European Journal of Medicinal Plants
4(1): 116-125, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Syzygium sp (Myrtaceae) Extracts: Inhibition of Alpha Amylase

Tuanny Cavatão de Freitas[1], Chrystian Araújo Pereira[1*] and Luciana Lopes Silva Pereira[1]

[1]Laboratório de Bioquímica, Instituto de Ciências Biológicas e Naturais, Universidade Federal do Triangulo Mineiro – UFTM, Zipe Code 38015.050, Uberaba, MG, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author TCDF performed the enzyme inhibition assays, statistical analysis and preparation of the samples, assisted in literature search and wrote the first draft of the manuscript. Author CAP designed the study, performed the literature search, conducted the achievement the samples, assisted enzymatic inhibition assays and statistical analyzes, and wrote the final version of the manuscript. Author Pereira LLSP assisted in the writing of the study, in literature search, in obtaining reagents and in writing of the manuscript final version. All authors read and approved the final manuscript

Original Research Article

Received 6th September 2013
Accepted 7th October 2013
Published 13th November 2013

ABSTRACT

Aims: The aims of this study were: a) prepare crude extracts of commercial teas *Syzygium* sp by different methods (maceration, decoction and infusion) in different proportions (1:20, 1:50 and 1:100) and b) submit them the inhibition assays digestive enzyme alpha-amylase before and after exposure to a simulated gastric fluid.
Place and Duration of Study: Laboratório de Bioquímica, Instituto de Ciências Biológicas e Naturais, Universidade Federal do Triângulo Mineiro, Brazil, between August 2012 and July 2013.
Methodology: Five samples of *Syzygium* sp teas were purchased commercially from pharmacies, drugstores and health food stores.
Results: In the first step - inhibition of enzymatic extracts of the teas without simulated gastric fluid - the results showed significant inhibition of alpha-amylase (mean 92.84%, CV = 3.86%). However, when these extracts were placed in contact with the simulated gastric fluid was observed decreased inhibition of alpha – amylase, suggesting a possible

**Corresponding author: Email: chrystian@icbn.uftm.edu.br;*

reduction of hypoglycemic potential of these teas under physiological conditions.
Conclusion: Aqueous extract *Syzygium* sp have high percentages of inhibition of the enzyme amylase, suggesting a potential hypoglycemic effect. Additional studies aiming at elucidating the chemical compounds present in the extracts of *Syzygium* sp using chromatographic techniques will be performed.

Keywords: Syzygium; diabetes; amylase; gastric fluid.

1. INTRODUCTION

One of the major health problems affecting humanity today is diabetes. Slowly progressive and silent, their losses bearer are numerous and serious. It is characterized as a chronic and extremely debilitating, particularly the association of hyperglycemia with comorbidities such as hypertension, obesity, and other circulatory problems that result in pathological metabolic syndrome [1,2].

Of universal occurrence affects people of all age groups in both sexes in all countries of the world. The adoption of unhealthy eating habits, reduction or absence of physical activity and sedentary lifestyle are the main causes of this epidemic progress, according to the consensus of scientific literature [3,2]. Besides all the health problems caused by diabetes, should be considered the economic aspect, exposed by the high costs of drug treatment [4]. Several drugs are available commercially for therapy, especially insulin and oral hypoglycemic agents, which represent large expenditures due to continuous use. Thus, the search for new drugs and therapeutic alternatives more accessible and less costly represent a promising path [3, 2]. Therefore, ethnopharmacological and ethnobotanical surveys related to the traditional use of plants and plant extracts for the population, can provide important information for the search for new treatments for diabetes.

An example is the species *Syzygium* sp (Myrtaceae), better known as Jamelão or jambolão, used for many years by the population, mainly in the form of teas, due to his alleged hypoglycemic and antidiabetic effects [5,6]. Despite the widespread use, the research that was submitted revealed no consensus about its effectiveness as well as its toxicity. Furthermore, studies have demonstrated that the hypoglycaemic effect were inconclusive as to the elucidation of the mechanism of action [7,8,9,10].

In this context, in 2009 the Ministry of Health included *Syzygium* sp in the first list of species potentially promising for therapeutic use, but still need more conclusive studies, called the National List of Medicinal Plants of Interest (RENISUS), whose goal is to guide and direct research with these species [11].

Thus, considering the great potential for use in therapy, the need for studies - especially those related to the effectiveness and mechanism of hypoglycemic action - and the lack of information related to your profile enzyme inhibition, the research objectives are: (a) prepare commercial extracts from teas *Syzygium* sp by different methods (maceration, decoction and infusion) in different proportions, and (b) use these extracts for the testing of the inhibition of digestive enzymes amylase, before and after exposure to a simulated gastric fluid.

