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Teas and herbal infusions as sources of melatonin and other bioactive non-nutrient components

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ABSTRACT

The consumption of teas and herbal infusions has increased in Europe and the USA in recent years. The goal of this work was to provide new knowledge on the contents of melatonin and other bioactive non-nutrient compounds of nineteen highly consumed herbal infusions. Melatonin was previously assessed in some medicinal plants alcoholic extracts but not described in herbal aqueous infusions as we reported for the first time. Noticeable melatonin contents were found in most of herbal infusions, showing chamomile and green tea the highest values. These studied herbal infusions could be considered as potential dietary sources of this antioxidant compound, and they also exhibited high levels of total phenolic compounds and total flavonoids (lemon balm revealed the highest contents). From results, the total phenolic compounds and total flavonoids were associated with the inhibition of lipase and α-glucosidase, as well as to the *in vitro* antioxidant capacity measured through five different methods (DPPH, ABTS, FRAP, ORAC, and deoxyribose assays). Thus, the studied teas and herbal infusions could be consided as suitable drinks herein validated for their bioactive compounds that may act as antioxidants and non-protein inhibitors of digestive enzymes, promoting health-promoting properties.

1. Introduction

Consumption of herbal infusions in Europe and the USA has increased in the last years (Izzo, Hoon-Kim, Radhakrishnan, & Williamson, 2016). It is known alternative medicine widely used medicinal plants for the prevention of some diseases and nowadays, consumers are becoming increasingly interested because of their health benefits. Studies indicated that medicinal plants possess more potent antioxidant activity than common fruits and vegetables. Antioxidant compounds present in medicinal plants could be used for inhibiting or preventing diseases consequence of oxidative stress (Li et al., 2013).

Although phenolic compounds are considered major contributors to plant foods antioxidant capacity, several authors described the role of other antiradical compounds with significant antioxidant properties, such as melatonin (*N*-acetyl-5-methoxytryptamine) (Aguilera et al., 2016; Reiter, Tan, & Galano, 2014). Besides its antioxidant and radical scavenging activity, melatonin exhibits anticancer properties and can prevent age-related cardiovascular disorders (Su et al., 2017). This indolamine has been quantified in some medicinal plants alcoholic extracts from feverfew (Murch, Simmons, & Saxena, 1997), St. John's wort (Murch, Rupasinghe, Goodenowe, & Saxena, 2004), fennel, anise (Manchester et al., 2000), Chinese medicinal plants (Chen et al., 2003), and Thai herbal teas (Padumanonda, Johns, Sangkasat, & Tiyaworanant, 2014), which showed high melatonin levels. Nevertheless, to the best of the authors' knowledge, no information about melatonin content in herbal aqueous infusions has been reported (Table 1).

Furthermore, medicinal plants contain certain compounds which act as inhibitors of the digestive enzymes, i.e. lipase, α-amylase or α-glucosidase (Dalar & Konczak, 2013; Ranilla, Kwon, Apostolidis, & Shetty, 2010). Their presence in herbal infusions, as opposed to alcoholic extracts, has not been studied thoroughly. These non-nutritive compounds can exert biological activities along their residence through the gastrointestinal tract, which could prevent or improve metabolic diseases such as diabetes and obesity (Jhong, Riyaphan, Lin, Chia, & Weng, 2015; Tucci, Boyland, & Halford, 2010). Hydrophilic compounds present in herbal infusions may reduce the

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Table 1
Botanical names, families, medicinally used parts and previously determine melatonin content of 19 selected medicinal plants.

