Industrial Crops and Products 70 (2015) 309–315

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Comparative analysis of 1-deoxynojirimycin contribution degree to α-glucosidase inhibitory activity and physiological distribution in *Morus alba* L

Chao Liu, Wei Xiang, Yan Yu, Zheng-Qin Shi, Xian-Zhi Huang, Li Xu*

College of Biotechnology, Southwest University, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 21 September 2014
Received in revised form 22 January 2015
Accepted 21 February 2015
Available online 29 March 2015

Keywords:
1-Deoxynojirimycin
Physiological distribution
α-Glucosidase inhibitory activity
Mulberry
Ethanol extracts

ABSTRACT

Natural α-glucosidase inhibitors offer an attractive strategy to manage postprandial hyperglycemia in patients with type 2 diabetes. 1-Deoxynojirimycin (DNJ), the main hypoglycemic constituents in *Morus alba* L., has been extensively researched. Here, for different parts of the mulberry tree, the DNJ content and the IC_{50} values of DNJ and ethanol extracts have been determined and compared. Possible metabolic pathways and storage sites for DNJ in mulberry trees have been investigated, and the kinetics of inhibition of α-glucosidase by DNJ and ethanol extracts of different parts of the mulberry tree have been measured. DNJ content was found to be highest in trunk bark, whereas α-glucosidase inhibitory activity was highest in both twig and trunk bark. Buds and roots are likely to be the topmost and bottommost sites of DNJ biosynthesis. DNJ is an insect antifeedant (not including silkworms) and may itself have antiviral activity, or be the precursor to a compound with antiviral activity. Ethanol extracts of twig, trunk and root bark are mixed-type inhibitors of α-glucosidase; extracts from other parts of the tree are competitive inhibitors. The study provides useful information about the physiological distribution of important active components, especially DNJ, in the mulberry tree and suggests how best to develop and use the crude drug resources provided by the tree.

1. Introduction

Globally, the incidence of type 2 diabetes (non-insulin-dependent diabetes mellitus) is increasing, at least in part because of increased living standards and the adoption of unhealthy diets. According to the World Health Organization, almost 350 million people suffer from diabetes; type 2 diabetes accounts for >90% of these cases (King et al., 1998; Shlomo et al., 2011; Shi and Hu, 2014). Finding an efficient therapy for this disease is, therefore, one of the major goals of medicinal chemists. α-Glucosidase, an enzyme found in the intestinal lumen and in the brush border membrane, plays a key role in carbohydrate digestion by degrading starch and oligosaccharides to monosaccharides before absorption. Inhibitors of α-glucosidase delay the degradation of starch and oligosaccharides and help control postprandial blood glucose levels in diabetic patients (Yang et al., 2012).

α-Glucosidase inhibitors, in addition to metformin, sulfonylureas and thiazolidinediones, have been recommended as the main oral antidiabetic drugs used to treat type 2 diabetes by type 2 diabetes practical targets and treatments, 3rd edn (Pan et al., 2009). Acarbose, voglibose and miglitol are examples of synthetic α-glucosidase inhibitors; although these drugs are effective in lowering blood glucose levels, they produce side effects including, flatulence, nausea, vomiting and diarrhea. A number of Chinese herbal medicines, including *Salvia miltiorrhiza* Bge. (Ma et al., 2011), *Commelina communis* (Makio et al., 2008; Kim and Kim, 1999), *Anemarrhenae rhizoma* (Hiroyuki et al., 2007), and *Angelica sinensis* (Luo et al., 2012) contain naturally occurring α-glucosidase inhibitors that may produce less side effects than the synthetic inhibitors. In China, the roots bark, leaves, twigs and fruits of mulberry tree have long been used as dried medicinal herbs and food colorants. Modern studies have shown that the main hypoglycemic constituents in mulberry are alkaloids (Luo et al., 2011; Hong and Shi, 2012; Asano and Yamashita, 2001), polyphenols (Abdel et al., 2005; Zhang et al., 2014), and polysaccharides (Asano et al., 1994; Kimura et al., 1995; Ding et al., 2007) in which DNJ is the most popular subject investigated. DNJ (moranoline) is an N-containing sugar that was first isolated from *Mori Cortex* (preparation of dry root bark of *Morus alba* L.) in 1976 and shown to be an effective inhibitor of intestinal α-glucosidase activity (Yagi et al., 1976).

* Corresponding author. Tel.: +86 23 68250191; fax: +86 23 68250191.
E-mail address: mulberry@swu.edu.cn (L. Xu).