2. MATERIALS AND METHODS

The analysis and the experiments were conducted in the Laboratório de Bioquímica, Instituto de Ciências Biológicas e Naturais, Universidade Federal do Triangulo Mineiro - UFTM, Brazil.

2.1 *Sygyzium sp* - Collection and Sample Preparation

Five samples (A, B, C *, D and E) of *Syzygium* sp teas were purchased commercially from pharmacies, drugstores and health food stores in the cities of Uberaba, Lavras and Araxá, all in the state of Minas Gerais, Brazil. At the time of purchase of the samples was requested a copy of the technical report issued by the supplier.

Macroscopically, the samples showed up in the form of small fragments of leaves and stems. The only exception was the sample C, which presented itself in the form of a fine powder, which according to the supplier's technical report was obtained from the leaves of *Syzygium* sp.

All samples were brought to the Laboratório de Bioquímica, Instituto de Ciências Biológicas e Naturais/UFTM where extractions were prepared in aqueous solution by different methods (maceration, decoction and infusion), described below. The aqueous extracts obtained were aliquoted, stored in "eppendorfs" and stored in a freezer until the experimental analyzes.

2.1.1 Decoction

Samples of commercial teas were immersed in water in proportions of 1:20, 1:50 and 1:100 and taken to heating in a closed container. Upon reaching the boiling point, the mixture remained at boiling for 10 minutes. After standing 30 minutes the mixture was filtered (organza fabric), and the crude extract obtained was used in the enzyme inhibition assays.

2.1.2 Maceration

Samples of commercial teas were immersed in water in the proportions of 1:20, 1:50 and 1:100 at room temperature and allowed to stand for 10 days. Following the macerate was filtered (organza fabric) and crude extract obtained was used in enzyme inhibition assays.

2.1.3 Infusion

Samples of commercial teas were immersed in boiling water in the proportions of 1:20, 1:50 and 1:100 over 10 minutes. Then the mixture was filtered (organza fabric) and crude extract obtained was used in the enzyme inhibition assays.

2.2 Obtaining Enzyme

For the enzymatic assays was used the enzyme porcine pancreatic α-amylase type VIB (Sigma ®).

2.4 Activity of α-amylase

The α-amylase activity was determined according to the methodology proposed by Noelting & Bernfeld [12]. In this test, 50 µL of sample of plant extract and 50 µL of enzyme α-amylase

were pre incubated for 20 minutes in a water bath at 37°C. The starch substrate was made 1% in Tris 0.05 mol L^{-1}, pH 7.0 plus NaCl 38 mmol L^{-1} and CaCl2 0.1 mmol L-^{1}. After addition of 100 mL of the substrate, the mixture was incubated for four time periods. The reaction was stopped by adding 200 µL of reagent 3,5 dinitro salicylic acid. Then the product was read in a spectrophotometer at 540 nm.

2.5 Determination of Inhibition

The inhibition of the enzyme was obtained by determining the slopes (absorbance vs. time) of enzyme activity assays control (without plant extract sample) and enzyme + inhibitor (with a sample of plant extract). The slope of the line is due to the formation of reaction product per minute, and the presence of the inhibitor causes a reduction in this slope. The percent of inhibition is calculated as the difference between the slopes (control and sample) divided by the slope of the control.

** Due to the form of presentation (powder) - different from the other samples - sample preparation C had to be adapted as the extraction ratios (1:100, 1:250 and 1:500) and separation of the extract (centrifugation rather than filtration).*

2.6 Preparation of Simulated Gastric Fluid

In order to simulate the process of digestion in the stomach in vitro, assays were also performed enzymatic activities in the presence of a simulated gastric fluid. To this end, the extracts were incubated with simulated gastric fluid prepared according to The United States Pharmacopeia – [13], for 1 hour in a water bath at 37°C. After this period, sample were neutralized with sodium bicarbonate salt to physiological pH and only then carried out the activity tests.

2.7 Statistical Analysis

The results were submitted to analysis of variance using the software SISVAR [14].

3. RESULTS AND DISCUSSION

Initially, a survey was conducted regarding the potential for inhibition of alpha-amylase by *Syzygium* sp samples, depending on the method of extraction and dilution.

