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Bioactive metabolites involved in the antioxidant, anticancer and anticalpain activities of *Ficus carica* L., *Ceratonia siliqua* L. and *Quercus ilex* L. extracts

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ABSTRACT

Ficus carica L., *Ceratonia siliqua* L. and *Quercus ilex* L. are natural products that are used as an advantageous rich source of bioactive compounds of high economic value because of its use in cosmetic, pharmaceutical and agriculture industries. These crops were studied for its phytochemical contents and were investigated for antioxidant activities and its effects on reactive oxygen species (ROS) production, calpain activity and antiproliferative effects. Results showed that extremely high total contents of phenolics, flavonoids and *ortho*-diphenols were detected in *Quercus ilex*, while proanthocyanidin level was higher in *Ficus carica*. *Ceratonia siliqua* pods showed more carotenoids and had the highest lightness value (L*). *Quercus ilex* and *Ceratonia siliqua* extracts were very effective in scavenging free radicals. The best hydroxyl radical scavenging activity was attributed to *Quercus ilex* with 80.51 ± 0.20% while nitric oxide assay showed no significant differences between extracts. *Ficus carica* presented a higher reducing ability with 638.23 ± 0.43 mg gallic acid equivalents/100 g, while *Quercus ilex* was very potent in reducing power assays. Percentages of metal chelating capacities of *Quercus ilex* and *Ficus carica* extracts were 87.87 ± 0.34 and 73.17 ± 0.16%, respectively. These two samples were also able to scavenge hydrogen peroxide efficiently. At 250 μg/mL, *Quercus ilex* presented the best xanthine oxidase inhibition, 89.81 ± 0.36%. *Ceratonia siliqua* and *Quercus ilex* extracts presented the best capacities of ROS inhibition and reduced cell viability in a concentration dependent manner. All samples decreased calpain activity. These extracts could be further exploited as a source of natural products with antioxidant and anticancer effects.

1. Introduction

Crop products represent a major focus for drug development and industry and it holds a significant share in drug-market all over the globe. *Ficus carica* is a tree that is classified in the Moraceae family, it represents one of the most important crop cultivated in the worldwide. Its figs (*Ficus carica*) harvested in July and August are vey consumed due to its high contents in sugar, source of energy, and antioxidants components like phenolic compounds and vitamins. Figs are also known for its various therapeutic properties such its use in traditional medicine for the treatment of cardiovascular, respiratory and anti-inflammatory disorders (Çalişkan and Aytekin Polat, 2011). Another tree native to the Mediterranean is *Ceratonia siliqua*. This evergreen plant is belonging to the Fabaceae family. It has been cultivated for a long time and the most used part is pods (*Ceratonia siliqua*). These are very exploited by industry to produce locust bean and carob bean gum (Ayaz et al., 2007). The genus *Quercus* has been the subject of intense research due to its important role in industry section. More than 300 species were found in the Mediterranean area. *Quercus ilex* roots barks are used in the Alge-

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rian folk medicine to treat gastropathies. Acorns (*Quercus ilex*) were mainly used for animal consumption and were found to be rich in tanins (Cantos et al., 2003; Karioti et al., 2010; Custódio et al., 2013).

To improve and maintain the products, manufacturers are actually looking for effective natural antioxidants with negligible side effects. Phenolic compounds are among the compounds that respond more to the satisfaction. The objectives are: (i) customer satisfaction and protect their health by consuming products containing natural antioxidants, (ii) to find another source of natural antioxidants for pharmaceutical and cosmetic industries, (iii) to find ingredients to improve the sensory and nutritional quality of products and its possible use for the formulation of new products (Feng et al., 2015).

The importance of antioxidants lies in its ability to inhibit the production of reactive oxygen species. These compounds are able to exercise a scavenger effect on free radical species derived from oxygen or nitrogen and neutralize non-radical species such as hydrogen peroxide. In some physiopathologic circumstances, there is an excessive production of free radicals leading to the occurrence of oxidative stress. This later is related to the appearance of several diseases such as cardiovascular disease, Alzheimer's and cancer (Park et al., 2011). Several studies were performed on antioxidants in the sense that the intake of these compounds leads to the improvement of health and reduces the rate of mortality associated with diseases caused by oxidative stress (Marrelli et al., 2012).

