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Variation of *Malva sylvestris* essential oil yield, chemical composition and biological activity in response to different environments across Southern Italy

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ABSTRACT

Mallow (*Malva sylvestris* L.) grows wild in many countries, and the amount of drug required as functional food or even pharmafood, due to its both medicinal and industrial importance, is produced almost entirely from wild harvest. We studied the effect of environment on crop yield, biological activity and chemical composition of the essential oils of different mallow samples, that were cultivated at sixteen experimental sites in South-central Italy (Molise) in different growing environments. GC–MS analysis of the essential oils revealed the presence of phenolics and fatty acids that were the main compounds in all the samples but their percentages in each plant were greatly different. Antioxidant activities of the essential oils were evaluated by DPPH radical-scavenging activity and FRAP assay. Antimicrobial activity was determined by using the broth dilution method. Samples were also evaluated for their anti-inflammatory properties verifying their inhibitory effects on nitric oxide production in LPS-stimulated RAW 264.7 cells. All essential oils inhibited NO production in cell supernatants in a dose-dependent manner. Moreover, antiproliferative activity was assessed on three human cancer cell lines: breast cancer cells MCF-7 and SKBr3, and melanoma cells C32. Our study demonstrates that the crop performance was greatly influenced by the pedo-climatic conditions, and particularly fertility of the environment and the crop management (water supply) that increased also mallow crop yield. The results of this study provide new knowledge to produce adequate quality of mallow oil.

1. Introduction

Malva sylvestris L. (Malvaceae), usually known as common mallow, is native of Europe, North Africa and Asia, and is present in all the Italian regions. Its traditional use has been documented since a long-time ago, although little clinical evidence is available. Already among the Romans, mallow was used for its emollient and laxative properties, and in popular medicine is broadly used for different inflammatory conditions in the form of infusions, decoctions, poultices, liniments, lotions, baths and gargles (Barros et al., 2010; Prudente et al., 2013). Edible uses are concerned with folk gastronomy: young leaves and flowers are eaten raw in salads, leaves and shoots are consumed in soups and as boiled vegetables, while juice is prepared with the entire plant, including the root (Guarrera and Leporatti 2007). Besides the folk culinary use, nowadays some other pharmacological and clinical effects are frequently mentioned. The ethnopharmacological literature has reported a long history of recognition for its potent anti-inflammatory, antioxidant, anticancer and antiulcerogenic properties (Gasparetto et al., 2012; Benso et al., 2015). It is also used as bronchodilator, expectorant, antitussive, anti-diarrheal and is highly recommended for acne and skin care, and as antiseptic, emollient and demulcent. (Coelho de Souza et al., 2004; Carvalho, 2005; Quave et al., 2008; Della Greca et al., 2009; Leporatti and Ghedira, 2009; Neves et al., 2009; Prudente et al., 2013). Recently, *M. sylvestris* ethanol extract demonstrated a very promising hypoglycemic activity in comparison to the commercial drug acarbose (Loizzo et al., 2016). Newly, mallow is playing an important role in sustainable agricultural practice, as mallow plant extract is effective in the control of com-

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mon bean anthracnose (Andrade Pinto et al., 2010) and exerts antifungal activity (Parveen et al., 2014). Phytochemical studies of this herb revealed the presence of polysaccharides, anthocyanins, coumarins, tannins, flavones, flavonols, anthocyanidines, leucoanthocyanidines, mucilage and terpenoids like sesquiterpenes, diterpenes, monoterpenes (Prudente et al., 2013). However, despite mallow blue tea prepared from flowers of *M. sylvestris* is one of the most common consumed herbal teas worldwide, used as a beverage but also in folk medicine and phytotherapy, only one previous paper describes the composition of the essential oil (Usami et al., 2013).

The phytochemical composition and biological activity of plants is strongly influenced by the environment, and this is particularly true for medicinal plants, as we reported in recent studies on sage (Russo et al., 2013) and chamomile (Formisano et al., 2015). Yield is the result of a complex interaction between genotype, soil, weather and management factors on growth and development processes (Peltonen-Sainio et al., 2010). Temperature, irradiation and precipitation directly and indirectly influence yield (Takashima et al., 2013). Temperature mainly determines the productivity and quality (Manukyan and Schnitzler, 2006). Water availability is a key to produce assimilates during different growth phases used both for vegetative dry matter production (Delfine et al., 2001) and either as reserve carbohydrates stored in the vegetative tissue (Tognetti et al., 2003). Particularly, in medicinal plants, a moderate water stress condition, while reducing the accumulation of dry matter, increases the yield of essential oil and modifies the composition in respect to the well watered control (Delfine et al., 2005; Formisano et al., 2015). In general, several studies have shown the effects of environment conditions on plants yield but few on medicinal plants and no one on mallow.

