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Variations in Tunisian wormwood essential oil profiles and phenolic contents between leaves and flowers and their effects on antioxidant activities

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ABSTRACT

This study is the first to investigate the chemical characterization of leaves and flowers essential oils and methanolic extract of *Artemisia absinthium* L. in Tunisia and their effect on the antioxidant activities. The yield of essential oil was higher in flowers (2.98%) than in leaves (1.87%). Qualitative and quantitative differences among the analyzed organ parts were revealed. 38 compounds in flower oils and 19 components in leaves oils were identified representing, respectively, 93.95% and 98.5% of the total oil composition. The two types of oils were dominated by chamazulene (flowers: 29.9%, leaves: 30.41%) and β-thujone (flowers: 19.66%, leaves: 25.75%). Flowers oils are characterized by camphor (16.16%) whereas leaves essential oils are distinguished by bornan-2-one (17.33%). According to our results chamazulene chemotype is probably specific to North African region for essential oil of *Artemisia absinthium* L. Essential oils show important antioxidant activities; however the highest antioxidant activities were recorded for methanol extract. In both cases leaves extract highlighted higher antioxidant capacity. Significant correlations were observed between the total phenols or flavonoid contents in methanol extracts and antioxidant activity estimated for both used tests even for leaves or for flowers extracts.

1. Introduction

Wormwood (*Artemisia absinthium* L.) is a medicinal and aromatic bitter herb, which has been used as a medicine from ancient times. It has traditionally been used as anthelmintic, choleretic, antiseptic, balsamic, depurative, digestive, diuretic, emmenagogue and in treating leukemia and sclerosis. The species was cited to be used externally as cataplasm of crushed leaves for snake and scorpion bites or decoction for wounds and sores applied locally as antiseptic and antifungal (Arnold et al., 1993). Ethnopharmacological literature documents the use of *A. absinthium* in Europe and Pakistan as an antiseptic, antispasmodic, febrifuge, cardiac stimulant, for the restoration of declining mental function and inflammation of the liver, and to improve memory (Guarrera, 2005).

Moreover, wormwood is an aromatic spice, widely employed as a flavoring agent in wine and other alcoholic beverages. It is also used in soft drinks and some foods, especially confectionery and desserts (Canadanovic-Brunet et al., 2005).

Extracts of wormwood have been used as a muscle relaxer that is occasionally added to liniments and as a mild sedative to treat anxieties (Dewick, 2001). Some studies strongly indicated the protective effect of extract of *A. absinthium* L. against acute liver injury which may be attributed to its antioxidative and/or immunomodulatory activities, and thereby scientifically support its traditional use (Amat et al., 2010). Others findings suggested that *A. absinthium* is neuroprotective and may prove to be useful adjunct in the treatment of stroke (Bora and Sharma, 2010). In other reports methanol extract of the plant species showed anti-diabetic and anti-cholesterolemic activities (Girija et al., 2011) and antimicrobial properties (Karaman et al., 2003). Moreover a recent study showed that *A. absinthium*'s extracts suppresses tumor necrosis factor alpha and accelerates healing in patients with Crohn's disease (Krebs et al., 2010).

Medicinal value of plants is related to their phytochemical components and their secondary metabolites such as essential oils, phenolic and flavonoids compounds (Mohammedi and Atik, 2011) and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Ghasemzadeh et al., 2012).

Wormwood essential oils have been widely used mainly due to their antimicrobial (Juteau et al., 2003), antiparasitic (Rücker et al., 1992), antihelmintic (Tariq et al., 2009) or hepatoprotective (Gilani and Janbaz, 1995) properties. Free radical scavenging activity of *A. absinthium* extracts have been reported both *in vitro* and *in vivo* (Astghik, 2003; Jasna et al., 2004). Antioxidant

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activity has been attributed specially to methanol extract of the species (Canadanovic-Brunet et al., 2005).

The phenolic and flavonoid compounds present in the plants are natural antioxidants (Oboh et al., 2008; Bahramikia et al., 2009). They also have anti-mutagenic and anti-cancerogenic properties (Kampa et al., 2004), cardioprotective (Caccetta et al., 2000), anti-inflammatory (Canadanovic-Brunet et al., 2006) and antimicrobial activity (Stanojević et al., 2010).

In humans, oxidative stress resulting in free radicals contribute to more than one hundred disorders including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis and cancer (Pourmorad et al., 2006). A number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively added to foodstuffs, although their use has begun to be questioned because of their toxicity (Canadanovic-Brunet et al., 2006), so there is considerable interest in preventive medicine and in the food industry in the development of natural antioxidants obtained from botanical sources, especially herbal plants (Djilas et al., 2003).

