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A systematic comparison of 25 Tunisian plant species based on oil and phenolic contents, fatty acid composition and antioxidant activity

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ABSTRACT

This study investigated 25 Tunisian plant species of 13 families based on their oil and total phenolic contents. The fatty acid profiles and antioxidant activity of phenolic extracts of these plant seeds were studied in order to identify species containing unusual fatty acids and potential antioxidants. The oil content varied from 4.2 to 66.5% of DW (Dry Weight). Fatty acid profiles were determined using gas chromatography analysis coupled to flame ionization detection and high resolution mass spectrometry (GC-FID and GC-HRMS). The results showed that plant seeds were mostly composed of classic fatty acids (palmitic, oleic, linoleic and linolenic acids), while unusual fatty acids such as long-chain and odd-chain fatty acids were minor components. However, the petroselinic acid was the major component of Apiaceae family seed oil, particularly in *Pimpinella saxifraga* and *Pimpinella major*. The phenolic content of seed methanolic extracts, measured by Folin–Ciocalteu assay, varied between 30 and 4700 mg GAE/100 g DW. The methanolic extracts exhibited a broad range of antioxidant activity, varying from 13 to 17,000 mg TEAC/100 g DW in the DPPH assay. In the ABTS assay, the antioxidant activity values similarly varied from 4 to 18,100 mg TEAC/100 g DW. The seeds of *Apium graveolens, Anethum graveolens*, *Pimpinella saxifraga* and *Lepidium sativum* had high oil content, interesting fatty acid profiles and their methanolic extracts displayed high antioxidant capacities. This investigation of Tunisian plants showed the potentialities of several species that could be scale-up cultivated for commercial purposes.

1. Introduction

The increasing use of plants as a source of oil has motivated researchers to explore the vegetal diversity and investigate new oil sources with classic and original fatty acid composition. Therefore, quantitative and qualitative analysis are required in order to characterize plants fat content. Quantifying the fat content of a seed is crucial in predicting its stability and ageing during storage especially for industrial uses (Seal et al., 2008).

Moreover, as well known, fatty acids are the elementary component of oil. Thus, physicochemical properties of vegetable oil are directly related to its fatty acid composition. This explains the use of specific oil in a particular field. For example, oils rich in polyunsaturated fats with high level of omega fatty acids have more nutritional value than oils containing large amounts of saturated fatty acids (SFA) (Mehmood et al., 2008). However, in renewable energy field, biodiesel production requires a vegetable oil including high amount of monounsaturated fatty acids, a low percentage of polyunsaturated fats, and a controlled proportion of saturated fatty acids (Fan et al., 2016). The oils containing fatty acids with unusual structures like oxygenated or long-chain fatty acids are interesting in polymer and plastic industry for production of coatings, plastics, urethane derivatives, surfactants, dispersants, cosmetics, lubricants, etc. (Carlsson, 2009; Montero de Espinosa and Meier, 2011).

Nevertheless, oils especially those containing large amounts of polyunsaturated fatty acids (PUFA) or unusual fatty acids are known for their sensibility to oxidation, which can be a major problem during their storage and industrial processing in many applications (Kousar Kalem et al., 2017). For example, in food processing, lipid oxidation contributes to the formation of oxidized products such as free radicals which lead to diverse undesirable chemical reactions inducing a loss in nutritional and organoleptic properties of foods (Wang et al., 2016). Some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are currently used to avoid or delay the oxidation processes. In spite of their efficiency, artificial antioxidants have been suspected to generate negative health effects such

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as toxic and carcinogenic risks (Kahl and Kappus, 1993). Nevertheless, it has been proved that some oils contain natural antioxidants such as olive oil which is rich in tocopherols, carotens and phenolic compounds essentially hydroxytyrosol, oleuropein and tyrosol (Servili and Montedoro, 2002). That's why, there is an increasing interest in studies of natural additives as potential antioxidants such as phenolic compounds (Chen and Xu, 2018). Phenolics are a large group of secondary metabolites widespread in plant kingdom. Plants are generally rich in phenolic compounds, such as phenolic acids, flavonoids, stilbenes, tannins, lignans, lignins and coumarins (Surveswaran et al., 2007; Sytar et al., 2018). They are well known by their antioxidant and free radical-scavenging activities which explains their increased use in oils or lipid containing products to prevent or delay oxidative deterioration (Lorenzo et al., 2017; Sellimi et al., 2017). Lately, few researches have been also interested in using natural antioxidants as stabilizers in polymers and biodiesel (Kirschweng et al., 2017; Varatharajan and Pushparani, 2017).