The current study therefore has the objective to study the effects of the environment and water availability on yield and on chemical composition of essential oils of *M. sylvestris* collected in different locations. Moreover, in order to valorize mallow plant as functional food or even pharmafood, here we also describe the antioxidant, antibacterial, anti-inflammatory and antiproliferative activities of the oils in comparison with their chemical composition. In fact, despite the widespread use of *M. sylvestris* for its astringent properties and its capacity to sooth tissue irritation and reduce inflammations, few studies have been made on different extracts to evaluate these biological effects (Conforti et al., 2008; Barros et al., 2010; Prudente et al., 2013; Loizzo et al., 2016) while no study has been ever carried out on the biological activities of *M. sylvestris* essential oil.

2. Materials and methods

2.1. Plant material

The study was conducted in the 2009–2010 growing season at sixteen experimental field sites in the Molise Region (South-central Italy). The fields were located in three different macro environment from the pedo-climatic point of view: flat land, low hill and high hill (Table 1).

The pedo-climatic environment of each experimental site was classified according to its fertility considering together: the texture of the soil, organic matter content, nutrient content, water availability and climate. The different sites, therefore, have been classified by their environmental fertility as low (L), medium (M) and high (H). Particularly, the site, and their experimental code, were (Table 1): Bagnoli del Trigno (high hill, H, M1), San Polo Matese (low hill, H, M2), Tavenna (low hill, H, M3), Termoli (flat land, H, M4), Bagnoli del Trigno (high hill, M, M5), Guglionesi (flat land, H, M6), Bagnoli del Trigno (high hill, M, M7), Acquaviva Collecroce (low hill, H, M8), Montaquila (low hill, H, M9), Campomarino (flat land, H, M10), Campomarino (flat land, H, M11), Bagnoli del Trigno (high hill, M, M12), Pietracupa (high hill, L, M13), Agnone (high hill, L, M14), Acquaviva Collecroce (low hill, L, M15) and Bagnoli del Trigno (high hill, H, M16). Guglionesi (M6) and Campomarino (M11) were the irrigated fields, while in the others sites were a rainfed crop. Molise region are positioned on the eastern side of the Apennines watershed, and have a typical Mediterranean climate of interior lands in south-central Italy.

The experiment was arranged in a block design with four replications (20 m2 each plot, consisted of 6 rows of mallow 4.44 m long spaced 75 cm apart) at each site. The used variety of mallow (cv mauritiana) were selected and certified. Before fertilizer applications, soil samples (0–30 cm) were taken from each block and analyzed according to standard procedures (SISS, 2000). The soil texture was characterized average as clay at high hill, and as clay-sand at low hill and flat land (Table 1). In general, organic matter contents at all sites averaged 1.35%. Total N content followed the ranking position observed for organic matter content. The soil profile was overall uniform, containing good amounts of available P (phosphorous, overall mean 26.5 $\mu g\, g^{-1}$) and medium quantities of exchangeable K (potassium, overall mean 151 $\mu g\, g^{-1}$). Soils had very low active CaCO3, and pH was average neutral; salinity was low.

The altitude of the experimental field sites was different, starting from 82 until 854 m a.s.l. The area has a typical Mediterranean climate of lands in southern Italy, with higher precipitation in high hill with respect to low hill and flat land, and more cold in high hill with respect to low hill and flat land (Table 1). A conventional meteorological station placed in each field site recorded climatic data. Overall, weather conditions reflected the specific orographic position (distance from the sea, Eat-West appearance, elevation above the sea level) of each experimental site.

Durum wheat (*Triticum durum* L.) was the previous crop in all cases. After ploughing (35 cm depth), 90 kg P ha-1, 90 kg K2O ha-1 and 100 kg N ha-1 were applied. Planting of mallow was done at 7 plants m-2 (Delfine, 2009). Each field was surrounded by a buffer strip to allow for uniform growing conditions. Weeds were manually controlled. Yield values were based on a hand made harvesting. The plant dry matter weight was obtained after oven drying at 35 °C for 72 h.

At each site, 50 sample plants were selected depending on the population size with a minimum distance of 100 m, then mixed for homogenization, and used in three replicates for essential oil extractions.

2.2. Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl); 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ); acetic acid; sodium acetate; hydrocloric acid; ferric chloride; sodium carbonate; Folin & Ciocalteau's phenolic reagent; sodium nitrite; aluminium chloride; sodium hydroxide; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX); gallic acid; quercetin; *tert*-butyl-4-hydroxy toluene (BHT) purchased from Sigma Chemical Co. (St. Louis, MO) and methanol purchased from Carlo Erba (Milano, Italy). Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium, phosphate buffered saline (PBS), fetal bovine serum, L-glutamine, penicillin/streptomycin, trypan blue, Griess reagent and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich S.p.A. (Milan, Italy).

Human cancer cell lines C32, MCF-7 and SkBr3 were obtained from American Type Culture Collection (Nos CRL-1585, HTB-22 and HTB-30, respectively). Murine monocytic macrophage cell line RAW 264.7 were purchased from ATCC no. CRL-2278, UK.