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Evaluation of total antioxidant potential of *Pistacia lentiscus* var. *chia* leaves extracts using UHPLC–HRMS

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ABSTRACT

In this study, *Pistacia lentiscus* var. *chia* leaves were dehydrated by freeze drying and the initial, final moisture content and the drying kinetics were examined. Soxhlet Extraction (SE), Microwave Assisted (MAE) and Ultrasound Assisted Extraction (UAE) were employed to recover extracts with high antioxidant activity, total phenolic and flavonoid content from fresh and dried leaves. Fresh leaves showed higher yield and antioxidant potential, with MAE extracts exhibiting the greatest extraction yield (48.11 ± 0.56% d.b.), followed by UAE (39.39 ± 1.13% d.b.) and SE (31.99 ± 1.55% d.b.). UAE extracts exhibited the highest antioxidant activity (IC_{50} = 37.13 ± 2.7 μg/mL), while SE extracts showed the highest total phenolic content (314.88 ± 0.01 mg GAE/g dry extract). UAE extracts of dried leaves exhibited total flavonoid content (106.5 ± 0.02 mg QE/g dry extract). Moreover, UHPLC-ESI–HMRS was performed to the extract with the highest antioxidant activity and confirmed the presence of isomers of galloyl quinic acid, quercetin and kaempferol glucosides, luteolin and neorehmannioside.

1. Introduction

Pistacia lentiscus var. *chia* of the Anacardiaceae family is an evergreen shrub with a strong characteristic aroma and green leaves, growing in the southern part of Chios Island. The plant is famous for producing a natural resin with characteristic aroma called mastiha, along with essential oil (from flowers, leaves and branches) and pressed oil extracted from mastiha berries (Barra et al., 2007). Mastic tree extracts have been used since Greek antiquity in folk medicine, mainly as anti-inflammatory, antiseptic and in treatment of various diseases, such as gastralgia and dyspepsia (Ljubuncic et al., 2005). Moreover, the aerial part has been used as a stimulant, for its diuretic properties and for the treatment of hypertension (Gardeli et al., 2008).

P. lentiscus products, nowadays, have a wide range of uses in food industry (Glampedaki and Dutschk, 2014) due to activities related to their secondary metabolites, such as flavonoids, polyphenols and phenolic acids. Phenolic compounds are a group of aromatic secondary plant metabolites widely spread throughout the plant kingdom and they have been reported to possess multiple biological effects such as antioxidant capacity (Dapkevicius et al., 1998; Proestos et al., 2005) and antimicrobial activity (Rauha et al., 2000). The main classes of polyphenols, phenolic acids and flavonoids, play a role in the prevention of human pathologies (Proestos and Komaitis, 2008; Tapiero et al., 2002). Moreover, flavonoids are a kind of highly effective antioxidant and less toxic than synthetic antioxidants such as BHA and BHT (Bimakr et al., 2011).

The leaves of the mastic tree are considered one of the main by-products during tree pruning and harvesting of mastic gum but have not yet been exploited. Therefore, no studies have been yet reported on the effective extraction of antioxidants and phenolic compounds from *P. letiscus* var. *chia* leaves. Succeeding effective (high extraction yield and concentration of bioactive compounds) recovery from a complex plant matrix is a difficult procedure due to co-extraction of other various compounds (Bimakr et al., 2011). Conventional extraction as heating, boiling, or refluxing can be used to extract flavonoids; however, the disadvantages are the loss of compounds due to ionisation, hydrolysis and oxidation during extraction as well as the long extraction time (Li et al., 2005). Therefore, in recent years, conventional techniques, such as Soxhlet Extraction (SE), tend to be replaced by various novel extraction techniques, such as Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) (Wang and

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Weller, 2006). These techniques are largely focused on finding technological solutions to diminish or even prevent the use of organic solvents in extraction processes to obtain more products with higher added value (Starmans and Nijhuis, 1996). Ultrasound Assisted Extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques (Huang et al., 2009; Wang et al., 2008). Moreover, UAE has been used for the extraction of plant components in order to shorten the extraction time, lower solvent consumption and increased extraction yields (Carrera et al., 2012).

Meanwhile, MAE is also considered a novel technique with better extraction yields, decreased extraction times and solvent consumption (Wang and Weller, 2006). Furthermore, MAE allows fast extractions, without the degradation of thermolabile compounds, with considerable savings in time and energy, and this technique is already used for the extraction of bioactive substances which are of interest for food and pharmaceutical industry (Karabegovi et al., 2013; Routray and Orsat, 2012).

In this study, conventional Soxhlet Extraction, Microwave and Ultrasound Assisted Extractions have been employed in order to recover bioactive compounds with high antioxidant activity from mastic tree leaves. Radical scavenging activity of the extracts of both fresh and dries mastic tree leaves was studied using the DPPH method. Total phenolic content of the extracts and total flavonoids content were also determined and the characterization of the bioactive compounds was made using by UHPLC–HRMS.

