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Polar constituents, protection against reactive oxygen species, and nutritional value of Chinese artichoke (*Stachys affinis* Bunge)

Alessandro Venditti [a], Claudio Frezza [b], Diana Celona [a], Armandodoriano Bianco [a], Mauro Serafini [b], Kevin Cianfaglione [c,d], Dennis Fiorini [e], Stefano Ferraro [e], Filippo Maggi [f,*], Anna Rita Lizzi [g], Giuseppe Celenza [g]

[a] Department of Chemistry, La Sapienza University, Rome, Italy
[b] Department of Environmental Biology, La Sapienza University, Rome, Italy
[c] EA 2219 Géoarchitecture, UFR Sciences & Techniques, Université de Bretagne Occidentale, Brest, France
[d] School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy
[e] School of Science and Technology, University of Camerino, Camerino, Italy
[f] School of Pharmacy, University of Camerino, Camerino, Italy
[g] Department of Biotechnological and Applied Clinical Science, University of L'Aquila, L'Aquila, Italy

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ABSTRACT

In the present work, we studied the chemical composition of Chinese artichoke (*S. affinis* tubers) by analyzing its polar constituents and its macro- and micro- nutrients. A total of nine compounds were isolated from the tuber ethanolic extract and structurally elucidated by Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry (MS). The marker compounds identified were oligosaccharide stachyose and the organic acid, succinic acid, as well as phenylethanoid and iridoid glycosides. The macronutrient profile was dominated by carbohydrates (36.9% dw), whereas potassium (2.36%) was the most abundant micro-nutrient. The tuber ethanolic extract was able to efficiently protect human cells (Caco-2, SHSY-5Y and K562) against *t*-BHP-induced oxidative damage.

1. Introduction

With approximately 300 species, the *Stachys* L. genus is one of the largest genera in the Lamiaceae family. The genus has a worldwide distribution but is most prevalent in Europe and East Asia. Many species of this genus have been widely used in traditional medicine, as long ago as the times of early Egyptian civilization. Aerial parts of several species have been used in infusions and decoctions to treat such problems as skin disorders, stomachache, ulcer, asthma, rheumatic pains and cancer (Gören, 2014).

Many species of the *Stachys* genus are used to prepare a wild tea, also known as 'mountain tea,' that is believed to be sedative, antispasmodic, diuretic and emmenagogic (Gören, 2014). In addition, several *Stachys* species are also used as a food, as their edible

* Corresponding author at: School of Pharmacy, University of Camerino, via S. Agostino 1, 62032 Camerino, Italy.
E-mail address: filippo.maggi@unicam.it (F. Maggi).

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tubers are an important source of carbohydrates (Łuczaj, Svanberg, & Köhler, 2011). In fact, the powdered form of some of these species is added to bread, and for this reason is called 'mayday flour'. This is the case with *S. affinis* Bunge (syn. *S. sieboldii* Miquel), well known as Chinese artichoke, and chorogi, an herbaceous plant native to China and Japan, where it has been extensively cultivated for its edible tubers. Since the nineteenth century this plant has been cultivated in Europe as well (Łuczaj et al., 2011), for instance in Italy, where it is known as 'tuberina'. In France it is known as 'Crosnes', alluding to the city of Crosne, in Essonne Province, where it was first cultivated in 1882. In the late twentieth century *S. affinis* was gradually forgotten in Europe, but recently Chinese artichoke has come back into fashion following an international trend of rediscovery and revaluation, and now is sold on the market and used in dishes.

In China, *S. affinis* has also been used as a traditional remedy to treat infections, colds, heart diseases, tuberculosis and pneumonia (Feng et al., 2015; Yamahara, Kitani, Kobayashi, & Kawahara, 1990).

Morphologically, *S. affinis* is similar to *S. palustris* L. (Hegi, Bergdolt, Zimmermann, & Süssenguth, 1927), although its tubers, also known as 'tubercules', are shorter and thicker (Fig. 1). The flavor of the tubercules is commonly considered to be delicate, with an artichoke-like flavor (Mercier & Perennes, 1982).

The *S. affinis* tubers are considered rich in proteins, carbohydrates, and vitamins. They are eaten raw (like topinambur), cooked or pickled, in salads, soups and as garnish (Mercier & Perennes, 1982); reduced into powder, they are used to prepare rice cookies. The tubers are rich in Fe^{2+} and thus are an ideal food for patients suffering from anaemia (Instituto Botanico Boreali-Occidentali Academiae Sinicae, 1983).

