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Schinus terebinthifolius vs *Schinus molle*: A comparative study of the effect of species and location on the phytochemical content of fruits

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ABSTRACT

The aim of this work was to evaluate the effects of location on both the protein, oil and phenolic compounds and the antioxidant capacity of *Schinus terebinthifolius* Raddi and *Schinus molle* L. fruits. Significant variability was detected. *Schinus terebinthifolius* fruits contained high levels of protein (17.5–26.2%), oil (13.88–14.08%) and phenolic compounds (32.39–35.23 mg GAE/g DW). *Schinus molle* samples had high antioxidant activity. Unsaturated fatty acids were the major fatty acids (63.52–69.07%). An HPLC analysis revealed noticeable differences between regions and species. This study highlights the value of *S. terebinthifolius* and *S. molle* fruits as an attractive source of bioantioxidants and may be used to improve the consistent index for estimating fruit richness in biomolecules for pharmaceutical, cosmetic, and food industries.

1. Introduction

The genus *Schinus*, which belongs to the Anacardiaceae family, includes approximately 30 species; most of them are native to South America (Machado et al., 2007). *Schinus terebinthifolius* Raddi and *Schinus molle* L. have received particular attention due to their nutritional importance, and their ornamental and medicinal properties (Cavalher-Machado et al., 2008). The former is native to South and Central America and also has been found in subtropical and tropical regions of the United States and Africa (Barbieri et al., 2014). The latter has been widely distributed in the Brazilian Atlantic forest and has been introduced to most tropical and subtropical areas of the world, as well as the Mediterranean (Gomes et al., 2013; Rhouma et al., 2009). In its native area, the fruits of *S. terebinthifolius* are used as a substitute for black pepper (Barbosa et al., 2007) and those of *S. molle* are marketed as pepper (pink peppercorn), greatly appreciated in Mediterranean cooking (Gomes et al., 2013).

In traditional medicine, *S. terebinthifolius* is used to treat many diseases such as bronchitis and urinary disorders and is also used as a febrifuge, analgesic, anti-inflammatory and antimicrobial (Barbosa et al., 2007; Cavalher-Machado et al., 2008; Gazzaneo et al., 2005). *Schinus molle* is used as an analgesic, antiseptic, antidepressant antibacterial, for respiratory and urinary infections, digestive and purgative diuretic, for toothache, against rheumatism and menstrual disorders (Barrachina et al., 1997; Duke, 2002; Machado et al., 2007).

Given their importance as a source of nutrients for both humans and farm animals and consumer preference for natural foods, vegetable proteins have increased in commercial value. Due to protein-calorie malnutrition affecting more than 170 million nursing mothers and children in developing countries (Iqbal et al., 2006) and the unknown safety of proteins introduced into crops via genetic modification, alternative sources of protein from underexploited plants should be investigated and used (Tlili et al., 2011a).

Vegetable oils are characterized by their biological properties and can be used directly in foods, or converted into many products such as soap, personal care products, adhesives and biodiesel. For this reason, vegetable oils have attracted much more attention nowadays and research has increased to explore new plant sources of oil, especially from non-conventional plants.

Due to the possible side effect of synthetic antioxidants, both food and pharmaceutical industries are paying more attention to natural antioxidants such as phenolic compounds. These secondary metabolites are extremely important because of their beneficial effects as antioxidants, anti-inflammatories and anticarcinogenics (Limmongkon et al., 2018; Tabart et al., 2012; Wenga and Yen, 2012). All these biomolecules (proteins, oils and phenolic compounds), including

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content and composition as well as antioxidant activity of plants, are influenced by several factors such as climatic conditions, soils, and genetic factors (Breene et al., 2007; Chirinos et al., 2013; Tlili et al., 2011b; Tlili et al., 2013)

To the best of our knowledge, there have been no reports regarding the effect of species and region on protein, oil and phenolic compounds content of *S. terebinthifolius* and *S. molle* fruits and the majority of studies are about essential oils of these species. Therefore, the goal of the present work was to study the: *i*) protein: content and fractions, *ii*) oil: level and fatty acid composition, *iii*) phenolic compounds: content and HPLC profile, and *iv*) antioxidant activity of *Schinus terebinthifolius* and *S. molle* fruits harvested from two Tunisian localities. This work could be helpful for the potential industrial use of *S. terebinthifolius* and *S. molle* fruits as a raw material source of these biomolecules.

2. Material and methods

2.1. Plant material

Mature fruits of *S. terebinthifolius* and *S. molle* were collected in March and April 2015 from two Tunisian regions Gafsa (Echabiba 34° 33′ and Zarroug 34° 26, respectively) and Tunis (El Kram 36° 50′ and El Manar 36° 49′, respectively). Species were identified by Dr. Ezzeddine Saadaoui and voucher specimens were deposited in the herbarium of the National Institute for Research in Rural Engineering Water and Forests (INRGREF, Tunisia). Samples were air-dried at room temperature and then ground for future analysis.

