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Sex-related differences in essential oil composition, phenol contents and antioxidant activity of aerial parts in *Pistacia lentiscus* L. during seasons

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ABSTRACT

Essential oil composition, total phenolic, flavonoid and condensed tannin contents were assessed in different aerial parts of the same females and males of *Pistacia lentiscus* over four harvesting times. Marked quantitative and qualitative in terpenic and phenolic composition depending on sex, plant part and collecting periods were observed. At the species level, the main major compounds of essential oils in the leaves and stems were α-limonene, α-pinene, germacrene-D and β-myrcene with female plant stems being rich in these compounds. Male flowers produced highest amounts of α-terpinen-4-ol and β-caryophyllene, whereas, fruits accumulate the highest level of β-myrcene. α-limonene and germacrene-D reached their highest contents at fruiting in females and at flowering in males. The highest total phenolic, flavonoid and condensed tannin contents were higher in male leaves and flowers, with marked decreasing of contents from the vegetative to the ripening period. The antioxidant activity of acetonic extracts assessed by the DPPH and FRAP assays differs significantly among aerial plant parts. The lowest averages of IC_{50} were observed in leaves at the vegetative and the flowering stages, respectively. The ferric reducing antioxidant ability (FRAP) for leaves and stems differs between sexes and organs with male extracts taken during the vegetative and flowering stages being more active. The revealed differences in chemicals and antioxidant activities among genders, organs and phenological stages should help to the optimization of *P. lentiscus use* in industrial and pharmaceutical fields according to the variation of its chemical composition.

1. Introduction

Angiosperms, with diverse life histories and mating strategies showed monoecious, andromonoecious, gynomonoecious, dioecious, androdioecious and gynodioecious forms. Dioecious occurred in 6% of species and 37% of the families (Pannell and Barrett, 1998). They presumably derive from monoecious ancestors via different evolutionary processes and pathways as a result of combination of selective force effects such asecological factors, resource availability and pollination vectors (Munagua-Rosas et al., 2011).

The dioecism associated or not with sex chromosome dimorphism (Charlesworth, 2002; Liu et al., 2004; Ming et al., 2007, 2011) was determined by a number of loci (Sather et al., 2010), growth regulators (Durand and Durand, 1990) and environmental factors (Lawton-Rauh et al., 2000; Nakamura, 2009; Barr and Fishman, 2011). Besides vegetative and reproductive traits, several differences may distinguish dioecious plants. Males and females may exhibit different responses to abiotic stresses (Rozas et al., 2009; Chen et al., 2010; Zhang et al., 2001; Juvany et al., 2014) and levels of genetic and secondary metabolite composition differences (McAdam et al., 2013). These compounds in both plant forms are involved in growth and development, attracting pollinators, defense against biotic and abiotic stresses (Vinod et al., 2007), and vary with ecological factors, plant phonological stages), and seasons (Carvalho et al., 2014)

The genus *Pistacia* (*Anacardiaceae*) grouped 11–16 dioecious species divided into two sections *Pistacia* and *Lentiscus* (Zohary, 1952; Noroozi et al., 2009; Al Saghir, 2010). *Pistacia lentiscus* L., an evergreen shrub, belongs to the section *Lentiscus* and is widely distributed in the Mediterranean region. It's represented by separated male and female individuals that reach 1–5 m in height. The within-population proportion of males and females differed according to the population-size and ecological factors (Verdu and Garcıa-Fayos, 1998). The species exhibits a high level of variation in morphological, anatomical, chemical and molecular traits depending on geographical origins and genders (Correia and Barradas, 2000; Barazani et al., 2003; Gratani et al., 2013; Werner et al., 2013).

The flowering period in males and females in Tunisia occurred between March and the end of April. Blooming time is from May to July

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and is preceded by the leaf development in March. Leaves are green leathery pinnate and have 6–18 leaflets. Flowers, strictly unisex, are grouped in panicles formed on branches of the previous growing season. Male flowers (8–10 per inflorescence) have 1–2 perianth lobes and 8–10 stamens with dark red anthers. Female flowers (4–20 per inflorescence) are greenish and have two bracteoles, 2–5 perianth lobes, and a unilocular ovary including one ovule.The fruiting period occurred in the middle and late summer (July-August) and maturation of fruits was completed in autumn (October). Fruits are usually one seeded drupes (4–6 mm), initially green, then red and become glossy black at full ripening (September-October). Fruit shedding occurred during the end of autumn (October-November). The growth of plants usually ends in August-September and the cycle of development concludes between November and December. Several males, in contrast to females develop during this period new branches and leaves. The vegetative growth ends after ripening.

The genus *Pistacia*, in Tunisia is represented, by the cultivated *Pistacia vera* L., and the spontaneous *P. atlantica* Desf., *P. terebenthus* L. (endangered and growing in scattered individuals) and *P. lentiscus* L. The latter is represented by numerous populations growing in degraded forests, maquis and garrigues, extending from the sub humid to the upper arid and lower arid bioclimates. The species is associated, according to ecological regions, with *Olea europea*, *Myrtus communis*, *Rubus ulmifolius*, *Ceratonia siliqua*, *Calicotome villosa,Pinus halepensis, Quercus suber* and *Quercus coccifera*.

