Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 547–556

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# Potentially antidiabetic and antihypertensive compounds identified from *Pistacia atlantica* leaf extracts by LC fingerprinting

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ARTICLE INFO

*Article history:*
Received 7 September 2017
Received in revised form
19 November 2017
Accepted 20 November 2017
Available online 24 November 2017

*Keywords:*
*Pistacia atlantica*
α-Amylase
α-Glucosidase
Angiotensin I-converting enzyme
Chromatographic fingerprints
Partial least square regression

ABSTRACT

The objective of this paper is to evaluate the variations in the ability of *Pistacia atlantica* leaves to inhibit enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and to hypertension (angiotensin converting enzyme-I (ACE-I)), depending on harvesting month, gender and growing region, as well as to identify the peaks in chromatographic fingerprints that potentially correspond to components with enzymatic inhibitory activities. In this study, LC fingerprints of *P. atlantica* leave extracts were developed. Peaks which were probably responsible for the anti-amylase, anti-glucosidase and anti-ACE-I activities were assigned. For the latter purpose, the relevant information was extracted, linking the chromatographic fingerprints with the activities using a linear multivariate calibration technique, i.e., Partial Least Squares (PLS) regression. Prior to the construction of the models, the fingerprints are aligned using a warping method, called Correlation Optimized Warping (COW). Besides COW, different other data pretreatment methods were applied and compared. Our findings revealed that the influence of the growing region and gender on the α-amylase, α-glucosidase and ACE-I inhibitory activities of *P. atlantica* leaves was less important than the harvest time. Thirteen common peaks were selected from the chromatograms and used as a dataset to model the biological activities. The peaks potentially responsible for the biological activity of the samples were indicated by studying the regression coefficients of the models. Seven peaks corresponding to possibly anti-amylase compounds were found, while 6 peaks were considered important for inhibiting the α-glucosidase activity. Furthermore, the regression coefficients of the hypertension model indicated eight peaks as being important for inhibiting the ACE-I activity. The contributions of individual phenolic compounds of *P. atlantica* leaves to the α-amylase, α-glucosidase and ACE-I inhibitory activities were also identified. This investigation showed that the extract of *P. atlantica* leaves provides a rational basis for the isolation and development of antidiabetic and antihypertensive agents.



## 1. Introduction

Diabetes mellitus (DM) is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The two major forms of diabetes are Type 1 (insulin-dependent DM) and Type 2 (noninsulin-dependent DM). Type I DM occurs when the human immune system destroys pancreatic β-cells, which are responsible for secreting insulin. Insulin concentration can efficiently be managed through continuous injection in timely dosages. Elevated postprandial blood glucose levels are widely recognized as one of the earliest disease markers in the prediction of subsequent microvascular and macrovascular complications that can progress to full symptomatic type 2 diabetes (T2DM) [2]. Type II DM accounts for 90% of the diabetic cases and typically begins as insulin resistance until the pancreas slowly loses its ability to produce insulin [3].

The major sources of blood glucose are dietary carbohydrates, such as starch, which is hydrolyzed by α-glucosidase and pancreatic α-amylase, in order to be absorbed by the small intestine. The α-amylase is involved in the breakdown of long chain carbohydrate

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https://doi.org/10.1016/j.jpba.2017.11.049
0731-7085/

(α-amylase), while α-glucosidase breaks down starch and disaccharides to glucose. The α-amylase and α-glucosidase inhibitors are potential targets in the development of lead compounds for the treatment of diabetes [4].

One of the long-term complications of diabetes is hypertension or high blood pressure. Hypertension and Type 2 diabetes are interrelated metabolic disorders [5] that strongly predispose an individual to atherosclerotic cardiovascular disease and to renal failure [6].

One of the most important intermediary factors for controlling hypertension is the action of the angiotensin converting enzyme-I (ACE-I) [7]. ACE-I, converts inactive angiotensin I in the body into angiotensin II. Angiotensin II stimulates the synthesis and release of aldosterone from the adrenal cortex, which then increases blood pressure via promoting sodium retention in the distal tubules [8]. As such, the inhibition of ACE is considered a useful therapeutic approach in the treatment of high blood pressure in both diabetic and nondiabetic patients [5].

Currently there are some antidiabetic (acarbose, miglitol and voglibose) and antihypertensive (captopril, ramipril, and imidaprilat) drugs, which act by inhibiting α-amylase, α-glucosidase and ACE, respectively. These drugs can cause side-effects such as abdominal discomfort, flatulence, and diarrhea, which reduce patient compliance and treatment effectiveness [9]. As a result, interest increased in research to discover natural inhibitory components that could replace these drugs [10].