The results of enzyme inhibition of extracts of commercial teas *Syzygium* sp by different extraction methods and in different proportions, shown in Table 1, demonstrated significant inhibition of the enzyme alpha-amylase, involved in the metabolism of carbohydrates, which may suggest a hypoglycemic activity. Of the 135 tests, only four deviated from the overall average of 92.84% (CV = 3.86%). They are: maceration B - 1:100, maceration D - 1:20, maceration D – 1:50 and maceration D - 1:100, whose percentage inhibition were, respectively, 76.68%, 80.04%, 69.46% and 45.99%.

Table 1. Percent inhibition[1] of alpha-amylase by aqueous extract of *Syzygium* sp obtained by decoction and infusion soaking in varying proportions

Sample	Method of extraction and dilution															
	Decoction						Maceration						Infusion			
	1:20		1:50		1:100		1:20		1:50		1:100		1:20		1:50		1:100	
A	94.78	± 3.58	97.61	± 1.19	96.66	± 0.97	95.15	± 3.14	93.19	± 2.35	92.42	± 2.44	95.49	± 2.24	96.71	± 1.97	93.92	± 1.01
B	94.10	± 3.70	97.64	± 1.57	95.06	± 0.87	92.70	± 7.59	88.55	± 10.80	76.68*	± 4.64	97.20	± 1.99	96.72	± 1.85	96.20	± 2.57
D	92.18	± 2.96	95.95	± 1.54	93.48	± 5.23	80.04*	± 6.38	69.46*	± 7.79	45.99*	± 5.97	94.98	± 3.30	95.65	± 1.50	95.62	± 2.59
E	95.43	± 1.89	95.73	± 1.08	95.46	± 3.73	92.58	± 2.80	94.56	± 1.86	92.54	± 5.46	95.48	± 1.38	94.23	± 1.18	96.87	± 1.47
	1:100		1:250		1:500		1:100		1:250		1:500		1:100		1:250		1:500	
C[2]	97.77	± 1.06	95.72	± 3.43	97.66	± 2.05	96.89	± 2.35	95.57	± 3.76	96.59	± 0.30	97.30	± 1.85	96.59	± 1.90	96.55	± 0.53

[1]*Data are the mean ± standard deviation of three replicates.*

[2]*Dilutions were adapted according to the form of presentation of the sample (powder), different from the other (leaves).*

** Significant difference (p = .05) by the Scott-Knott test.*

As in our findings, Pereira et al [15] working with infusions of green and black teas found alpha-amylase inhibition of 42.19% and 73.44%, respectively. The authors suggested that such inhibition may be due to the presence of polyphenolic compounds in teas, since, according Salunkhe et al [16], the hydroxylated aromatic ring characteristic of these phenolic compounds, interacts with proteins causing a complexion. Corroborating this hypothesis, several authors recently confirmed the inhibition of alpha-amylase by phenolic compounds [17,18,19,20].

Regarding the potential hypoglycemic effect and potential use of inhibitors of glycolytic enzymes as an aid in the treatment of obesity and diabetes, several authors have shown promising results. Gunawan-Puteri et al [18] observed potent inhibitory activity of amylase in leaf extracts of *Phyllanthus urinaria* and *Artemisia vulgaris*. According to the authors, this activity may allow the exploration of the use of *P. Urinary* in treating patients with diabetes.

In vitro study indicated that the compound trilobatina obtained from extracts of leaves of *Lithocarpus polystachyus* Rehd had strong inhibitory activity against alpha-glucosidase and moderate against alpha-amylase, and present a moderate antioxidant activity and a strong potential sweetener. According to the authors, these features, when combined into a compound, are potentially useful for managing hyperglycemia induced by glucose and provide the biochemical logic to other animal and clinical studies [21].

Gonçalves et al [17], have shown that phenolic compounds extracted from *Vitis vinifera* were effective in inhibiting the enzyme alpha-amylase. The authors suggested that this inhibition in the gut may contribute to the decrease in starch hydrolysis and consequently lead to a reduction in postprandial glycemia.

After verifying the ability of extracts *Sygyzium sp* inhibit the enzyme alpha-amylase, analyzes were performed to assess the behavior of these extracts against a simulated gastric fluid. The results are shown in Table 2.

The results showed decreased inhibition of amylase when the extracts were placed in contact with the simulated gastric fluid. There was a decrease and variation in the percentages of amylase inhibition, suggesting a possible decrease in hypoglycemic potential of these teas under physiological conditions. The acidic pH of gastric fluid may be the cause of the decrease of the inhibitory activity of the extracts of *Syzygium* sp*;* this can be explained by a conformation change, chemical reaction or other mechanism that causes the inactivation of inhibitors present.