2. Materials and methods

2.1. Plant material

Leaves of *P. lentiscus* var. *chia* were collected in February 2013 at Chios, Greece, during the harvest of mastic gum. Pruning of leaves and branches is considered a necessary step in the collection process of mastic gum, therefore, the leaves used in this study were considered as agricultural by-product. The leaves collected were cleaned in order to remove damaged, diseased, or pest-infected ones and stored at −30 °C until further use.

2.2. Chemicals

All reagents and solvents used in the extractions were of analytical grade. Gallic acid, quercetin, dimethyl sulfoxide (DMSO), methanol, sodium acetate (CH_3COONa), Folin–ciocalteu reagent, sodium carbonate (Na_2CO_3) and 2,2-diphenylpicrylhydrazyl (DPPH) were purchased from Sigma–Aldrich. Ethanol was purchased from Fisher scientific, UK and aluminium chloride ($AlCl_3$) from Merck.

2.3. Drying of mastic tree leaves

For the freeze drying experiment, 30 randomly selected fully expanded mastic tree leaves were selected per sample forming a thin layer approximately 1 mm high. All mastic leaves samples were frozen at −30 °C for 72 h, in a biomedical freezer (SANYO, MDF-236, Osaka, Japan) and then were dehydrated for 24 h using a laboratory freeze-dryer (Leybold–Heraeus GT 2A, Koln, Germany) under vacuum of 0.055–0.065 mbar. During freeze drying process the procedure is carried out in two stages: first the material is frozen (−30 °C) and then the ice is removed by sublimation, directly from the solid to the gaseous phase (Krokida et al., 2003) and the moisture content was measured during the process. The dried products were stored at −30 °C in plastic containers until the extraction. All drying experiments were conducted in triplicate.

2.3.1. Drying kinetics

The weight of the samples was measured regularly during drying in order to examine the drying kinetics. A first order kinetic model describing the moisture transfer during drying is considered:

$$-\frac{dX}{dt} = k(X - X_e) \tag{1}$$

where X is the material moisture content on dry basis during drying (kg water/kg dry solids), X_e is the equilibrium moisture content of dehydrated material (kg water/kg dry solids), k is the drying rate (min^{-1}) and t is the time of drying (min) (Krokida et al., 2003). The drying rate is determined as the slope of the falling rate-drying curve. At zero time, the moisture content (dry basis) of the dry material X (kg water/kg dry solids) is equal to X_i, and Eq. (1) is integrated to give the following expression:

$$X = X_e - (X_e - X_i)e^{-kt} \tag{2}$$

2.3.2. Moisture content determination

The moisture content of the fresh and dehydrated products was performed according to AOAC (1980) (Cunniff, 1998) and was determined in a vacuum oven (Sanyo Gallenkamp PLC, Leicester, England) maintained at 70 ± 0.2 °C, until constant weight. The moisture content calculation was based on the following equation:

$$M_{(wet.basis)} = \frac{(m_w - m_d)}{m_w} \tag{3}$$

where M is the moisture content on wet basis (g/g), mw is the wet weight (g) and md is the dried weight of the sample (g). Experiments were conducted in triplicate.

2.4. Extraction experiments

Fresh and freeze dried *P. lentiscus* var. *chia* leaves were extracted using three different extraction methods: Soxhlet Extraction (SE), Microwave Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE). The fresh leaves were chopped in diameter less than 1 mm, while the freeze dried leaves were grounded (d < 450 μm). All the experiments were conducted in triplicate.

2.4.1. Soxhlet Extraction (SE)

A Soxhlet Extraction apparatus was used, consisting of a condenser, a soxhlet chamber and an extraction flask (100 mL). Fresh or dried mastic leaves (20–25 g) were placed into an extraction thimble with 100 mL of the desired solvent (ethanol or water) in the extraction flask. The solvent were refluxed for 3–4 h till the completion of three extraction circles.

2.4.2. Microwave Assisted Extraction (MAE)

Microwave Assisted Extraction has been carried out on a Microwave Digestion System (Start D, Milestone, Sorisole, Italy). Samples of fresh or dried mastic leaves (0.6 g) were extracted with 12 mL H_2O using fluctuating radiation to keep the temperature steady. Temperature was set at 100 °C and power at 400 W for a total duration of 60 min according to the conditions suggested by Nkhili et al. (2009).

2.4.3. Ultrasound Assisted Extraction (UAE)

Ultrasound Assisted Extraction was carried out in an ultrasound bath (ULTRAsonik cleaner, model 28H, 2.8 L, 105-W power; Ney Dental, Inc., Bloomfield, Connecticut). Samples of fresh or dried mastic leaves (20–25 g) were placed in a beaker with 250 mL of ethanol or water, which then was settled in the ultrasonic bath (operating at 40 kHz frequency and temperature 25 °C) for 60 min.