Pistacia lentiscus is traditionally used in the treatment of burns, asthma, hypertension, hyperuricemia and arthritis. The main components and pharmacological properties of the species have been reviewed by Nahida et al. (2012) and Bozorgi et al. (2013). Mastic gum produced only from stem male plants of *P. lentiscus* var. *Chia* is the most important economically product used in pharmaceutical, cosmetic and perfume industries. It is found to be effective in preventing and treatment of various cancers (Chadzopulu et al., 2011), gastrointestinal diseases (Balan et al., 2007) and showed neuroprotective (Quartu et al., 2012), antibacterial (Marone et al., 2001) and antidiabetic (Rehman et al., 2015) and anti-inflammatory properties. Seed fatty oils were known for their hepatoprotective effects (Haouli et al., 2015; Maameri et al., 2015).

Multiple studies have been reported on the chemical composition of the essential oils and phenols of aerial parts of *P. lentiscus* from different regions. Several chemotypes with major compounds such as α-pinene (Boelens and Jimenez, 1991; Aouinti et al., 2014),

β-myrcene (Castola et al., 2000; Amhamdi et al., 2009), terpinen-4-ol (Ben Youssef et al., 2005), limonene (Gardeli et al., 2008), germacrene-D and car-3-ene (Congiu et al., 2002) have been identified. The oil exhibits various levels of antioxidant (Klibet et al., 2016) and antibacterial (Hafsé et al., 2013) activities.The main identified polyphenols from the leaves are gallic acid and its derivatives, flavonols and favonoid glycosides and anthocyanes (Romani et al., 2002; Benhammou et al., 2008). The change in the chemical composition both in oils and phenols from different aerial parts has been attributed to environmental factors, seasons (Ait Said et al., 2011), phenological stages (Carvalho et al., 2014) and sex (Juvany et al., 2014; Yaniv and Dubai, 2014). Since, there are no earlier studies available where all these sources of variation and their interaction have been examined regarding the chemical changes of the same plants, the present study deals with the variation of the essential oil composition, phenols and tannin contents and antioxidant activities in *P. lentiscus* genotypes according to plant sex and organs sampled at different periods over the growth cycle of plants. This work is within a large investigation program on the genetics and the chemical diversity of the species in Tunisia.

The main following questions are approached: 1) Do essential oil, phenol contents and antioxidant activities of different organs differ between genders? and 2) Is there similar patterns of variation in the production of these secondary metabolites between the two sexes according to the phonological stage of plants?

2. Material and material

2.1. Plant material

Samples were collected in 2009 and 2010 from six plants (three females and three males), growing in Ezzit Djebel Mountain (latitude 35°49′N, longitude 10°59′E, located at 60 km from Tunis). The site belongs to the sub-humid bioclimatic zone with a rainfall ranging between 500 and 600 mm/year and is situated at an altitude of 350 m. The chosen plants grow in an area that does not exceed 300m^2 to minimize the effect of ecological factors. Samples were harvested from the same plants at four phenological stages: vegetative dormancy (December, 2009), full flowering (April, 2010), early (August, 2010) and late fruiting (October, 2010). Leaves, stems, flowers and fruits unripe (green), ripe (red and black) were taken from the 10–20 cm shoot parts of randomly chosen branches (dichoblastes) that had not floured in 2009. Organs were assessed for their essential oil composition, phenolic, condensed tannin contents and antioxidant activity.

The samples were kept for drying during 7 days at room temperature, then ground to powder before analysis.

2.2. Chemicals

Folin-Ciocalteu reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6 tripyridyl-s-triazine), gallic acid, quercetin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium carbonate, $AlCl_3$, $FeCl_3{\cdot}6H_2O$ and $FeSO_4{\cdot}7H_2O$ were purchased from Sigma–Aldrich (St. Louis, MO). All solvents and reagents used were of the highest purity.

2.3. Isolation of oils and phenols

Phenolic extracts from each of organ at each development stage were obtained by magnetic stirring for 12 h of 2.5 g of dry organ powder with 25 ml of aqueous acetone (80:20,v/v). Extracts were kept at 4 °C for 24 h, filtered through a Whatman No. 4 filter paper, and evaporated to dryness under vacuum. They were stored at 4 °C until analysis.

The essential oils have been extracted from 100 g air-dried leaves, stems and flowers by hydrodistillation for 3 h, using a Clevenger-type apparatus. Oil yields were then estimated on the basis of the dry weight of plant material. Oils were recovered directly, from above the distillate without adding any solvent, and stored in the dark at 4 °C.

2.4. Essential oils identification

The essential oils were analyzed by Gas chromatography–mass spectrometry (GC–MS) using an HP 5975C mass spectrometer (Agilent Technologies) with electron impact ionization (70 eV). AHP-5MS capillary column (30 m × 250 m coated with 5% phenylmethyl silicone, 95% dimethyl polysiloxane, 0.25 m film thickness) was used. The oven temperature was programmed to rise from 60 to220 °C at a rate of 4 °C/min; the transfer line temperature was 230 °C.The carrier gas was He with a flow rate of 0.8 ml/min and a splitratio of 50:1. Scan time and mass range were 1 s and 50–550 *m/z*, respectively.

The identification of oil components was assigned by comparison of their retention indices (RI) relative to (C8–C22) n-alkanes with those of literature or with those of authentic compounds available in the author's laboratory. Further, identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra (Adams, 2001). The determination of the percentage composition was based on peak area normalization without using correction factors.