbosa TK, Rossi SL, Nascimento RC. Utilização das plantas medicinais por diabéticos do município de Goioerê – PR. Rev. Saúde e Pesquisa, 2011; 4(2):223-231.

Ferreira AC, Neto JC, Da Silva AC, Kuster RM, Carvalho DP. Inhibition of thyroid peroxidase by *Myrcia uniflora flavonoids*. Chem. Res. Toxicol., 2006; 19(3):351-355.

Ferreira DF. 2000. Análises estatísticas por meio do SISVAR para Windows versão 4.0. In: Reunião Brasileira da Sociedade Internacional de Biometria. [ONLINE] Available at: http://www.dex.ufla.br/~danielff/softwares.htm. [Accessed 22 december 2013].

Ferreira EA. 2010. Efeitos hipolipemiante e hepatoprotetor da 2'4'6'- trihidroxiacetofenona isolada de *Myrcia Multiflora*. [ONLINE] Available at: https://repositorio.ufsc.br/bitstream/handle/123456789/94430/285377.pdf?sequence=1. [Accessed 10 november 2013].

Freitas TC, Pereira CA, Pereira LLS. *Syzygium sp.* (Myrtaceae) Extracts: Inhibition of Alpha Amylase. European J Med Plants, 2014; 4(1):116-125.

Funke I, Melzig MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of α-amylase activity. Braz. J. Pharmacog., jan/mar 2006; 16(1):1-5.

Gobbo-Neto L, Lopes NP. Plantas Medicinais: Fatores de Influência no Conteúdo de Metabólitos Secundários**.** Quim Nova, 2007; 30(2):374-381.

Khalil NM, Pepato MT, Brunetti IL. Free Radical Scavenging Profile and Myeloperoxidase Inhibition of Extracts from Antidiabetic Plants: *Bauhinia forficata* and *Cissus sicyoides*. Biol Res., 2008; 41:165-171.

Kandra L, Gyémánt G, Zajácz A, Batta G. Inhibitory effects of tannin on human salivary α-amylase. Biochem Bioph Res Co., jul 2004; 319(4):1265-1271.

Malta DC, Morais Neto OL, Silva Junior JB. Apresentação do plano de ações estratégicas para o enfrentamento das doenças crônicas não transmissíveis no Brasil, 2011 a 2022. Epidemiol. Serv. Saúde, oct/ dec 2011; 20(4):425-438.

Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira ICFR. Decoction, infusion and hydroalcoholic extract of *Origanum vulgare L.*: Different performances regarding bioactivity and phenolic compounds. Food Chem., 2014; 158(1):73-80.

Matsuda H, Morikawa T, Yoshikawa M. Antidiabetogenic constituents from several natural medicines. Pure Appl Chem., 2002; 74(7):1301-1308.

Ministério da Saúde do Brasil. 2007. Evolução dos Gastos do Ministério da Saúde com Medicamentos. [ONLINE] Available at: http://portal.saude.gov.br/portal/arquivos/pdf/estudo_gasto_medicamentos.pdf. [Accessed 8 november 2013].

Ministério da Saúde do Brasil. 2012. Vigilância de Doenças Crônicas Não Transmissíveis. [ONLINE] Available at: http://portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idtxt=31877&janela=1. [Accessed 7 november 2013].

Nickavar B, Abolhasani L. Bioactivity-Guided Separation of an α-Amylase Inhibitor Flavonoid from *Salvia virgata.* Iranian Journal Pharmaceutical Reserch**,** 2013; 12(1):57–61.

Noelting G, Bernfeld P. Sur les enzymes amylolytiques. III. La -amylase: dosage d'activité et controle de l'absence d' -amylase. Helv Chim Acta, 1948; 31(1):286-290.

Pepato MT, Baviera AM, Vendramini RC, Perez MPMS, Kettelhut IC, Brunetti IL. *Cissus sicyoides* (princess vine) in the long-term treatment of streptozotocin-diabetic rats. Biotechnol Appl Bioc., 2003; 37:15-20.