http://dx.doi.org/10.1016/j.indcrop.2015.02.046

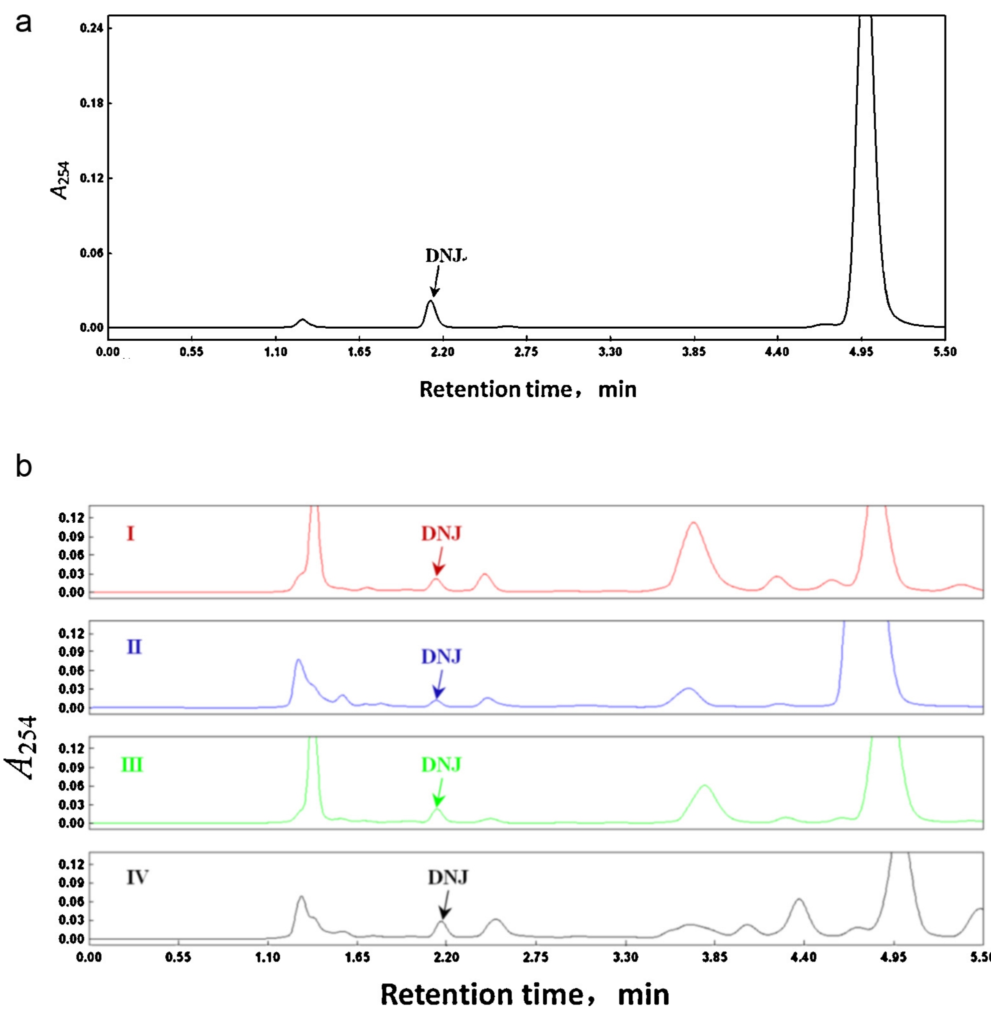

Fig. 1. Standard chromatograms of DNJ (a) and mulberry extracts (b) in which I, II, III and IV stands for the chromatograms of twig bark, fruits, root wood and leaves, respectively.

Previous studies have described DNJ analysis (Kimura et al., 2004) and extraction (Liu et al., 2014; Vichasilp et al., 2012), and have compared the effects of different growth periods (Ouyang et al., 2009), habitats (Meng et al., 2008) and cultivars (Kazuhisa et al., 2008; Fang et al., 2012), on the DNJ content and hypoglycemic activity (Asano et al., 1994; Abdel et al., 2005; Zhang et al., 2014) of mulberry extracts. The distribution of DNJ in different parts of the mulberry tree and the relationship between DNJ content and α-glucosidase inhibitory activity in ethanol extracts of the different parts of mulberry tree have, however, never been reported. In the present study, we have investigated the distribution of DNJ in mulberry trees and investigated the α-glucosidase inhibitory activity of different ethanol extracts. We have also clarified the inhibition kinetics of the different extracts. Our results will allow better exploitation and utilization of the active components of the mulberry tree.

2. Materials and methods

2.1. Plant materiasl and chemicals

Nine separate parts (young shoots, leaves, fruits, twig bark, trunk bark, root bark, twig wood, trunk wood, and root wood) of Hongguo 2 mulberry tree were collected from the Jiuyuan Mulberry Planting Professional Cooperatives of Chongqing, China, and identified by Prof. Maode Yu (College of Biotechnology, Southwest University). α-Glucosidase, DNJ, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), 9-fluorenylmethyl chloroformate (FMOC-Cl) and acetonitrile (HPLC grade) were purchased from Sigma–Aldrich (St. Louis, MO, US). Dimethyl sulfoxide and glycine were purchased from KeLong Chemical (Chengdu, China).

2.2. Preparation of extracts

Ultrasonic-assisted extraction (30 min × 3350 W), using aqueous HCl (0.05 mol L^{-1}, 40 mL g^{-1}) (Kim et al., 2003), was used to extract DNJ from dried powders for determination of DNJ content in different parts of the mulberry tree. Dried powders (30 g) from different parts of the tree were also extracted with 70% aqueous ethanol (1000 mL) using ultrasonic-assisted technology (30 min × 3350 W). The crude extracts obtained after evaporation of the solvent were dissolved in dimethyl sulfoxide–water (40:60, v/v) to give a solution (50 mg mL^{-1}) for determination of DNJ content as well as solutions (0.013–19.2 mg mL^{-1}) for determination of α-glucosidase inhibitory activity. Standard solutions (0–240 μg mL^{-1}) of DNJ were used for the determination of DNJ content in the powdered mulberry samples and standard solutions (0.078–40 μg mL^{-1}) of DNJ were used for the determination of α-glucosidase inhibitory activity.