Tubers of *S. affinis* are a rich source of stachyose (Łuczaj et al., 2011), an oligosaccharide (tetrasaccharide composed of glucose, fructose and galactose), which is not digested by humans and is fermented by gut bacteria. For this reason, in China these tubers are eaten in the fermented state (Hu, 2005). This process transforms oligosaccharides into substances that are digestible. It has been shown that stachyose exerts noteworthy hypoglycemic effects in diabetic rats (Zhang et al., 2004). In another study, the polysaccharide fraction extracted from tubers of *S. affinis* exhibited high scavenging activity toward superoxide anion, hydroxyl, and ATBS radicals (Feng et al., 2015).

Despite the fact that *S. affinis* has been consumed as a vegetable for centuries, little is known about its secondary metabolites and nutritional value. To the best of our knowledge, phenylethanoid glycosides such as acteoside (syn. of verbascoside) and stachysoside C are the main secondary metabolites that have been characterized in its tubers thus far (Yamahara et al., 1990). In particular, acteoside is considered to be a promising antinephritic agent (Hayashi, Nagamatsu, Ito, Yagita, & Suzuki, 1996). The *S. affinis* methanolic extract was proven to protect mice from lethal effects of potassium cyanide (Yamahara et al., 1990) and to inhibit hyaluronidase activity (Takeda, Fujita, Satoh, & Kakegawa, 1985). Of considerable interest is the observation that the tuber aqueous extract alleviated learning and memory dysfunction associated with dementia and Alzheimer's disease in mice through an antioxidant mechanism (Harada, Tsujita, Ono, Miyagi, Mori & Tokuyama, 2015).

In the attempt to provide new insights into the secondary metabolism and nutritional properties of Chinese artichoke, in the present work we have performed a phytochemical analysis of the ethanolic extract obtained from tubers, and analyzed the tuber nutritional profile in terms of its content of macro- and micronutrients. Moreover, the cytotoxic and anti-reactive oxygen species (ROS) activities of *S. affinis* ethanolic extract were evaluated on a panel of human cell lines.

Fig. 1. Tubers of *Stachys affinis*.

2. Material and methods

2.1. Plant material

Tubers of *S. affinis* were collected in December 2014 in the town of Gazoldo degli Ippoliti, province of Mantova (geographic coordinates: 45°11′25″N, 10°34′40″E) at an altitude of 35 m a.s.l. where it was cultivated. A plant voucher specimen was authenticated by one of us (K. Cianfaglione) and deposited in the *Herbarium Universitatis Camerinensis* (CAME, included in the online edition of Index Herbariorum, c/o School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy) under the codex CAME 27797. After their collection, tubers were washed with tap water to remove debris and then dried in a ventilated oven at 35 °C for 3 days. Afterwards, they were reduced into a powder in a mill (blender MFC model DCFH 48; IKA-WERK, Staufen, Germany) and sieved (2 mm pore diameter).

2.2. Chemicals

During the phytochemical analysis, several solvents and reagents were used: ethanol 96% for the extraction procedure; *n*-butanol, distilled water and methanol as mixtures at different concentrations among them all, to be used as eluting systems for the chromatographic separations on silica gel 60 (70–230 mesh ASTM) columns; deuterated solvents such as CD_3OD (deuteromethanol) and D_2O (deuterium oxide) for the analysis of samples by NMR Spectroscopy; and RS purity grade methanol for the analysis of samples by Mass Spectrometry. All the natural solvents were at RPE purity grade, and were purchased from Sigma Aldrich (Milan, Italy), as were the deuterated solvents, while silica gel was bought from Fluka Analytical (St. Louis, MO, USA). Potassium hydroxide, hexane and ethanol used for the analysis of fatty acid composition were purchased from Sigma–Aldrich; Supelco 37 Component FAME Mix was purchased from Supelco (Bellefonte, PA, USA). Anhydrous sodium sulfate was purchased from Fluka-Riedel-deHaën (Milan, Italy) and methanol from Panreac Quimica SA (Barcelona, Spain). Deionized water (>18 MΩ cm resistivity) was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). Dimethyl sulfoxide (DMSO), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], *tert-butyl*-hydroperoxide (*t*-BHP) were purchased from Sigma Chemical Co. 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) was from Cayman Chemical. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, and foetal bovine serum were from Euroclone.