The biosynthesis of phenolic compounds and oils could be affected by culture environment and conditions. In fact, plants growing under stress conditions such as drought are known by their developed resistance. A high content of natural antioxidants was observed in these plants due to several resistance responses including an increase in protective pigment biosynthesis such as tannin and phenolic compounds (Estiarte et al., 1994; Northup et al., 1995). Moreover, previous studies proved that biosynthesis of unusual fatty acids such as behenic and eicosenoic acids had been enhanced under drought conditions (Anwar et al., 2006; Dwivedi et al., 1996). In their study, Bouaziz et al. (2009) investigated the antioxidant and antimicrobial potential of 25 Saharan plants which are used in folk medicine. Regueb, a small rural and agricultural region in Sidi Bouzid (Tunisia) characterized by a semi-arid to arid climate could be also an important source of vegetal diversity with interesting oil content and antioxidant capacity (Fautras, 2017; You et al., 2016), which are usually used in ethnomedecine. For example, *Lepidium sativum* is a plant widely used in traditional medicine in the treatment of kidney and respiratory diseases while *Peganum harmala* is used in the remediation of rheumatism, diabetes, hypertension, respiratory diseases, and toothache (Borgi et al., 2011). Previous studies investigated the potential of these plants but not in Regueb region (Fatma et al., 2016; Oszmiański et al., 2013). To the best of our knowledge, no data was previously recorded in literature concerning the oil and antioxidant potentialities of *Cirsium vulgare, Crocus longiflorus, Cleome amblyocarpa*, *Pimpinella major* and *Pimpinella saxifraga*. Furthermore, as far as we know, the phenolic extract antioxidant ability of *Retema sphaerocarpa* and *Ruta graveolens* recorded as medicinal plants of arid zones by UNESCO (1960), hadn't been previously reported. Highlighting the presence of natural potential antioxidants and unusual fatty acids in Regueb plants will increase the value of this semi-arid to arid region in order to enhance its agricultural exploitation in commercial purposes and improve living conditions.

The present study aims to quantify and identify the fatty acid composition of seed oils belonging to 25 Tunisian species from Regueb region using GC-FID and GC-HRMS analysis. This study also proposes to determine the total phenolic content of seed methanolic extracts by Folin–Ciocalteu assay, to evaluate the antioxidant capacity by using ABTS and DPPH tests and to investigate the relationship between the total phenolic content and the antioxidant activity.

2. Materials and methods

2.1. Plants collect

The seeds belonging to 25 Tunisian species of 13 families were collected from Regueb, Sidi Bouzid in central Tunisia where the climate is semi-arid to arid with an annual rainfall of 200 −300 mm (Fig. 1). Seeds were randomly collected from plants, to obtain an average of 100 g, between April and November 2016 and the species were identified in Faculty of Sciences of Sfax (Tunisia).

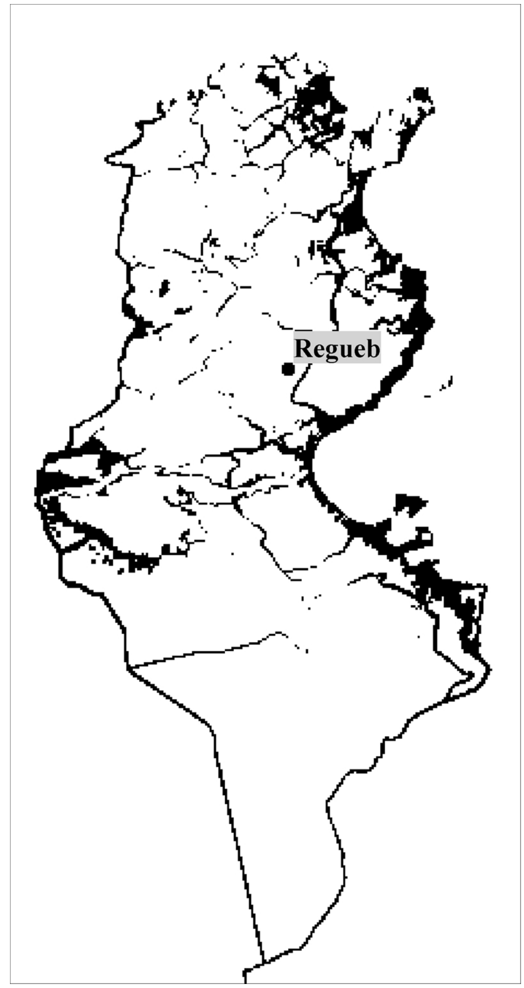

Fig. 1. Geographic location of the collected plants.

2.2. Oil extraction

The oil extraction was performed using Precellys Homogenizer according to the method described by La Russa et al. (2012) with some modifications. The dried plant seeds (100 mg) were grounded with diethyl ether (1 mL) by Precellys Bead Mill Homogenizer (Precellys, Bertin, France) (5 min, 4500 rpm) at room temperature (25 ± 2 °C). Then, the organic phase was separated from residual materials by centrifugation for 3 min at 3000 rpm. The pellets were re-extracted three times in order to maximize oil extraction. All of organic phases were recuperated and evaporated by nitrogen flux to eliminate diethyl ether and recuperate oil.

2.3. Fatty acid methyl esters (FAME) preparation

FAME were prepared by transesterification of oil triglycerides according to Bligh and Dyer (1959) works with some modifications. The extracted oil (5 mg) was dissolved in 100 µL diethyl ether, then the methylation reaction was catalyzed by 5 µL of TMAH (tetramethylammonium hydroxide). The mixture was shaked for 10 min (450 rpm) in order to enhance the transesterification. The reaction was then stopped by the addition of 50 µL decane. After that, the mixture was centrifuged (3 min, 3200 rpm) and the superior phase containing FAME was recuperated. The methyl esters of the fatty acids were dissolved in heptane solvent (1:20, v/v) and analyzed by GC-FID and GC-HRMS.

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