	Herbal Infusions	Scientific name	Family	Tissues used for infusion	Melatonin content (ng/g)	References
Nervous System	Lemon balm	*Melissa officinalis* L.	Lamiaceae	Stems, leaves, and flowers	–	–
	Linden	*Tilia platyphyllos Scop.*	Malvaceae	Inflorescences	410 (methanol extract)	(Gomez, Hernández, Cerutti, & Silva, 2015)
	Passionflower	*Passiflora incarnata* L.	Passifloraceae	Leaves and flowers	–	–
	St. John's wort	*Hypericum perforatum* L.	Clusiaceae	Flowers	2000–5,230,000 (percloric acid extracts)	(Murch et al., 2004)
	Valerian	*Valeriana officinalis* L.	*Caprofoliaceae*	Roots, rhizomes, and stolons	–	–
	Black Tea	*Camellia sinensis* L.	Theaceae	Leaves	Not detected (ethanol extract)	(Kocadağlı et al., 2014)
	Green Tea	*Camellia sinensis* L.	Theaceae	Leaves	Not detected (ethanol extract)	(Kocadağlı et al., 2014)
	Red Tea	*Camellia sinensis* L.	Theaceae	Leaves	–	–
Digestive System	Boldo	*Peumus boldus molina*	Monimiaceae	Leaves and cortex	–	–
	Chamomile	*Matricaria chamomilla* L.	Asteraceae	Flower heads	–	–
	Fennel	*Foeniculum vulgare miller*	Apiaceae	Fruits, seeds, root, and leaves	28 (ethanol extract)	(Manchester et al., 2000)
	Green Anise	*Pimpinella Anisum* L.	Apiaceae	Fruits	7 (ethanol extract)	(Manchester et al., 2000)
	Pennyroyal	*Mentha pulegium* L.	Lamiaceae	Leaves	–	–
	Senna	*Cassia Angustifolia Vahl*	Fabaceae	Leaves and fruits	–	–
Cardiovascular System	Hawthorn	*Crataegus oxyacanthal*	Rosaceae	Floral tops, leaves, fruits, and cortex	–	–
	Horsetail	*Equisetum arvense* L.	Equisetaceae	Stem	–	–
	Olive Tree	*Olea europaea* L.	Oleaceae	Leaves and fruits	–	–
	Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Leaves, stems, and flowers	–	–
	Thyme	*Thymus vulgaris* L.	Lamiaceae	Leaves and flowers	–	–

proteases action; the occurrence of these non-protein proteases inhibitors has not been described to date. These compounds can present likewise bioactivity in relation to cancer prevention of inflammation reduction (de Mejia & Dia, 2010).

Because of the health-related potential properties of herbal infusions, the aim of this work was to provide new knowledge on the content of melatonin and other bioactive non-nutrient components of nineteen highly consumed teas and herbal infusions. To achieve this goal, we assessed the content of melatonin, phenolic compounds, and inhibitors against the digestive lipase, α-glucosidase, α-amylase, trypsin, and chymotrypsin enzymes. The *in vitro* antioxidant capacity was determined following different methodologies (DPPH, ABTS, FRAP, ORAC, and deoxyribose assays) to study the relation between the mentioned bioactive compounds, herbal infusions scavenging activity and inhibition of digestive enzymes.

2. Materials and methods

2.1. Botanicals

Nineteen commercial medicinal plants provided by a local company were selected (Table 1) and were stored in drying conditions at −20 °C. They were received as dried powders prepared in sachets to be infused.

2.2. Preparation of herbal infusions

Infusions were prepared in triplicate from the selected plants adding 1 g of plant material into 50 mL of boiling water, then extracted for 5 min, and filtered using a paper filter (11 μm, Whatman).

2.3. Experimental procedures

2.3.1. Melatonin

Melatonin was assessed using a modified method based on the descriptions of Aguilera et al. (2015). Herbal infusions were purified using solid phase extraction (SPE, C-18 cartridge, Waters) as follows: first, a conditioning step was carried out with 1 mL of MeOH and 1 mL of water to activate the column, followed by sample application (1 mL of sample) and washing step with 2 mL of 10% MeOH; finally, 1 mL of MeOH was used for the elution step. The obtained extracts were evaporated to dryness by using an evaporator centrifuge (Speed Vac SC 200, Savant, USA). The residues were dissolved in mobile phase ($MeOH/H_2O$ (80:20, v/v) + 0.1% formic acid). Melatonin was determined by HPLC-ESI-MS/MS triple quadrupole (Varian 1200 L with API-ES between 10 and 1500 Da range mass). The standard addition method was used. Infusions were spiked with different known amounts of a standard melatonin solution (0–10 nM). Samples and standard solutions were analysed by triplicate. Melatonin content was expressed as ng/g dry weight (DW). The detection (LOD) and quantification (LOQ) limits were calculated as the analyte concentrations that gave rise to peak heights with signal-to-noise ratios of 3 and 10, respectively. LOD and LOQ were 11.7 and 39.1 pg/mL, respectively.

2.3.2. Content of total phenolic compounds (TPC)

They were determined by Folin-Ciocalteu colorimetric method according to Singleton, Orthofer, and Lamuela-Raventós (1998). Total phenolic content was expressed as mg GAE/g DW and analysis were carried out in triplicate for each herbal infusion.

2.3.3. Total flavonoids content (TFC)

The content was quantified according to Xu and Chang (2007) using the aluminium chloride method. Total flavonoid content was calculated with a calibration curve of catechin and the results were expressed as mg CAE/g DW.

2.3.4. Lipase inhibition

The inhibitory effect of the lipase was determined using an enzymatic kit (Lipase-PS™, Trinity Biotech, Jamestown, NY) adapted to micromethod. Serum pancreatic lipase catalyzes the hydrolysis of a natural 1,2-diglyceride to form monoglyceride and fatty acid.