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In vitro anti-cholinesterase and anti-hyperglycemic activities of flowers extracts from seven pomegranate varieties

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ABSTRACT

Punica granatum (pomegranate, Punicaceae family) has been known as medicinal plant with potential biological activities. The present study was carried out to investigate the cholinesterase and hyperglycemic inhibitory activities of the flowers of pomegranate collected in South of Tunisia. The plant extracts were prepared with the ethanol solvent and the biological activities were variety and concentration dependent. Espagnoule variety with the lowest IC_{50} value of 2.70 ± 0.10 μg/mL exhibited the strongest acetylcholinesterase inhibitory activity. It was found that Rafrafi ($IC_{50} = 4.0 \pm 0.11$ μg/mL) and Garsi varieties ($IC_{50} = 4.5 \pm 0.21$ μg/mL) exhibited the strongest butyrylcholinesterase inhibitory activity as compared with the reference drug, galanthamine (3.74 ± 0.28 μg/mL). For anti-hyperglycemic activity, the Rafrafi variety was the most potent against α-glucosidase activity with the lowest IC_{50} value of 29.77 ± 1.50 μg/mL. Only Zaghwani variety exhibited lower anti-α-amylase activity ($IC_{50} = 185.92 \pm 2.00$ μg/mL) whereas other pomegranate varieties were not active.

1. Introduction

Alzheiemr's disease (AD) is a progressive neurodegenerative disease that causes problems with memory, behavior and thinking (Cummings and Cole, 2002). The prevalence of AD doubles every 5 years after the age of 60 and it is characterized by a shortage of acetylcholine (AChE) and butyrylcholine (BuChE) (Orhan et al., 2006). Treatments for AD include psychosocial interventions, disease-modifying treatments, psychotropic agents and especially cholinesterase inhibitors that block the hydrolysis of the two chemical neurotransmitters, AChE and BuChE (Giacobini, 2002). However, cholinesterase inhibitors such as donepezil, galanthamine and tacrin are reported to possess side effects: nausea, vomiting, cramping, diarrhea, etc. (Qin et al., 2013). On the other hand, several studies discuss the relationship between AD and diabetes mellitus (Arvanitakis et al., 2004). A recent investigation suggests a causal link between sugar imbalance and AD (Ronnemaa et al., 2008). Also, high BuChE activities were reported for patients with type I or type II diabetes (Rustemeijer et al., 2001). Diabetes mellitus, a chronic metabolic disorder caused by defects in both insulin action and/or insulin secretion. Diabetes mellitus is characterized by postprandial and fasting hyperglycemia and it is associated with long term alteration in fat, protein, oxidative stress and carbohydrate metabolism (Zanatta et al., 2007). According to a previous study, the total number of people with diabetes is projected to rise from 171 millions in 2000 to 366 millions in 2030 (Wild et al., 2004). The increasing in blood glucose level, which is an important predictor of microvascular and macrovascular complications, is mainly a consequence of the diabetes (Laakso, 1999). Two key enzymes α-amylase and α-glucosidase are responsible for digestion of dietary carbohydrates to glucose. Salivary and pancreatic α-amylases hydrolyze starch molecules to produce oligosaccharides and disaccharides. Further digestion takes place in the small intestinal brush border by α-glucosidases, which catalyzes the cleavage of glucose from disaccharides as the final step to release glucose (Gupta et al., 2003). One therapeutic approach to control the blood glucose level and in this way to treat diabetes is to decrease the post-prandial hyperglycemia by inhibition of carbohydrate enzymes (Rhabasa-Lhoret and Chiasson, 2004). However, the synthetic inhibitors are reported to cause increasing in the incidence of renal tumors (Charpentier et al., 2000). Consequently, the search for effective and safe cholinesterase and carbohydrate enzymes inhibitors from natural materials was increased. *Punica granatum* (pomegranate, Punicaceae family) has been known as medicinal plant with potential biological activities (anti-inflammatory, anti-cancer, anti-oxidant,

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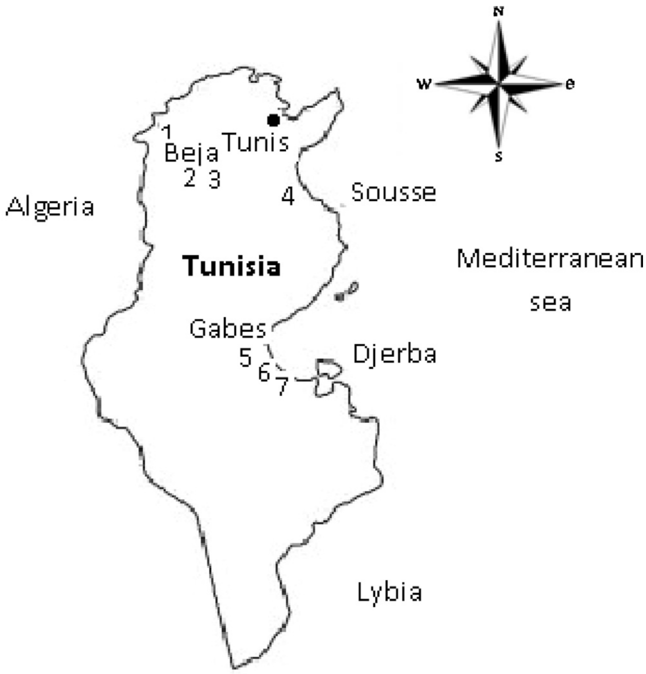

