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Effects of geographical origin and extraction methods on total phenolic yield of olive tree (*Olea europaea*) leaves

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ABSTRACT

Olive tree (*Olea europaea*) leaves were extracted with methanol by homogeniser-assisted extraction (HAE) and ultrasound-assisted extraction (UAE) methods, which are novel technologies used in this field. The leaves were hand-picked from three olive trees of the same cultivar grown in the same region. In order to observe the effect of the geographical origin, the following six different sites in Anatolia were chosen: Bursa, Mardin, Ayvalik, Kas, Tekirdag and Canakkale. The results were presented by means of the extract yields and total phenolic contents expressed in gallic acid equivalent (GAE) per gram of dried leaf. The extract yields of HAE varied from 102.27 to 443.16 mg/g-dried leaf. Total polyphenols content in leaves ranged from 10.11 to 61.66 mg-GAE/g-dried leaf. As regards UAE, the extract yield changed between 88.75 and 350.82 mg/g-dried leaf, while total phenolic content varied from 7.35 to 38.66 mg-GAE/g-dried leaf. The greatest amounts of extract and total polyphenol were observed in olive leaves cultivated in Bursa through HAE. In addition, the kinetics of the total phenolic content through UAE were described by the film theory model.

1. Introduction

Researches into finding new uses for olive products, particularly by-products of olive oil industry, are of great value not only to the economy, but also to the environment where olives are grown and to the human health. Since leaves represent around 10% of the total weight of olives arriving to the mill, it is worth obtaining high added-value compounds from those materials [1]. Since ancient times, olive leaf extract has been a folk remedy against fevers and malaria [2–5]. It is of great value for their antioxidant properties. In addition, it shows antimicrobial activity, which makes it possible to use it as food additive. It also has a capability of preventing hypertension [6]. Recent studies revealed that olive leaf extract has anti-HIV activity by blocking the HIV virus entry to host cells [7]. Those properties of olive leaves are mostly attributed to their polyphenols, since polyphenols are classified as secondary metabolites having redox potential, which allow them to act as reducing agents, hydrogen donators, metal chelators and singlet oxygen quenchers [8,9].

A quick, simple and cost-effective method is of great necessity for screening natural plant extracts for their phenolic contents and antioxidant activity. The combination of effective extraction methods and low-cost raw materials represents an environmental and economical alternative to conventional extraction methods, which requires much more time and solvent consumption. It is of great value to criteria like global warming, disposal, consumer and worker health. The application of some supports such as stirring and ultrasound will also contribute to the extraction procedure.

Phenolic profile of olive leaves is known to be affected by several agronomical and technological factors such as leaf age, degree of ripeness, geographical origin, cultivar, phonological stage during sampling, proportion of brunches on the tree, moisture content, degree of contamination with soil and industrial processes employed for extraction [10,11]. Several studies have been carried out to investigate some of the effects mentioned above [12–14]. In our previous work, the effect of obtaining methods through supercritical-CO_2 extraction and soxhlet methods on the phenolic profile was evaluated [15]. In this study, olive tree (*Olea europaea*) leaves were extracted with methanol by homogeniser-assisted extraction (HAE) and ultrasound-assisted extraction (UAE) methods. Furthermore, in order to observe the effect of geographical origin, the following six different sites in Anatolia (Turkey) were chosen: Bursa, Mardin, Ayvalik, Kas, Tekirdag and Canakkale.

2. Experimental

2.1. Plant materials

In order to observe the effect of the geographical origin, the following six different sites in Anatolia were chosen: Bursa,

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Mardin, Ayvalik, Kas, Tekirdag and Canakkale. These origins are located in various sites of Turkey: Tekirdag, Canakkale and Bursa in Marmara, Ayvalik in Agean, Kas in Mediterranean and Mardin in south-east Anatolian of Turkey. Bursa and Mardin are presenting both the terrestrial and Mediterranean climate while the others show different climate properties lying in the humid and/or windy air with different altitudes. After collecting, the leaves were dried and stored at ambient temperature in the dark. Before extraction processes, they were ground into particles with an average diameter between 0.9 and 2.0 mm.

2.2. Chemicals

Methanol was provided from Merck and were of >99.8% mass fraction purity. Folin-Ciocalteu reagent, sodium carbonate and gallic acid were purchased from Sigma–Aldrich. 18 mΩ deionised water from a Millipore Milli-Q water purification system was used to prepare mixtures analyses.

2.3. Methods

2.3.1. Homogeniser-assisted extraction

500 mg of dried and ground leaf samples were extracted three times with 10 mL of solvent by blending in a homogeniser (Heidolph, Silent Crusher M) at 28,000 × *g* for 30 s (three times at 10 s intervals). The mixture was centrifuged (Nüve, CN 180) at 5000 × *g* for 25 min. Extracts were filtered through a 0.45 μm syringe filter and stored at −80 °C until the analysis for the biochemical measurements. For the extract yields, the solvent was removed from a certain quantity extract in a rotary evaporator (Buchi, Switzerland).