Pereira da Silva CS, Proença CEB. Uso e disponibilidade de recursos medicinais no município de Ouro Verde de Goiás, GO, Brasil. Acta Bot. Bras., 2008; 22(2):481-492.

Pereira LLS, Santos CD, Sátiro LC, Marcussi S, Pereira CA, Souza SP. Ação inibitória e estabilidade do extrato de farinha de feijão branco sobre enzimas digestivas na presença de fluído gástrico simulado. Rev. Bras. Farm., 2011; 92(4):367-372.

Pereira LLS, Souza SP, Silva MC, Carvalho GA, Santos CD, Corrêa AD, Abreu CMP. Atividade das glicosidases na presença de chá verde e de chá preto. Rev. Bras. Pl. Med., 2010; 12(4):516-518.

Rosa RL, Barcelos ALV, Bampi, G. Investigação do Uso de Plantas Medicinais no Tratamento de Indivíduos com Diabetes Melito na Cidade de Herval D' Oeste – SC. Rev. Bras. Pl. Med., 2012; 14(2):306-310.

Salunkhe DK, Chavan JK, Kadam SS. 1990. Dietary tannins: consequences and remedies. Boca Raton, United States: CRC Press.

Santos HB, Modesto-Filho J, Diniz MFFM, Vasconcelos THC, Pereira FSB, Ramalho JÁ, Dantas JG, Santos EB. Avaliação do efeito hipoglicemiante de *Cissus sicyoides* em estudos clínicos fase II. Rev. Bras. Farmacog., jan/ mar 2008; 18(1):70-76.

Santos MM, Nunes MGS, Martins RD. Uso empírico de plantas medicinais para tratamento de diabetes. Rev. Bras. Pl. Med., 2012; 14(2): 327-334.

Santos RA. 2005. Estudo fitoquímico e atividade alelopática de *Myrcia guianensis.* [ONLINE] Available at: http://livros01.livrosgratis.com.br/cp147970.pdf. [Accessed 11 november 2013].

Schmidt MI, Duncan BB, Azevedo e Silva G, Menezes AM, Monteiro CA, Barreto SM, Chor D, Menezes PR. Chronic non-communicable diseases in Brazil: burden and current challenges. The Lancet, jun 2011; 377:1949 – 1961.

Sociedade Brasileira de Diabetes. 2003. Consenso brasileiro sobre diabetes 2002: diagnóstico e classificação do diabetes melito e tratamento do diabetes melito do tipo 2. [ONLINE] Available at: http://www.nutritotal.com.br/diretrizes/files/55--Consenso_diabetes.pdf. [Accessed 5 november 2013].

Sociedade Brasileira de Diabetes. 2007. Diretrizes da Sociedade Brasileira de Diabetes: tratamento e acompanhamento do diabetes mellitus. [ONLINE] Available at: http://www.anad.org.br/profissionais/images/diretrizes_SBD_2007.pdf. [Accessed 5 november 2013].

Souza PM. 2011. Atividade de inibição enzimática por espécies vegetais do bioma cerrado. [ONLINE] Available at: http://repositorio.unb.br/bitstream/10482/9355/1/2011_PaulaMonteirodeSouza.pdf. [Accessed 11 november 2013].

The United States Pharmacopeia (USP) – The National Formulary NF 18 (Pharmacopeial Convention Ing) Rockvile, United States. 1995.

Viana GSB, Medeiros ACC, Lacerda AMR, Leal LKAM, Vale TG, Matos, FJA. Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*. BMC Pharmacology, jun. 2004; 4(9).

How to cite this article:

Angélica Rosa de Oliveira, Chrystian Araújo Pereira. Inhibition of alpha-amylase by "insulin plant" (*Myrcia sphaerocarpa* DC) extracts: an alternative for the treatment of diabetes mellitus?. J App Pharm Sci, 2015; 5 (05): 089-093.