Fig. 1. Map of Tunisia indicated the origin sites of the seven studied pomegranate varieties (Zaghwani (1, Zaghwan), Espagnoule (2, Esslouguia), Zehri (3, Testour), Chetoui (4, Sidi Bou Ali), Gabsi (5, Kattena), Rafrafi (6, Zerkine) and Garsi (7, Mareth)) cultivated in the germplasm of zerkine, Gabes.

anti-diabetic, anti-microbial activities, etc.). The effect of flower extract on α-glucosidase activity was studied (Li et al., 2005). Also, *P. granatum* fruit hull extracts were found to exhibit interesting anti-α-glucosidase and anti-α-amylase activities (Prashanth et al., 2001; Gholamhoseinian et al., 2008). However, for our knowledge, there is no study in the literature for anti-α-amylase activity of pomegranate flowers. We have reported the significant influence of extraction solvent on acetylcholinesterase and butyrylcholinesterase inhibitory activity of pomegranate leaves (Bekir et al., 2013a) whereas the effect of pomegranate flower on these cholinesterase activities is still unknown. Recently, we have examined the chemical composition (phenolics, flavonoids, tannins and anthocyanins contents) from pomegranate flowers. Most of the ethanol extracts have good DPPH and ABTS scavenging activities and were found to effectively inhibit 5-lipoxygenase activity and MCF-7 proliferation (Bekir et al., 2013b).

This paper reports on a first study on the effect of variety factor on acetylcholinesterase and butyrylcholinesterase activities of pomegranate. The effect of variety factor to inhibit α-glucosidase and α-amylase activities was also studied.

2. Materials and methods

2.1. Plant material

Seven pomegranate varieties were studied in this work: Chetoui (CH), Espagnoule (ES), Gabsi (GB), Garsi (GR), Rafrafi (RA), Zaghwani (ZG) and Zehri (ZH). These studied varieties were cultivated in the germplasm located at the experimental field station of Zerkine in the province of Gabès, south of Tunisia (Fig. 1) (Mars and Marrakchi, 1999). The flowers of pomegranate were collected at full bloom stage (May 2010) and were dried at 45 °C.

Table 1
Chemical composition of flower from 7 pomegranate varieties (Bekir et al., 2013b).

Varieties	Polyphenols (GAE)[a]	Flavonoids (QE)[a]	Tannins (CE)[a]	Anthocyanins (CGE)[a]
Chetoui	330.9 ± 11.3	29.5 ± 0.8	30.6 ± 0.6	0.70 ± 0.03
Espagnoule	298.7 ± 4. 8	20.4 ± 0.9	15.7 ± 0.8	0.50 ± 0.02
Gabsi	305.8 ± 3.4	28.5 ± 0.6	29.9 ± 0.9	0.50 ± 0.02
Garsi	281.7 ± 5.5	21.8 ± 0.1	16.0 ± 0.0	0.60 ± 0.03
Rafrafi	324.4 ± 8.9	13.7 ± 0.1	24.2 ± 1.8	0.50 ± 0.01
Zaghwani	285.0 ± 7.3	22.0 ± 0.7	14.1 ± 0.8	0.40 ± 0.02
Zehri	313.0 ± 1.8	16.0 ± 0.8	16.0 ± 0.8	0.30 ± 0.02

[a] mg g^{-1} dry weight.

2.2. Preparation of ethanolic extracts

The dried and powdered flower (5 g) was extracted with ethanol (80%) at 35 ± 2 °C under stirring for 2 h. After extraction, each ethanol extract was filtered through a filter paper (fine pore, 0.45 μm) and concentrated in vaccum (2 mBar) for 4 h at 50 ± 1 °C in a rotavapour (Heidolph, Germany). Resulting extracts which contain water were lyophilised and were stored at 4 °C until analysis.

2.3. Determination of chemical composition biological activities

Chemical composition assays (total phenolics, total flavonoids, total condensed tannins and total anthocyanins) and anti-Alzheimer activity (anti-acetylcholinesterase and butyrylcholinesterase assays) were cited in Bekir et al. (2013a). The inhibition of α-glucosidase and α-amylase activities were evaluated according to Kammoun El Euch et al. (2015).

2.4. Statistical analysis of data

All data were expressed as means ± standard deviations of triplicate measurements. Standard deviations (SD) did not exceed 5% for the majority of the values obtained. The confidence limits were set at $P < 0.05$.

3. Results and discussion

3.1. Chemical composition

In the current study, we carried out a preliminary screening on chemical composition of flower extracts from seven pomegranate varieties (Bekir et al., 2013b) (Table 1). Thus, the total phenolic contents (TPC: 281.70 ± 5.54–330.89 ± 11.32 mg GAE/g dw), total flavonoid contents (TFC: 16.0 ± 0.8–29.5 ± 0.8 mg QE/g dw), condensed tannins contents (CTC: 14.1 ± 0.8–30.6 ± 0.6 mg CE/g dw) and total anthocyanin contents (TAC: 0.3 ± 0.02–0.7 ± 0.03 mg CGE/g dw) are shown. We suggest that the chemical composition of pomegranate flowers correlates with the biological activities evaluated in this work.

3.2. Anti-Alzheimer activities

In this work, we investigated cholinesterase activities of pomegranate flowers against AChE and BuChE. According to our knowledge, no studies on cholinesterase inhibition activity of pomegranate flowers reported up to date.

The flower extracts inhibited AChE in a dose-dependent manner (Table 2). Screening showed significant reduction in the AChE activity at extracts concentrations of 250 μg/mL. Espagnole variety with the lowest IC_{50} value of 2.70 ± 0.10 μg/mL exhibited the strongest AChE inhibitory activity. This inhibitory activity was six-fold lower than the reference drug galanthamine (0.45 ± 0.03 μg/mL). It is worthy to note that Espagnole pomegranate variety was found