Plants have been used ethno-medicinally in the treatment of diabetes and hypertension for several centuries. Several inhibitors of α-amylase, α-glucosidase and ACE-I have been isolated from medicinal plants to serve as alternative drugs with increased potency and less adverse effects than existing synthetic drugs [7,11]. *Artemisia dracunculus* L. has been reported to have hypoglycaemic effects. According to [12] 4,5-di-*O*-caffeoylquinic acid, 6-demethoxycapillarisin, and 2′,4′-dihydroxy-4-methoxydihydrochalcone were isolated from the ethanolic extract of *Artemisia dracunculus* L., which showed inhibitory effects towards the enzyme aldose reductase, a key enzyme involved in the mechanisms implicated in the development of various secondary complications of diabetes. Methyl caffeate is another natural molecule, isolated from the methanolic extract of *Solanum torvum Swartz*. fruit, which shows an antidiabetic effect in streptozotocin-induced diabetic rats [13]. *Salacia reticulate*, belonging to the family Celastacea, has been traditionally used in India, Sri Lanka, China, and other Southeast Asian countries for treating symptoms associated with Type 2 DM. The α-glucosidase inhibitors, such as salacinol, kotalanol, de-*O*-sulphated-salacinol, and de-I-sulphated-kotalanol were isolated from this plant [14], the inhibitory effects of which are similar to those of voglibose and acarbose, widespread antidiabetic drugs. Other plants, inhibiting the α-amylase and α-glucosidase are *Myrcia* species (*M. sphaerocarpa, M. speciosa,* and *M. salicifolia)* [15], *Phyllanthus* species (*P. acidus*, *P. emblica* and *P. nirur)* [16] and *Andromachia igniaria* [17].

Analytical techniques, such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), have been used to isolate, identify, and determine individual bioactive compounds [18]. Multivariate chemometric methods have been applied as tools to extract maximum useful information from chromatographic fingerprints. Successful attempts to predict the antioxidant and biological activities of herbal products using multivariate calibration techniques in combination with chromatographic fingerprints have been described. Reference [19] developed and reported a fast fingerprints strategy for the determination of the total antioxidant capacity of Chinese green tea, using either partial least squares (PLS) or uninformative variable elimination PLS (UVE-PLS) as modeling techniques. Similar studies followed for the cytotoxic activity of *Mallotus* species [20].

*Pistacia atlantica* is a medicinal plant that is traditionally used as a sedative and anxiolytic herbal medicine. In Algeria, the leaves of *P. atlantica* have been employed as anti-diabetic and anti-hypertension remedies [21]. Surprisingly, their

chemical and phytochemical properties have been little investigated. Most studies focused on the extract of *P. atlantica* leaves [22,23]. However, the inhibition of α-amylase, α-glucosidase and ACE-I by both genders of *P. atlantica* leaves, harvested at different months has never been investigated. Hence, the aim of this study was to examine the effect of the harvest months, gender and growing region on the α-amylase, α-glucosidase and ACE-I inhibitory activities. Another goal was to model the anti-diabetic and anti-hypertensive activities of *P. atlantica* leaves extract as a function of the fingerprints using the multivariate calibration technique PLS. The regression coefficients of the resulting models were evaluated to indicate and identify the peaks in the fingerprints that potentially correspond to components in the samples with enzymatic inhibitory activities.

## 2. Theory

### 2.1. Data preprocessing

Prior to the chemometric treatment of the data, the fingerprints obtained from *P. atlantica* extracts are organized in an $n \times p$ matrix **X**, with $n$ fingerprints constituting the rows and $p$ data points per fingerprint, the columns. After aligning the peaks by correlation optimized warping (COW), other pretreatment methods, including column centering, normalization followed by column centering, and standard normal variate (SNV) followed by column centering, were applied and compared.

Along the time axis, peak shifts may occur due to fluctuations in flow rate, temperature, and injection related parameters [24]. Consequently, in **X**, equivalent of corresponding peaks is not found in the same columns, which of course affects the data analysis, providing less accurate results. To resolve this problem, a peak alignment or warping technique is recommended. In this work, the COW algorithm developed by Vest Nielsen et al. [25] was used, which optimizes the correlation coefficient between a sample and the target chromatogram. The target chromatogram was selected as that having the highest correlation with all chromatograms in the sample set. COW aligns chromatograms towards a target by means of piecewise linear stretching and compressing (warping) segments of the time axis. Each segment in the sample chromatogram is elongated or compressed by adding or removing data points to obtain the best correlation between the peaks in the sample chromatogram and those in the corresponding segment of the target chromatogram. The correlation coefficient between the segment of the target chromatogram (T) and that of the aligned chromatogram (S) is calculated using (Eq. (1)) [25,26].

$$r = \frac{(\boldsymbol{w_T} - mean(\boldsymbol{w_T}))(\boldsymbol{w_S} - mean(\boldsymbol{w_S}))}{std(\boldsymbol{w_T})\, std(\boldsymbol{w_S})} \quad (1)$$

Where $\boldsymbol{w_T}$ and $\boldsymbol{w_S}$ are the segments of target and aligned chromatograms; $mean(\boldsymbol{w_T})$ and $mean(\boldsymbol{w_S})$ are the mean values of target and aligned chromatograms, respectively; $std(\boldsymbol{w_T})$ and $std(\boldsymbol{w_S})$ are the standard deviation values of target and aligned chromatograms, respectively.

The performance of the warping is determined by evaluating the sum of all correlation coefficients. The major advantage of COW is that it is assumed to preserve the peak shape and area after alignment. A more detailed description of the COW method can be found in the literature [24,25,27].