2.2. Protein analysis

2.2.1. Kjeldhal method

Protein contents were measured according to the AOAC official method using a Kjeldhal apparatus (Association of Official Agricultural Chemists, 1984). 100 mg of dry sample was digested for 1 h at 450 °C with 10 ml of concentrated H_2SO_4 and 200 mg of digestion mixture (8 g K_2SO_4 + 20 g $CaSO_4$ + 2 g selenium). The end of digestion was indicated by the clear color of the solution. Then, distilled water was used to made up the solution to 30.0 ml in a volumetric flask. To estimate nitrogen level, 10 ml of 2% boric acid solution was first put in a beaker with a few drops of methyl red as an indicator. After that, 10 ml of the digested mixture, 30 ml of 40% NaOH solution, and 10 ml of distilled water were placed to the distillation chamber. The ammonium borate liberated was titrated with H_2SO_4 (0.01N). Protein was calculated according to the following formula:

% protein = %N × 6.25 (where N is Nitrogen).

2.2.2. Protein fractionation

Protein fractions were fractionated using Osborne method as reported by Venkatachalam et al. (2008) and Wang et al. (2016) with some modifications. Briefly, 100 mg of sample was stirred with 10 ml of distilled water at room temperature for 2 h. Then, the homogenate was centrifuged at 10000 rpm for 20 min at 4 °C. The supernatant that contained the soluble albumin is recovered for the protein assay. The precipitate was redissolved in Tris HCl solution (100 mM, pH 8.1) and stirred vigorously at room temperature for 2 h. Then, after centrifugation under the same conditions as above the supernatant was used to estimate the globulin content and the precipitate was dispersed in 55% isopropanol solution to extract prolamin fraction. After that, the final obtained precipitate was stirred with 0.2N acetic acid solution to extract glutelin fraction. Finally, protein contents were determined using the Bradford method.

2.3. Lipid analysis

2.3.1. Oil extraction

Oil content was determined according to the International Organization for Standardization (ISO) method 659:1998 (ISO, 1999). Soxhlet apparatus was used to extract oil from about 25 g of the dry powder with petroleum ether for 4 h. Then, a rotary evaporator was used to concentrate the solvent at 50 °C under reduced pressure. Finally, the obtained oil was dried using a stream of nitrogen and then stored at −20 °C for further analysis.

2.3.2. Fatty acid analysis

The composition of fatty acid in the oil was determined by gas chromatography as fatty acid methyl esters (FAMEs) using the method described by Cecchi et al. (1985). The FAMEs were separated and identified using a HP 6890 gas chromatograph with a split/splitless injector equipped with a flame ionization detector, and a 30 m HP Innowax capillary column with an internal diameter of 250 µm and a film thickness of 0.25 µm. The temperatures of the injector and the detector were maintained at 250 °C and 275 °C, respectively; the oven was programmed to rise from 50 to 180 °C at a rate of 4 °C/min, from 180 to 220 °C at 1.33 °C/min and to stabilize at 220 °C for 7 min. The carrier gas was the nitrogen. The identification of fatty acids was performed by comparing retention times of the FAMEs with those of co-injected authentic standards (SUPELCO PUFA-3). HP Chemstation software was used for the integration and the analyses of fatty acid peaks.

2.4. Phenolic compounds analysis

2.4.1. Methanolic extracts

The powder (1 g) was stirred vigorously with 10 ml of methanol for 48 h and the obtained solution was filtered (Whatman no.1). The precipitate was once again extracted and filtered. The final extract was concentrated for further analysis (Mejri et al., 2017; Pires et al., 2018).

2.4.2. Total phenolic content

Total phenol content was estimated using the method described by Tlili et al. (2011b). Briefly, 200 µl of methanolic extract was mixed with 1 ml Folin- Ciocalteu reagent. The mixture was placed for 5 min in the dark and then 0.8 ml of sodium carbonate 7% was added. After 30 min in the darkness, the absorbance was measured at 760 nm. Total phenolic compounds were estimated using a gallic acid standard curve (concentration range: 50–200 µg/ml). Results are expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

2.4.3. Total flavonoid contents

To 500 µl of methanolic extract we added 1500 µl of distilled water. At t = 0 we added 75 µl of $NaNO_2$ (7%), after 5 min we added 75 µl of $AlCl_3$ (10%) and at 6 min we added 500 µl of NaOH (1N) and 250 µl of distilled water, respectively (Tlili et al., 2014a). After incubation for 15 min at room temperature, the absorbance was read at 510 nm. Total flavonoids were calculated using quercetin standard curve (concentration range: 100–750 µg/ml). Total flavonoids content is expressed as milligrams of quercetin equivalent (QE) per gram dry weight (mg QE/g DW).

2.4.4. Condensed tannins contents

50 µl of the methanolic extract was stirred with 3 ml of methanol vanillin solution (4%) and 1.5 ml of concentrated H_2SO_4. The mixture was allowed to stand for 15 min, and the absorbance was read at 500 nm (Tlili et al., 2014a). The quantity of proanthocyanidins, determined using a catechin calibration curve (100–750 µg/ml), was expressed as mg catechin equivalent per gram dry weight (mg CE/g DW).

2.4.5. HPLC analysis

HPLC analyses were performed using a Varian Prostar HPL