Pereira et al [15] evaluated the inhibition potential of green and black teas on the enzymes alpha-amylase, alpha and beta-glucosidases observed that both inhibited the enzymes. However, the authors found that after exposure of teas to a simulated gastric fluid inhibition of the enzyme alpha-amylase disappeared. According to the authors, after drinking tea, the passage through the gastrointestinal tract may lead to structural changes in the inhibitor due to the acidic pH of the stomach, inactivating it. As for glycosidases, black tea kept inhibition even after exposure to simulated gastric fluid, suggesting that it may aid in carbohydrate-restricted diet.

Table 2. Percent inhibition[1] of alpha-amylase by aqueous extract of *Syzygium* sp obtained by decoction and infusion soaking in varying proportions, before and after exposure to a simulated gastric fluid

		Method of extraction and dilution								
		Decoction					Maceration	Infusion		
Sample	**ENZYME ASSAY**	**1:20**	**1:50**	**1:100**	**1:20**	**1:50**	**1:100**	**1:20**	**1:50**	**1:100**
	Before the fluid	97.50%	96.77%	95.83%	57.58%	40.48%	11.36%	92.16%	78.79%	28.00%
A	After the fluid	40.00%	22.58%	33.33%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	Before the fluid	90.91%	72.73%	81.82%	40.43%	0.00%	14.89%	64.04%	73.58%	25.00%
B										
	_____	64.00%	18.18%	36.36%	23.21%	1.79%	26.79%	32.69%	11.54%	2.04%
	After the fluid									
	Before the fluid	93.15%	95.24%	86.05%	47.37%	20.59%	0.00%	93.33%	52.94%	62.96%
D	After the fluid	28.13%	7.14%	0.00%	15.56%	50.00%	12.50%	0.00%	0.00%	0.00%
	Before the fluid	82.50%	87.50%	62.96%	96.97%	50.00%	62.96%	96.67%	94.59%	89.83%
E	After the fluid	0.00%	53.33%	15.79%	0.00%	12.12%	0.00%	0.00%	0.00%	9.43%
		1:100	**1:250**	**1:500**	**1:100**	**1:250**	**1:500**	**1:100**	**1:250**	**1:500**
	Before the fluid	90.00%	96.23%	98.18%	85.19%	87.76%	85.71%	85.37%	98.53%	94.12%
C[2]	After the fluid	78.05%	56.60%	20.29%	9.62%	6.12%	0.00%	72.09%	7.04%	17.11%

[1] Enzymatic inhibition assays of the enzyme alpha-amylase before and after exposure to simulated gastric fluid, simultaneously performed for each dilution.
[2]Dilutions were adapted according to the form of presentation of the sample (powder), different from the other (leaves).

Working with extracts of white bean flour, Pereira et al [22] observed inhibition of the enzyme alpha-amylase from 79.1% to 81.81% before and after exposure to simulated gastric fluid, respectively. The authors suggested that the inhibitor still stable after passage through the stomach during digestion and found that extracts of white bean flour proved to be a potential source of reducing the absorption of carbohydrates in the diet and, therefore, a promising adjuvant for the treatment of obesity and diabetes.

Migliato et al. [23], demonstrated high levels of phenolic compounds in extracts of fruits of *Syzygium cumini*. The presence of phenolic compounds in the leaves of *Syzygium* sp may be responsible for the inhibition found in our work.

The results of this study reveal a high ability to inhibit alpha-amylase by extracts of *Syzygium* sp (Jambolão), suggesting a potential use as hypoglycemic and antidiabetic. Despite this promising potential mechanisms of action and physiological effects in the treatment of diabetes are not yet determined, thus requiring, studies related to the identification of bioactive compounds and biological assays to evaluate efficacy and toxicity.

4. CONCLUSION

Syzygium sp aqueous extracts obtained by decoction, maceration and infusion in the proportions of 1:20, 1:50 and 1:100, show high percentage inhibition of the amylase enzyme, suggesting a potential hypoglycemic effect thereof.

Moreover, when exposed to a simulated gastric fluid, the same extracts showed significant decreases in the percentage inhibition of the amylase enzyme.