Despite the large body of literature on the antioxidant and phenolic contents of figs, carob pods and acorns, several aspects of its biological activities remain unexplored. In that framework, the aims of this work were focused on the determination of the antioxidants composition of *Ficus carica* figs, *Ceratonia siliqua* pods and *Quercus ilex* acorns, to quantify and identify the main carotenoids present of these, and to investigate its antioxidant activities. There are several antioxidants which differ according to the types, structures, mode of action and the reactivity and that is why several tests on the antioxidant activity were performed. Other complementary assays are also used to estimate the total antioxidant capacity of the studied extracts. Having in mind the potential of *Ficus carica*, *Ceratonia siliqua* and *Quercus ilex* as source of bioactive compounds and the lack of bioactivity reports on *Quercus ilex*, this work also evaluated for the first time the anti-inflammatory and anticalpain activities of its extracts and its cytotoxic effects on human glioblastoma cancer cells.

2. Material and methods

2.1. Chemicals

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) and lucigenin were provided from Sigma-Aldrich (France) and the Eagle's Minimal Essential Medium (EMEM) was purchased from Invitrogen. L-glutamine, sodium pyruvate, Fetal Bovine Serum (FBS) and trypsin- ethylene diamine tetra-acetic acid (EDTA) were purchased from Invitrogen (Scotland-UK). Antibiotics streptomycin and penicillin were obtained from GIBCO (Cergy- Pontoise, France). The chromatographic solvents were methanol, acetonitrile, ethyl acetate (HPLC grade, procured from Merck, Darmstadt,Germany). Water was purified in a NANOpure®DIamond™system (Barnsted Inc., Dubuque, IO). Standards β-carotene, pheophytins a and b, luetin, β-cryptoxanthin, Chlorophylls a and b, antheraxanthin and zeinoxanthin were obtained from Sigma-Aldrich (Germany).

2.2. Samples preparation

Samples were harvested from three locations in Bejaia city (Algeria): carob pods (*Ceratonia siliqua*) from Ighil-Yesli, holm oak acorns (*Quercus ilex*) from Aichoune and white figs (*Ficus carica*) from Beni Maouche. The collection was done in clean places, away from pollution impact. Each specimen within the harvest site had been a random sampling on several samples. Botanical identification was made by the member of laboratory of Botany (University A. Mira of Bejaia). Voucher specimens were preserved at the Herbarium of Natural History Museum of Aix-en-Provence, France (*Ceratonia siliqua*: D-PH-2013-37-8; *Ficus carica*: D-PH-2013-37-9 and *Quercus ilex*: D-PH-2013-37-10). Healthy and uninfected samples were selected. Seeds were removed from ripe carob pods and pulp was dried in a ventilated oven (40 °C) until a constant weight of samples. Mature acorns were husked manually to separate the kernels and pericarp and the pulp was crushed and milled to a powder. The resulting millings from the studied crops were sifted in order to select the powders having a diameter smaller than 500 μm. Dried figs were cut and triturated into small pieces. Powders from the studied crops were retained and conserved in smoked glass, sealed and stored away from light and moisture for subsequent uses.

2.3. Extraction procedure

The procedure of extraction was to mix samples (1 g) with ethanol (50 mL) in glass vials and left maceration for 24 h at room temperature under continuous stirring. Solutions were centrifuged ($6800 \times g/20$ min) and extraction was repeated three times. Obtained extracts were combined and stored at 4 °C until the analyses were realized.

2.4. Antioxidant components

2.4.1. Total phenolic compounds

Total phenolic compounds content was determined using Singleton and Rossi (1965) method and the results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW) with the use of calibration curve obtained with gallic acid ($y = 0.0015 + 0.1483x$; $r^2 = 0.999$).

2.4.2. Total flavonoids content

Flavonoids content was quantified using the method reported by Huang et al. (2004). Amounts of flavonoids were deduced from a standard curve ($y = -0.0008 + 0.1162x$; $r^2 = 0.994$) and calculated in mg quercetin equivalents (QE)/100 g dry weight (DW).

2.4.3. Flavonols content

Total flavonols in the studied samples were assayed by the method reported by Adedapo et al. (2008) and the amounts were expressed as mg of quercetin equivalents per 100 g of dry weight (mg EQ/100 g DW). Quercetin was used to establish the calibration curve ($y = -0.0003 + 2.2549x$; $r^2 = 0.999$).

2.4.4. Proanthocyanidins content

Proanthocyanidins content was determined by the method reported by Maksimović et al. (2005) using butanol-HCl and the amounts were expressed as mg (+)-catechin equivalent (CE) 100 g^{-1} DW ($y = -0.1488 + 2.078x$; $r^2 = 0.999$).

2.4.5. Ortho-diphenols content

Ortho-diphenols contents were determined by the method described by Tovar et al. (2002) and were expressed as mg equivalents of gallic acid (EGA)/100 g DW ($y = -0.0087 + 0.0848x$; $r^2 = 0.998$).

2.4.6. Ascorbic acid content

Ascorbic acid contents were determined using the method described by Mau et al. (2005) and the amounts were expressed as