2.3.2. Ultrasound-assisted extraction

Ultrasound-assisted extraction was conducted in an ultrasonic bath (Protech) with a frequency of 50 Hz, at 25 °C. 500 mg of dried and ground leaves and 10 mL of solvent were sealed in an erlenmayer flask and placed into the bath. The mixture was centrifuged (Nüve, CN 180) at 5000 × *g* for 25 min. After centrifugation, the supernatant was filtered through a 0.45 μm syringe filter and stored at −80 °C until analysis for the biochemical measurements. For the extract yields, the solvent was removed from a certain quantity extract in a rotary evaporator (Buchi, Switzerland).

2.3.3. Total phenols determination

The concentration of the total polyphenols in extracts was measured by UV-spectrophotometry (Optima, SP-300), based on colorimetric oxidation/reduction reaction. The total phenolic content was determined according to the Folin-Ciocalteu method by the following procedure of Malik and Bradford [16]. Folin-Ciocalteu reagent was used as oxidising agent. To 10 μL Folin-Ciocalteu reagent of extract, 190 μL of water was added. 1 mL of Folin-Ciocalteu reagent and 800 μL of Na_2CO_3 (75%, w/v) were added. The samples were incubated for 30 min. The absorbance was measured at 760 nm. The amount of total phenolic content was expressed in gallic acid equivalent per gram of dried leaf (mg GAE/g dried matter). A calibration curve was calculated using pure gallic acid concentrations ranging from 0.053 to 0.425 mg/mL with a regression coefficient of 0.9994.

2.3.4. Kinetic of ultrasound-assisted extraction

The kinetics of the UAE process of the olive leaves were modelled by employing the film theory. It is based on two-stage extraction mechanism, which is two parametric. The first phase defines the washing stage (with washing coefficient) and the other specifies the slow extraction (with the slow extraction coefficient) as states below [17,18]:

$$\frac{C(t)}{C_e} = 1 - (1 - b) \cdot e^{-kt} \quad (1)$$

The linearised form of Eq. (1) can be indicated as follow:

$$\ln\left(1 - \frac{C(t)}{C_e}\right) = \ln(1 - b) - k \cdot t \quad (2)$$

where $C(t)$, concentration of the variable at time t; C_e, concentration of the variable when the equilibrium is reached; t, extraction time; b, washing coefficient based on the film theory; k, slow extraction coefficient based on the film theory.

2.3.5. Statistical analysis

Three replicate extractions were carried out for each of the samples followed by a minimum of three spectrophotometric measurements from each extract. Statistical analysis on the means of triplicate experiments was carried out using the ANOVA procedure of the InStat® software, version 3.0 (GraphPad, San Diego, CA, USA). Tukey's test of significance between means was used for illustration of significance. The relationship between the experimental data and the calculated quantities was evaluated through the correlation coefficient (r) and the root-mean-square deviations ($rmsd$) according to the following equation [19]:

$$rmsd = \sqrt{\frac{\sum_{i=1}^{n} (C_{i,\mathrm{exp}} - C_{i,\mathrm{cal}})^2}{n}} \quad (3)$$

where n is the number of the experiments, $C_{i,\mathrm{exp}}$ refers to the concentration value of experiment i and $C_{i,\mathrm{cal}}$ is the calculated concentration value of the i.

3. Results and discussions

3.1. Effect of geographical origin

The first phase of this comparative study involves the extract yield of olive leaves and the analysis of the total phenolic content of those with respect to differences in geographical location. Figs. 1 and 2 represent the variations of the extract yield and the total phenolic content of the leaves of olive trees grown at six different geographical locations by means of HAE and UAE.

There were clear differences in both extract yield and total phenolic content in leaves of different geographical locations. However, some cultivars were found statistically the same ($P > 0.05$).

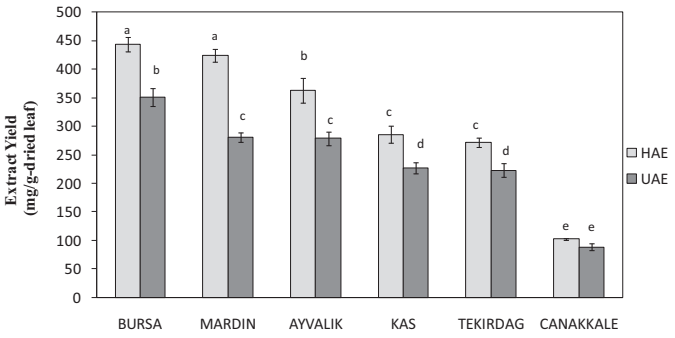

Fig. 1. Extract yield as *mg* per *g of dried leaf* from six different geographical origins through HAE and UAE. Data are expressed as the mean ($n = 3$) ± S.D. Values for each season not sharing a common letter were significantly different from each cultivar at $P < 0.001$.