Additional studies aiming at elucidating the chemical compounds present in the extracts of *Syzygium* sp using chromatographic techniques will be performed.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

We are grateful to Profa. Dra. Olga Luisa Tavano (UFTM, Brazil) and Profa. Dra. Silvana Marcussi (UFLA, Brazil) for his kindness in providing some reagents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Liberatore Junior RDR, Demartini AAC, Ono AHA, Andrade GC. Prevalence of obesity in children and adolescents with type 1 diabetes mellitus. Revista Paulista de Pediatria. 2008;26(2):142-5.
2. Brazilian Society of Diabetes (SBD) (2012). Accessed 14 March 2012. Available: http://www.diabetes.org.br.
3. Negri G. Diabetes mellitus: plants and natural active principles hypoglycemic. Braz. J. Pharm. Sci . 2005;41(2):121-42.
4. Lyra R, Silva RS, Montenegro JrRM, Matos MVC, Cezar NJB, Silva LM. Prevalence of diabetes mellitus and associated factors in an urban adult population of low education and income of Brazilian northeastern hinterland. Arq Bras Endocrinol Metabol. 2010;54(6):560-66.
5. Matos FJA. Medicinal plants - Guide for selection and use of plants used in herbal medicine in northeastern Brazil. Impr. Universitaria / Issues UFC, Fortaleza, 2002.
6. Migliato KF, Baby AR, Zag V, et al. Pharmacological Action of *Syzygium cumini* (L.) Skeels. Acta Farm. Bonaerense. 2006;25(2):310-4.
7. Helmstadter A. Syzygium cumini (L.) SKEELS (Myrtaceae) against diabetes –125 years of research. Pharmazie. 2008:63:91–101.
8. Pepato MT, Folgado VBB, Kettelhut IC, Brunetti IL. Lack of antidiabetic effect of Eugenia jambolana leaf decoction on rat streptozotocin diabetes. Braz. J. Med. Biol. Res. 2001;34(3):389-95.
9. Schoenfelder et al. Hypoglycemic and hypolipidemic effect of leaves from Syzygium cumini (L.) Skeels, Myrtaceae. in diabetic rats. Braz. J. Pharmacog. 2010;20(2):222-27.
10. Vizzotto M. Characterization of the functional properties of jambolão / Marcia Vizzotto, Marina Couto Pereira. - Pellets: Embrapa Temperate Climate, 2008. 26 p. (Embrapa Temperate Climate. Bulletin Research and Development, 79) .006.
11. MINISTÉRIO DA SAÚDE (2009). Fitoterapia. Accessed March 15, 2012. Available: http://www.portal.saude.gov.br/portal/saude/profissional/area.cfm?id_area=1336.
12. 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):286-90.
13. USP. The United States Pharmacopeia – The National Formulary NF 18 (Pharmacopeial Convention Ing) Rockvile, MD.Janeiro 1995.
14. Ferreira D F. Statistical analyzes through SISVAR for windows version 4.0. In: Brazilian Meeting of the International Society of Biometrics. 45., 2000, São Carlos. Program Summary and ... São Carlos: UFSCar, 2000. p. 235.
15. Pereira LLS, Souza SP, Silva MC, Carvalho GA, Santos CD, Correa AD, Abreu CMP. Glucosidase activity in the presence of green tea and black tea. Rev. Bras. Pl. Med. 2010;12(4):516-18.
16. Salunkhe D K, Chavan J K, Kadam S S. Dietary tannins: consequences and remedies. Boca Raton: CRC Press; 1990.
17. Gonçalves R, Mateus N, Freitas V. Inhibition of a-amylase activity by condensed tannins. Food Chem. 2011;125:665-72.
18. Gunawan – Puteri M DPT, Kato E, Kawabata J J. a- Amylase inhibitors from an Indonesian medicinal herb, *Phyllanthus urinatia*. Food Agric. 2012;92:606-09.
19. Wang H, Dub YJ, Song HC. a- Glucosidase and a- amylase inhibitory activities of guava leaves. Food Chem. 2010;123:6-13.

20. Wongsa P, Chaiwarit J, Zamaludien A. In vitro screening of phenolic compounds, potential inhibition against a-amylase and a-glucosidase of culinary herbs in Thailand. Food Chem. 2012;131: 964-71.
21. Dong Hua-Qiang, Li M, Zhu F, Liu Fu-Lai, Huang JB. Inhibitory potential of trilobatin from Lithocarpus polystachyus Rehd against a-glucosidase and a-amylase linked to type 2 diabetes. Food Chem. 2012;130:261-66.
22. Pereira LLS, Santos CD, Satiro LC, Marcussi S, Pereira CA, Souza SP. Inhibitory activity and stability of the extract of white bean flour on digestive enzymes in the presence of simulated gastric fluid. Braz. J. Pharm. 2011;92(4):367-72.
23. Migliato KF, Carvalho ES, Sacramento LVS, Mello JCP, Baby AR, Velasco MVR, Salgado HRN. Total polyphenols from Syzygium cumini (L.) skeels fruit extract. Braz. J. Pharm. Sci. 2009;45(1):12-26.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=281&id=13&aid=2505