In recent times, the field of modern medicines has increasingly focused interest in synthesizing drugs from plant origin. The valorization of natural resources such as plant extracts and products of plant secondary metabolism, particularly used by the popular tradition is for a great interest due to their potential as a source of natural antioxidants and biologically active compounds (Celiktas et al., 2007). Efforts have been made in many regions of the world to identify plants having medicinal properties effective against various modern diseases. Tunisia, located in the Mediterranean area, has a large number of medicinal and aromatic species. More than 500 species out of 2103 (approximately 25% of the total flora) are considered for therapeutic use (Le Floc'h, 1983).

Even investigations on chemistry and biological activities of *A. absinthium* L. originating from different area in the world have been reported previously (Baykan Erel et al., 2012); there is no report regarding this species in Tunisia. In this paper, the chemical composition of the essential oils and total phenolic and flavonoid contents of methanolic extracts obtained separately from leaves and flowers of *A. absinthium* L. originated from Tunisia was assessed for the first time. Furthermore, the antioxidant activity of essential oils and methanol extracts was tested based on two complementary tests and discussed according to essential oil chemical profiles and the phenolic and flavonoid contents for the methanol extract of the two analyzed plant organs.

2. Materials and methods

2.1. Plant material

Flowers and leaves of the studied species were collected separately at floral stage of the plant from the region of Bizerte (North East of Tunisia). Collective sample was constituted of a mixture of a randomized collected 35 individual plants. Before analyses, aerial parts were air-dried at room temperature for two weeks. A voucher specimen (ARTABS-2012) of the studied species was deposited at the Herbarium of the Faculty of Sciences of Tunis for future reference.

2.2. Essential oil extraction and identification

The essential oils have been extracted from 100 g air-dried leaves and flowers separately by hydrodistillation for 3 h, using a Clevenger-type apparatus. Essential oil yields were estimated on the basis of the dry weight of plant material. The essential oils were dried using anhydrous sodium sulphate and then stored in sterile tubes at 4 °C until analyses.

The essential oils composition was identified by GC–FID and GC–MS analyses. GC analysis was performed in triplicate by an Agilent 6980 gas chromatograph equipped with a flame ionization detector and split-splitless injector attached to HP-INNOWAX polyethylene glycol capillary column (30 m × 0.25 mm). One microliter of the sample (dissolved in hexane as 1/50 (v/v)) was injected into the system.

The identification of the essential oil was performed using a Hewlett Packard HP5890 series II GC–MS equipped with a HP5MS column (30 m × 0.25 mm). The carrier gas was helium at 1.2 ml/min. Each sample (1 μl) was injected in the split mode (1:20), the program used was isothermal at 70 °C, followed by 50–240 °C at a rate of 5 °C/min, then held at 240 °C for 10 min. The mass spectrometer was an HP 5972 and the total electronic impact mode at 70 eV was used. Oil components were identified by comparison of their retention indices determined with reference to a homologous series of C9–C24 n-alkanes and with those of authentic standards. Identification was confirmed by comparison of their mass spectra with those recorded in NIST08 and W8N08 libraries. Component relative percentages were obtained from GC–FID peak areas without correction factors.

2.3. Methanolic extract preparation

For each sample, 2 g of the air-dried aerial parts, finely ground in a mortar grinder mill, was extracted with 50 ml methanol (80% (v/v)) for 24 h in a water bath shaker maintained at room temperature. The extract was filtered using a 0.45 μm Millipore filter and stored in a brown bottle at 4 °C prior to further analysis.

2.4. Total phenolic and flavonoid contents

The total phenolic content of leaves and flowers was assessed using the Folin–Ciocalteu reagent, following Singleton and Rosi's (1965) method based on the reduction of a phosphowolframate–phosphomolybdate complex by phenolics to blue reaction products. An aliquot of each diluted sample extract (0.5 ml) was mixed with 2 ml Folin–Ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate solution (7.5%) was added. After incubation (90 min) in dark, the absorbance at 760 nm was read *versus* the prepared blank. The total phenolic content of the plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. All samples were analyzed in three replications.

Flavonoid contents were estimated according to aluminum chloride colorimetric method (Djeridane et al., 2006). One milliliter of diluted extract was mixed with 1 ml of 2% AlCl3 methanolic solution. After incubation at room temperature for 15 min, the absorbance was measured at 430 nm. Total flavonoids were expressed as mg rutin equivalent/g DW (mg CE/g DW), through the calibration curve of rutin (0–400 μg/ml range). All samples were analyzed in three replications.

2.5. Antioxidant activity of essential oils and methanol extracts

2.5.1. Free radical-scavenging activity

The DPPH radical scavenging capacity was measured according to Hanato et al. (1988). 1 ml of plant extract (at different concentrations in methanol) was mixed with 0.5 ml of 0.2 mM DPPH methanolic solution. The reaction was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm against a blank (methanol solution). The scavenging activity was estimated using the following equation: Scavenging effect (%) = $[100 \times (A_c - A_S/A_c)]$, where A_c is the